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ORIGINAL ARTICLE

Factors Affecting Healthcare Employee Satisfaction and Their Relationship to Patient Satisfaction in Community Health Centres

Alva Cherry Mustamu[1, 2]

Abstract

Background/Aim: In the realm of healthcare services, the satisfaction of medical professionals in their workplace environment has become a central concern closely associated with the perceived satisfaction levels of patients. This research investigated the intricate relationship between employee satisfaction and patient satisfaction within the context of community health centres. The study aimed to identify specific factors of employee satisfaction, such as compensation, supervision and work environment, that potentially influence the level of patient satisfaction.

Methods: Employing a cross-sectional descriptive design, a total of 162 employees and 276 patients from 13 community health centres participated in this study, conducted between June and September 2023. The participants were selected based on specific criteria, including age above 17, fluency in Indonesian and ability to complete the questionnaires. The study utilised two questionnaires: one measuring patient satisfaction with aspects of healthcare service and the other gauging employee satisfaction concerning management practices in community health centres. Both questionnaires employed Likert scale measurements to gather responses.

Results: The findings indicated a significant influence of investigated factors on patient satisfaction. Notably, compensation of employees emerged as a crucial factor significantly affecting patients satisfaction, aligning with previous research emphasising its importance in healthcare settings. Conversely, other factors such as supervision, work relationships, nature of work, opportunities for status change and work interactions with colleagues of employees did not exhibit a significant impact on patient satisfaction.

Conclusions: The study findings conformed the importance of compensation of employees a pivotal factor in designing strategies to improve patient satisfaction in community health centres. While other factors like supervision and work interactions did not demonstrate statistically significant correlations, this suggests the potential for more complex relationships that warrant further investigation. Identifying specific factors that influence patient satisfaction in community health centres can significantly contribute to enhancing healthcare service quality and overall patient satisfaction in the future.

Key words: Employee satisfaction; Patient satisfaction; Community health centres; Compensation; Work environment.

1. Department of Nursing, Ministry of Health Polytechnic Sorong, West Papua, Indonesia.
2. Quality Assurance and Education Development Centre, Health Polytechnic of the Ministry of Health of the Republic of Indonesia, Sorong City, West Papua Province, Indonesia.

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Corresponding author:
ALVA CHERRY MUSTAMU
E: alvamustamu@poltekkessorong.ac.id
E: alvamustamu@gmail.com

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Introduction

In the realm of healthcare services, the satisfaction of medical professionals in their workplace environment has become a central concern closely associated with the perceived satisfaction levels of patients.[1-3] Within healthcare facilities, factors such as service quality, treatment costs, facilities and communication have been recognised as primary drivers of patient satisfaction.[4-7] Meanwhile, specific aspects of medical professional satisfaction, such as compensation, supervision, working conditions and career development opportunities, also play a significant role in shaping the performance and services provided by healthcare workers.[8-10] Primary healthcare centres, particularly community health centres, hold a pivotal role in delivering healthcare services to the community.[11-13] Thus, a profound understanding of the factors influencing patient satisfaction in the community health centre environment becomes highly crucial.

Although previous research has provided an overview of the relationship between medical staff satisfaction and patient satisfaction, there is still room to further explore the correlation and influence of certain factors within the community health centre setting.[14-19] The limitations in understanding provide a platform for deeper research to delve into the specific role of medical staff satisfaction factors in influencing patient satisfaction in community health centres. The understanding of the significance of the community health centre environment in influencing patient satisfaction is also an aspect that has not been entirely disclosed in previous literature.[20-27] Hence, this research is expected to fill this knowledge gap by exploring the complexity of the relationship between medical staff satisfaction and patient satisfaction, particularly within the community health centre environment.[28, 29]

The aim of this study was to analyse the impact of specific factors related to medical staff satisfaction, such as compensation, supervision and the work environment, on patient satisfaction in community health centres.

Methods

This study employed a descriptive cross-sectional research design. The research participants consisted of 162 employees and 276 patients from 13 community health centres between June and September 2023 in Teluk Wondama Regency, West Papua Province, Indonesia. They were selected using a combination of purposive sampling and cluster sampling techniques. Purposive sampling ensured that participants met specific criteria, including: patients aged 17 and above, capable of speaking and understanding the Indonesian language, not excessively confused or unwell to complete the questionnaire and willing to participate in the study, along with all employees willing to be respondents. Respondents who did not fully complete the questionnaire and the heads of the health centres were excluded from this study. Cluster sampling was employed to select diverse community health centres as clusters and then participants within these clusters. This approach facilitated the inclusion of relevant individuals and varied healthcare facilities, enhancing the representativeness and generalisability of the study findings. This study employed the age of 17 as the cut-off age for respondents due to considerations related to policies or regulations in the research environment in Indonesia, where the age of 17 is often regarded as the age of majority or the age at which individuals are considered to have attained a sufficient level of cognitive maturity to participate in research. Additionally, at this age, individuals tend to be more independent in utilising healthcare services without the need for parental or guardian presence. The sample size calculation was conducted with a confidence level of 95 %. Each respondent required approximately 10 minutes to complete the entire questionnaire.

Measurement - questionnaires and variables

The Patient Satisfaction Questionnaire regarding community health centre services was designed by researcher to measure the anticipated patient needs when receiving services at community health centres. This scale was developed using the patient satisfaction domains towards community health centre services, consisting of: Service quality, Treatment costs, Facilities and Communication. Participant responses were provided using a 5-point Likert scale. Each domain comprised three questions with five response options: very dissatisfied (1), dissatisfied (2), moderately satisfied (3), satisfied (4) and very satisfied (5). Furthermore, the questionnaire for this research has undergone validity and reliability testing.

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The Employee Satisfaction Questionnaire regarding community health centre management was designed by researcher to measure how well the needs of employees could be facilitated by the management of the community health centres. This scale was developed using domains of employee satisfaction with the community health centre workforce, including: Compensation, Supervision, Nature of work, Work interactions with colleagues, Working environment, Career development opportunities and Job security. Participant responses were gathered using a 5-point Likert scale. Each domain consisted of three questions with five response options: very dissatisfied (1), dissatisfied (2), moderately satisfied (3), satisfied (4) and very satisfied (5). Furthermore, the questionnaire for this research has undergone validity and reliability testing.

Data collection and analysis

The data collection process commenced by obtaining permission from the thirteen heads of the community health centres. Subsequently, the purpose and objectives of the research were explained, followed by requesting informed consent and questionnaire completion for the community health centre employees. For patient respondents, data were collected after they received services, preceded by an explanation of the research and signing of informed consent.

The collected data were analysed using the *Jamovi* software.[30] The analysis involved examining the relationship between employee satisfaction domains and patient satisfaction using Pearson correlation and multiple linear regression. The statistical analysis conducted in this study focused on examining the demographics of employees and patients at the community health centre, as well as their satisfaction levels and the correlation between employee satisfaction and patient satisfaction. Descriptive statistics were used to analyse demographic data, including age, gender, education, employment status, marital status, family dependents and other relevant factors among employees and patients. Additionally, correlation analyses were performed to investigate the relationship between employee satisfaction factors and patient satisfaction, as well as the influence of specific demographic background factors of employees on patient satisfaction. The analysis revealed intriguing differences in demographics between employees and patients, with notable variations observed in age, gender, education, employment status and other factors. Furthermore, regression analyses were employed to assess the influence of employee satisfaction factors and demographic background factors on patient satisfaction at the community health centres. These analyses provided insights into the factors that contribute to patient satisfaction and highlighted the significance of employee satisfaction, employment status and health insurance in influencing patient satisfaction levels.

Validity and reliability analysis

The questionnaire validity tests indicated a range of values ranging from 0.630 to 0.776 with a Cronbach's α value of 0.972 for patient satisfaction and 0.780 to 0.791 with a Cronbach's α value of 0.908 for employee satisfaction.

Results

The demographic data of employees and patients at the community health centre revealed intriguing differences between these two groups (Table 1). The average age of employees was approximately 39.8 years, ranging from 24 to 59 years old, while patients had an average age of around 40.6 years, with an age range between 17 and 65 years. A majority of the employees were females, accounting for 52.5 %, whereas among the patients, the percentage of females reached 50.4 %. In terms of educational attainment, a substantial proportion of employees held a Bachelor's degree (55.6 %), while the majority of patients had completed their high school education (24.3 %). Other differences emerged concerning employment status; a significant portion of employees had non-permanent employment (51.2 %), while the majority of patients had permanent jobs (29.7 %). Furthermore, noticeable differences were observed in marital status and family dependents between the two groups. Most employees were married (54.3 %) and had family dependents (54.3 %), whereas the majority of patients were unmarried (45.7 %) and had family dependents (52.2 %).

From the data (Table 2), it is observed that the overall average employee satisfaction level stood at 82.8 ± 4.15, indicating a relatively high satisfaction level tendency. Regarding the various domains of satisfaction, the survey results exhibited variability in employee satisfaction lev-

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Table 1: Sociodemographic data of the sample

Variable, N (%)	Employees	Patients
Age, mean ± SD (Min-Max)	39.8 ± 9.94 (24-59)	40.6 ± 13.8 (17-65)
Work experience, mean ± SD (Min-Max)	13.3 ± 7.17 (1-25)	-
Gender		
Female	85 (52.5)	139 (50.4)
Male	77 (47.5)	137 (49.6)
Highest education attainment		
No education	-	52 (18.8)
Elementary school (SD)	-	50 (18.1)
Junior high school (SMP)	-	59 (21.4)
Senior high school (SMA)	-	67 (24.3)
College/University	-	48 (17.4)
Diploma III	72 (44.4)	-
Bachelor's degree	90 (55.6)	-
Employment status		
Unemployed	-	95 (34.4)
Permanent employment	79 (48.8)	82 (29.7)
Temporary employment	83 (51.2)	99 (35.9)
Current position		
Administrative officer	14 (8.6)	-
Pharmacist	15 (9.3)	-
Midwife	29 (17.9)	-
Nutritionist	15 (9.3)	-
Environmental health officer	22 (13.6)	-
Laboratory analyst	22 (13.6)	-
Nurse	45 (27.8)	-
Family responsibilities		
No	74 (45.7)	144 (52.2)
Yes	88 (54.3)	132 (47.8)
Marital status		
Single	74 (45.7)	84 (30.4)
Married	88 (54.3)	103 (37.3)
Widowed/Divorced	-	89 (32.2)
Dependent children		
No	74 (45.7)	-
Yes	88 (54.3)	-
Experience of training or special certification in health sector		
No	2 (1.2)	-
Yes	160 (98.8)	-
Work experience in the health sector		
No	2 (1.2)	-
Yes	160 (98.8)	-
Distance from community health centre		
Not close	84 (51.9)	-
Close	78 (48.1)	-
Additional workload apart from work at community health centres		
No	135 (83.4)	-
Yes	27 (16.6)	-
Health insurance		
No	1 (0.6)	142 (51.4)
Yes	161 (99.4)	134 (48.6)
Frequency of visits to community health centres per year		
Once	-	140 (50.7)
More than 1 time per year	-	136 (49.3)
Chronic medical conditions		
No	-	127 (46.0)
Yes	-	149 (54.0)

CONTENT

els across different job aspects. For instance, the average score for Compensation stood at 12.1, ranging from 9 to 15, indicating a relatively high level of satisfaction regarding the compensation received by the employees. However, aspects such as Supervision, Nature of work, Work interactions with colleagues, Working environment, Career development opportunities and Job security showed variations in satisfaction scores, although generally maintaining a relatively positive range between 9 to 15.

Table 2: Scores for the overall average employee satisfaction level and satisfaction domains at community health centres

Variables	Mean	SD	Min	Max
Employee satisfaction	82.8	4.15	73	93
Domains				
Compensation	12.1	1.55	9	15
Supervision	11.7	1.41	9	15
Nature of work	11.8	1.49	9	15
Work interactions with colleagues	11.8	1.55	9	15
Working environment	11.9	1.56	9	15
Career development opportunities	11.7	1.44	9	15
Job security	11.8	1.46	9	15

SD: standard deviation; Min: minimum; Max: maximum; Values are based on Likert 5 scale.

The overall average patient satisfaction rate was approximately 48, with a standard deviation of 2.98, indicating that, generally, patient satisfaction fell within a moderate range (Table 3). Upon examining the satisfaction domains, it is evident that the quality of service, cost of treatment, facilities and communication all had average scores above 11 (within the range of 9 to 15). This suggests that patients tended to provide positive assessments regarding these aspects in the services they receive at the community health centre.

Table 3: Scores for the overall average patient satisfaction level and service-related satisfaction domains at community health centres

Variables	Mean	SD	Min	Max
Patient satisfaction	48.0	2.98	40	57
Domains				
Quality of service	12.1	1.37	9	15
Cost of treatment	11.9	1.36	9	15
Facilities	11.9	1.37	9	15
Communication	12.1	1.36	9	15

SD: standard deviation; Min: minimum; Max: maximum; Values are based on Likert 5 scale.

Table 4 presents the correlation results between employee satisfaction and patient satisfaction at the community health centre. It is shown that the correlation between employee satisfaction and patient satisfaction had a Pearson's r value of -0.169, with a p-value of 0.031. This indicates a statistically significant yet weak correlation between employee satisfaction and patient satisfaction at the community health centre. Furthermore, the table also illustrates the correlation between various aspects of employee satisfaction (such as compensation, supervision, nature of work, work interactions with colleagues, working environment, career development opportunities and job security) with various aspects of patient satisfaction (quality of service, cost of treatment, facilities and communication). In some cases, there were significant correlations between specific aspects of employee satisfaction and specific aspects of patient satisfaction. For instance, compensation showed a significant positive correlation with the quality of service, cost of treatment, facilities and patient communication. However, several other aspects of employee satisfaction, such as the nature of work, work interactions with colleagues and the working environment, did not demonstrate significant correlations with patient satisfaction.

The table 5 indicates that within the gender category, the correlation between this variable and patient satisfaction domains (quality of service, treatment cost, facilities and communication) was relatively weak with correlation coefficients ranging from -0.058 to 0.069 and not statistically significant ($p > 0.05$). Conversely, the age variable showed a significant correlation with treatment cost (Pearson's $r = 0.195$, $p = 0.013$) and the highest education level had a significant correlation with treatment cost (Pearson's $r = 0.160$, $p = 0.041$). Employment status revealed a significant correlation with treatment cost (Pearson's $r = 0.165$, $p = 0.035$). Additional work burden besides work at the community health centre did not exhibit a significant correlation with patient satisfaction domains. Furthermore, other variables such as family dependents, marital status, dependent children, specific health sector training, previous work experience in the health sector, proximity to the health centre and having health insurance did not show significant correlations with patient satisfaction domains.

Employee's compensation had a significant influence on patient satisfaction, with a regression coefficient (b) of 0.408 (95 % CI: 0.098 to 0.717) (Table 6). This indicates that for each one-unit increase in employee compensation satisfaction,

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Table 4: Correlation between employee satisfaction and patient satisfaction at the community health centre

Employee satisfaction domain	Patient satisfaction				
	Corr¶	Quality of service	Cost of treatment	Facilities	Communication
Compensation	r	0.191 *	0.098	0.165*	0.155
	p	0.015	0.216	0.036	0.049
Supervision	r	0.018	-0.128	-0.080	-0.15
	p	0.824	0.105	0.314	0.057
Nature of work	r	-0.025	-0.116	-0.204**	-0.059
	p	0.751	0.141	0.009	0.456
Work interactions with colleagues	r	-0.005	-0.088	-0.092	-0.118
	p	0.949	0.263	0.246	0.135
Working environment	r	-0.104	-0.027	0.036	0.024
	p	0.186	0.735	0.653	0.760
Career development opportunities	r	0.061	0.044	-0.170*	-0.035
	p	0.444	0.582	0.031	0.662
Job security	r	-0.114	-0.107	-0.133	-0.098
	p	0.148	0.175	0.093	0.213
Employee satisfaction	r		**-0.169**		
	p		**0.031**		

*¶: Pearson's correlation, r: correlation coefficient, p: p-value; * p < 0.05, ** p < 0.01;*

Table 5: Correlation between employee demographics and patient satisfaction domains at community health centres

Employee demographics	Patient satisfaction				
	Corr¶	Quality of service	Cost of treatment	Facilities	Communication
Gender	r	0.061	-0.038	0.069	-0.058
	p	0.440	0.627	0.380	0.462
Age	r	0.015	0.195*	-0.037	0.076
	p	0.854	0.013	0.638	0.339
Highest education attainment	r	-0.038	0.160*	0.019	-0.015
	p	0.631	0.041	0.815	0.854
Length of employment	r	0.049	-0.083	-0.009	0.009
	p	0.537	0.296	0.912	0.910
Employment status	r	0.074	0.165*	0.053	0.129
	p	0.352	0.035	0.506	0.103
Family dependents	r	0.065	-0.031	0.096	0.005
	p	0.408	0.694	0.226	0.948
Marital status	r	-0.007	-0.034	0.018	-0.022
	p	0.926	0.672	0.817	0.782
Dependent children	r	-0.039	-0.009	0.026	0.077
	p	0.623	0.913	0.740	0.328
Health sector training or special certification experience	r	-0.076	-0.045	-0.120	-0.073
	p	0.336	0.566	0.130	0.358
Health sector work experience	r	0.067	0.073	0.044	-0.138
	p	0.395	0.358	0.582	0.080
Distance from the community health centre	r	0.117	-0.129	-0.027	0.037
	p	0.139	0.101	0.738	0.644
Additional work burden besides work at the community health centre	r	0.028	-0.027	0.140	0.000
	p	0.720	0.729	0.076	1.000
Health insurance	r	-0.114	-0.061	-0.112	-0.051
	p	0.148	0.439	0.155	0.518

*¶: Pearson's correlation, r: correlation coefficient, p: p-value; * p < 0.05, ** p < 0.01;*

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Table 6: The influence of specific employee satisfaction factors on patient satisfaction at community health centres

Predictor	Estimate	SE	t	p	b	95 % CI
Intercept	52.1898	4.908	10.634	<0.001		
Compensation	0.4078	0.158	2.580	0.011	0.4078	(0.098, 0.717)
Supervision	-0.1168	0.174	-0.671	0.503	-0.1168	(-0.456, 0.222)
Nature of work	-0.2264	0.161	-1.409	0.161	-0.2264	(-0.543, 0.090)
Work interactions with colleagues	-0.1555	0.154	-1.013	0.313	-0.1555	(-0.456, 0.145)
Working environment	-0.0658	0.149	-0.443	0.658	-0.0658	(-0.359, 0.227)
Career development opportunities	0.0575	0.165	0.347	0.729	0.0575	(-0.268, 0.383)
Job security	-0.2571	0.167	-1.544	0.125	-0.2571	(-0.595, 0.081)

SE: standard error, t: test value; p: p-value, b: regression coefficient, CI: confidence interval;

Table 7: The influence of specific sociodemographic factors of employees on patient satisfaction in community health centres

Predictor	Estimate	SE	t	p	b	95 % CI
Intercept	51.8881	4.5345	11.443	<0.001		
Gender	0.3769	0.5035	0.749	0.455	0.3769	-0.614 - 1.368
Age	0.0394	0.0246	1.606	0.110	0.0394	-0.008 - 0.087
Highest education attainment	0.2128	0.4911	0.433	0.665	0.2128	-0.753 - 1.179
Length of employment	-0.0108	0.0347	-0.312	0.756	-0.0108	-0.078 - 0.056
Employment status	1.0240	0.4978	2.057	0.041	1.0240	0.044 -2.004
Family dependents	0.1456	0.4893	0.298	0.766	0.1456	-0.814 - 1.106
Marital status	-0.0860	0.4971	-0.173	0.863	-0.0860	-0.666 - 0.494
Dependent children	0.1547	0.4819	0.321	0.749	0.1547	-0.796 - 1.106
Health sector training or special certification experience	-3.2796	2.2188	-1.478	0.142	-3.2796	-7.645 - 1.086
Health sector work experience	0.4221	0.4872	0.866	0.388	0.4221	-0.534 - 1.378
Distance from the community health centre	0.1050	0.5010	0.210	0.834	0.1050	-0.887 - 1.097
Additional work burden besides work at the community health centre	0.6441	0.4887	1.318	0.190	0.6441	-0.317 - 1.605
Health insurance	-6.4882	3.1577	-2.055	0.042	-6.4882	-12.685 - -0.292

SE: standard error, t: test value; p: p-value, b: regression coefficient, CI: confidence interval;

there was an associated increase of 0.408 units in patient satisfaction. However, other factors such as supervision, nature of work, work interactions with colleagues, working environment, career development opportunities and job security did not show a significant influence on patient satisfaction, as the regression coefficients (b) for these factors had statistically insignificant values. Nevertheless, these results suggest that employee compensation may be one of the important factors in influencing patient satisfaction at community health centres, while other factors may require further research for full understanding.

The Table 7 presents the influence of specific demographic background factors of employees on patient satisfaction in community health centres. The regression analysis results indicate that several demographic variables had varying degrees of influence on patient satisfaction. Employment status showed a statistically significant positive influence on patient satisfaction (b = 1.024, p = 0.041), suggesting that employees with permanent or stable employment status tended to contribute positively to patient satisfaction levels. Conversely, health insurance status demonstrated a statistically significant negative influence on patient satisfaction (b = −6.488, p = 0.042), indicating that employees without health insurance coverage may have lower levels of patient satisfaction.

Other demographic variables such as gender, age, highest education attainment, length of employment, family dependents, marital status, dependent children, health sector training or special certification experience, health sector work experience, distance from the community health centre and additional work burden besides work at the community health centre, did not exhibit statistically significant influences on patient satisfaction. For instance, gender (b = 0.377, p = 0.455), age (b = 0.039, p = 0.110), highest education attainment (b = 0.213, p = 0.665) and other variables showed no significant associations with patient satisfaction levels. These findings

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suggest that while certain demographic factors such as employment status and health insurance coverage may significantly impact patient satisfaction, other factors such as gender, age and education level may not play significant roles in determining patient satisfaction levels in community health centres.

Discussion

In this study, various aspects related to employee and patient satisfaction at community health centres were examined. This includes analysing each domain of employee and patient satisfaction as well as the sociodemographic factors of employees as service providers. The primary objective of this research was to identify specific factors of employee satisfaction that potentially influence the level of patient satisfaction within the community health centres environment.

The findings indicate that compensation and health insurance, which is also part of the compensation package, significantly influence patient satisfaction at the community health centres. However, other factors such as supervision, relationships with colleagues, nature of work, opportunities for status change and work interactions with colleagues did not demonstrate a significant influence on patient satisfaction.

These findings are consistent with prior research showing variability in the impact of employee satisfaction factors on patient satisfaction, especially in unique healthcare service environments such as community health centres.[27, 28, 31-33] This study confirms that compensation (salary, wages, benefits including health insurance) is a crucial factor influencing patient satisfaction, while other factors shown less significant or statistically insignificant impacts.

Employee compensation factors such as salary, wages, benefits including health insurance, significantly impact patient satisfaction in healthcare service environments such as community health centres.[12, 23, 34, 35] Several aspects explain why adequate compensation for employees positively affects patient satisfaction. Firstly, good compensation can enhance employee motivation and performance. When employees feel that their salary corresponds to their contributions and responsibilities, they tend to be more motivated to perform their duties better. This implies that they might provide better, more responsive and caring services to meet patient needs, thereby enhancing patient satisfaction.[36-41]

Secondly, adequate compensation can improve employee retention and workforce quality.[42-51] Satisfied employees with their compensation tend to stay longer in healthcare institutions, reducing employee turnover that can affect patient care continuity.[52, 53] It also enables employees to gain broader experiences, enhancing their patient care skills, which ultimately can positively affect patient satisfaction.[54-56] Additionally, employees satisfied with their compensation tend to feel more emotionally committed to their work, which can enhance the quality of services provided to patients.[57-59]

These findings underline the need to further consider compensation factors in designing strategies to improve patient satisfaction at Community health centres. While factors like supervision and work interactions did not show significant correlation, further research is necessary to unveil more complex relationships that could offer new insights into enhancing patient satisfaction.

There is potential for developing new models to enhance healthcare service quality. One such model could integrate key employee satisfaction factors into a more comprehensive framework. This model could account for the complex relationship between various employee satisfaction factors such as compensation, supervision, interactions with colleagues and others with patient satisfaction.[60-65] Moreover, this model could also involve employee demographic background variables such as age, education, employment status and health insurance to provide a more complete picture of factors influencing patient satisfaction.

Conclusion

Upon meticulous analysis, the research has revealed that employee's compensation significantly impacts patient satisfaction, unlike other factors such as supervision, interpersonal relationships in the workplace, the nature of work, prospects for career progression

and interactions among colleagues, which did not exhibit a substantial effect. Furthermore, this study also indicates that the environment within the healthcare centre, which has previously received limited research attention, does not notably influence patient satisfaction. These findings emphasise the necessity of reassessing strategies and directing attention toward compensation-related aspects while formulating policies to enhance patient satisfaction within the healthcare centre. Nonetheless, these findings open avenues for further exploration into additional factors influencing patient satisfaction and for developing a more comprehensive model to improve healthcare services extended to the community. Besides offering new insights, the findings of this research are anticipated to serve as a foundation for developing models and recommendations aimed at enhancing the healthcare service system in community health centres.

Ethics

The study was approved by the Ethics Committee of the Ministry of Health Polytechnic Slide below the supervision of the Ministry of Health of the Republic of Indonesia, decision No DM. 4.1/1/007/2023), dated 7 June 2023. Written informed consent was obtained from patients prior to their participation in the study and for publishing of the anonymised data. Additionally, the completion of the questionnaires was done anonymously. Permission to conduct the study was also obtained from the community health centre management. The study was organised and implemented based on the adherence to the Ethical Principles for Medical Research Involving Human subjects (The Declaration of Helsinki, 8th Revision, 2013).

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Conflicts of interest

The author declares that there is no conflict of interest.

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Data access

The data that support the findings of this study are available from the corresponding author upon reasonable individual request.

Author ORCID numbers

Alva Cherry Mustamu (ACM):
0000-0002-6682-514X

Author contributions

Conceptualisation, methodology, software, validation, formal analysis, investigation, resource management, data curation, original draft writing, revision, visualisation: ACM

References

1. Soriano-Vázquez I, Cajachagua Castro M, Morales-García WC. Emotional intelligence as a predictor of job satisfaction: the mediating role of conflict management in nurses. Front Public Health. 2023 Nov 10;11:1249020. doi: 10.3389/fpubh.2023.1249020.
2. Saray Kiliç H, Pazar B, İbrahimoğlu Ö, Naldan ME. Quality of care perceived by patients during cystoscopy and affecting factors. Medicine. 2023 Dec 15;102; e36314: doi: 10.1097/md.0000000000036314.
3. Alowais SA, Alghamdi SS, Alsuhebany N, Alqahtani T, Alshaya AI, Almohareb SN, et al. Revolutionizing

CONTENT

healthcare: the role of artificial intelligence in clinical practice. BMC Med Educ. 2023 Sep 22;23(1):689. doi.org/10.1186/s12909-023-04698-z.
4. Baryakova TH, Pogostin BH, Langer R, McHugh KJ. Overcoming barriers to patient adherence: the case for developing innovative drug delivery systems. Nat Rev Drug Discov. 2023 May;22(5):387–409. doi: 10.1038/s41573-023-00670-0.
5. Aiken LH, Lasater KB, Sloane DM, Pogue CA, Fitzpatrick Rosenbaum KE, Muir KJ, et al. Physician and nurse well-being and preferred interventions to address burnout in hospital practice: factors associated with turnover, outcomes, and patient safety. JAMA Health Forum. 2023 Jul 7;4(7):e231809. doi: 10.1001/jamahealthforum.2023.1809.
6. Lewis TP, McConnell M, Aryal A, Irimu G, Mehata S, Mrisho M, et al. Health service quality in 2929 facilities in six low-income and middle-income countries: a positive deviance analysis. The Lancet Glob Health. 2023 Jun 1;11(6):e862–70. doi: 10.1016/s2214-109x(23)00163-8.
7. Janerka C, Leslie GD, Gill FJ. Development of patient-centred care in acute hospital settings: A meta-narrative review. Int J Nursing Stud. 2023 Apr 1;140:104465. doi: 10.1016/j.ijnurstu.2023.104465
8. Berhanu KZ. Mediating role of job satisfaction on the relation between staff development and performance. Cogent Educ. 2023 Dec 31;10(1):2207408. doi.org/10.1080/2331186X.2023.2207408
9. Li P, Bastone A, Mohamad TA, Schiavone F. How does artificial intelligence impact human resources performance. Evidence from a healthcare institution in the United Arab Emirates. J Innov Know. 2023 Apr 1;8(2). doi: 10.1016/j.jik.2023.100340
10. Zhou Q, Sacramento C, Martinaityte I. Work meaningfulness and performance among healthcare professionals: The role of professional respect and participative management. J Business Res. 2023 Aug 1;163:113908. doi: 10.1016/j.jbusres.2023.113908.
11. Freijser L, Annear P, Tenneti N, Gilbert K, Chukwujekwu O, Hazarika I, et al. The role of hospitals in strengthening primary health care in the Western Pacific. Lancet Reg Health West Pac. 2023 Apr 1;33:100698. doi: 10.1016/j.lanwpc.2023.100698.
12. El Mouaddib H, Sebbani M, Mansouri A, Adarmouch L, Amine M. Job satisfaction of primary healthcare professionals (public sector): A cross-sectional study in Morocco. Heliyon. 2023 Sep 1;9(9):e20357. doi: 10.1016/j.heliyon.2023.e20357.
13. Getahun GK, Kinfe K, Minwuyelet Z. The role of community-based health insurance on healthcare seeking behavior of households in Addis Ababa, Ethiopia. Prev Med Rep. 2023 Aug 1;34:102234. doi: 10.1016/j.pmedr.2023.102234.
14. You W, Cusack L, Donnelly F. A lack of nurse autonomy impacts population health when compared to physician care: an ecological study. Sci Rep. 2023 Jul 25;13(1):12047. doi: 10.1038/s41598-023-38945-6.
15. Tian Y. A review on factors related to patient comfort experience in hospitals. J Health Popul Nutr. 2023 Nov 8;42(1):125. doi:10.1186/s41043-023-00465-4.
16. Čadek M, Täuber S, Lawrence BJ, Flint SW. Effect of health-care professionals' weight status on patient satisfaction and recalled advice: a prospective cohort study. eClinicalMedicine. 2023 Mar 1;57. doi: 10.1016/j.eclinm.2023.101855.
17. Guo S, Zhang H, Chang Y, Zhang J, Chen H, Zhang L. The relationship between presenteeism among nurses and patients' experience in tertiary hospitals in China. Heliyon. 2023 Dec 1;9(12):e22097. doi: 10.1016/j.heliyon.2023.e22097.
18. Randa MB, Phale JM. The effects of high nurses' turnover on patient care: perspectives of unit managers in critical care units. Int J Africa Nurs Sci. 2023 Jan 1;19:100580. doi: 10.1016/j.ijans.2023.100580.
19. Li L, Cui X, Feng W. Enhancing patient satisfaction in cross-regional healthcare: a cross-sectional study in the knowledge-based healthcare landscape. J Knowl Econ. 2023 Dec 11; doi:10.1007/s13132-023-01685-z
20. Iyer R, Park D, Kim J, Newman C, Young A, Sumarsono A. Effect of chair placement on physicians' behavior and patients' satisfaction: randomized deception trial. BMJ. 2023 Dec 15;383:e076309. doi: 10.1136/bmj-2023-076309.
21. Hudson DM, Evans R. A Service evaluation of what satisfies our patients in magnetic resonance imaging. J Radiol Nurs. 2023 Dec 1;42(4):477–85. doi: 10.1016/j.jradnu.2023.07.007.
22. Arboleda AM. Reprint of: satisfaction with life and perception of healthcare services. Int J Hosp Manag. 2023 Jul 1;112:103516. doi:10.1016/j.ijhm.2023.103516.
23. Nazarian-Jashnabadi J, Rahnamay Bonab S, Haseli G, Tomaskova H, Hajiaghaei-Keshteli M. A dynamic expert system to increase patient satisfaction with an integrated approach of system dynamics, ISM, and ANP methods. Exp Sys Appl. 2023 Dec 30;234:121010. doi:10.1016/j.eswa.2023.121010
24. Arboleda AM. Satisfaction with life and perception of healthcare services. Int J Hosp Manag. 2023 Apr 1;110:103450. doi:10.1016/j.ijhm.2023.103450.
25. Godovykh M, Pizam A. Measuring patient experience in healthcare. Int J Hosp Manag. 2023 Jul 1;112:103405. doi:10.1016/j.ijhm.2022.103405.
26. Wu Q, Li N, Cai X, He Y, Du Y. Impact of indoor environmental quality (IEQ) factors on occupants' environmental perception and satisfaction in hospital wards. Building Env. 2023 Nov 1;245:110918. doi:10.1016/j.buildenv.2023.110918.
27. Shen X, Zhang H, Li Y, Qu K, Zhao L, Kong G, et al. Building a satisfactory indoor environment for healthcare facility occupants: A literature review. Building Env. 2023 Jan 15;228:109861. doi:10.1016/j.buildenv.2022.109861.
28. Hsu W, Chao YC, Yang CH. Factors influencing home care workers' loyalty in long-term nursing services. Humanit Soc Sci Commun. 2023 Jun 9;10(1):1–13. doi:10.1057/s41599-023-01819-3.
29. Arsat N, Lah NASN, Thomas DC, Soong SF, Chong LT, Sawatan W, et al. The effect of work setting and demographic factors on caring behaviour among nurses in the public hospitals and public health services, Sabah, Malaysia. BMC Nursing. 2023 Jun 8;22(1):194. doi:10.1186/s12912-023-01359-w.
30. Jamovi. about – jamovi [Internet]. [Cited: 8-Jun-2023]. Available at: https://www.jamovi.org/about.html.
31. Abdullahi AK, Mosanya AU, Bello N, Musa MK. Evaluation of job satisfaction among pharmacists working in public health facilities. Expl Res Clin Social Phar. 2023

CONTENT

Dec 1;12:100338. doi:10.1016/j.rcsop.2023.100338.
32. Rakhshani T, Fararouei M, Kianizadeh T, Kamyab A, Jeihooni AK. Investigating the factors affecting health care workers' intention to remain in villages. Heliyon. 2023 Apr 1 ;9(4):e15542. doi:10.1016/j.heliyon.2023.e15542.
33. Al-Bsheish M, Jarrar M, Al-Mugheed K, Samarkandi L, Zubaidi F, Almahmoud H, et al. The association between workplace physical environment and nurses' safety compliance: A serial mediation of psychological and behavioral factors. Heliyon. 2023 Nov 1;9(11):e21985. doi:10.1016/j.heliyon.2023.e21985.
34. Chee SY. Deinstitutionalized senior-friendly accommodations: A phenomenological exploration of deindividualized institutional life of older adults in senior living facilities. Int J Hosp Manag. 2023 May 1;111:103476. doi:10.1016/j.ijhm.2023.103476.
35. Etemad-Sajadi R, Heo CY, Clergue V. Instilling the core tenets of hospitality in healthcare services: The role of service assurance and social presence. Int J Hosp Manag. 2023 May 1;111:103492. doi:10.1016/j.ijhm.2023.103492.
36. Dorta-Afonso D, Romero-Domínguez L, Benítez-Núñez C. It's worth it! High performance work systems for employee job satisfaction: The mediational role of burnout. Int J Hosp Manag. 2023 Jan 1;108:103364. doi:10.1016/j.ijhm.2022.103364.
37. Wood S, Leoni S, Ladley D. Comparisons of the effects of individual and collective performance-related pay on performance: A review. Human Res Manag Review. 2023 Dec 1;33(4):100982. doi:10.1016/j.hrmr.2023.100982.
38. Jones DB, Tonin M, Vlassopoulos M, Winichakul KP. Paying for what kind of performance? Performance pay, multitasking, and sorting in mission-oriented jobs. Games Econ Behavior. 2023 Nov 1;142:480–507. doi:10.1016/j.geb.2023.08.002.
39. Liaquat M, Ahmed G, Ismail H, Ul Ain Q, Irshad S, Sadaf Izhar S, et al. Impact of motivational factors and green behaviors on employee environmental performance. Res Globalization. 2024 Jun 1;8:100180. doi:10.1016/j.resglo.2023.100180.
40. Camilleri AR, Dankova K, Ortiz JM, Neelim A. Increasing worker motivation using a reward scheme with probabilistic elements. Org Behavior Human Dec Proc. 2023 Jul 1;177:104256. doi:10.1016/j.obhdp.2023.104256.
41. Siraj N, Hágen I. Performance management system and its role for employee performance: Evidence from Ethiopian SMEs. Heliyon. 2023 Nov 1;9(11):e21819. doi:10.1016/j.heliyon.2023.e21819.
42. Fahim MGA. Strategic human resource management and public employee retention. Review Econ Polit Sci. 2018 Jan 1;3(2):20–39. doi:10.1108/REPS-07-2018-002.
43. Geisler M, Berthelsen H, Muhonen T. Retaining social workers: the role of quality of work and psychosocial safety climate for work engagement, job satisfaction, and organizational commitment. Human Serv Org Manag, Leadership Gov. 2019 Jan 1 ;43(1):1–15. doi:10.1080/23303131.2019.1569574.
44. Khalid K, Nawab S. Employee participation and employee retention in view of compensation. SAGE Open. 2018 Oct 1;8(4):2158244018810067. doi:10.1177/2158244018810067.
45. Fulmer IS, Gerhart B, Kim JH. Compensation and performance: A review and recommendations for the future. Pers Psych. 2023;76(2):687–718. doi:10.1111/peps.12583.
46. Bourini I, Jahmani A, Mumtaz R, Al-Bourini FA. Investigating the managerial practices' effect on Employee-Perceived Service Quality with the moderating role of supportive leadership behavior. Eur Res Manag Business Econ. 2019 Jan 1;25(1):8–14. doi:10.1016/j.iedeen.2018.11.001.
47. Florek-Paszkowska AK, Hoyos-Vallejo CA. A comprehensive bibliometric analysis and future research directions in the nexus of sustainable business practices and turnover intention. Clean Resp Cons. 2023 Dec 1;11:100146. doi:10.1016/j.clrc.2023.100146.
48. Hosen S, Hamzah SR, Arif Ismail I, Noormi Alias S, Faiq Abd Aziz M, Rahman MM. Training & development, career development, and organizational commitment as the predictor of work performance. Heliyon. 2024 Jan 15;10(1):e23903. doi:10.1016/j.heliyon.2023.e23903.
49. Cachón-Rodríguez G, Blanco-González A, Prado-Román C, Del-Castillo-Feito C. How sustainable human resources management helps in the evaluation and planning of employee loyalty and retention: Can social capital make a difference? Eval Program Plan. 2022 Dec 1;95:102171. doi:10.1016/j.evalprogplan.2022.102171.
50. Torquati JC, Raikes H, Huddleston-Casas CA. Teacher education, motivation, compensation, workplace support, and links to quality of center-based child care and teachers' intention to stay in the early childhood profession. Early Child Res Quart. 2007 Apr 1;22(2):261–75. doi:10.1016/j.ecresq.2007.03.004.
51. Abebe A, Assemie A. Quality of work life and organizational commitment of the academic staff in Ethiopian universities. Heliyon. 2023 Apr 1;9(4):e15139. doi:10.1016/j.heliyon.2023.e15139.
52. Fadare OO, Witry MJ, Gaither CA, Doucette WR, Schommer JC. What drives job satisfaction among community pharmacists? An application of relative importance analysis. Exp Res Clinical Soc Pharm. 2023 Mar 1;9:100237. doi:10.1016/j.rcsop.2023.100237.
53. Kim BJ, Choi CJW. Impact of compensation and willingness to keep same career path on burnout among long-term care workers in Japan. Human Res Health. 2023 Aug 17;21(1):64. doi:10.1186/s12960-023-00845-1.
54. Guerrero JG, Ali SAA, Attallah DM. The acquired critical thinking skills, satisfaction, and self confidence of nursing students and staff nurses through high-fidelity simulation experience. Clin Simul Nurs. 2022 Mar 1;64:24–30. doi:10.1016/j.ecns.2021.11.008.
55. Altinay L, Alrawadieh Z, Tulucu F, Arici HE. The effect of hospitableness on positive emotions, experience, and well-being of hospital patients. Int J Hosp Manag. 2023 Apr 1;110:103431. doi:10.1016/j.ijhm.2023.103431.
56. 56. Hannawa AF, Wu AW, Kolyada A, Potemkina A, Donaldson LJ. The aspects of healthcare quality that are important to health professionals and patients: A qualitative study. Pat Educ Couns. 2022 Jun 1 ;105(6):1561–70. doi:10.1016/j.pec.2021.10.016.
57. Lu M, Al Mamun A, Chen X, Yang Q, Masukujjaman M. Quiet quitting during COVID-19: the role of psychological empowerment. Humanit Soc Sci Commun. 2023 Aug 8;10(1):1–16. doi:10.1057/s41599-023-02012-2.
58. Susanto P, Hoque ME, Jannat T, Emely B, Zona MA, Is-

CONTENT

lam MA. Work-life balance, job satisfaction, and job performance of smes employees: the moderating role of family-supportive supervisor behaviors. Front Psychol. 2022;13. doi:10.3389/fpsyg.2022.906876.
59. Kim YH, Shin SI, Kim HK, Jun M, Wreen M. Advanced practice nurses' organization commitment: impact of job environment, job satisfaction, and person-organization fit. Asian Nurs Res. 2023 May 1;17(2):91–101. doi:10.1016/j.anr.2023.03.002.
60. Kossyva D, Theriou G, Aggelidis V, Sarigiannidis L. Outcomes of engagement: A systematic literature review and future research directions. Heliyon. 2023 Jun 1 ;9(6):e17565. doi:10.1016/j.heliyon.2023.e17565.
61. Haleem A, Javaid M, Pratap Singh R, Suman R. Exploring the revolution in healthcare systems through the applications of digital twin technology. Biomed Technol. 2023 Dec 1;4:28–38. doi:10.1016/j.bmt.2023.02.001.
62. Möckli N, Espinosa JA, Simon M, Meyer-Massetti C, Zúñiga F. Clarifying the muddy concept of home healthcare coordination: A comprehensive theoretical framework. Heliyon. 2023 Mar 1;9(3):e14243. doi:10.1016/j.heliyon.2023.e14243.
63. Paul M, Maglaras L, Ferrag MA, Almomani I. Digitization of healthcare sector: A study on privacy and security concerns. ICT Express. 2023 Aug 1;9(4):571–88. doi:10.1016/j.icte.2023.02.007.
64. Alshurideh MT, Al Kurdi B, Alzoubi HM, Akour I, Obeidat ZM, Hamadneh S. Factors affecting employee social relations and happiness: SM-PLUS approach. J Open Innov Technol Market Complex. 2023 Jun 1;9(2):100033. doi:10.1016/j.joitmc.2023.100033.
65. Fernández-Castro M, López M, Martín-Gil B, Rodríguez-Soberado P, Rivas-González N, Muñoz-Alvaredo L, et al. Nurses' evidence-based practice competence and hospital practice environment after specific training under the Best Practice Spotlight Organization® Programme. A cross sectional study. Nurse Educ Today. 2023 Jul 1;126:105808. doi:10.1016/j.nedt.2023.105808.

CONTENT

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ORIGINAL ARTICLE

Clinical and Pharmacological Analysis of Patients With Acute Coronary Syndrome Under 45 Years of Age: A Prospective Cohort Study

Saad Badai Nashtar,[1] Muhammed Mutar Nazzal,[2] Hayder Mohammed Faeq,[2] Afroz Abidi[3]

Abstract

Background/Aim: Acute coronary syndrome (ACS) in young people is a problem of growing concern. There is an increasing need to evaluate this disease and predict its sequelae for better prevention and management. Aim of this study was to analyse clinical and pharmacological aspects of ACS hospitalised patients for a better evaluation and prediction.

Methods: The study included questionnaire based data taken from 225 patients (207 males, 18 females) admitted to Ibn Al-Nafees tertiary cardiac centre. Socio-economic, clinical and pharmacological data were obtained from all patients with follow up from time of admission to discharge.

Results: Male to female ratio was 11.5:1, anterior infarction was the predominant site (54.6 %), mortality rate was 1.3 %. Ejection fraction (LVEF) was below 45 % in 66.7 % of the patients, majority (70.7 %) with ischaemic hypokinesia. Smoking was the most common risk factor (77.3 %). Heart failure (HF) was the most common complication (57.3 %). There were significant relationship between HF occurrence and number of risk factors, LVEF, anterior site of ACS and number of echo findings. There were non-significant increase in relative risk of HF with each risk factor, positive troponin and pre-admission pain duration. Prediction tests showed an ascending positive slope of HF risk with number of risk factors, duration of admission and age.

Conclusion: There was a high rate of HF occurrence in this study which is mostly attributed to major wall damage due to blockage of the main coronary artery. Analysis demonstrated a good survival rate but high rate of HF occurrence urges for more consideration of guideline-directed management.

Key words: Acute coronary syndrome; Myocardial infarction; Heart failure; Ticagrelor; Clopidogrel; Antiplatelets.

1. Department of Pharmacology, Al-Kindy College of Medicine, University of Baghdad, Baghdad, Iraq.
2. Ibn Al-Nafees Cardiac Care Hospital, Al-Rusafa Health Directorate, Baghdad, Iraq.
3. Department of Pharmacology, Era's Lucknow Medical College, Lucknow, Uttar Pradesh, India.

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Corresponding author:
SAAD BADAI NASHTAR
E: saadbadai@kmc.uobaghdad.edu.iq

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Introduction

Acute coronary syndrome (ACS) is a subset of coronary heart disease which includes myocardial symptomatic clinical changes with or without electrocardiogram (ECG) changes and cardiac troponin[1] - unstable angina, non-ST-segment elevation myocardial infarction (NSTEMI) and ST-segment elevation myocardial infarction (STEMI).[2] ACS is responsible for a significant number of inpatient admissions worldwide, with 175,000 inpatient admissions in the United Kingdom in 2012.[3] ACS is more common in males than females, with a male to female ratio of 2.19:1.[4]

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ACS is caused by the rupture of an atherosclerotic plaque in the coronary artery, leading to the formation of a blood clot that obstructs blood flow to the heart muscle. Many factors can precipitate ACS, including physical or emotional stress, drug use and spontaneous implantable cardioverter defibrillator shocks.[5] Treatment for ACS includes medications such as a bullous dose of acetylsalicylic acid (300 mg), heparin, antiplatelet therapy (clopidogrel or ticagrelor) and nitroglycerin, as well as invasive procedures such as percutaneous coronary intervention (PCI) and coronary artery bypass grafting (CABG).[6]

Coronary artery disease (CAD) is a major cause of morbidity and mortality worldwide due to obstructive atherosclerotic coronary changes. There are two main forms of CAD: chronic stable ischaemic heart disease where coronary arteries slowly and progressively narrow over the years. The other type is ACS. STEMI involves complete occlusion of coronary artery due to atherosclerotic plaque, characterised by ST- segment elevation and raised cardiac biomarkers.[7] The commonly predicted risk factors of CAD are hyperlipidaemia, hypertension, smoking, diabetes, obesity, age factor including females above 55 years and males above 45 years and positive family history.[8]

The common symptoms of CAD include chest discomfort or anginal pain, shortness of breath or dyspnoea, dizziness or lightheaded, palpitations, nausea, stomach discomfort or vomiting and sometimes weakness. Females may demonstrate atypical symptoms. Over the years, CAD can vitiate the heart and lead to complications like arrhythmias most likely atrial fibrillation, cardiac arrest, cardiogenic shock and heart failure (HF).[9]

Treatment and prevention for CAD embraces lifestyle changes, risk factor prevention and medications. The minimally invasive coronary revascularisation procedure like PCI or coronary angioplasty helps to improve blood flow to the heart. CABG is another surgical method to create a new path for the blood to flow around blockages. Medications include nitrates, beta blockers, angiotensin-converting-enzyme (ACE) inhibitors, angiotensin II receptor blockers (ARBs), statins and antiplatelet agents. High and moderate risk patients typically undergo surgery within 24-48 h. To evade high morbidity and mortality related with ACS, it must be quickly and appropriately diagnosed and managed.[10, 11]

The age of onset of ACS varies widely, with patients ranging from 18 to 69 years old, with a median age of 28 years.[4] It is well known that ACS risk increases with age. Therefore, it occurs more often in older than younger age groups. Although atherosclerotic changes appear early in arteries but serious symptoms of disease usually needs many years to be clinically prominent.[12] The cut off age of 45 years old had been suggested by most researchers to define ACS in young patients.[13]

There are increasing indicators of concern regarding the rates of ACS among young people but data still deficient about disease management outcomes which urge to more study of this subject.[14] About 6-10 % of cardiac infarctions in United States occurs in patients below 45 years old.[15] Regarding Iraqi data a study conducted in Duhok City revealed that in 36 out of 380 (9.47 %) ACS patients were less than 45 years age[16] while another study conducted in Sulaimaniyah showed that patients below 45 years old were 21.6 %.[17]

The main objective of this study was to analyse different clinical and pharmacological aspects related to ACS in hospitalised patients under 45 years of age and their effects in cure and prediction of complication occurrence.

Methods

This was a prospective observational cohort study conducted in the Critical Care Unit of Ibn Al-Nafees Cardiac Care Tertiary Centre, Al-Rusafa Health Directorate, Baghdad-Iraq. Study included 225 patients (207 males, 18 females) admitted for a period of 18 months from January 2020 to June 2021. Data collection was done through a detailed well-organised questionnaire forms. Data were obtained directly from the patients and some missed data were obtained from the clinical records. Inclusion criteria included: patients lower than 45 years age from both sexes, presented with typical chest pain and approved to be ACS, treated within the adopted regime by giving an urgent loading dose of antiplatelet (ticagrelor 180 mg or clopidogrel 300 mg) in addition to acetylsalicylic acid 300 mg and other needed medications, then being admitted urgently for PCI and later followed-up until their discharge.

Prescriptions of all patients of either sex diag-

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nosed with ACS from the time the patient was admitted were collected and documented in a soft and hard copy of case record form (CRF) including the patient's particulars and drug details. The prescriptions were analytically scrutinised using the WHO core prescribing indicators. Pregnant females and patients with other interrelated cardiac disease like arrhythmias, endocarditis and rheumatic heart disease and other non-complying with inclusion criteria were excluded. All patients were diligently followed from the time of admission till discharge. The past medical history of the patient was also reviewed.

Socio-economic class was classified into low, intermediate and high according to a specific questions including residence, occupation, income and other related information. Other data collected directly from the patients, their relatives and CRF.

Data analysis was done by Statistical Package for Social Science (SPSS) version 24. Analysis and interpretation of data was implemented by using descriptive statistics to obtain frequencies and proportions. Inferential statistics were conducted to obtain odds ratio, relative risk, Chi-square test and logistic regression wherever needed. Figures and tables were used for interpretation and displaying of data. At 95 % confidence interval (CI), $p < 0.05$ was considered statistically significant in all tests.

Results

In this study, socio-demographic data of the patients which are listed in Table 1 had shown that majority of the patients (70.7 %) were between 36-45 years. Majority of the patients were male (92 %) with male to female ratio 11.5:1, married (80.9 %) and from low socio-economic class (88.0 %). Most of them were employed (53.4 %) and college graduated (54.2 %).

Regarding clinical findings, it was found that majority of the infarction sites were anterior (54.6 %). Left ventricular ejection fraction (LVEF) was above 55 % in (37.3 %) of the patients. Echo findings revealed that ischaemic hypokinesia (70.7 %) and left ventricular (LV) systolic dysfunction (61.3%) were the most presenting features. Coronary angiography demonstrated that

Table 1: Socio-demographic characteristics of the patients with the acute coronary syndrome

Socio-demographic variables	N	%
Age		
≤ 25	4	1.8
26-35	62	27.5
36-45	159	70.7
Sex		
Male	207	92.0
Female	18	8.0
Marital status		
Married	180	80.0
Single	40	17.8
Divorced	5	2.2
Socio-economic class		
Low	198	88.0
Intermediate	18	8.0
High	9	4.0
Employment		
Employed	120	53.4
Unemployed	105	46.6
Education		
Illiterate	24	10.9
Primary school	38	16.8
Elementary school	35	15.5
College	122	54.2
High degree	6	2.6

N: number of patients; %: percentage of total number of patients;

left anterior descending artery (61.8 %) was the most frequently blocked artery. Nause and vomiting (49.8 %) was the most frequent presenting symptom after chest pain which was excluded because it occurred in all patients and adopted as a major inclusion criterion. Smoking (77.3 %) was the most frequent risk factor. Troponin test was negative in (58.7 %) of the patients. HF was the most common complication in more than half of the patients (57.3 %). Majority of the patients (91.1 %) presented to the hospital within 1 day of symptom appearance and about half (50.6 %) were discharged after 4 days of hospital admission. Details are listed in Table 2.

Table 3 demonstrates the relationship between the major complication (HF) and different clinical features of the patients. The link between HF occurrence and risk factors number was highly significant ($p < 0.00001$). The same result was obtained in relation to type of ACS, LVEF, echo findings and duration of hospital admission.

Variables included were only those which were related to HF (213 out of 225 patients). Chi-square test was done, p-value was significant (< 0.05).

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There was no significant relationship between age (p = 0.153), sex (p = 0.292) and employment (p = 0.880) of the patients with occurrence of HF (Table 4). On the other hand, there was a highly significant relationship between marital status and HF (p < 0.00001).

Table 2: Clinical findings of patients with acute coronary syndrome under 45 years of age

Variable		N	%
Type of ACS (infarction site)			
	Anterior	123	54.6
	Inferior	63	28.0
	Posterior	0	0.0
	Lateral	6	2.7
	Stable angina	3	1.3
	Unstable angina	30	13.3
Left ventricular ejection fraction (LVEF)			
	> 55 %	84	37.3
	45-55 %	69	30.7
	35-44 %	60	26.7
	< 35 %	12	5.3
Echo findings*			
	Normal	21	9.3
	LVDD	42	18.7
	LVSD	138	61.3
Hypokinesia (ischemia)		159	70.7
	RVD	15	6.7
	MR	22	9.7
	TR	4	1.7
	AR	5	2.2
	HHD	17	7.5
	LV thrombosis	3	1.3
	MVP	3	1.3
	LVH	18	8.0
Findings of coronary angiography*			
	LMB	9	4.0
	LAD	139	61.8
	CX	48	21.3
	RCA	74	33.3
	OM	3	1.3
	Diagonal	0	0.0
	Ramus	7	3.1
	None	54	24.0
Clinical symptoms (other than chest pain)			
	Epigastric pain	22	9.8
	Dyspnoea	42	18.6
	Nausea and vomiting	112	49.8
	Diaphoresis	6	2.7
	None	43	19.1
Risk factors*			
	Diabetes mellitus	60	26.7
	Smoking	17	77.3
	Hypertension	75	33.3
	Dyslipidaemia	12	5.3
	Stress	129	57.3
	Family history	36	16.0
	COVID-19	18	8.0
	None	3	1.3
Troponin			
	Positive	97	1.3
	Negative	128	58.7
Complications			
	None	84	37.4
	Arrhythmia	9	4.0
	HF	129	57.3
	Death	3	1.3
Time of admission after pain			
	Within 1 day	205	91.1
	2 days	18	8.0
	More than 2 days	2	0.9
Duration of admission (days)			
	2	18	8
	3	54	24
	4	114	50.6
	5	24	10.7
	6	15	6.7

**more than one feature could be presented in the patient. ACS: acute coronary syndrome; LVD: left ventricular diastolic dysfunction; LVSD: left ventricular systolic dysfunction; RVD: right ventricular dysfunction; MR: Mitral regurgitation; TR: tricuspid regurgitation; AR: aortic regurgitation; HHD: hypertensive heart disease; LV: left ventricle; MVP: mitral valve prolapse; LVH: left ventricular hypertrophy; LMB: left marginal branch; LAD: left anterior descending; CX: circumflex; RCA: right coronary artery; OM: obtus marginal; HF: heart failure; N: number of patients; %: percentage of total number of patients;*

Table 3: Heart failure (HF) probability in relation to different clinical variables

Variables	HF-Yes	HF-No	Total	p-value
Number of risk factors				
1	3	24	27	0.00001*
2	63	30	93	
3 and more	51	27	78	
None	12	3	15	
Site of ACS				
Anterior	84	24	108	0.000012*
Inferior	33	30	63	
Lateral	3	6	9	
Stable/ unstable angina	9	24	33	
LVEF				
> 55 %	3	78	81	< 0.00001*
45-55 %	66	3	69	
< 44 %	60	3	63	
Echo findings				
1	9	33	42	< 0.00001*
2	84	24	108	
3 and more	34	6	40	
None	2	21	23	
Duration of admission (days)				
2	2	16	1	< 0.00001*
3	18	36	54	
4	84	22	109	
5	16	5	21	
6	9	5	15	
Total	**129**	**84**	**213**	

Variables included are only those which were related to heart failure (213 out of 225 patients). ACS acute coronary syndrome; LVEF: left ventricular ejection fraction;
**Chi-square test was done, p-value was significant (< 0.05).*

CONTENT

Table 4: Heart failure (HF) patient complication probability according to socio-demographic variables

Socio-demographic variables		HF-Yes	HF-No	Total	p-value
Age / years					
	22-33	18	18	36	0.1530
	34-45	111	66	177	
Sex					
	Male	120	81	201	0.2920
	Female	9	3	12	
Marital status					
	Married	119	56	175	0.0001*
	Single / divorced	10	28	38	
Employment					
	Employed	72	46	118	0.8800
	Unemployed	57	38	95	
Total		**129**	**84**	**213**	

**Chi-square test was done, p-value was significant (< 0.05);*

Table 5: Relative risk and odd ratio of heart failure (HF) according to risk factors, troponin value, pre-admission pain time and antiplatelet drug administered

Socio-demographic variables		Total	HF-Yes	HF-No	RR	95 % CI	p-value	OR
Smoking								
	Yes	**177**	108	69	1.22	0.898 - 1.658	0.200	1.57
	No	**48**	24	24				
Dyslipidaemia								
	Yes	**12**	6	6	0.87	0.486 - 1.542	0.620	0.73
	No	**213**	123	90				
Hypertension								
	Yes	**75**	48	27	1.18	0.946 - 1.484	0.130	1.51
	No	**150**	81	69				
Diabetes mellitus								
	Yes	**60**	39	21	1.19	0.945 - 1.503	0.140	1.55
	No	**165**	90	75				
Troponin								
	Positive	**97**	60	37	1.15	0.917 - 1.435	0.230	1.39
	Negative	**128**	69	59				
Pre-admission pain duration								
	Within 1 day	**205**	120	85	1.30	0.791 - 2.141	0.300	1.73
	More than 1 day	**20**	9	11				
Antiplatelet drug on admission								
	Ticagrelor	**72**	47	25	1.15	0.923 - 1.427	0.210	1.43
	Ticagrelor + ASA	**153**	87	66				

HF: heart failure; RR: relative risk; CI: confidence interval; OR: odd ratio; ASA: acetylsalicylic acid;

Relative risk and odds ratio of HF according to different variables are listed in Table 5 which revealed no significant association between presence of all these variables and occurrence of HF although some numbers suggested an increased risk and odds ratio but still not significant.

Binary logistic regression was employed to determine predictors of HF as a complication of ACS in the following factors: age, duration of hospital admission and number of risk factors of the patients, as demonstrated in Figures 1, 2 and 3, respectively. There was a positive association between these factors and number of patients with HF.

CONTENT

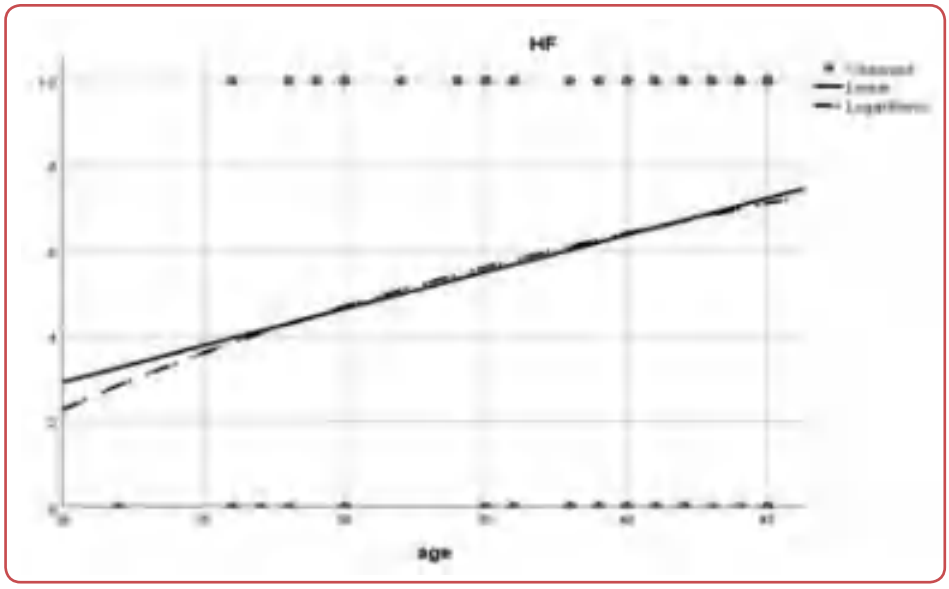

Figure 1: Logistic regression between dependent factor (heart failure) and independent factor (age)

p-value: linear 0.089, logistic 0.094;

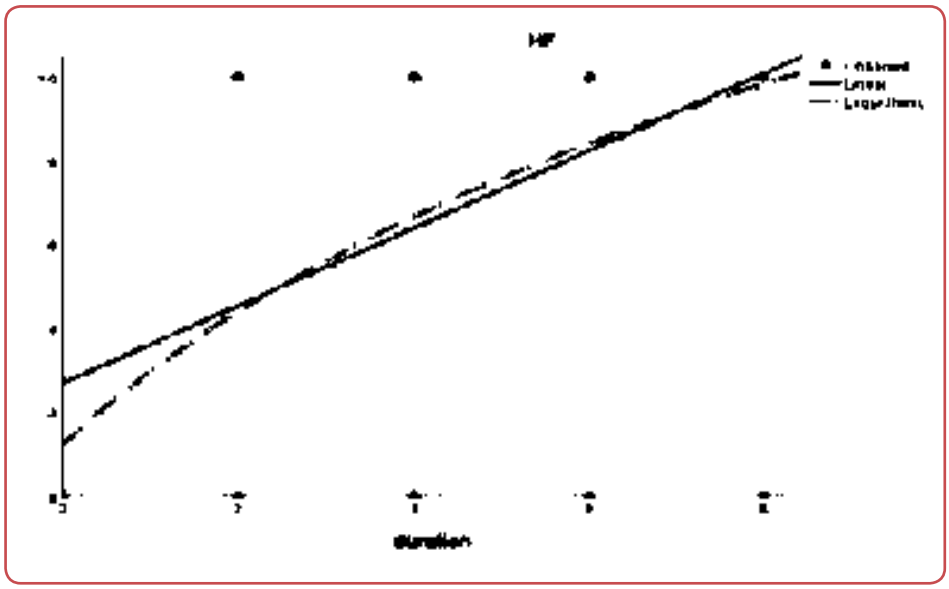

Figure 2: Logistic regression between dependent factor (heart failure) and independent factor (duration of hospital admission)

p-value: linear 0.002, logistic 0.000;

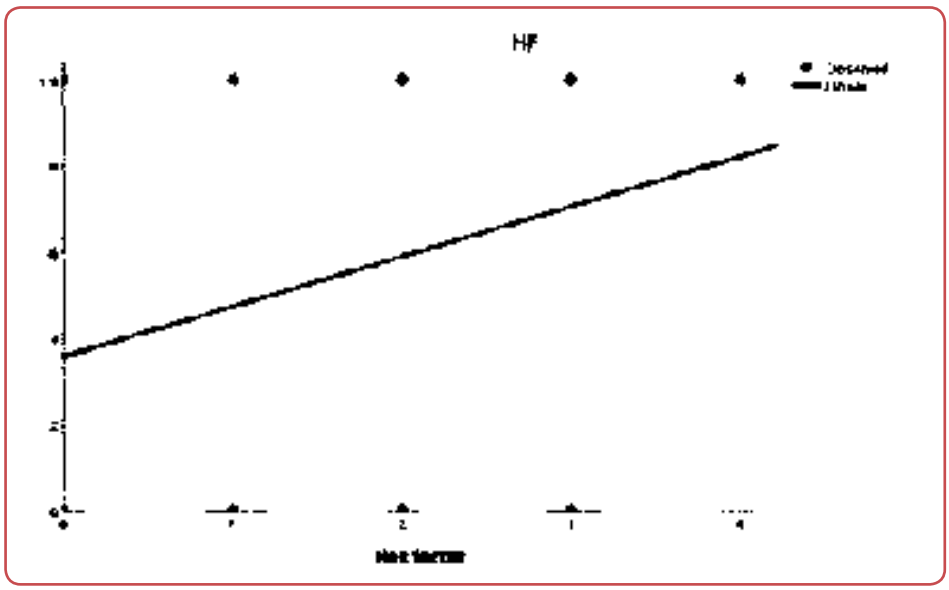

Figure 3: : Logistic regression between dependent factor (heart failure) and independent factor (number of risk factors

p-value: linear 0.065, logistic 0.017

CONTENT

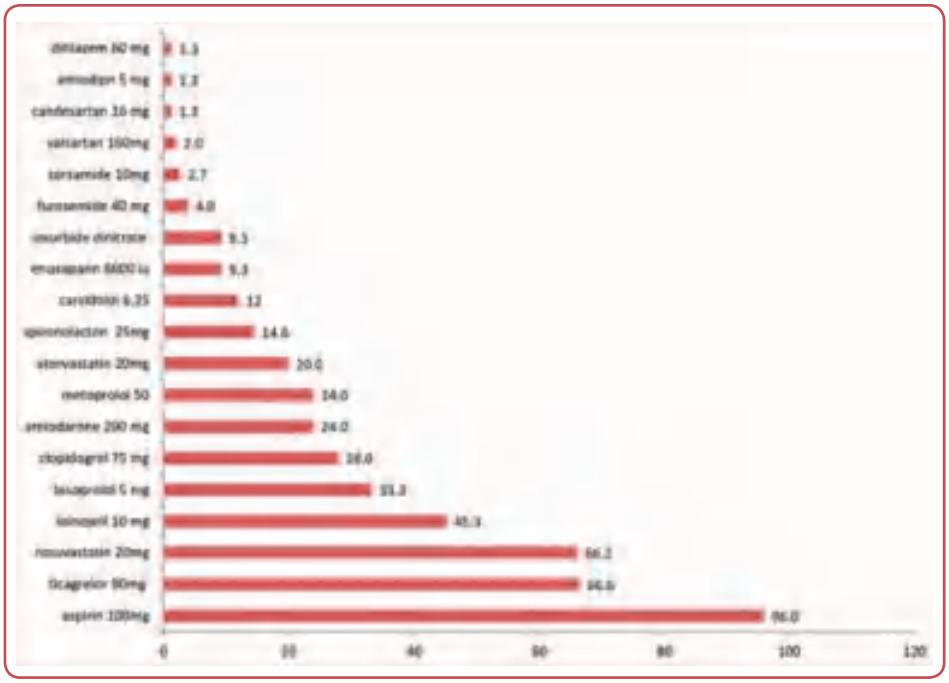

Figure 4: Percentage ranking of discharge medications prescribed to the patients

The prescribed medications on discharge were antiplatelet drugs, 66.6 % of the patients received ticagrelor 90 mg while 28.0 % of them received clopidogrel 75 mg and 96.0 % received acetylsalicylic acid 100 mg. Bisoprolol 5 mg was the most prescribed beta blocker agent (33.3 %). Among statins rosuvastatin was the most prescribed drug (66.2 %) followed by atorvastatin 40 mg (20.0 %). Between ACEI and ARBS lisinopril 5 mg was the most prescribed (45.3 %) (Figure 4).

Discussion

This study analysed 225 patients of ACS during a period of 18 months in the cardiac care unit of Ibn Al-Nafees Tertiary Care Hospital in Baghdad. All of them were under 45 years of age but 70.7 % were in the age group 36-45 years (mean age was 38.17 ± 6.32 years) this results seems to be near to the mean age calculated by another comparable study conducted in Malaysia on 282 patients which was (39.2 ± 5.1 years),[18] majority of them were males (92 %). These data complied with most other studies as ACS is more common in older age group and in male population due to physiological mechanisms and presence of risk factors.[17, 19] Regarding male to female ratio (11.5:1) in this study, this can be explained by far less exposure of females in sampled community to risk factors like smoking, stress and alcohol than males. In addition, oestrogen tends to confer young females a protective effect against coronary atherosclerosis.[20]

Most of the patients (53.4 %) were employed linking work related stress factors to ACS. Married patients were in majority (80.2 %) which goes against many studies which have shown that unmarried people are more likely to develop coronary artery disease[21] but this can be explained by link of low socio-economic status of the majority of the patients (88.0 %) and psychological stress as the second important risk factor (57.3 %) which imposes familial responsibilities as an additional psychological burden on the patients below 45 years of age. Regarding risk factors, smoking (77.3 %) and stress (57.3 %) were the most common risk factors in patients which is correlated with the study of Wang et al.[22] Zupancic study concluded that psychological stress is associated with ACS especially in patients with previously silent disease.[23]

Many studies suggested hypertension as the first risk factor in ACS patients[24, 25] but the probable reason for these factors retreat in this study is the young age of the patients sample since hypertension tends to occur in older age which is associated with structural changes in arteries.[26] Family history of ACS was found in 16 % of the patients in this study which was lower than that of another Iraqi study in a comparable sample which found that it was 24 %.[27]

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Echo findings demonstrated that ischaemic heart disease patients were maximum 61.3 % and LV systolic dysfunction was more than LV diastolic dysfunction. Echo finding can help in early detection and diagnosis of ACS and guide in proper management thereby decreasing mortality. These finding can be used as independent predictors and can help in identifying high risk-patients, patients who need medical therapy or surgical intervention and follow them to improve their outcome. High percentage of LV ischaemic damage occurred mostly due to high percentage of blockage in left anterior descending artery (the largest coronary artery) (61.8 %) which lead to systolic and diastolic dysfunction and that explains the high proportion of HF complication (57.3 %) of patients in the current study which was higher than the Malaysian study (35.4 %). Incidence of HF among patients hospitalised for an acute MI varies among studies, starting from 14-36 %.[28] In spite of the lower mortality rate in this study than the global rate, HF occurrence was much higher.

As for complications, 37.4 % of the patients escaped with no complications but most of the patients (57.3 %) developed HF. Mortality rate was 1.3 % which is lower than the global rate of mortality that was estimated by 7 %.[29] This is mostly due to rapid coronary intervention and young age of the patients. Arrhythmia occurred in 4 % of the patients which was lower in comparison to the Malaysian study (18.4 %).

For HF as a major complication in this study, relations with different parameters were assessed to predict the future probability of occurrence. Regarding the relation between HF occurrence with number of risk factors per each patient there was an extremely significant difference among groups since its occurrence was higher in patients with 2 and more risk groups in comparison to none and single risk groups which imposes a strong relationship between number of risk factors and HF as a complication of ACS. This finding suggests clearly that ACS in young people is mostly an outcome of more than one independent risk factor synergism.

The type of ACS was significantly associated with HF occurrence ($p < 0.000012$) as 84/108 (about 87 %) of patients with anterior type ended with HF. Anterior wall infarction is associated with a higher risk of adverse remodelling and HF.[30] The higher risk of HF associated with anterior MI is caused by the greater magnitude of irreversible LV damage, as compared with other MI locations.[31] On the other hand LVEF was extremely significant in association between HF and reduced ejection fraction results, this suggest higher area of LV wall damage due to blockage of main anterior coronary artery. Also, there were extremely significant association between 2 and more echo findings and HF occurrence which can be explained by the previously mentioned causes. In addition and for the same reasons patients who developed HF had significantly higher duration of hospital stay.

Relative risk and odds ratio of developing HF were higher but with non-significant manner in all factors except dyslipidaemia which was associated with lower risk, this seems to be due to the low effect of dyslipidaemia as an independent risk factor of ACS in young population. Regarding troponin, pre-admission pain duration and antiplatelet drug(s) on admission there was a non-significant increase of relative risk and odd ratio predicting a non-significant increase in HF occurrence in presence of these factors.

HF risk prediction was assessed with logistic regression test in regard to the following independent factors (age, duration of hospital admission and number of risk factors). The results have shown that there is a positive correlations between increased number of HF patients and increase of each of these independent factors consolidating the previous results of relation between HF occurrence and these factors in the form that it can be expected HF more often as a complication of ACS in the presence of these factors. Improving prediction of ACS patients at risk of HF development is needed since timely initiation of guideline-directed HF therapy can reduce the risk of further LV remodelling, morbidity and mortality.[31-33]

In light of the above results the discharge treatment strategy should concentrate on the prevention of post MI complication development especially HF alongside with inhibition of further atherosclerotic process development. It is recommended to consider an urgent interventional management for the ischaemic patients with anterior ischemia and those who have more than 2 risk factors prior to MI development.

In the current study, beta blocker drugs (metoprolol, bisoprolol and carvedilol) were prescribed for 69.3 % of the discharged patients which

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seems to be covering all patients with HF but not all the guidelines directed indications which imposes an urgent revision of beta blockers prescription policy in this centre. Beta blockers are one of the fundamental guideline-recommended therapies that must be considered as a first line therapy in patients with ventricular tachycardia or fibrillation in the acute and sub-acute phase of an ACS since it plays a crucial role in inhibition of matrix metalloproteinases (MMPs) responsible for tissue remodelling.[34]

P2Y12 inhibitors anti-platelets (ticagrelor and clopidogrel) were prescribed for 94.6 % of the discharged patients while acetylsalicylic acid was prescribed for 96 % of them. All patients should be put under antiplatelet therapy of both a P2Y12 inhibitor drug and acetylsalicylic acid unless one of them or both were contraindicated. Dual antiplatelet therapy of both P2Y12 inhibitor and acetylsalicylic acid, is the standard therapeutic strategy in patients with ACS who underwent to PCI according to the current guidelines in order to optimise antiplatelet effects.[35] Most of the patients were prescribed ticagrelor (66.6 %) in comparison to those prescribed clopidogrel (28 %) and this goes with results of the studies which gives superiority to ticagrelor on clopidogrel.[36, 37]

Statins (HMG-CoA reductase inhibitors) specifically atorvastatin and rosuvastatin were prescribed in 86.6 % of the patients. Current European and American guidelines recommend the administration of high-potency statins as early as possible in ACS.[38] Antihypertensive drugs especially ACI and ARBs were prescribed for less than half of the patients and the most common agent used was lisinopril (45.3 %). The rationale for such percentage is that most of the patients (66.7 %) had no history of hypertension. But this pattern is not going with rationale with regard to the majority of the patients who developed HF.

Beyond beta blockers and ACEIs, there were no prominent guideline-directed prescription with regard to HF especially HF with reduced ejection fraction (HFrEF) in which ejection fraction is less than 40 %.[39] About 13 % of the patients in the current study ended with HFrEF and this need to be treated according to current guidelines which include in addition to beta blockers and ACEIs, mineralocorticoid receptor antagonists (eplerenone and spironolactone), sodium-glucose co-transporter-2 (SGLT2) inhibitors (dapagliflozin and empagliflozin), angiotensin receptor-neprilysin inhibitors (ARNIs) (sacubitril).[19] All these agents were minimally or not prescribed. More consideration should be paid to the goal of management of ACS complications especially HF and HFrEF alongside with the goal of ACS recurrence inhibition.

The present research highlights the importance of rational drug prescribing in ACS patients. Early detection, avoiding risk factors, patient awareness and education especially if family history is present, multidisciplinary approach for treating such patients can improve the prognosis in long term and improve survival rate. Drug therapy prescribing should be in accordance with guidelines in order to further reduce the complications, decrease the economic burden on the patient and incidence of drug interactions and adverse reactions.

Conclusion

There was a high rate of HF occurrence in this study which is mostly attributed to major wall damage due to blockage of the main coronary artery. Analysis demonstrated a good survival rate which suggests a good submission to therapeutic guidelines but high rate of HF occurrence urges for more consideration of guideline-directed management.

Ethics

The study was approved by the Al-Kindy College of Medicine Scientific Unit Ethics Committee, decision No KCM-N521-23, dated 22 June 2023. All participants gave a written and oral consent for use of their anonymised medical data in this study.

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CONTENT

Conflicts of interest

The authors declare that there is no conflict of interest.

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Data access

The data that support the findings of this study are available from the corresponding author upon reasonable individual request.

Author ORCID numbers

Saad Badai Nashtar (SBN):
0000-0001-9545-0204
Muhammed Mutar Nazzal (MMN):
0009-0008-6390-3764
Hayder Mohammed Faeq (HMF):
0009-0009-1174-261X
Afroz Abidi (AA):
0000-0002-3451-0514

Author contributions

Conceptualisation: MMN, SBN
Methodology: MMN, SBN
Software: SBN, HMF
Formal analysis: SBN, AA
Investigation: MMN, HMF
Data curation: MMN, HMF
Writing - original draft: SBN, AA
Writing - review and editing: SBN
Supervision: MMN
Project administration: SBN

References

1. Byrne RA, Rossello X, Coughlan JJ, Barbato E, Berry C, Chieffo A, et al; ESC Scientific Document Group. 2023 ESC Guidelines for the management of acute coronary syndromes. Eur Heart J. 2023 Oct 12;44(38):3720-826. doi: 10.1093/eurheartj/ehad191.
2. Thygesen K, Alpert JS, Jaffe AS, Simoons ML, Chaitman BR, White HD, et al; Writing Group on the Joint ESC/ACCF/AHA/WHF Task Force for the Universal Definition of Myocardial Infarction; ESC Committee for Practice Guidelines (CPG). Third universal definition of myocardial infarction. Eur Heart J. 2012 Oct;33(20):2551-67. doi: 10.1093/eurheartj/ehs184.
3. Tan YC, Sinclair H, Ghoorah K, Teoh X, Mehran R, Kunadian V. Gender differences in outcomes in patients with acute coronary syndrome in the current era: A review. Eur Heart J Acute Cardiovasc Care. 2016 Nov;5(7):51-60. doi: 10.1177/2048872615610886.
4. Naji AA, Abbas AA, Hashem RB. Evaluation of serum RANKL level in acute coronary syndrome. Al-Kindy Coll Med J. 2014;10(2):45-8.
5. Freedman JE, Loscalzo J. Platelet-monocyte aggregates: bridging thrombosis and inflammation. Circulation. 2002 May 7;105(18):2130-2. doi: 10.1161/01.cir.0000017140.26466.f5.
6. Gilutz H, Shindel S, Shoham-Vardi I. Adherence to NSTEMI guidelines in the emergency department: regression to reality. Crit Pathw Cardiol. 2019 Mar;18(1):40-6. doi: 10.1097/HPC.0000000000000165.
7. Singh A, Museedi AS, Grossman SA. Acute coronary syndrome. 2023 Jul 10. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan–. PMID: 29083796. Available at: https://pubmed.ncbi.nlm.nih.gov/29083796/.
8. Karthikeyan T, Raja M, Radha D, Gaur T A, Geetha J, Sakthivadivel V. Risk factors and inflammatory markers in acute coronary syndrome-ST elevation myocardial infarction (STEMI). Horm Mol Biol Clin Investig. 2023 Mar 20;44(2):115-20. doi: 10.1515/hmbci-2021-0106.
9. Dauriz M, Morici N, Gonzini L, Lucci D, Di Chiara A, Boccanelli A, et al. Fifteen-year trends of cardiogenic shock and mortality in patients with diabetes and acute coronary syndromes. Am J Med. 2020 Mar;133(3):331-9.e2. doi: 10.1016/j.amjmed.2019.07.044.
10. Guedeney P, Collet JP. Diagnosis and management of acute coronary syndrome: what is new and why? Insight From the 2020 European Society of Cardiology Guidelines. J Clin Med. 2020 Oct 28;9(11):3474. doi: 10.3390/jcm9113474.
11. Poudel N, Alurkar VM, Jha GS, Kafle R, Sapkota S, Lamsal L. Profile of acute coronary syndrome in young people: a hospital based observational study in western Nepal. BJHS. 2018;3(1):361-5. doi: 10.3126/bjhs.v3i1.19759.
12. Choudhury L, Marsh JD. Myocardial infarction in young patients. Am J Med. 1999;107(3):254–61. doi: 10.1016/S0002-9343(99)00218-1.
13. Avezum A, Makdisse M, Spencer F, Gore JM, Fox K, Montalescot G, et al. Impact of age on management and outcome of acute coronary syndrome: observations from the Global Registry of Acute Coronary Events (GRACE). Am Heart J. 2005 Jan;149(1):67-73. doi: 10.1016/j.ahj.2004.06.003.

CONTENT

14. Dakhil Z. 67 young patients with non-ST elevation acute coronary syndromes: baseline characteristics, in-hospital management and outcomes. Heart. 2021;107:A55-A56. doi: 10.1136/heartjnl-2021-BCS.67.
15. Chouhan L, Hajar HA, Pompisiello JC. Comparison of thrombolytic therapy for acute myocardial infarction in patients aged <35 and >55 years. Am J Cardiol. 1993;71:157–9. doi: 10.1016/0002-9149(93)90731-q.
16. Mohammad AM, Abdulhaleem BH, Habeeb QS. First 24 h' outcomes of acute coronary syndrome in Iraq. Med J Babylon. 2020;17:154-8. doi: 10.4103/MJBL.MJBL_15_20.
17. Jalal Khaznadar AA, Salh RW. Impact of age on risk factors and clinical manifestations of acute coronary syndrome: observations from the coronary care unit of Sulaimani, Iraq. Hosp Pract Res. 2020;5(1):28-34. doi: 10.34172/hpr.2020.06.
18. Che-Muzaini CM, Norsa'adah B. Complications of acute coronary syndrome in young patients. Iran J Public Health. 2017 Jan;46(1):139-40. PMID: 28451542.
19. Swaroop G. Post-myocardial infarction heart failure: a review on management of drug therapies. Cureus. 2022 Jun 8;14(6):e25745. doi: 10.7759/cureus.25745.
20. Mendelsohn ME, Karas RH. The protective effects of estrogen on the cardiovascular system. N Engl J Med. 1999 Jun 10;340(23):1801-11. doi: 10.1056/NEJM199906103402306.
21. Wong CW, Kwok CS, Narain A, Gulati M, Mihalidou AS, Wu P, et al. Marital status and risk of cardiovascular diseases: a systematic review and meta-analysis. Heart. 2018 Dec;104(23):1937-48. doi: 10.1136/heartjnl-2018-313005.
22. Wang W, Zhao D, Sun JY, Wang WH, Cheng J, Liu J, et al. [Risk factors comparison in Chinese patients developing acute coronary syndrome, ischemic or hemorrhagic stroke: a multi-provincial cohort study]. Zhonghua Xin Xue Guan Bing Za Zhi. 2006 Dec;34(12):1133-7. Chinese. PMID: 17274911.
23. Zupancic ML. Acute psychological stress as a precipitant of acute coronary syndromes in patients with undiagnosed ischemic heart disease: a case report and literature review. Prim Care Companion J Clin Psychiatry. 2009;11(1):21-4. doi: 10.4088/pcc.08r00623.
24. Desta DM, Nedi T, Hailu A, Atey TM, Tsadik AG, Asgedom SW, et ak. Treatment outcome of acute coronary syndrome patients admitted to Ayder Comprehensive Specialized Hospital, Mekelle, Ethiopia; A retrospective cross-sectional study. PLoS One. 2020 Feb 13;15(2):e0228953. doi: 10.1371/journal.pone.0228953.
25. Mohanan PP, Mathew R, Harikrishnan S, Krishnan MN, Zachariah G, Joseph J, et al. Presentation, management and outcomes of 25 748 acute coronary syndrome admissions in Kerala, India: results from the Kerala ACS Registry. Eur Heart J. 2013 Jan;34(2):121-9. doi: 10.1093/eurheartj/ehs219.
26. Pinto E. Blood pressure and ageing. Postgrad Med J. 2007 Feb;83(976):109-14. doi: 10.1136/pgmj.2006.048371.
27. Mirza AJ, Taha AY, Khdhir BR. Risk factors for acute coronary syndrome in patients below the age of 40 years. Egypt Heart J. 2018 Dec;70(4):233-5. doi: 10.1016/j.ehj.2018.05.005.
28. Fox KA, Eagle KA, Gore JM, Steg PG, Anderson FA; GRACE and GRACE2 Investigators. The Global Registry of Acute Coronary Events, 1999 to 2009--GRACE. Heart. 2010 Jul;96(14):1095-101. doi: 10.1136/hrt.2009.190827.
29. Ahmad WAW, Zambahari R, Ismail O, Sinnadurai J, Rosman A, Piaw CS, et al. Malaysian National Cardiovascular Disease Database (NCVD)–Acute Coronary Syndrome (ACS) registry: How are we different? CVD Prev Control 2011;6(3):81– 9. doi: 10.1016/j.cvdpc.2011.04.004.
30. Gaudron P, Eilles C, Kugler I, Ertl G. Progressive left ventricular dysfunction and remodeling after myocardial infarction. Potential mechanisms and early predictors. Circulation. 1993 Mar;87(3):755-63. doi: 10.1161/01.cir.87.3.755.
31. Jenča D, Melenovský V, Stehlik J, Staněk V, Kettner J, Kautzner J, et al. Heart failure after myocardial infarction: incidence and predictors. ESC Heart Fail. 2021 Feb;8(1):222-37. doi: 10.1002/ehf2.13144.
32. 32. Ponikowski P, Anker SD, AlHabib KF, Cowie MR, Force TL, Hu S, et al. Heart failure: preventing disease and death worldwide. ESC Heart Fail. 2014 Sep;1(1):4-25. doi: 10.1002/ehf2.12005.
33. Lewis EF, Velazquez EJ, Solomon SD, Hellkamp AS, McMurray JJ, Mathias J, et al. Predictors of the first heart failure hospitalization in patients who are stable survivors of myocardial infarction complicated by pulmonary congestion and/or left ventricular dysfunction: a VALIANT study. Eur Heart J. 2008 Mar;29(6):748-56. doi: 10.1093/eurheartj/ehn062.
34. DeLeon-Pennell KY, Meschiari CA, Jung M, Lindsey ML. Matrix metalloproteinases in myocardial infarction and heart failure. Prog Mol Biol Transl Sci. 2017;147:75-100. doi: 10.1016/bs.pmbts.2017.02.001.
35. Gragnano F, Capolongo A, Terracciano F, Gargiulo G, De Sio V, Cesaro A, et al. Escalation and de-escalation of antiplatelet therapy after acute coronary syndrome or PCI: available evidence and implications for practice. J Clin Med. 2022 Oct 23;11(21):6246. doi: 10.3390/jcm11216246.
36. Wallentin L, Becker RC, Budaj A, Cannon CP, Emanuelsson H, Held C, et al; PLATO Investigators; Freij A, Thorsén M. Ticagrelor versus clopidogrel in patients with acute coronary syndromes. N Engl J Med. 2009 Sep 10;361(11):1045-57. doi: 10.1056/NEJMoa0904327.
37. Wiviott SD, Braunwald E, McCabe CH, Montalescot G, Ruzyllo W, Gottlieb S, et al; TRITON-TIMI 38 Investigators. Prasugrel versus clopidogrel in patients with acute coronary syndromes. N Engl J Med. 2007 Nov 15;357(20):2001-15. doi: 10.1056/NEJMoa0706482.
38. Ibanez B, James S, Agewall S, Antunes MJ, Bucciarelli-Ducci C, Bueno H, et al; ESC Scientific Document Group. 2017 ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation: The Task Force for the management of acute myocardial infarction in patients presenting with ST-segment elevation of the European Society of Cardiology (ESC). Eur Heart J. 2018 Jan 7;39(2):119-77. doi: 10.1093/eurheartj/ehx393.
39. Bloom MW, Greenberg B, Jaarsma T, Januzzi JL, Lam CSP, Maggioni AP, et al. Heart failure with reduced ejection fraction. Nat Rev Dis Primers. 2017 Aug 24;3:17058. doi: 10.1038/nrdp.2017.58.

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ORIGINAL ARTICLE

Clinical Characteristics and Hospitalisation Outcomes of Hypoglycaemia in Hospitalised Patients With Type 2 Diabetes Mellitus

Swarna Krishnamoorthy,[1] Viswanathan Pandurangan,[2] Sivaprakash Varadan,[2] Sowmya Gopalan,[2] Sudagar Singh RB[2]

Abstract

Background/Aim: Strict glycaemic control delays the onset as well the progression of diabetes related microvascular complications. The major roadblock in achieving the target glycated haemoglobin (HbA_1c) and blood glucose levels is hypoglycaemia. The aim of this study was to assess the clinical characteristics and outcomes of hypoglycaemia in the type 2 diabetes mellitus (T2DM) hospitalised patients.

Methods: This was an observational study done for nine months in T2DM patients who had documented hypoglycaemia (blood glucose < 70 mg/dL) during the hospital stay. T2DM patients with hypoglycaemia on admission, hypoglycaemia due to anti-diabetic drug overdose, intensive care unit (ICU) patients with hypoglycaemia were excluded from the study. Eligible patients were categorised into two groups as symptomatic and asymptomatic hypoglycaemia. Clinical features, risk factors, hospitalisation outcome were compared between the symptomatic and asymptomatic hypoglycaemia group.

Results: Two hundred patients were enrolled in this study (n = 89, symptomatic group and n = 111, asymptomatic hypoglycaemia). Hypoglycaemic episode in past was significantly associated with symptomatic hypoglycaemic events during hospitalisation [34 (38.2 %) vs 27 (24.3 %)], p = 0.01. Admission blood glucose levels (mg/dL), HbA_1c (%) were significantly higher in symptomatic hypoglycaemia group [(225.93 vs 178.72, p = 0.008), (8.55 ± 2.49 vs 7.72 ± 1.82, p = 0.007)], respectively. The blood glucose level during the hypoglycaemia episode was significantly higher in patients with asymptomatic hypoglycaemia group (56.38 ± 9.51 vs 44.22 ± 11.21 mg/dL, p < 0.001). Patients with $HbA_1c \leq 6$ % were significantly higher in asymptomatic hypoglycaemia (n = 12, 10.8 % vs n = 2, 2.24 %, p = 0.02). Majority recovered fully without complications and got discharged (n = 155, 77.5 %).

Conclusion: In presented study, symptomatic hypoglycaemic patients had significantly higher admission blood glucose levels and HbA_1c %. Patients with HbA_1c < 6 % were significantly higher in asymptomatic group. Past history of hypoglycaemia was significantly associated with symptomatic hypoglycaemia during hospitalisation.

Key words: Blood glucose; Glycated haemoglobin; Hypoglycaemia; Symptoms; Type 2 diabetes mellitus.

1. Department of Hepatology, Christian Medical College, Vellore, India.
2. Department of General Medicine, Sri Ramachandra Institute of Higher Education and Research (SRIHER), Porur, Chennai, India.

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Corresponding author:
VISWANATHAN PANDURANGAN
E: viswa.smc@gmail.com
T: +91 9789907448

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Introduction

Strict glycaemic control is a major goal in treating patients with diabetes. The UK Prospective Diabetes Study (UKPDS) and The Diabetes Control and Complication Trail (DCCT) have shown reduced diabetes related microvascular complications like neuropathy, retinopathy and nephropathy with tighter blood glucose control and intensive therapy.[1, 2] Glycated haemoglobin (HbA_1c) target of less than 7 is recommended by American diabetes association (ADA). In practice, the major limiting factor in achieving desirable glycaemic control and target HbA_1c is hypoglycaemia.[3] However misconceived notion among doctors is that hypoglycaemia is uncommon in type 2 diabetes mellitus (T2DM) compared to type 1 diabetes mellitus. Early in the course of T2DM, hypoglycaemia is relatively rare as glucose counter-regulatory responses like secretion of glucagon, adrenaline is preserved. Over time with increasing duration of diabetes and beta cell failure, functional beta cell reserve decreases and there is failure of counter-regulatory responses. Occurrence of hypoglycaemic events in later course of T2DM is common.[4, 5] Moreover, recurrent episodes of hypoglycaemia cause hypoglycaemic unawareness and hypoglycaemia associated autonomic failure (HAAF).[6] Hypoglycaemic unawareness is due to attenuated adrenal response where patients may not experience the symptoms corresponding to the fall in blood glucose levels and sometimes the threshold that stimulates the response, drops below neuroglycopenia associated glucose levels. Blunted counterregulatory response and sympathoadrenal failure are components of HAAF. For the severity of hypoglycaemia, patients with HAAF have decreased epinephrine and glucagon response and subsequent development of neuroglycopenic symptoms. This sets a vicious cycle and causes sympathoadrenal failure later.

India holds second position worldwide with approximately 74-75 million people living with T2DM and projected number is 125 million by the year 2045.[7, 8] However, the data on prevalence, demographic characteristics, risk factors of hypoglycaemia in type-2 diabetic patients is inconsistent and sparse. Elderly people, patients with multiple comorbidities are frequently prone for hypoglycaemic episodes. Hypoglycaemia is considered as a significant risk factor for adverse cardiovascular events like myocardial infarction, arrythmias.[9] Fear of hypoglycaemia in patients and treating doctors naturally drives towards less intensified regimen resulting in sub-optimal glycaemic control. Despite all the threats posed by hypoglycaemia, still it remains a complication overlooked in clinical practice. Sulphonylureas drugs and insulin are known to cause hypoglycaemia. Risk factors of hypoglycaemia include elderly patients, co-prescription of certain antibiotics like fluroquinolones, HbA_1c levels. Hence, this study intended to study the clinical features, risk factors of in-patient hypoglycaemic events and its outcome in hospitalised patients with T2DM. It was intended to assess whether admission blood glucose, HbA_1c, antidiabetic drugs used, blood glucose levels during the hypoglycaemic event had any significant association between symptomatic and asymptomatic group.

The aim of the study was to compare the clinical characteristics, risk factors, glycaemic status, complications, length of stay and outcome of in-patient hypoglycaemia between the groups of symptomatic and asymptomatic hypoglycaemia patients with T2DM.

Methods

This was a prospective observational study done for a period of nine months between November 2020 and July 2021. Study was conducted in a tertiary care hospital in Southern India. Patients were enrolled from general medicine wards. Patients with T2DM above 18 years who were admitted in medical wards and subsequently during hospital stay had hypoglycaemia (blood glucose level < 70 mg/dL) were included in this study. T2DM patients who had hypoglycaemia after hospitalisation were enrolled. Non-inclusion criteria were: i) patients less than 18 years of age, ii) non-diabetic patient, whose capillary blood glucose (CBG) < 70 mg/dL, iii) pregnant patients, v) patients admitted with overdose of glucose lowering drugs, vi) patients who did not give consent for the study, vii) T2DM patients with hypoglycaemia on admission, viii) T2DM patients with sepsis who had hypoglycaemia on arrival to emergency room.

Informed consent was taken from all the participants prior to the study. Institutional ethics committee (IEC) approval was obtained and

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study followed the ethical standards for human subjects. Study was conducted in patients whose bedside capillary blood glucose (CBG) was < 70 mg/dL at any point of time during hospitalisation. The nursing staff were instructed to immediately notify patients with a blood glucose < 70 mg/dL by point of care testing. These patients were approached after reviewing medical records and confirming the diagnosis of diabetes. Patient's symptoms and signs during hypoglycaemic episodes were noted. Patients were categorised into two group as symptomatic and asymptomatic hypoglycaemia. Each patient was included only once in the study and symptoms were collected during the first hypoglycaemia episode. Only the first hypoglycaemic episode was included in this study and detailed information of study patients including age, sex, duration of diabetes, risk factors of hypoglycaemia (*nil per os*, decreased food intake, use of fluroquinolone, vomiting, kidney disease, liver disease and use of beta blockers), prior history of hypoglycaemia, blood glucose value during hypoglycaemic episode and treatment regimen were recorded using a standard proforma. HbA_1c, blood glucose value on admission, serum electrolytes (sodium, potassium, chloride, bicarbonate), renal function tests, serum albumin levels, the total leucocyte count done on admission were noted. Patients were followed up until discharge and the outcomes studied were duration of stay in the hospital, any adverse cardiovascular events (arrythmias, acute coronary syndrome or heart failure), nosocomial infections, acute kidney injury and mortality. Clinical characteristics, admission blood glucose, HbA_1c, CBG during the episodes and the outcomes studied were compared between the two groups (symptomatic and asymptomatic hypoglycaemia).

Symptomatic hypoglycaemia was defined as an event during which typical symptoms of hypoglycaemia were accompanied by a measured glucose concentration of ≤ 70 mg/dL. Asymptomatic hypoglycaemia was defined as an event with a measured glucose concentration of ≤ 70 mg/dl without the symptoms of hypoglycaemia. Severe hypoglycaemia was defined as measured glucose concentration < 40 mg/dL.[10] Adrenergic symptoms included sweating, anxiety, trembling, dry mouth, hand coldness, palpitations, nausea and increased appetite. Neurological symptoms were confusion, blurred vision, headache, slurred speech, numbness around lips.

Statistical analysis

Parameters were expressed as either number (percentage) or mean (standard deviation - SD) as appropriate. Descriptive data were expressed as frequency and percentage analysis for categorical variables, mean and SD for continuous variables. For continuous variables, depending on normality of data, Student's t-tests or non-parametric Wilcoxon tests were used to compare between the groups with symptomatic and asymptomatic hypoglycaemia. For discrete variables, χ^2 tests or Fisher's exact tests was used. Statistical significance was set at $p < 0.05$. Statistical analysis was done using *MEDCALC* software 2021 version.

Results

This study included a total of 200 patients with T2DM who developed hypoglycaemia during hospitalisation. Out of 200, 111 (55.5 %) patients did not experience any symptoms during hypoglycaemia episode (asymptomatic group) and 89 (45.5 %) patients were symptomatic during the episode. Fifteen percent of patients had hypoglycaemia in ICU (n = 30) and 85 % (n = 170) of patients had hypoglycaemia in wards.

The age group most commonly affected was found to be between 55-64 years in both symptomatic (n = 35, 39.3 %) and asymptomatic (n = 34, 30.6 %) group. Hypoglycaemic event occurred predominantly in patients aged more than 55 years in symptomatic (n = 64, 71.8 %) and asymptomatic group (n = 67, 60.3 %). Symptomatic patients were older than asymptomatic group, though not statistically significant (60.23 ± 11.74 vs 56.61 ± 14.69 years of age), p = 0.054. Table 1 shows the baseline characteristics of study patients. Age, gender, out-patient treatment had no significant difference between the symptomatic group and asymptomatic group.

Patients with diabetes duration of 1-5 years had higher episodes of asymptomatic hypoglycaemia compared to symptomatic group [31 (27.9 %) vs 12 (13.5 %)] which was found to be significant (p = 0.04). Patients with hypoglycaemic episode previously was found to have significantly higher symptomatic hypoglycaemic events during hospital stay [34 (38.2 %) vs 27 (24.3 %)], p = 0.01.

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Table 1: Socio-demographic and clinical characteristics of patients

N	Parameter	Symptomatic group (N = 89)	Asymptomatic group (N = 111)	p-value
1.	**Age** (years) (Mean ± SD)	60.23 ± 11.74	56.61 ± 14.69	0.054
2.	**Gender, N (%)**			
	Male	52 (58.0 %)	62 (56.0 %)	0.770
	Female	37 (42.0 %)	49 (44.0 %)	
3.	**Duration of diabetes** (years) (Mean ± SD)	10.96 ± 7.56	9.24 ± 7.04	0.098
4.	**Hypoglycaemic episode in past**			
	Present, N (%)	34 (38.2 %)	27 (24.3 %)	0.040
	Absent, N (%)	55 (61.8 %)	84 (75.7 %)	
5.	**Treatment details, N (%)**			
	OHAs only	49 (55.0 %)	53 (47.7%)	0.310
	Both OHAs and insulin	21 (23.5 %)	26 (23.4%)	0.980
	Insulin only	8 (9.0 %)	19 (17.1%)	0.090
6.	**Admission blood glucose** (mg/dL) (Mean ± SD)	225.93 ± 139.02	178.72 ± 94.76	0.008
7.	**HbA_1c** (%) (Mean ± SD)	8.55 ± 2.49	7.72 ± 1.82	0.007
8.	**BUN** (mg/dL) (Mean ± SD)	19.04 ± 14.16	16.45 ± 12.16	0.160
9.	**Serum creatinine** (mg/dL) (Mean ± SD)	1.25 ± 0.94	1.17 ± 0.96	0.550
10.	**Serum albumin** (g/dL) (Mean ± SD)	3.22 ± 0.61	3.21 ± 0.68	0.910

Descriptive data were expressed as N (%) for categorical variables, mean and standard deviation (SD) for continuous variables; p value < 0.05 was considered statistically significant; OHA: oral hypoglycaemic agent; BUN: blood urea nitrogen;

Table 2: Risk factors associated with hypoglycaemia

N	Risk factors	Symptomatic group (N = 89)	Asymptomatic group (N = 111)
1.	Reduced food intake than usual, N (%)	20 (22.5 %)	20 (22.5 %)
2.	*Nil per os* (NPO), N (%)	19 (21.3 %)	19 (21.3 %)
3.	Prior renal disease, N (%)	18 (20.2 %)	18 (20.2 %)
4.	Prior liver disease, N (%)	9 (10.1 %)	9 (10.1 %)
5.	History of vomiting, N (%)	11 (12.4 %)	11 (12.4 %)
6.	Antibiotic use, N (%)	27 (30.3 %)	27 (30.3 %)
7.	Use of β-blockers, N (%)	15 (16.9 %)	15 (16.9 %)

*Data were expressed as N (%); p value < 0.05 was considered statistically significant. *Patients who were on NPO for upper gastrointestinal endoscopy, colonoscopy or ultrasonography of abdomen during the episode of hypoglycaemia;*

The observed risk factors associated with hypoglycaemia in study included decreased food intake than usual, *nil per os* (NPO), vomiting episodes, prior renal disease, underlying liver disease, use of antibiotics (any fluroquinolone), infections and history of beta blockers intake. Table 2 shows risk factors associated with hypoglycaemic episode.

Antibiotic intake was found to be slightly more in asymptomatic group (n = 36, 32.4 %) than symptomatic group (n = 27, 30.3 %). More patients in asymptomatic group (n = 30, 27 %) were on beta blockers compared to symptomatic group (n = 15, 16.9 %) though association was not statistically significant (p = 0.09). In symptomatic patients (n = 89), adrenergic symptoms were experienced by 39 (43.8 %), 34 (38.2 %) had pure neuroglycopenic symptoms and 16 (18.0 %) had both adrenergic as well as neuroglycopenic symptoms. The most common adrenergic symptom observed was sweating (n = 40, 44.9 %). The most common neuroglycopenic symptom observed was drowsiness (n = 36, 40.4 %). Other adrenergic symptoms experienced by patients included palpitations (n = 23), anxiety (n = 20), trembling of hands (n = 17), increased appetite (n = 16) and dry mouth (n = 2) as shown in Figure 1. Neuroglycopenic symptoms observed were confusion (n = 16), blurring of vision (n = 14), seizures (n = 10), slurred speech (n = 8) and perioral numbness (n = 4). Figure 2 shows neuroglycopenic symptoms.

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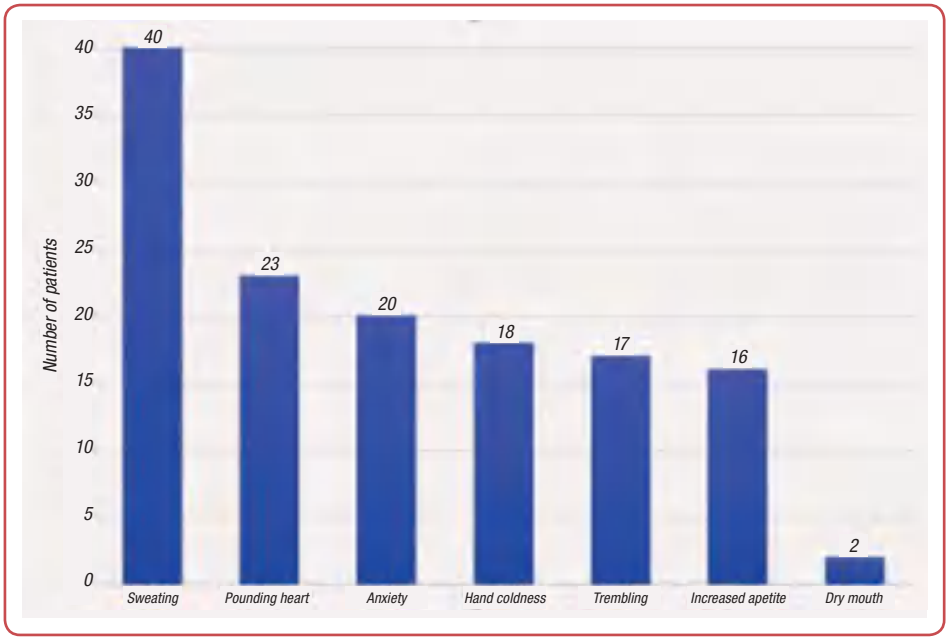

Figure 1: Adrenergic symptoms in patients with hypoglycaemia

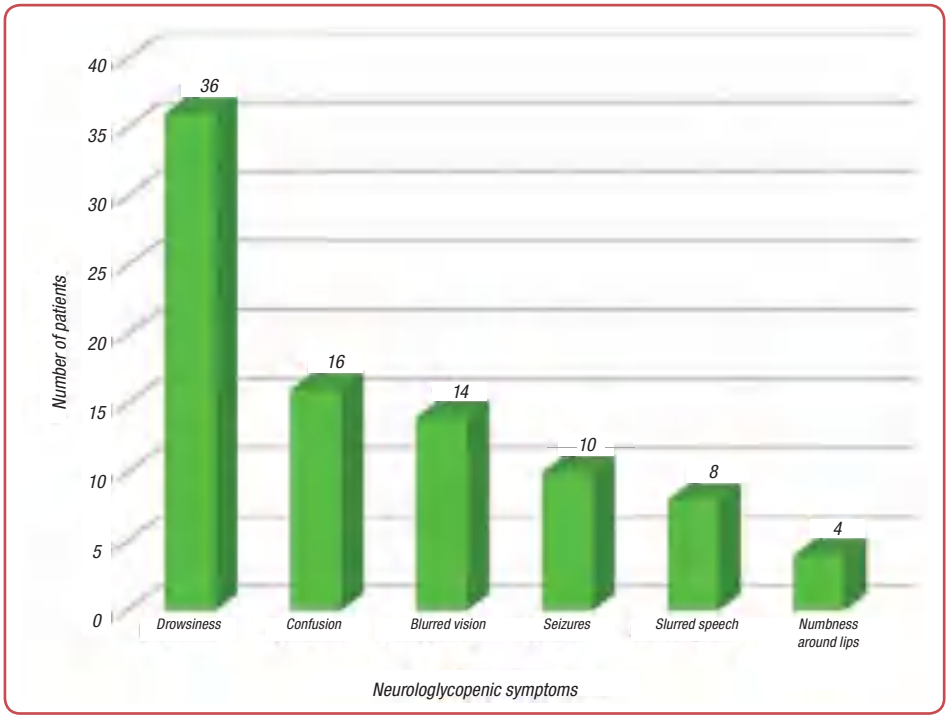

Figure 2: Neuroglycopenic symptoms in patients with hypoglycaemia

Majority in symptomatic group were only on regular insulin (n = 31, 34.8 %), whereas in asymptomatic group most were on combination of regular insulin and intermediate acting insulin (n = 45, 40.5 %) during hospital stay prior to hypoglycaemic event. It is to be noted that 9 (10.1 %) patients in symptomatic group and 8 (7.2 %) patients were not on any treatment for diabetes prior to hypoglycaemic episode as the monitored blood glucose level during stay did not require medications. There was no significant difference in the glucose lowering medication used during the hospital stay between the two groups (Table 3).

Mean admission blood glucose (mg/dL) was significantly higher in symptomatic group than asymptomatic group (225.93 vs 178.72, p = 0.008) whereas mean HbA_1c (%) was significantly lower in asymptomatic group (7.72 ± 1.82) when compared to symptomatic group (8.55 ± 2.49), p = 0.007. Admission blood glucose (mg/dL) was predominantly in the range of 101-200 mg/dL in

CONTENT

Table 3: Comparison of in-patient treatment among the study groups

N	Inpatient treatment	Symptomatic group (N = 89)	Asymptomatic group (N = 111)	p-value
1.	OHA only (metformin, sulphonylurea or both), N (%)	13 (14.6 %)	14 (12.6 %)	0.68
2.	Regular insulin only, N (%)	31 (34.8 %)	37 (33.3 %)	0.82
3.	Regular + neutral protamine hagedorn (NPH), N (%)	29 (32.5 %)	45 (40.5 %)	0.24
4.	OHA + regular insulin, N (%)	4 (4.5 %)	4 (3.6 %)	0.74
5.	OHA+ NPH + regular insulin, N (%)	2 (2.2 %)	1 (0.9 %)	0.43
6.	Long-acting insulin (glargine), N (%)	1 (1.1 %)	2 (1.8 %)	0.69
7.	No treatment, N (%)	9 (10.1 %)	8 (7.2 %)	0.46

Data were expressed as N (%); p value < 0.05 was considered statistically significant; OHA: oral hypoglycaemic agent;

Table 4: Blood glucose values during the hypoglycaemic episodes

N	Capillary blood glucose (CBG)	Symptomatic group (N = 89)	Asymptomatic group (N = 111)	p-value
1.	CBG (Mean ± SD)	44.22 ± 11.21	56.38 ± 9.51	< 0.0001
2.	60-69 mg/dL, N (%)	5 (5.6 %)	45 (40.5 %)	< 0.0001
3.	50-59 mg/dL, N (%)	27 (30.3 %)	44 (39.6 %)	0.1700
4.	40-49 mg/dL, N (%)	26 (29.2 %)	16 (14.4 %)	0.0100
5.	Below 40 mg/dL, N (%)	31 (34.8 %)	6 (5.4 %)	< 0.0001

Descriptive data were expressed as N (%) for categorical variables, mean and standard deviation (SD) for continuous variables; p value < 0.05 was considered statistically significant;

both symptomatic (n = 39, 43.8 %) and asymptomatic patients (n = 61, 55 %), which was found to be not significant (p = 0.11). The majority in both the groups had HbA_1c between 7.1-10.0 %. More patients (n = 12, 10.8 %) in the asymptomatic group had $HbA_1c \leq 6$ % compared to the symptomatic group (n = 2, 2.24 %), p = 0.02. Table 4 shows CBG values of study patients during the hypoglycaemic event.

In the symptomatic group, severe hypoglycaemia (CBG < 40 mg/dL) was observed in 31 patients (34.8 %) which was statistically significant (p < 0.0001) on comparison with asymptomatic group (n = 6, 5.4 %). Severe hypoglycaemia < 50 mg/dL was seen in 64 % (n = 57) of patients in the symptomatic group compared to 19.8 % (n = 22) in the asymptomatic group. Majority of patients with symptomatic hypoglycaemia had glucose value less than 40 mg/dL (n = 31, 34.8 %) during hypoglycaemia episode. Elevated serum blood urea nitrogen (BUN) was observed more in the symptomatic group (n = 29, 32.6 %) than asymptomatic group (n = 25, 22.5 %), though not significant p = 0.16. Similarly, 33.7 % of symptomatic patients with hypoglycaemia had serum creatinine more than 1.1 mg/dL compared to 27 % in asymptomatic group. No significant difference was observed in mean serum sodium (mmol/L), total leucocyte count (cells/mm^3), serum potassium (mmol/L) between symptomatic and asymptomatic group [133.79 ± 5.43 vs 135.14 ± 10.41 (p = 0.27), 11052.58 ± 5571.45 vs 11269.1 ± 5010.46 (p = 0.77), 4.2 ± 0.64 vs 4.16 ± 0.74 (p = 0.69), respectively].

Median duration of stay in the hospital for both symptomatic and asymptomatic group was eight days and there was no significant difference in length of stay between the two groups. Table 5 shows outcomes of study patients including complications.

Out of 89 symptomatic patients, 64 (72 %) improved and were discharged without any complications. Twenty-five patients (28 %) developed complications during the course in hospital, of which three patients (3.37 %) died and 22 recovered. The most common complications observed was nosocomial infection (n = 17, 19.1 %), followed by acute kidney injury (n = 12, 13.5 %), seizure (n = 10, 11.2 %) and cardiovascular events (n = 2, 2.24 %) in symptomatic group. Out of 111 asymptomatic patients, a majority of 91 (81.9 %) got improved and were discharged without any complications. Twenty patients (18 %) in asymptomatic group developed complications during the course in hospital, of which 14 (15.5 %) recovered, 2 (2.2 %) patients died and 4 (4.4 %) were lost to follow up as they were discharged against medical advice. The complications most observed were acute kidney injury (n = 18, 16.2 %), followed

CONTENT

Table 5: Outcomes including complications observed in patients

N	Outcome	Symptomatic group (N = 89)	Asymptomatic group (N = 111)	p-value
1.	Discharge from the hospital without any complications, N (%)	64 (71.9 %)	91 (81.9 %)	0.0900
2.	- Patients who developed complications, N (%)	25 (28.0 %)	20 (18.0 %)*	-
	- Patients who recovered after developing complications, N (%)	22 (24.7 %)	14 (15.5 %)	
3.	Adverse cardiovascular events, N (%)	2 (2.2 %)	4 (3.6 %)	0.5800
4.	Nosocomial infection, N (%)	17 (19.1 %)	13 (11.7 %)	0.1500
5.	Acute kidney injury, N (%)	12 (13.5 %)	18 (16.2 %)	0.5900
6.	Seizure, N (%)	10 (11.2 %)	0 (0.0 %)	0.0003
7.	Duration of stay in hospital (days)			
	< 5 days, N (%)	15 (16.9 %)	15 (13.5 %)	0.5000
	5-9 days, N (%)	42 (47.2 %)	57 (51.3 %)	0.5600
	10-15 days, N (%)	22 (24.7 %)	32 (28.8 %)	0.5100
	> 15 days, N (%)	10 (11.2 %)	7 (6.3 %)	0.2100
8.	Death, N (%)	3 (3.4 %)	2 (1.8 %)	0.4800

**4 patients were lost to follow up as they were discharged against medical advice; p value < 0.05 was considered statistically significant;*

by nosocomial infection (n = 13, 11.7 %) and cardiovascular events (n = 4, 3.6 %) in asymptomatic group. Of note, ten patients (11.2 %) in symptomatic group had seizure but none in the asymptomatic group which was significant (p = 0.0003).

Discussion

Presented study targeted on T2DM patients because hypoglycaemia in T2DM is multifactorial and depends on type of therapy (insulin, insulin secretagogues or insulin sensitiser), presence of comorbid conditions and medications. Hypoglycaemia is a complication in hospitalised T2DM patients which causes morbidity and managing in-patient hypoglycaemia still remains a challenge still remains.[10] In presented study, hypoglycaemic events predominantly occurred in patients aged 55 years and older and symptomatic patients were significantly elderly patients. ADA report on impact of hypoglycaemia in elderly states geriatric population are vulnerable to hypoglycaemic events due to decline in renal function, impaired hepatic metabolism of anti-diabetic medications and a decrease in beta-receptor function.[11] Elderly people naturally have long duration of diabetes which also results in absent glucagon response due to diminished paracrine crosstalk between alpha and beta cells.[12] Hence, the American geriatric society recommends target HbA_1c of 8 % in elderly.[13] One of the definitive risk factors which predicts recurrent episodes is past history of hypoglycaemia.[14] Presented study highlights the significant difference between symptomatic and asymptomatic group with regards to prior episode of hypoglycaemia, admission blood glucose and HbA_1c. Criner et al observed significant difference in recurrent hypoglycaemia between symptomatic (13 %) and asymptomatic group (44 %).[14] Marked variation exists in published studies with regards to defining cut off for hypoglycaemia related events. As per ADA 2020, level-1 hypoglycaemia is present when measured blood glucose is < 70 mg/dL, level-2 hypoglycaemia is defined as blood glucose < 54 mg/dL. Severe hypoglycaemia (level-3) is present when severe impairment in mental status or cognitive decline requiring physical assistance for correction of hypoglycaemia.[15] Severe hypoglycaemia occurs with measured blood glucose level < 40mg/dL.[10] Sweating (40 %) and drowsiness (36 %) were the common adrenergic and neuroglycopenic symptom respectively in presented study. Symptomatic group of patients had significantly lower CBG at the time of hypoglycaemia and 34.8 % of symptomatic group had severe hypoglycaemia (CBG < 40 mg/dL). Shriraam et al reported weakness (76.2 %) and dizziness (74 %) as common hypoglycaemic symptom in T2DM patients and 23 % had severe hypoglycaemia in their study.[16] Patients with CBG between 60-69 mg/dL predominantly didn't manifest symptoms (n = 45) whereas only 5 developed symptoms in presented study. Hence clinical practitioners and health care providers should actively seek for patient reported events, low blood glucose values or hypoglycaemic unawareness in every visit. Reported rates of severe hypoglycaemia in published studies varies from 0.7-12/100 person-years.[17]

CONTENT

One of the existing myths in management of diabetes is that higher HbA_1c doesn't cause hypoglycaemia in T2DM.[4] Presented study observed HbA_1c was significantly associated with in-hospital hypoglycaemia in T2DM patients in both symptomatic and asymptomatic group and mean HbA_1c levels was significantly higher in symptomatic group (8.5 %) than asymptomatic group (7.7 %). The diabetes and ageing study by Lipska et al showed hypoglycaemia occurrence across all levels of glycaemic control and 19 % of the people who reported hypoglycaemia had HbA_1c > 9 % (n = 187 out of 985 patients with hypoglycaemia).[18] In the Freemantle study, 1 % increase in HbA_1c was reported to have significant increase in frequency of hypoglycaemic episodes.[19] All these findings highlight the significance of occurrence of hypoglycaemia in T2DM patients despite higher HbA_1c.

Hypokalaemia was observed more in asymptomatic group (n = 14, 12.6 %) than symptomatic group (n = 5, 5.6 %) in presented study. Kang et al in their study observed nearly 22 % patients having hypokalaemia during severe hypoglycaemia.[20] This was associated with high blood pressure and tachycardia possibly reflecting sympathetic drive as a response to hypoglycaemia which secondarily induces hypokalaemia by activation of beta adrenoreceptors. Elevated creatinine was noted in 30 % of total patients in presented study. A study done by Carreira et al found hypoglycaemia was 4.2 times higher with acute kidney injury (AKI) and duration of AKI more than 5.5 days is a predictor of hypoglycaemia and mortality.[21] Renal function must be evaluated in all patients with hypoglycaemia, particularly in elderly patients as age related decline in glomerular filtration rate happens and AKI is a precipitating factor for hypoglycaemia.[22] Elderly patients preferably should have a target HbA_1c of 8 % rather than stringent glycaemic control which carries risk of serious hypoglycaemia. Presented study didn't find any significant difference between the two group with regards to length of stay, complications reported including adverse cardiovascular event or mortality possibly due to low event rate observed and smaller sample size and hence causality cannot be claimed from presented findings. In this study, 6 patients had adverse cardiovascular event. ACCORD trial reported hypoglycaemia was three times higher in intensive arm and trail was stopped early in view of serious adverse cardiovascular events and subsequent mortality, though increase in mortality was not directly linked to hypoglycaemia.[23] ADVANCE study also concluded severe hypoglycaemia is associated with higher macrovascular events (Hazard ratio, HR: 2.88), death from cardiovascular cause (HR: 2.68).[24] In presented study, 10 (11 %) of patients who had seizures in the symptomatic group got recovered and discharged from the hospital. Diagnosis of those patients who had seizures include acute on chronic kidney disease (n = 2), bronchial asthma (n = 2), angioedema (n = 1), accelerated hypertension (n = 1), diabetic foot ulcer (n = 2), urinary tract infection (n = 2).

Limitations of this study are that it was not assess the temporal pattern of hypoglycaemia (daytime vs nocturnal events) and recurrent hypoglycaemia in study patients were not studied. For each patient included in this study, the first hypoglycaemic episode only was counted and subsequent number of hypoglycaemic episodes following the first event were not assessed. The clinical characteristics with hospitalised T2DM patients without hypoglycaemia were not compared. Observed event rate of complications is smaller and causality or association with hypoglycaemia cannot be established from this study. Levels of hypoglycaemia as per ADA was not assessed between the groups.

Conclusion

This study showed that symptomatic in-hospital hypoglycaemia in T2DM patients was significantly associated with prior episode of hypoglycaemia and admission blood glucose levels. HbA_1c levels and severe hypoglycaemia were significantly higher in symptomatic group of T2DM patients. Results showed that risk of hypoglycaemia was seen at all levels of glycaemic status in patients with T2DM. Study also highlights that even with sub-optimal HbA_1c levels > 7 %, hypoglycaemia can occur in hospitalised patients with T2DM. Predominant episodes of hypoglycaemia were asymptomatic in hospitalised patients. General practitioners and clinicians should routinely enquire about symptoms of hypoglycaemia in each visit including patient recalled events as well as documented hypoglycaemia without any symptoms.

CONTENT

Ethics

Study approval was obtained from Institutional Research Ethics Committee, SRIHER, India (Decision No: CSP-MED/19/NOV/57/192, dated 29 January 2020).

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None.

Conflicts of interest

The authors declare that there is no conflict of interest.

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Data access

The data that support the findings of this study are available from the corresponding author upon reasonable individual request.

Author ORCID numbers

Swarna Krishnamoorthy (SK):
0009-0003-5246-5096
Viswanathan Pandurangan (VP):
0000-0002-2721-9657
Sivaprakash Varadan (SV):
0000-0003-4719-4570
Sowmya Gopalan (SG):
0000-0003-3117-433X
Sudagar Singh R B (SRB):
0000-0002-8625-4305

Author contributions

Conceptualisation: SK, VP, SV
Methodology: SK, VP, SV, SG, SRB
Validation: SK, VP, SV, SG, SRB
Formal analysis: SK, VP, SV
Investigation: SK, VP, SV
Data curation: SK, VP, SV
Writing - original draft: . SK, VP, SV, SG, SRB
Writing - review and editing: SG, SRB
Supervision: SG, SRB

References

1. UK Prospective Diabetes Study (UKPDS) Group: Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet. 1998;352(9131):837-53. doi: 10.1016/S0140-6736(98)07019-6.
2. Nathan DM, Genuth S, Lachin J, Cleary P, Crofford O, Davis M, et al. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus (Diabetes Control and Complications Trial Research Group). N Engl J Med. 1993;329:977-86. doi:10.1056/NEJM199309303291401.
3. Viswanathan M, Joshi SR, Bhansali A. Hypoglycemia in type 2 diabetes: Standpoint of an experts' committee (India hypoglycemia study group). Indian J Endocrinol Metab. 2012;16(6):894-8. doi:10.4103/2230-8210.102986.
4. Heller SR, Peyrot M, Oates SK, Taylor AD. Hypoglycemia in patient with type 2 diabetes treated with insulin: it can happen. BMJ Open Diabetes Res Care. 2020;8(1):e001194. doi:10.1136/bmjdrc-2020-001194.
5. Zammitt NN, Frier BM. Hypoglycemia in type 2 diabetes: pathophysiology, frequency, and effects of different treatment modalities. Diabetes Care. 2005;28:2948-61. doi:10.2337/diacare.28.12.2948.
6. Cryer PE: The barrier of hypoglycemia in diabetes. Diabetes. 2008;57:3169-76. doi:10.2337/db08-1084.
7. Olujide OP, Olujide ME, Leonardi-Bee J, Chattopadhyay K. Content and quality of clinical practice guidelines for the management of type 2 diabetes in India: A systematic review. Endocrinol Diabetes Metab. 2023;6:405. doi:10.1002/edm2.405.
8. Maiti S, Akhtar S, Upadhyay AK, Mohanty SK. Socioeconomic inequality in awareness, treatment and control of diabetes among adults in India: Evidence from National Family Health Survey of India (NFHS), 2019-2021. Sci Rep. 2023;13(1):2971. doi:10.1038/s41598-023-29978-y.
9. Chopra S, Kewal A. Does hypoglycemia cause cardiovascular events? Indian J Endocrinol Metab. 2012;16:102-4. doi: 10.4103/2230-8210.91203.
10. Cruz P. Inpatient hypoglycemia: the challenge remains. J Diabetes Sci Technol. 2020;14(3):560-6. doi: 10.1177/1932296820918540.

CONTENT

11. Seaquist ER, Anderson J, Childs B, Cryer P, Dagogo-Jack S, Fish L, et al; American Diabetes Association; Endocrine Society. Hypoglycemia and diabetes: a report of a workgroup of the American Diabetes Association and the Endocrine Society. J Clin Endocrinol Metab. 2013 May;98(5):1845-59. doi: 10.1210/jc.2012-4127.
12. Segel SA, Paramore DS, Cryer PE. Hypoglycemia-associated autonomic failure in advanced type 2 diabetes. Diabetes. 2002;51:724-33. doi:10.2337/diabetes.51.3.724.
13. Brown AF, Mangione CM, Saliba D, Sarkisian CA; California Healthcare Foundation/American Geriatrics Society Panel on Improving Care for Elders with Diabetes. Guidelines for improving the care of the older person with diabetes mellitus. J Am Geriatr Soc. 2003 May;51(5 Suppl Guidelines):S265-80. doi: 10.1046/j.1532-5415.51.5s.1.x.
14. Criner KE, Kim HN, Ali H, Kumar SJ, Kanter JE, Wang L, et al. Hypoglycemia symptoms are reduced in hospitalized patients with diabetes. J Diabetes Complications. 2021;35:107976. doi: 10.1016/j.jdiacomp.2021.107976.
15. American Diabetes Association. Standards of medical care in diabetes-2020 abridged for primary care providers. Clin Diabetes. 2020;38(1):10-38. doi:10.2337/cd20-as01.
16. Shriraam V, Mahadevan S, Anitharani M, Jagadeesh NS, Kurup SB, Vidya TA, et al. Reported hypoglycemia in Type 2 diabetes mellitus patients: Prevalence and practices-a hospital-based study. Indian J Endocrinol Metab. 2017;21(1):148-53. doi: 10.4103/2230-8210.196002.
17. Silbert R, Salcido-Montenegro A, Rodriguez-Gutierrez R, Katabi A, McCoy RG. Hypoglycemia among patients with type 2 diabetes: epidemiology, risk factors, and prevention strategies. Curr Diab Rep. 2018;18(8):53. doi: 10.1007/s11892-018-1018-0.
18. Lipska KJ, Warton EM, Huang ES, Moffet HH, Inzucchi SE, Krumholz HM, et al. HbA1c and risk of severe hypoglycemia in type 2 diabetes: the Diabetes and Aging Study. Diabetes Care. 2013 Nov;36(11):3535-42. doi: 10.2337/dc13-0610.
19. Davis TM, Brown SG, Jacobs IG, Bulsara M, Bruce DG, Davis WA. Determinants of severe hypoglycemia complicating type 2 diabetes: the Fremantle Diabetes Study. J Clin Endocrinol Metab. 2010;95:2240-7. doi:10.1210/jc.2009-2828.
20. Kang MY. Blood electrolyte disturbances during severe hypoglycemia in Korean patients with type 2 diabetes. Korean J Intern Med. 2015, 30:648-56. doi: 10.3904/kjim.2015.30.5.648.
21. Carreira A, Castro P, Mira F, Melo M, Ribeiro P, Santos L. Acute kidney injury: a strong risk factor for hypoglycaemia in hospitalized patients with type 2 diabetes. Acta Diabetol. 2023;60(9):1179-85. doi:10.1007/s00592-023-02112-0.
22. Saito N, Toyoda M, Ono M, Kaneyama N, Kimura M, Fukagawa M. Importance of the evaluation of renal function for the prevention of hypoglycemia in elderly diabetes patients. Tokai J Exp Clin Med. 2020;45(3):139-43. http://mj-med-u-tokai.com/pdf/450309.pdf.
23. ACCORD Study Group; Buse JB, Bigger JT, Byington RP, Cooper LS, Cushman WC, Friedewald WT, et al. Action to control cardiovascular risk in diabetes (ACCORD) trial: design and methods. Am J Cardiol. 2007 Jun 18;99(12A):21i-33i. doi: 10.1016/j.amjcard.2007.03.003.
24. Zoungas S, Patel A, Chalmers J, de Galan BE, Li Q, Billot L, et al; ADVANCE Collaborative Group. Severe hypoglycemia and risks of vascular events and death. N Engl J Med. 2010 Oct 7;363(15):1410-8. doi: 10.1056/NEJMoa1003795.

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ORIGINAL ARTICLE

Effectiveness of Counterpressure Technique With a Birth Ball on Cervical Dilatation and Reduction of Labour Pain and Uterine Contractions: A Prospective Cohort Study

Adriana Egam,[1] Vera Iriani Abdullah,[1] Mariana Isir,[1] Fitra Duhita,[1] Alva Cherry Mustamu[2]

1. Department of Midwifery, Ministry of Health Polytechnic Sorong, West Papua, Indonesia.
2. Department of Nursing, Ministry of Health Polytechnic Sorong, West Papua, Indonesia.

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Corresponding author:
ADRIANA EGAM
E: adrianaegam23@gmail.com

Abstract

Background/Aim: Non-pharmacological interventions play a crucial role in managing labour pain and facilitating labour progression. The counterpressure technique is a commonly utilised method purported to alleviate pain and enhance cervical dilation during childbirth. However, its effectiveness remains a subject of debate, necessitating further investigation. This study aimed to evaluate the efficacy of the counterpressure technique in managing labour pain and influencing labour progression among women in labour.

Method: A randomised controlled trial was conducted involving participants in active labour. The intervention group received the counterpressure technique, while the control group received standard care. Pain levels, uterine contractions and cervical dilation were assessed and compared between the two groups.

Results: Analysis revealed no significant difference in pain levels or uterine contractions between the intervention and control groups. However, there was a significant increase in cervical dilation in the intervention group compared to the control group ($p = 0.034$, Cohen's $d = -0.586$).

Conclusion: Despite the counterpressure technique's limited impact on pain relief and uterine contractions, it significantly facilitated cervical dilation during labour. These findings contribute to understanding of non-pharmacological interventions in childbirth and underscore the importance of evidence-based approaches to labour management. Further research is warranted to elucidate the underlying mechanisms of the counterpressure technique and optimise its implementation in clinical practice.

Key words: Labour pain; Obstetric labour complications; Counterpressure technique; Birth ball; Non-pharmacological pain management.

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Introduction

The management of labour pain has become a primary focus in modern obstetric practice. In recent decades, the increase in knowledge and understanding of the labour process has transformed how we approach labour pain management.[1-3] Now, it is not only about assisting mothers through this process but also ensuring that their experience is as positive as possible. Thus, the management of labour pain has become central in modern obstetric practice.[2, 3]

The use of non-pharmacological techniques has gained popularity in reducing labour pain without significant side effects.[2, 4-8] These techniques

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CONTENT

encompass various approaches, ranging from relaxation techniques and breathing exercises to the use of physical aids such as birth balls. The primary advantage of these techniques is that they do not entail significant side effects commonly associated with pharmacological interventions, making them an appealing choice for many women.[9]

One technique that has attracted attention is the use of a birth ball as an adjunct in reducing labour pain. The birth ball is a simple yet effective tool that can help alleviate labour pain. By sitting or lying on the ball, women can relieve pressure on the lower back and pelvis, which are often sources of pain during labour.[10, 11] Additionally, the ball can aid in positioning and descent of the baby, facilitating the labour process.[12-14]

Previous research indicates that the counterpressure technique is also effective in reducing the intensity of pain during labour. Counterpressure involves applying pressure to specific areas on the lower back of women during contractions, which can help alleviate pain.[2, 15, 16] Studies have shown that this technique can significantly reduce pain intensity during labour, providing additional benefits for women who choose to use these non-pharmacological techniques.[2, 4, 17, 18]

However, limited information is available regarding the combined effects of the counterpressure technique with the use of a birth ball in managing labour pain.[10, 15, 19] Although both techniques have been proven effective independently, questions remain about how they can work together.

The use of a birth ball has become increasingly popular in recent years as a tool to help reduce labour pain.[10, 20, 21] These balls, often made of rubber and filled with air, can be used in various positions to alleviate pain and facilitate the labour process. Many mothers report that using a birth ball helps them feel more comfortable during labour and gives them more control over their experience.[22-24]

Counterpressure technique is another non-pharmacological technique that can be used to help reduce labour pain.[2, 7] This technique involves applying pressure to specific areas on the back or hips during contractions, which can help alleviate pain. The effectiveness of this technique may vary depending on the individual, but many women report a significant decrease in labour pain when using this technique.

There is some evidence to suggest that the combination of counterpressure technique and the use of a birth ball can provide additional benefits in reducing labour pain.[10] Both techniques can be used together to provide physical and emotional support during labour. However, more research is needed to fully understand how these two techniques can work together to reduce pain.

The duration of labour can vary significantly between individuals and even between different labours for the same woman. Some studies have shown that the use of techniques such as counterpressure and birth ball can help speed up the labour process, but these results are not consistent across all studies.[10, 25, 26] Therefore, it is important to conduct more research to determine whether there are significant differences in the duration of labour between groups using these combination techniques and the control group.

Like all childbirth interventions, the use of a birth ball and counterpressure technique carries potential risks and benefits. Potential benefits include pain reduction, increased control over the labour process and a more positive childbirth experience. Potential risks may include physical injury if the ball is not used correctly.[27] However, with proper supervision and instruction, these risks can be minimised.

The management of labour pain has become a primary focus in modern obstetric practice, with an increase in non-pharmacological techniques such as the use of a birth ball and counterpressure.[1-3] While both techniques are effective independently, little is known about their combined effects. Previous research has shown their individual effectiveness, but not their combined impact.[27] Understanding this interaction is crucial, as it may affect labour duration. It's also important to consider the potential risks and benefits, including pain reduction and increased control over the labour process.

The aim of this study was to evaluate the effectiveness of using a combination of counterpressure technique with the use of a birth ball in reducing labour pain and to understand its impact on the duration of labour and the mother's experience during the childbirth process. This research aimed to provide a better understanding of labour pain management and to contribute to improvements in clinical practice in caring for pregnant women.

CONTENT

Methods

This study employed a prospective cohort design to investigate the effectiveness of combining the counterpressure technique with the use of a birth ball in reducing labour pain. A cohort design was chosen for its ability to observe changes over time and evaluate the cause-effect relationship between intervention variables and observed outcomes. This study was approved by the Ethics Commission of the Health Polytechnic Ministry of Health Sorong, ensuring adherence to ethical standards and the protection of participants' rights and welfare. Informed consent was obtained from all participants prior to their inclusion in the study.

The population consisted of pregnant women with a gestational age over 37 weeks visiting the Malawili Community Health Centre. Participants were selected based on specific inclusion and exclusion criteria to ensure valid and reliable outcomes. Inclusion criteria were: gestational age over 37 weeks, age range of 18 to 40 years, absence of significant medical complications such as hypertension or gestational diabetes and no history of severe preeclampsia or spinal problems. Exclusion criteria included any medical conditions or injuries that would contraindicate the use of counterpressure or a birth ball during labour.

Pain intensity was measured using a validated visual analogue scale (VAS), detailed in the appendices. The VAS ranges from 0 (no pain) to 10 (worst pain imaginable), providing a quantifiable measure of pain. Participants received detailed instructions and demonstrations on using the VAS for self-reporting pain levels.

Participant recruitment took place through antenatal clinics and maternity hospitals from September to November 2023. Simple random sampling was used to assign participants to either the intervention group or the control group. The intervention group utilised a birth ball and the counterpressure technique, while the control group received standard care without analgesia, reflecting the hospital's protocol and the preference of some patients.

Procedure

During labour, trained midwives educated participants on the counterpressure technique, which involved applying steady pressure to specific points on the lower back or hips during contractions. Participants were trained to apply the pressure themselves or with the help of a partner, under midwife supervision. Detailed instructions for using the birth ball were also provided, emphasising correct positioning and movements to relieve lower back and pelvic pressure and to facilitate the baby's descent.

The control group received standard care, which did not include analgesia due to either hospital protocols or patient preferences. This approach allowed for a clear comparison of the intervention's effectiveness against the standard non-pharmacological practices.

Participants were randomly assigned to the intervention or control group using a computer-generated randomisation list, ensuring an unbiased distribution and enhancing the validity of the results.

Statistical analysis

Statistical analysis involved comparing the intervention and control groups using Wilcoxon and Mann-Whitney tests, facilitated by the *Jamovi* statistical software. These non-parametric tests were chosen to handle the ordinal nature of the VAS pain scores and potential non-normal distribution of data.

Results

Characteristics of respondents

A total of 60 respondents participated in this study, with 28 assigned to the control group and 32 to the intervention group using a simple random sampling technique. The slight imbalance in group sizes resulted from the random sampling process. The characteristics of the respondents are detailed in Table 1.

There were no significant differences between the control and intervention groups in terms of age, gestational age, delivery history, education, occupation, religion or monthly income (Table 1). This indicates that the groups were well matched and any observed differences in outcomes are likely due to the intervention itself.

CONTENT

Table 1: Characteristics of respondents (women in labour)

Variable	Control (n = 28)	Intervention (n = 32)	p-value
Age, Mean, SD (Min-Max)	31.5, 2.70 (27-35)	30, 2.15 (27-35)	0.422
Gestational age, Mean, SD (Min-Max)	40.1, 1.56 (37-42)	39.5, 1.65 (37-42)	0.498
Delivery history			
Nulliparous	14 (46.7 %)	16 (53.3 %)	1.000
Multiparous	14 (46.7 %)	16 (53.3 %)	
Education			
No schooling	4 (44.4 %)	5 (55.6 %)	
Elementary school	8 (57.1 %)	6 (42.9 %)	
Junior high school	9 (64.3 %)	5 (35.7 %)	0.282
High school	5 (27.8 %)	13 (72.2 %)	
University	2 (40.0 %)	3 (60.0 %)	
Occupation			
Unemployed	16 (57.1 %)	12 (42.9 %)	0.128
Employed	12 (37.5 %)	20 (62.5 %)	
Religion			
Protestant Christian	10 (45.5 %)	12 (54.5 %)	
Catholic	8 (50.0 %)	8 (50.0 %)	0.952
Islam	10 (45.5 %)	12 (54.5 %)	
Monthly income of the family			
Below relative minimum wage	19 (50.0 %)	19 (50.0 %)	0.496
Above relative minimum wage	9 (40.9 %)	13 (59.1 %)	

Intervention: counterpressure technique with birth ball;

Comparison of pain levels between intervention and control group

Table 2 shows that before applying the counterpressure technique, there was no significant difference in pain levels between the control group (mean = 6.79, SD = 1.50) and the intervention group (mean = 6.06, SD = 1.92), with a p-value of 0.173. This indicates that both groups had similar baseline pain levels, ensuring the validity of subsequent comparisons regarding the effectiveness of the intervention.

Table 2: Pain before counterpressure technique in women in labour

Parameter	Control (mean ± SD)	Intervention (mean ± SD)	p-value
Pain (pre-test)	6.79 ± 1.50	6.06 ± 1.92	0.173

Intervention: counterpressure technique; SE: standard error;

The results presented in Figure 1 indicate that there was no significant difference in pain levels between the control group (mean: 3.11 ± 1.31) and the intervention group (mean: 3.09 ± 1.38), with a p-value of 0.958. The effect size measured using Cohen's d was also very small (0.00994), suggesting that the counterpressure technique did not have a significant impact on reducing pain in the intervention group compared to the control group.

Comparison of uterine contraction after counterpressure technique

The analysis in Figure 2 revealed no significant difference in uterine contractions between the control group (32.2 ± 7.20) and the intervention group (32.8 ± 6.90), with a p-value of 0.700. Additionally, the analysis indicated that the standard mean difference between the two groups was 1.82 with a Cohen's d effect size of 0.0592. These findings suggest that the application of the counterpressure technique did not result in a significant difference in uterine contractions compared to the control group, although there was a slight non-significant increase observed in the intervention group.

Comparison of cervical dilatation after counterpressure technique

The analysis in Figure 3 revealed a significant difference in cervical dilatation between the control group (5.50 ± 1.29) and the intervention group (6.25 ± 1.27), with a p-value of 0.034. Additionally, the standard error of the difference between the two groups was 0.331 and the Cohen's d effect size was -0.586. These findings suggest that

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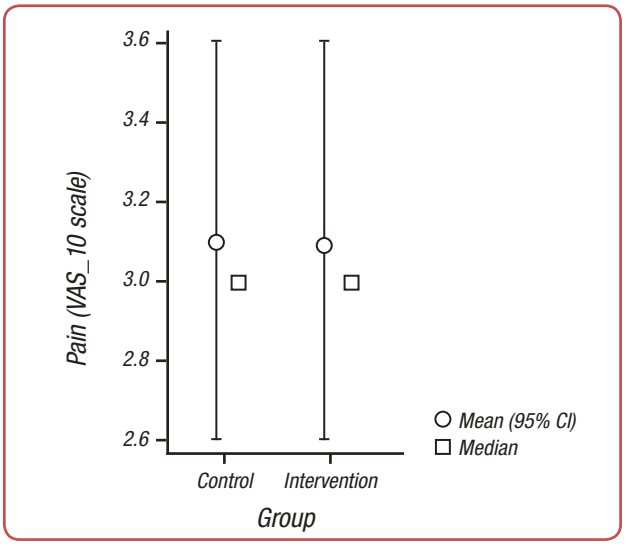

Figure 1: Pain after counterpressure technique in women in labour

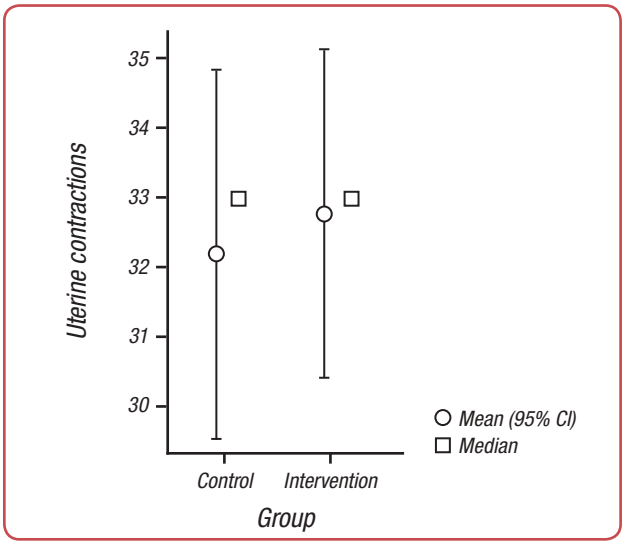

Figure 2: Uterine contraction after counterpressure technique in women in labour

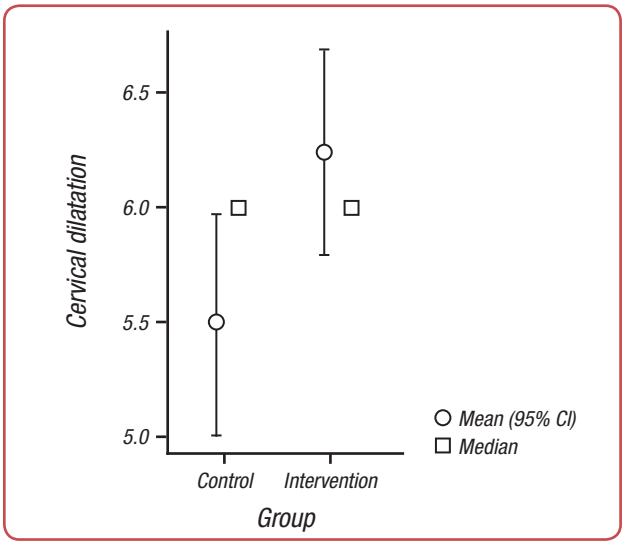

Figure 3: Cervical dilatation after counterpressure technique in women in labour

the application of the counterpressure technique resulted in a significant difference in cervical dilatation compared to the control group, with the intervention group exhibiting a higher mean cervical dilatation.

Discussion

The findings of this study offer valuable insights into the efficacy of the counterpressure technique in managing labour pain and influencing labour progression. This result suggests that while the counterpressure technique may not directly impact pain or uterine contractions, it may have a notable effect on cervical dilatation, potentially facilitating labour progression.

The examination of pain levels between the intervention and control groups in this study contributes to the ongoing discourse regarding the efficacy of the counterpressure technique in managing labour pain. Presented findings, which revealed no significant difference in pain levels ($p = 0.958$) and a minimal effect size (Cohen's $d = 0.00994$), align with prior research conducted by Ahmed et al, who similarly reported comparable pain scores between groups utilising the counterpressure technique and those who did not. This consistency in findings suggests that while the counterpressure technique is widely used as a non-pharmacological approach to pain management during labour, its effectiveness in significantly reducing pain remains uncertain.[28]

However, it is essential to interpret these results within the context of existing literature, acknowledging variations in study methodologies and participant demographics that may influence outcomes. Despite the non-significant findings, the counterpressure technique may still hold value as part of a comprehensive pain management strategy during childbirth, particularly in combination with other evidence-based interventions.

The examination of uterine contractions in this study provides valuable insights into the potential effects of the counterpressure technique on labour dynamics. Presented findings, which indicated no significant difference in uterine contractions between the intervention and control groups ($p = 0.700$, Cohen's $d = 0.0592$) are consistent with prior research in this domain. For in-

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stance, Sriayuningtyas et al conducted a similar investigation and also reported comparable uterine contraction patterns between groups utilising the counterpressure technique and those who did not.[29] Moreover, this study builds upon existing literature by offering a nuanced analysis of the effect size, which, while small, underscores the subtle influence of the counterpressure technique on uterine activity during labour. Although the observed increase in uterine contractions in the intervention group was not statistically significant, it is noteworthy within the context of non-pharmacological interventions for labour pain management.

The analysis of cervical dilation differences between the intervention and control groups in this study provides a deep understanding of the effectiveness of the counterpressure technique in influencing labour progression. Presented findings indicate a significant difference in cervical dilation between the two groups ($p = 0.034$, Cohen's $d = -0.586$), with the intervention group exhibiting a higher average cervical dilation compared to the control group. These results are consistent with prior research indicating that the application of the counterpressure technique significantly contributes to increased cervical dilation during labour. For instance, a study conducted by Wahyuni et al demonstrated that the group receiving the counterpressure technique intervention experienced a significant increase in the rate of cervical dilation compared to the control group.[30] This analysis also highlights a moderate effect size, suggesting that although the influence of the counterpressure technique on cervical dilation is significant, its impact is not overwhelmingly large. Nonetheless, these findings provide robust support for the benefits of the counterpressure technique in facilitating labour progression, which is relevant for enhancing the well-being of both mothers and babies during the childbirth process. Integrating the findings from the comparisons of pain levels, uterine contractions and cervical dilatation, this study contributes to understanding of the multifaceted effects of the counterpressure technique on labour outcomes. Despite the non-significant differences observed in pain levels and uterine contractions between the intervention and control groups, a significant disparity emerged in cervical dilatation, suggesting a nuanced impact of the counterpressure technique on labour progression.

The influence of counterpressure technique in facilitating cervical dilation during childbirth can be explained through several complex hormonal and physiological mechanisms. Firstly, as a woman enters active labour, her body naturally releases the hormone oxytocin. Oxytocin is the primary hormone responsible for uterine contractions that drive the birthing process. The use of counterpressure technique can stimulate oxytocin release by applying concentrated pressure to specific points on the body, such as the lower back or waist area. This stimulation can enhance oxytocin production and increase the strength and frequency of uterine contractions, which in turn can expedite the cervical dilation process.

Furthermore, counterpressure technique can also alleviate tension in the pelvic and surrounding muscles. During childbirth, tension in these muscles can impede the baby's movement towards the birth canal and hinder cervical dilation. By applying pressure to specific points, counterpressure technique can help reduce this muscle tension, facilitating more space for the baby to move and allowing the cervix to soften and dilate more easily. Additionally, counterpressure technique can influence the body's response to stress and comfort. Pressure stimulation at specific points on the body has been known to stimulate the parasympathetic nervous system, which is responsible for the body's relaxation response. By stimulating this relaxation response, counterpressure technique can help reduce stress and tension that may impede labour progression. As a result, the body becomes more prepared for the birthing process and cervical dilation can occur more efficiently.

Overall, the hormonal and physiological mechanisms behind the effectiveness of counterpressure technique in facilitating cervical dilation during childbirth are complex and involve intricate interactions between the hormonal, nervous and muscular systems of the body. Further research is needed to better understand these mechanisms and to optimise the use of counterpressure technique in clinical practice to improve childbirth outcomes.

While the technique may not directly alleviate pain or influence uterine activity, its association with increased cervical dilatation implies a potential role in facilitating labour advancement. These findings underscore the complexity of non-pharmacological interventions in labour management and highlight the need for a comprehensive approach to understanding their effects. Additional-

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ly, recognising the limitations of this study, such as sample size and contextual factors, opens avenues for future research to explore the underlying mechanisms and optimise the utilisation of the counterpressure technique in clinical practice. By addressing these gaps, future studies can further enhance our knowledge of effective strategies for promoting maternal and foetal well-being during childbirth, ultimately contributing to evidence-based obstetric care.

Conclusion

While the counterpressure technique did not significantly reduce pain levels or influence uterine contractions, it was associated with a significant increase in cervical dilation compared to the control group. This finding underscores the potential role of the counterpressure technique in facilitating labour advancement. The significance of these findings lies in their contribution to the existing body of knowledge regarding non-pharmacological interventions in childbirth. The counterpressure technique showed promise in enhancing cervical dilation and further research is warranted to elucidate its underlying mechanisms and optimise its implementation in clinical practice. Moreover, future studies should explore the synergistic effects of the counterpressure technique with other pain management strategies and evaluate its long-term effects on maternal and neonatal outcomes. Ultimately, these findings have implications for both research and practice, highlighting the need for evidence-based approaches to labour management and suggesting avenues for improving maternal and foetal well-being during childbirth.

Ethics

The study was approved by the Ethics Committee of the Ministry of Health Polytechnic Sorong below the supervision of the Ministry of Health of the Republic of Indonesia, decision No DM. 4.1/1/172/2023, dated 19 August 2023. Written informed consent was obtained from patients prior to their participation in the study and for publishing of the anonymised data. The study was organised and implemented based on the adherence to the Ethical Principles for Medical Research Involving Human subjects (The Declaration of Helsinki, 8th Revision, 2013).

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Conflicts of interest

The authors declare that there is no conflict of interest.

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Data access

The data that support the findings of this study are available from the corresponding author upon reasonable individual request.

Author ORCID numbers

Adriana Egam (AE):
0009-0001-1784-3413
Vera Iriani Abdullah (VI):
0000-0003-1690-0794
Mariana Isir (MI):
0009-0003-9551-2614
Fitra Duhita (FD):
0000-0002-1813-1178
Alva Cherry Mustamu (ACM):
0000-0002-6682-514X

CONTENT

Author contributions

Conceptualisation: AE, VA, MI, FD
Methodology: AE, VA, MI, FD
Validation: AE, VA, MI, FD
Formal analysis: AE, VA, MI, FD
Investigation: AE, VA, MI, FD
Resources: AE, VA, MI, FD
Data curation: AE, VA, MI, FD
Writing - original draft: AE, VA, MI, FD
Writing - review and editing: ACM
Visualisation: ACM
Supervision: AE, VA, MI, FD
Project administration: AE, VA, MI, FD
Funding acquisition: AE, VA, MI, FD

References

1. Solomon ET, Kassie FY, Mekonnen DG, Mihret MS, Abate AT, Dessie AA. Knowledge, attitude, and practice towards labor pain management and associated factors among skilled birth attendants working at hospitals found in Central, West, and North Gondar zones, Northwest Ethiopia, 2019: A multicenter cross-sectional study. Pain Res Manag. 2021 May 13;2021:8835677. doi: 10.1155/2021/8835677.
2. Nori W, Kassim MAK, Helmi ZR, Pantazi AC, Brezeanu D, Brezeanu AM, et al. Non-pharmacological pain management in labour: a systematic review. J Clin Med. 2023 Jan;12(23):7203. doi: 10.3390/jcm12237203.
3. Pietrzak J, Mędrzycka-Dąbrowska W, Wróbel A, Grzybowska ME. Women's knowledge about pharmacological and non-pharmacological methods of pain relief in labour. Healthcare. 2023 Jan;11(13):1882. doi: 10.3390/healthcare11131882.
4. Chang CY, Gau ML, Huang CJ, Cheng H min. Effects of non-pharmacological coping strategies for reducing labour pain: A systematic review and network meta-analysis. PLoS ONE. 2022 Jan;17(1):e0261493. doi: 10.1371/journal.pone.0261493.
5. Barut S, Sabancı Baransel E, Çelik OT, Uçar T. The trends and hotspots of research on non-pharmacological interventions for labour pain management: a bibliometric analysis. J Psychosom Obstet Gynecol. 2024 Dec;45(1):2322614. doi: 10.1080/0167482X.2024.2322614.
6. Parkies LE, Murray D, Okafor UB. Pharmacological labour pain interventions: South African midwives' perspective. BMC Nurs. 2024 Mar;23:176. doi: 10.1186/s12912-024-01844-w.
7. Elgzar WT, Alshahrani MS, Ibrahim HA. Non-pharmacological labour pain relive methods: utilization and associated factors among midwives and maternity nurses in Najran, Saudi Arabia. Reprod Health. 2024 Jan;21(1):11. doi: 10.1186/s12978-023-01737-2.
8. Mwakawanga DL, Mselle LT, Chikwala VZ, Sirili N. Use of non-pharmacological methods in managing labour-pain: experiences of nurse-midwives in two selected district hospitals in eastern Tanzania. BMC Pregnancy Childbirth. 2022 Apr;22(1):376. doi: 10.1186/s12884-022-04707-x.
9. Toussaint L, Nguyen QA, Roettger C, Dixon K, Offenbächer M, Kohls N, et al. Effectiveness of progressive muscle relaxation, deep breathing, and guided imagery in promoting psychological and physiological states of relaxation. Evid-Based Complement Altern Med. 2021 Jul;2021:5924040. doi: 10.1155/2021/5924040.
10. Jha S, Vyas H, Nebhinani M, Singh P, T D. The effect of birthing ball exercises on labour pain and labour outcome among primigraviade parturient mothers at a tertiary care hospital. Cureus. 2023;15(3):e36088. DOI:10.7759/cureus.36088.
11. Grenvik JM, Rosenthal E, Wey S, Saccone G, De Vivo V, De Prisco Lcp A, et al. Birthing ball for reducing labour pain: a systematic review and meta-analysis of randomized controlled trials. J Matern Fetal Neonatal Med. 2022 Dec;35(25):5184-93. doi: 10.1080/14767058.2021.1875439.
12. Costa AC, Prata JA, Oliveira KR, Silva CRF da, Progianti JM, Mouta RJO, et al. Freedom of movement and positioning in childbirth with non-invasive technologies of nursing care. Cogitare Enferm. 2023 Mar;28:e84830. doi: 10.1590/ce.v28i0.89445.
13. Berta M, Lindgren H, Christensson K, Mekonnen S, Adefris M. Effect of maternal birth positions on duration of second stage of labour: systematic review and meta-analysis. BMC Pregnancy Childbirth. 2019 Dec;19(1):466. doi: 10.1186/s12884-019-2620-0.
14. Kamath P, Pai M, Shenoy R, Karkada S, D'souza S, Noronha J. Effectiveness of a peanut ball device during labouron maternal and neonatal outcomes: protocol for a randomised controlled trial. F1000 Research. 2022 Nov;11:717. doi: 10.12688/f1000research.109537.2.
15. Rosmiarti, Marlin R, Murbiah. Reduction of labour pain with back massage. Enferm Clín. 2020 Jun;30:209-12. doi: 10.1016/j.enfcli.2019.11.056.
16. Mohan M, Varghese L. Effect of foot reflexology on reduction of labour pain among primigravida mothers. Int J Ther Massage Bodyw. 2021 Mar;14(1):21-9. doi: 10.3822/ijtmb.v14i1.386.
17. Matabane SE, Musie MR, Mulaudzi MF. Childbirth preparation: knowledge of the use of non-pharmacological pain relief methods during childbirth in Tshwane District, South Africa: a cross-sectional study. Nurs Rep. 2024 Mar;14(1):1-11. doi: 10.3390/nursrep14010001.
18. Williams EL, Khan FM, Claydon VE. Counter pressure maneuvers for syncope prevention: A semi-systematic review and meta-analysis. Front Cardiovasc Med. 2022 Oct;9:1016420. doi: 10.3389/fcvm.2022.1016420.
19. Sari N, Runjati, Fatmasari D, Pujiyanto TI. Practices of counter pressure and birth ball exercise combination to increase β-endorphin hormone levels in labour pain. Dordrecht (NA): Atlantis Press; pp. 314-317. doi: 10.2991/ahsr.k.200723.079.
20. Zuarez-Easton S, Erez O, Zafran N, Carmeli J, Garmi G, Salim R. Pharmacologic and nonpharmacologic options for pain relief during labour: an expert review. Am J Obstet Gynecol. 2023 May;228(5, Supplement):S1246-59. doi: 10.1016/j.ajog.2023.03.003.
21. Wang J, Lu X, Wang C, Li X. The effectiveness of delivery ball use versus conventional nursing care during delivery of primiparae. Pak J Med Sci. 2020;36(3):550-4. doi: 10.12669/pjms.36.3.1440.
22. Stulz V, Dashputre A, Reilly H. Midwives' experiences using a peanut ball for women during labour: A qualitative study. Midwifery. 2023 Oct;125:103797. doi: 10.1016/j.midw.2023.103797.
23. Taavoni S, Abdolahian S, Haghani H, Neysani L. Effect of birth ball usage on pain in the active phase of labour: a randomized controlled trial. J Midwifery Womens Health. 2011;56(2):137-40. doi: 10.1111/j.1542-2011.2010.00013.x.

CONTENT

24. O'Brien D, Coughlan B, Thompson S, Carroll L, Sheehy L, Brosnan M, et al. Exploring midwives' experiences of implementing the Labour Hopscotch Framework: A midwifery innovation. Eur J Midwifery. 2022;6:18. doi: 10.18332/ejm/146081.
25. Sönmez T, Apay SE. Effect of different birth balls used at the first stage of labour on birth outcomes and maternal satisfaction: a randomized controlled trial. Clin Exp Health Sci. 2023 Sep;13(3):600-7. doi: 10.33808/clinexphealthsci.1164952.
26. Shen HC, Wang H, Sun B, Jiang LZ, Meng Q. Birthing ball on promoting cervical ripening and its influence on the labour process and the neonatal blood gas index. World J Clin Cases. 2021 Dec;9(36):11330-7. doi: 10.12998/wjcc.v9.i36.11330.
27. Anwar KK, Yustiari Y, Naningsi H, Asi M. The effect of counter pressure and birthball on the time of the first stage of labour and prevention of perineal rupture. Poltekita J Ilmu Kesehat. 2023 Nov;17(3):647-54. doi: 10.33860/jik.v17i3.3042.
28. Ahmed MA, Ahmed RHS, Ali AH, Salah Shalaby N, El-Shamy FF, Gaber Salem S. Effect of counter-pressure versus effleurage massages on labour pain intensity among parturient women. Egypt J Health Care. 2021;12(3):408-20. doi: 10.21608/ejhc.2021.190770.
29. Sriayuningtyas E, Galaupa R. The effect of counter pressure and kneading techniques on reducing the intensity of labour pain in the first stage of active phase. Int J Health Pharm. 2022;2(4):676-9. doi: 10.51601/ijhp.v2i4.86.
30. Wahyuni NS, Tridiyawati F. Effectiveness of rebozo technique and massage counter technique on labour pain in active phase 1 At Pmb Bd Itoh 2023. Int J Health Pharm. 2024;4(1):168-75. doi: 10.51601/ijhp.v4i1.293.

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ORIGINAL ARTICLE

Rare Occurrence of *RHD* Null Alleles With Del Expression Among Serologically D-Negative Blood Donors

Marko Lilić,[1, 2] Gordana Guzijan,[3, 4] Snežana Jovanović Srzentić[5, 6]

Abstract

Background/Aim: An investigation into the diversity of serologically D-negative blood donors in the Republic of Srpska entity of Bosnia and Herzegovina forms the basis of this research. The primary purpose of the study was the examination of *RHD* variants over a period of five years.
Methods: A comprehensive depiction of the *RHD* distribution in D-negative blood donors is achieved through a combination of serological observations and DNA testing (PCR-SSP with fluorometric signal detection), involving 74,149 blood donors. The adsorption/elution method was used to confirm the Del phenotype.
Results: A small fraction (0.31 %) of the serologically D-negative blood donors was found to contain eight different *RHD* alleles. The Del phenotype of the *RHD*01N.03* and *RHD*01EL.44* alleles was highlighted, challenging the common perception that these alleles are associated exclusively with a D-negative expression.
Conclusion: The importance of molecular methods in analysing and understanding Del variants, which typically elude conventional serological assays, is underscored by the findings. A group of donors seemingly having the *RHD*01* allele but who lacked D antigen expression was encountered, hinting at the potential presence of still unidentified, possibly geographically restricted, *RHD* variants or alterations in other genes responsible for the expression of Rh proteins in the erythrocyte membrane.

Key words: Blood group antigens; Polymerase chain reaction; Blood donors; Phenotype; Genetic variation.

1. Department of Biology, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia.
2. School of Medicine, University of Mostar, Mostar, Bosnia and Herzegovina.
3. Institute for Transfusion Medicine of the Republic of Srpska, Banja Luka, The Republic of Srpska, Bosnia and Herzegovina.
4. Medical Faculty, University of Banja Luka, Banja Luka, The Republic of Srpska, Bosnia and Herzegovina.
5. Blood Transfusion Institute of Serbia, Belgrade, Serbia.
6. Medical Faculty, University of Belgrade, Belgrade, Serbia.

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Corresponding author:
MARKO LILIĆ
E: marko.lilic@vz.ht.hr

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Introduction

Recombination between the two Rh genes are not uncommon and result in hybrid alleles.[1-3] Several other genetic events also influence the expression of Rh antigens (weak, partial, Del or null phenotype).[1, 2] In this paper, as suggested previously, Del was used for the phenotype and DEL for the allele designation.[4]

In Del individuals, D antigen expression is quantitatively very low.[4] They are usually typed as D-negative in routine serological testing and can only be detected by the adsorption/elution technique.[4-6] Only with routine molecular screening for Del variants the carriers could be successfully detected and not mistyped as D-negative.[7, 8] Anti-D alloimmunisation with Del-mistyped red blood cells (RBC) has been reported, although rarely.[3, 4, 6, 9-13]

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The frequency of Del has been well documented for large populations.[6] It is significant in Asian populations, where the frequency of Del variants among serologically D-negative donors is 1:5–1:8 in comparison with 1:350–1:2,000 for the populations of European origin.[14] The *RHD*01EL.01* allele is the most prevalent DEL allele in Asian populations, as exemplified by Chinese[15, 16] and Korean.[17, 18] Pregnant women with this allele do not receive RhIg and patients could be transfused with D-positive RBCs.[6] The most common alleles with Del expression in populations of European origin are *RHD*11* and *RHD*01EL.08*.[4, 19-21]

Adsorption/elution testing can assist in Del detection, particularly when molecular methods are not available.[13] The RhCE phenotypes could be significant for Del detection because of their strong association with C+/E+ phenotypes in many populations.[5,14,18,21-25] With the advancement of molecular techniques, the detection of Del variants is becoming increasingly feasible, underscoring the importance of integrating both methodologies into standard blood typing procedures, as illustrated in Asia, where Del transfusion may lead to alloimmunisation.[12, 26]

The primary focus of this study involved the examination of *RHD* variants within the population of D-negative blood donors over a period of five years from the Republic of Srpska entity of Bosnia and Herzegovina. Through the assessment of Rh phenotype associations, the presence of RhD epitopes and the analysis of different *RHD* alleles, objective was to strengthen and enhance the safety protocols in blood transfusion procedures.

Methods

Participants

A prospective study was conducted at the Institute for Transfusion Medicine of the Republic of Srpska in Banja Luka, Bosnia and Herzegovina, with 74,149 unrelated healthy regular blood donors. The samples have been collected for five years (2017–2021). They represented the majority of the blood donors in the study period of the entity the Republic of Srpska from Bosnia and Herzegovina.

Initial routine serological testing was performed for all samples. If the result was D-negative in direct agglutination, for participants with C+/E+ indirect antiglobulin test (IAT) was performed. If the results of both tests were negative, samples were screened for the presence of *RHD* exons 3, 5 and 10. Every sample reactive at the screening assay was further tested by specific PCR-SSP (PCR-sequence-specific primers) assays.

The study was approved by the Ethical Board of the Clinical Centre of Banja Luka. The research was conducted ethically, with all study procedures being performed in accordance with the requirements of the World Medical Association's Declaration of Helsinki. Written informed consent was obtained from each participant for study participation and data publication.

Serological testing

For D antigen determination, two direct agglutination methods were used:

- tube method using anti-D monoclonal IgM/IgG, anti-D blend (TH-28/MS-36, anti-D blend 175 2-415 1E4, *CE Immunodiagnostika*, Germany);
- gel method and ID-Cards „DiaClon ABO/Rh for Donors" (monoclonal anti-D:ESD-1M, 175-2, *BioRad*, USA).

Verification of weak D by IAT: ID-DiaClon Anti-D (reagent containing monoclonal IgG anti-D for confirmation of D weak by IAT), Coombs Anti-IgG (ID-Cards containing anti-human globulin rabbit anti-IgG for verification of D weak by IAT), LISS/Coombs (ID-Cards of 6 microtubes containing anti-IgG and anti-C3d), all manufactured by *BioRad*.

The RhCE antigens were also detected by two methods:

- tube method using human monoclonal anti-C test reagent (clones: P3X25513GB+MS24), anti-E (clone 906), anti-c (clone 951) and anti-E (clones: P3GD512+MS63), all by *Diagast*, France;
- DiaMed-MP Test C, c, E, e, K (*BioRad*) consisting of microplates with dried antibodies: anti-C cell line MS-24; anti-c MS-33; anti-E MS-260; anti-e MS-63 and anti-K MS-56.

DNA testing

DNA was extracted from 200 µL of whole blood sample by using the Ready DNA Isolation Spin kit (*Inno-train Diagnostik*, Germany). The donor-

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derived DNA samples were tested for the presence of *RHD* sequences by three RBC-FluoGene assays (RBC-FluoGene CDE, RBC-FluoGene D weak/variant, RBC-FluoGene CDE eXtend), which were based on PCR-SSP, with the fluorometric results evaluation involving the fluorescence reading of TaqMan probes by the FluoVista instrument according to the manufacturer's instructions (*Inno-train Diagnostik*).

Adsorption/elution test

In serologically D-negative, C+/E+ participants where *RHD* sequence was confirmed by DNA testing, D antigen was later re-examined in the Blood Transfusion Institute of Serbia (BTIS), Belgrade, Serbia, by adsorption of human polyclonal anti-D antibodies (produced "in house" from anti-D test sera of human origin by the Department for Production of Diagnostic Test Reagents of BTIS). Afterwards, RBCs were washed five times and subsequently eluted (DiaCidel, *Bio-Rad*) for antibody identification. Anti-D from the eluate was detected by the gel method using NaCl and Liss/Coombs ID-cards by ID-DiaCell IP-IIP-IIP and ID-DiaCell I-II-III screening test red cells (both *Bio-Rad*). Antibody specificity was determined using ID-DiaPanel P and ID-DiaPanel (both *Bio-Rad*).

Statistical methods

Absolute frequencies of antigens and alleles were collected by direct counting. The Pearson's chi-squared test was used to determine significance, set at $p < 0.05$ as statistically significant, in frequency comparisons. Proportion 95 % confidence interval (CI) was calculated using the Wilson score interval. IBM SPSS software version 18.0 was used.

Results

Initially, 74,149 blood donors were routinely serologically tested for D antigen. There were 12,827 serologically D-negative donor samples (17.30 %), both in direct agglutination as well as in IAT. Among these, 481 were D-negative, C+ or E+ (0.65 % of all samples, or 3.75 % of D-negative samples). The distribution of the main RhCE antigens among these 481 donors was as follows: C 35.86 % (345/962), c 64.14 % (617/962), E 15.28 % (147/962) and e 84.72 % (815/962). The frequencies of serologically determined Rh phenotypes in D-negative blood donors are given in Table 1. Homozygous *RHCE* genotype was determined in three D-negative blood donors with C+/E+ (0.62 %): *RHCE*02/*02* in two and *RHCE*03/03* in one.

In the serologically D-negative blood donor population with C+/E+, there were mostly *RHD*-negative donors (441/481, 91.68 %), whose final result of *RHD* deletion (homozygous *RHD*01N.01*) was defined by the negative result in the *RHD* screening assay. In each of the remaining samples (40/481, 8.32 %), some *RHD* sequence was detected; therefore, there were 40 *RHD*-positive samples. Summary frequencies for *RHD* variants in this study are presented in Table 2.

Table 1: The frequencies of Rh phenotypes in serologically D-negative blood donors

Serologically determined Rh phenotypes	Donors (N)	Frequency in D-negative C+/E+ blood donors (N = 481)	Frequency in all D-negative blood donors (N = 12,827)
ddccee	12,346	NA	96.250 %
ddCcee	333	69.231 %	2.596 %
ddCCee	2	0.416 %	0.016 %
ddCcEe	8	1.663 %	0.062 %
ddccEe	137	28.482 %	1.068 %
ddccEE	1	0.208 %	0.008 %
Other D-negative phenotypes	0	0.000 %	0.000 %
Total	12,827	100.000 %	100.000 %

NA: not applicable;

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Table 2: Summary frequencies of RHD variants in blood donors

Blood donors' subgroup	Total blood donors	Frequency of blood donors with *RHD* variants	95 % confidence interval[a]
All blood donors	74,149	0.054 % or 1:1,853	0.040 % – 0.073 %
D-negative	12,827	0.312 % or 1:321	0.229 % – 0.424 %
D-negative, C+/E+ blood donors	481 including: • 441 RHD-negative • 40 *RHD-positive*	8.316 % or 1:12	6.166 % – 11.130 %

a: Proportion confidence interval was calculated using the Wilson score interval;

Table 3: Distribution of detected RHD alleles (N = 40) with the associated Rh phenotype

Rh phenotype	Result designation	*RHD*11*	Seemingly *RHD*01*	*RHD*01N.03*	*RHD*01W.1*	*RHD*01W.2*	*RHD*01W.3*	*RHD*01W.14*	*RHD*05.05*	*RHD*01EL.44*	No conclusive result, *RHD*-positive	Total
Ccee	A	11	15	3	1	1	-	1	1	1	2	**36**
	B	27.500 %	37.500 %	7.500 %	2.500 %	2.500 %		2.500 %	2.500 %	2.500 %	5.000 %	**90.000 %**
	C	2.287 %	3.119 %	0.624 %	0.208 %	0.208 %		0.208 %	0.208 %	0.208 %	0.416 %	**7.484 %**
	D	0.086 %	0.117 %	0.023 %	0.008 %	0.008 %		0.008 %	0.008 %	0.008 %	0.016 %	**0.281 %**
ccEe	A	-	1	-	-	-	-	-	-	-	1	**2**
	B		2.500 %								2.500 %	**5.000 %**
	C		0.208 %								0.208 %	**0.416 %**
	D		0.008 %								0.008 %	**0.016 %**
Ccee	A	-	-	-	-	-	1	-	-	-	-	**1**
	B						2.500 %					**2.500 %**
	C						0.208 %					**0.208 %**
	D						0.008 %					**0.008 %**
CcEe	A	1	-	-	-	-	-	-	-	-	-	**1**
	B	2.500 %										**2.500 %**
	C	0.208 %										**0.208 %**
	D	0.008 %										**0.008 %**
Total	A	12	16	3	1	1	1	1	1	1	3	**40**
	B	30.000 %	40.000 %	7.500 %	2.500 %	2.500 %	2.500 %	2.500 %	2.500 %	2.500 %	7.500 %	**100.000 %**
	C	2.495 %	3.326 %	0.624 %	0.208 %	0.208 %	0.208 %	0.208 %	0.208 %	0.208 %	0.624 %	**8.316 %**
	D	0.094 %	0.125 %	0.023 %	0.008 %	0.008 %	0.008 %	0.008 %	0.008 %	0.008 %	0.023 %	**0.312 %**

A – absolute number in each cell designates the number of samples;
B – the first percentage is the frequency in all RHD positive donors (N = 40);
C – the second percentage is the frequency in serologically D-negative C+/E+ donors (N = 481);
D – the third percentage is the frequency in all serologically D-negative donors (N = 12827);
The minus sign denotes no alleles within the Rh phenotype.

Eight different *RHD* alleles were found in this study. There were 19 samples that need further clarification. Out of these, genotyping of three samples did not reveal any known *RHD* allele and they remained unresolved (without a conclusive result, labelled as "*RHD*-positive"). The remaining 16 samples were designated as seemingly *RHD*01*, indicating just the presence of all *RHD* exons, as all the exons' respective reference SNVs (single nucleotide variant) were detected in these samples. Table 3 shows the distribution of the *RHD* genotyping results linked to the associated Rh phenotype.

The donors with hybrid *RHD-RHCE* alleles (*RD*01N.03* and *RHD*01EL.44*) were afterwards serologically tested by adsorption/elution. All four donors gave positive result, thus indicating

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the Del expression of both respective alleles. The same adsorption/elution technique showed positive result in the donor with *RHD*05.05* and selected donors with *RHD*11*.

Discussion

In this study, the diversity of serologically D-negative blood donors in the Institute for Transfusion Medicine of the Republic of Srpska that collects blood donor samples from the whole entity of the Republic of Srpska in Bosnia and Herzegovina was described, so this study may be considered population-wide research. After initial study in 2019,[21] this research focused on the Rh diversity of the serologically D-negative blood donor population in the period of 5 years.

Three of eight different *RHD* alleles were previously determined in our country (*RHD*11*, *RHD*01W.1* and *RHD*01W.3*)[21] and the five remaining were encountered locally for the first time. As established for other European populations, *RHD*11* is the most frequent allele with the Del phenotype also in the studied population.[4, 19-21] The *RHD*01W.2* and *RHD*01W.14* alleles were not encountered within their most common haplotype (cDE),[27] as both donors had the Ccee phenotype. All but one *RHD*11* donors had the Ccee phenotype (the remaining one had CcEe), which validated the established observation of *RHD*11* Del expression linkage to *CDe* haplotype.[27] All eight alleles were previously described in neighbouring Croatia.[22, 28-30] All but *RHD*05.05* and *RHD*01EL.44* alleles were detected in neighbouring Serbia.[21]

Interestingly, while in authors' initial study, with a smaller sample size,[21] the frequency of *RHD* alleles in D-negative C+/E+ donors was relatively high (9.8 %), the comparison with results of this study (8.3 %) yields a nonsignificant difference, p = 0.645. The difference in comparison between the results in this study and the same ratio from Serbia[21] is not significant (p = 0.688), but it is significant with the percentage values from the two Croatian donor studies (first study[30]: 3.59 %, p < 0.001; second study[22]: 1.74 %, p = 0.013).

The three rare *RHD* alleles in the focus of this study were *RHD*05.05*, *RHD*01N.03* and *RHD*01EL.44* (*RHD*DEL44*). The allele *RHD*05.05* was originally described in Asian populations, but also in Austria and Greece.[31, 32] It has been reported in Dalmatia, the coastal region by the Adriatic Sea in Croatia;[29] therefore, its occurrence was not completely inconceivable. This allele codes for the DHK (DYO) antigen, first described in Asia.[33, 34] The phenotype varies from D-negative through weak D to D-positive.[35] Since there was an anti-D reactivity detected in the eluate from the donor's RBCs, the donor's status was changed to D-positive.

This is the first publication with any *RHD* null allele other than *RHD*01N.01* detected in Bosnia and Herzegovina. The *RHD*01N.03* allele is infrequently detected among serologically D-negative individuals. In the Han Chinese population, it was the most common after the homozygous *RHD* deletion.[36] In a Croatian study, it was present in almost half of all *RHD*-positive D-negative blood donors.[30] Three samples in this study were negative in IAT, but anti-D was identified after adsorption/elution technique. The haplotype association of *RHD*01N.03* is predicted[37] to be with the *RHCE*02 (*Ce)* allele. The designation *RHD*01N.03* was chosen in this paper, as it was the result indicated by the FluoGene software. For clarification purposes, since all three donors with this allele have the Ccee phenotype, it is impossible to distinguish the *RHD*01N.03* (*RHD*D-CE(2-9)-D*) allele from the *RHD*01N.04* (*RHD*D-CE(3-9)-D*) allele with the used molecular test system due to the exon 2 sequence equivalence of *RHD* and *RHCE*02* (*RHCE*Ce*).

The *RHD*DEL44* allele has been sporadically registered globally and to the authors' knowledge, this research represents the first description of any DEL allele in Bosnia and Herzegovina. In Europe, the reports came from Austria,[19] Switzerland,[38, 39] Portugal[40] and the most recent one from Croatia.[22] Outside Europe, the allele presence was documented in China,[41-43] Australia,[7] India,[44] Argentina,[45] Thailand[46] and Oceania.[47] It is a hybrid allele, with the gene structure *RHD*D-CE(4-9)-D*.[1, 27, 35, 47] Its expression varies as either Del or D-negative. It is mostly reported as D-negative,[3, 4, 27, 35, 43] but ISBT categorises it within the DEL alleles (hence the official name) and not the null allele group.[1] It has just been reported that the allele of the same gene structure can even express a C antigen.[47] Recently, there was a description of the *RHD*DEL44* allele and its expression within the blood donors in northwestern Croatia.[22] It seems likely that there could exist at least a central or southeastern European basin of this allele with detectable antigens on the RBCs accounting for the Del expression. Both the results of the Croatian study[22] and this study

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suggest the need to verify the expression of this allele, thereby preventing its reporting as a null allele by default solely due to its hybrid structure. *RHD*DEL44* in presented donor was most probably associated in haplotype[37] with the *RHCE*02* (**Ce*) allele.

For the four donors with the rare hybrid alleles (*RHD*01N.03* and *RHD*01EL.44*) their Del expression was indicated. Accordingly, their statuses have been changed to D-positive, so their blood products can be properly administered in the future. Both alleles are commonly interpreted as having a D-negative expression[30, 36] which is further inconsistent considering their official nomenclature.[1, 3] The *RHD*01N.03* allele has a structure of *RHD*D-CE(2-9)-D*, which suggests it should typically behave as a null allele.[1] The *RHeference* database[35] has numerous references to the D-negative phenotype and only one to the Del phenotype like presented in this study, from Thailand.[48] Although *RHD*01EL.44* is catalogued as the DEL allele by ISBT, its Del phenotype was observed only originally in China[41, 42] and Croatia.[22] The phenotype of this allele could not be verified for the carrier in Oceania[47], as it was a case of heterozygous *RHD* genotype of *RHD*DEL44* with *RHD*01* in *trans* position.

Satisfactory conclusive results were not obtained for three donor samples. It is confirmed they definitely do have *RHD* sequences. However, the final result was not clear due to discrepancies and their evaluation is still pending. The most problematic but possibly exciting results involve the group of 16 donors (3.33 %), all *RHD*-positive, seemingly *RHD*01*. All reference SNVs detecting each of the 10 *RHD* exons were present in all 16 samples and consistently, no other SNV from any other *RHD* allele than *RHD*01* was detected. The authors share genuine reservations that these really are reference sequences of the *RHD* gene and that there are intact standard D antigens on RBCs of these donors. Serological reactivities in both assays were negative. It can be confirmed that data of these 16 blood donors were subsequently examined and evidence of any blood relationship among them was not found. Consequently, it could be the case of a rare *RHD* variant, probably a geographically specific one. The high frequency of these samples in serologically D-negative C+/E+ donors (3.33 %) corroborates that hypothesis. PCR assays based on SNV detection disclose very little of the entire *RHD* sequence.[49] Therefore, there could be many sequence variants that remained undetected by the PCR setup in this study. There could also be some variation in the genes required for expression of Rh proteins in the RBC membrane (like *RHAG*).[50] Regrettably, in our region, there are no sequencing opportunities and it is also not financially feasible for our institutions to have them all sequenced remotely. The authors are keen to contribute these samples to any future research involving whole-gene *RHD* sequencing (both exons and introns of the *RHD* gene) that would be willing to explore and finally identify the underlying molecular causes of the absence of D antigen expression in these seemingly *RHD*01* donors.

This research demonstrates the importance of verifying the expression of large hybrid Rh alleles. Although alloimmunisation occurrences are extremely rare, blood bank facilities should take all precautions to make sure that D-negative units truly are D-negative, especially for the blood donor population. Molecular Rh examination already marks an important stage, but the results displayed in this research shed new light on the canon for two different large hybrid alleles. A supporting view is articulated by Srivastava et al[47] where, in addition, the importance of hybrid allele's breakpoint regions to the expression and stability of mRNA transcripts is discussed, as well as the need to distinguish different expression patterns of hybrid alleles by blood transfusion services.

Conclusion

The *RHD* diversity in 74,149 serologically D-negative blood donors was demonstrated by the *RHD* frequency of 0.31 % in this study, which also challenges the common perception for specific alleles, like *RHD*01N.03* and *RHD*01EL.44*, that they do not express D antigen. This underlines the critical role of molecular techniques in identifying Del variants, which are often undetectable by standard serological tests. The presence of donors with seemingly *RHD*01* allele variants points towards the possibility of undiscovered *RHD* polymorphisms or other genetic factors affecting Rh protein expression. These insights reinforce the need for molecular screening in blood donation to ensure safety. Furthermore, this highlights the study's contribution to the broader understanding of *RHD* allele variations and their implications for blood transfusion compatibility.

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Ethics

The study was approved by the Ethical Board of the University Clinical Centre of Banja Luka, Banja Luka, the Republic of Srpska, approval No 01-9-382.2/15, dated 29 September 2015.

Acknowledgement

None.

Conflicts of interest

ML is employed as a scientific advisor by a local distributor of *Inno-Train Diagnostik*, which was not involved in this research in any way, particularly scientifically, financially, or in writing the paper. All other authors declare that there is no conflict of interest.

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Data access

The data that support the findings of this study are available from the corresponding author upon reasonable individual request.

Author ORCID numbers

Marko Lilić, (ML):
0000-0003-4017-4622
Gordana Guzijan (GG):
0000-0003-2973-6823
Snežana Jovanović Srzentić (SJS):
0009-0009-0358-0040

Author contributions

Conceptualisation: ML
Methodology: ML, GG, SJS
Validation: ML, GG, SJS
Formal analysis: ML, GG, SJS
Investigation: ML, SJS
Resources: GG
Data curation: GG
Writing – original draft: ML
Writing – review and editing: ML, GG, SJS
Visualisation: ML
Supervision: ML, GG
Project administration: GG

References

1. (ISBT 004) RHD blood group alleles v6.4 31-JUL-2023 [Internet]. Amsterdam, The Netherlands: International Society for Blood Transfusion. c2023. [Cited: 17-Jan-2024]. Available from: https://www.isbtweb.org/resource/004rhd.html.
2. Flegel WA. Molecular genetics and clinical applications for RH. Transfus Apher Sci. 2011;44(1):81-91. doi: 10.1016/j.transci.2010.12.013.
3. Wagner FF. Considerations on terminology and database organization for blood group genotyping data. Ann Blood. 2023;8:1. doi: 10.21037/aob-21-42.
4. Wagner FF. Serology and molecular biology of DEL: a narrative review. Ann Blood. 2023;8:28. doi: 10.21037/aob-22-16.
5. Nuchnoi P, Thongbus J, Srisarin A, Kerdpin U, Prachayasittikul V. Clinical and laboratory update on the DEL variant. Lab Med. 2014;45(4):285-90. doi: 10.1309/LMTUZ00O7VFTGCEB.
6. Flegel WA, Wagner FF. DEL. Blood Transfus. 2020;18(3):159-62. doi: 10.2450/2020.0296-19.
7. Scott SA, Nagl L, Tilley L, Liew YW, Condon J, Flower R, et al. The RHD(1227G>A) DEL-associated allele is the most prevalent DEL allele in Australian D- blood donors with C+ and/or E+ phenotypes. Transfusion. 2014;54(11):2931-40. doi: 10.1111/trf.12701.
8. Sandler SG, Flegel WA, Westhoff CM, Denomme GA, Delaney M, Keller MA, et al. It's time to phase in RHD genotyping for patients with a serologic weak D phenotype. Transfusion. 2015;55(3):680-9. doi: 10.1111/trf.12941.
9. Kim KH, Kim KE, Woo KS, Han JY, Kim JM, Park KU. Primary anti-D immunization by DEL red blood cells. Korean J Lab Med. 2009;29(4):361-5. doi: 10.3343/kjlm.2009.29.4.361.
10. Yasuda H, Ohto H, Sakuma S, Ishikawa Y. Secondary anti-D immunization by Del red blood cells. Transfusion. 2005;45(10):1581-4. doi: 10.1111/j.1537-2995.2005.00579.x.
11. Wagner T, Körmöczi GF, Buchta C, Vadon M, Lanzer G, Mayr WR, et al. Anti-D immunization by DEL red blood cells. Transfusion. 2005;45(4):520-6. doi: 10.1111/j.0041-1132.2005.04256.x.

CONTENT

12. Shao CP, Wang BY, Ye SH, Zhang WL, Xu H, Zhuang NB, et al. DEL RBC transfusion should be avoided in particular blood recipient in East Asia due to allosensitization and ineffectiveness. J Zhejiang Univ Sci B. 2012;13(11):913-8. doi: 10.1631/jzus.B1100348.
13. Kim TY, Hong YJ, Kim MJ, Kim H, Kim TS, Park JS, et al. Recommendations Regarding practical DEL typing strategies for serologically D-negative Asian donors. Transfus Med Hemother. 2020;47(1):88-93. doi: 10.1159/000500098.
14. Wagner FF. RHD PCR of D-Negative blood donors. Transfus Med Hemother. 2013;40(3):172-81. doi: 10.1159/000351604.
15. Shao CP, Maas JH, Su YQ, Köhler M, Legler TJ. Molecular background of Rh D-positive, D-negative, D(el) and weak D phenotypes in Chinese. Vox Sang. 2002;83(2):156-61. doi: 10.1046/j.1423-0410.2002.00192.x.
16. Chen JC, Lin TM, Chen YL, Wang YH, Jin YT, Yue CT. RHD 1227A is an important genetic marker for RhD(el) individuals. Am J Clin Pathol. 2004;122(2):193-8. doi: 10.1309/3XMF-2NV5-707T-JE7X.
17. Kim JY, Kim SY, Kim CA, Yon GS, Park SS. Molecular characterization of D- Korean persons: development of a diagnostic strategy. Transfusion. 2005;45(3):345-52. doi: 10.1111/j.1537-2995.2005.04311.x.
18. Hong YJ, Kim H, Song EY, Cho D, Park KU, Han KS. Development of real-time PCR and melting curve analysis for the rapid detection of DEL with RHD (c.1222T>C) or RHD (c.1227G>A). Clin Lab. 2016;62(10):1995-2000. doi: 10.7754/Clin.Lab.2016.160320.
19. Polin H, Danzer M, Gaszner W, Broda D, St-Louis M, Pröll J, et al. Identification of RHD alleles with the potential of anti-D immunization among seemingly D- blood donors in Upper Austria. Transfusion. 2009;49(4):676-81. doi: 10.1111/j.1537-2995.2008.02046.x.
20. Flegel WA, von Zabern I, Wagner FF. Six years' experience performing RHD genotyping to confirm D- red blood cell units in Germany for preventing anti-D immunizations. Transfusion. 2009;49(3):465-71. doi: 10.1111/j.1537-2995.2008.01975.x.
21. Guzijan G, Jovanovic Srzentic S, Pavlovic Jankovic N, Djilas I, Lilic M. Implementation of molecular RHD typing at two blood transfusion institutes from southeastern Europe. Transfus Med Hemother. 2019;46(2):114-20. doi: 10.1159/000496751.
22. Lilić M, Jaklin G, Gojčeta K, Raos M, Golubić Ćepulić B. [Molecular determination of the RHD allele in the population of RhD-negative blood donors in northwestern Croatia]. 8. hrvatski transfuziološki kongres, Zagreb, 4.-7.11.2021. Liječ Vjesn. 2021;143(Supl. 2):52-53. Croatian.
23. Seo MH, Won EJ, Hong YJ, Chun S, Kwon JR, Choi YS, et al. An effective diagnostic strategy for accurate detection of RhD variants including Asian DEL type in apparently RhD-negative blood donors in Korea. Vox Sang. 2016;111(4):425-30. doi: 10.1111/vox.12450.
24. Luettringhaus TA, Cho D, Ryang DW, Flegel WA. An easy RHD genotyping strategy for D- East Asian persons applied to Korean blood donors. Transfusion. 2006;46(12):2128-37. doi: 10.1111/j.1537-2995.2006.01042.x.
25. Orzińska A, Guz K, Polin H, Pelc-Kłopotowska M, Bednarz J, Gieleżyńska A, et al. RHD variants in Polish blood donors routinely typed as D-. Transfusion. 2013;53(11 Suppl 2):2945-53. doi: 10.1111/trf.12230.
26. Ohto, H, Flegel, WA, Safic Stanic, H. When should RhD-negative recipients be spared the transfusion of DEL red cells to avoid anti-D alloimmunization? Transfusion. 2022;62(11):2405–8. doi: 10.1111/trf.17122.
27. Wagner FF, Flegel WA. The Rhesus Site. Transfus Med Hemother. 2014;41(5):357-63. doi: 10.1159/000366176.
28. Safic Stanic H, Kruhonja Galic Z, Dogic V, Bingulac-Popovic J, Jukic I. Serological and molecular characterisation of the most prevalent weak D variants in Croatian population. Transfusion Medicine. 2023;33(2):132-6. doi: 10.1111/tme.12944.
29. Lukacevic Krstic J, Dajak S, Bingulac-Popovic J, Dogic V, Mratinovic-Mikulandra J. Anti-D reagents should be chosen accordingly to the prevalence of D variants in the obstetric population. J Clin Lab Anal. 2018;32(3):e22285. doi: 10.1002/jcla.22285.
30. Safic Stanic H, Dogic V, Herceg I, Jagnjic S, Bingulac-Popovic J, Babic I, et al. D variants in the population of D-negative blood donors in the north-eastern region of Croatia. Transfus Med. 2021;31(1):43-7. doi: 10.1111/tme.12726.
31. Polin H, Danzer M, Hofer K, Gassner W, Gabriel C. Effective molecular RHD typing strategy for blood donations. Transfusion. 2007;47(8):1350-5. doi: 10.1111/j.1537-2995.2007.01278.x.
32. Koutsouri T, Chaikali A, Giannopoulos A, Douramani P, Flesiopoulou I, Panos P, et al. Frequency distribution of RHD alleles among Greek donors with weak D phenotypes demonstrates a distinct pattern in central European countries. Transfusion Med. 2019;29:468-70. doi: 10.1111/tme.12623.
33. Omi T, Takahashi J, Tsudo N, Okuda H, Iwamoto S, Tanaka M, et al. The genomic organization of the partial D category DVa: the presence of a new partial D associated with the DVa phenotype. Biochem Biophys Res Commun. 1999;254(3):786-94. doi: 10.1006/bbrc.1998.0121.
34. Hyodo H, Ishikawa Y, Kashiwase K, Ogawa A, Watanabe Y, Tsuneyama H, et al. Polymorphisms of RhDVa and a New RhDVa–Like Variant Found in Japanese Individuals. Vox Sanguinis. 2000;78:122-5. doi: 10.1046/j.1423-0410.2000.7820122.x.
35. Floch A, Téletchéa S, Tournamille C, de Brevern AG, Pirenne F. A Review of the literature organized into a new database: RHeference. Transfus Med Rev. 2021;35(2):70-7. doi: 10.1016/j.tmrv.2021.04.002.
36. Ye SH, Wu DZ, Wang MN, Wu XY, Xu HG, Xu H, et al. A comprehensive investigation of RHD polymorphisms in the Chinese Han population in Xi'an. Blood Transfus. 2014;12(3):396-404. doi: 10.2450/2013.0121-13.
37. Daniels G. Rh and RHAG Blood Group Systems. In: Daniels G, editor. Human blood groups. 3rd ed. Chichester, UK: Wiley Blackwell; 2013. p.182-258.
38. Gowland P, Gassner C, Hustinx H, Stolz M, Gottschalk J, Tissot JD, et al. Molecular RHD screening of RhD negative donors can replace standard serological testing for RhD negative donors. Transfus Apher Sci. 2014;50(2):163-8. doi: 10.1016/j.transci.2014.02.009.
39. Crottet SL, Henny C, Meyer S, Still F, Stolz M, Gottschalk J, et al. Implementation of a mandatory donor RHD screening in Switzerland. Transfus Apher Sci. 2014;50(2):169-74. doi: 10.1016/j.transci.2014.02.011.
40. Pereira J, Salvado R, Martins N, Ribeiro ML. RHD null alleles in the Portuguese population. Transfus Med. 2009;19(Suppl. 1):25, PO13.

CONTENT

41. Li Q, Hou L, Ye LY, Yue DQ, Zhu ZY. Molecular basis of the RHD gene in blood donors with DEL phenotypes in Shanghai. Vox Sang. 2009;97(2):139-46. doi: 10.1111/j.1423-0410.2009.01181.x.
42. Xu W, Zhu M, Wang BL, Su H, Wang M: Prospective evaluation of a transfusion policy of RhD-positive red blood cells into DEL patients in China. Transfus Med Hemother. 2015;42(1):15-21. doi: 10.1159/000370217.
43. Ye L, Yue D, Wo D, Ding X, Guo S, Li Q, Guo Z, Zhu Z. Molecular bases of unexpressed RHD alleles in Chinese D– persons. Transfusion. 2009;49(8):1655-60. doi: 10.1111/j.1537-2995.2009.02181.x.
44. Kulkarni SS, Gogri H, Parchure D, Mishra G, Ghosh K, Rajadhyaksha S, et al. RHD-positive alleles among D-C/E+ individuals from India. Transfus Med Hemother. 2018;45(3):173-7. doi: 10.1159/000479239.
45. Trucco Boggione C, Nogués N, González-Santesteban C, Mufarrege N, Luján Brajovich M, Mattaloni SM, et al. Characterization of RHD locus polymorphism in D negative and D variant donors from Northwestern Argentina. Transfusion. 2019;59(10):3236-42. doi: 10.1111/trf.15504.
46. Thongbut J, Laengsri V, Raud L, Promwong C, I-Na-Ayudhya C, Férec C, et al. Nation-wide investigation of RHD variants in Thai blood donors: Impact for molecular diagnostics. Transfusion. 2021;61(3):931-8. doi: 10.1111/trf.16242.
47. Srivastava K, Bueno MU, Flegel WA. Breakpoint regions of an RHD-CE(4-9)-D allele and a rare JK allele in a Pacific Islander individual. Blood Transfus. 2023 Aug 25. doi: 10.2450/BloodTransfus.534.
48. Thongbut J, Raud L, Férec C, Promwong C, Nuchnoi P, Fichou Y. Comprehensive molecular analysis of serologically D-negative and weak/partial D phenotype in Thai blood donors. Transfus Med Hemother. 2020;47(1):54-60. doi: 10.1159/000499087.
49. Daniels G. An overview of blood group genotyping. Ann Blood. 2023;8:3. doi: 10.21037/aob-21-37.
50. Wen J, Verhagen OJHM, Jia S, Liang Q, Wang Z, Wei L, et al. A variant RhAG protein encoded by the RHAG*572A allele causes serological weak D expression while maintaining normal RhCE phenotypes. Transfusion. 2019;59(1):405-11. doi: 10.1111/trf.14969.

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ORIGINAL ARTICLE

Prevalence and Risk Factors of Psychological Distress Among Indonesian Incarcerated Male Juveniles

Prasetyo Aji Nugroho,[1, 2] Somporn Rungreangkulkij,[1] Suriadi Jais[3]

Abstract

Background/Aim: Adolescence is a developmental period that is rife with emotional problems as adolescents lack psychological maturity. Juvenile offenders who are incarcerated may be more vulnerable to psychological distress. Aim of this study was to analyse the prevalence and risks of psychological distress among male juveniles (MJs) incarcerated in Indonesia.

Method: The participants of this study were 206 male juvenile offenders (MJOs) aged 12-17 from 28 Special Child Development Institutions (Lembaga Pemasyarakatan Khusus Anak; LPKA) in Indonesia. The participants' socio-demographic data was gathered while the strength and difficulties questionnaire (SDQ) was used to assess the extent of their psychological distress.

Results: Of the 47.6 % of participants that reported psychological distress, peer relationship problems (64.6 %) was the most severe, followed by conduct problems (51.5 %). The binary logistic regression results revealed that education level ($p = 0.005$) and psychological trauma ($p < 0.001$) correlated with psychological distress and that they were responsible for 37.5 % of psychological distress. Therefore, education level and psycho-trauma significantly affect the extent of the psychological distress that MJOs in Indonesia experience.

Conclusion: Juveniles require support to further their formal or informal education. Furthermore, healthcare providers could develop appropriate interventions to manage specific traumatic events as well as prevent or improve the mental health of MJOs.

Key words: Incarceration; Male juvenile; Psychological distress.

1. Department of Psychiatric and Mental Health Nursing, Faculty of Nursing, Khon Kaen University, Khon Kaen, Thailand.
2. Department of Psychiatric and Mental Health Nursing, School of Nursing, Institut Teknologi dan Kesehatan Muhammadiyah Kalimantan Barat, Barat, Indonesia.
3. Graduate Nursing School, Institut Teknologi dan Kesehatan Muhammadiyah Kalimantan Barat, Barat, Indonesia.

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Corresponding author:
PRASETYO AJI NUGROHO
E: prasetyoaji.nu@kkumail.com

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Introduction

Male juveniles (MJs) commit three times as many crimes as female juveniles.[1] When incarcerated, male juvenile offenders (MJOs) lose many of their freedom; such as the ability to engage in heterosexual relationships, recreational areas, furthering education and social activities.[2] As such, incarceration can often lead to low self-esteem and negative perceptions of one's self, leading to psychological distress.[3] Two-thirds of juvenile offenders suffer from at least one diagnosable psychological disorder.[4] Psychological disorders are psychopathological conditions; such as signs, symptoms and problematic traits; that adversely affect the cognition, emotions and behaviours of individuals as well as their quality of life and ability to socialise, work and engage in other im-

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portant lifestyle activities for a duration exceeding two weeks.[5-9] Psychological distress can often lead to mental illnesses and mental disorders. A global meta-analysis concluded that juvenile offenders experience mental illnesses while a systematic review and meta-regression analysis reported that MJOs have diagnosable conduct disorders (61.7 %), attention deficit hyperactivity disorder (ADHD) (17.3 %), major depression (10.1 %), post-traumatic stress disorder (PTSD) (8.6 %) and psychotic illnesses (2.7 %).[10] A study on psychological distress among 17-year-old Australian MJOs who are incarcerated or in community based-programmes found that most incarcerated MJOs are significantly more likely to screen positive for depressive symptoms and psychosis than their peers in community based-programmes.[11] Another study of psychiatric morbidity among Nigerian MJOs aged ≥ 15 incarcerated at a juvenile correctional facility showed that 62.5 % experienced psychiatric disorders with disruptive behaviour disorders (40.8 %), substance abuse disorders (15.8 %), anxiety disorders (14.2 %), psychosis (6.7 %) and mood disorders (5.0 %).[12-13] A South Korean study, similarly, found that 90.8 % of MJOs aged ≤ 19 have at least one diagnosable psychiatric disorder while 75.1 % have psychiatric comorbidities.[13]

Therefore, as MJOs clearly experience psychological distress, it warrants further examination and the development of management methods. The factors that contribute to psychological distress among incarcerated juveniles are age (≤ 20 years old), low levels of education, incarcerations of ≤ 1 year, being violent offenders, adhering to the Christian faith, experiencing traumatic events, misbehaviours and family factors.[14-24] Agustine examined psychological distress among MJOs in Jawa Barat Province, Indonesia using the strength and difficulties questionnaire (SDQ). The study found that 14.2 % of the 56 participants experienced psychological distress.[25] Agustine then conducted a pilot study involving 27 participants from the Sungai Raya Special Child Development Institution (*Lembaga Pemasyarakatan Khusus Anak*; LPKA) using the SDQ, which revealed that 11 % experienced psychological distress. Although multiple studies have examined many aspects of psychological distress among MJOs across the globe, Indonesian studies have only examined the prevalence of psychological distress. As such, this present study is the first domestic study aimed to examine the overall prevalence of psychological distress in a large population that is representative of Indonesian MJOs. It also identified the risk factors of psychological distress among MJOs incarcerated in Indonesia. As the mental health characteristics of MJOs differ from that of female juvenile offenders and there are more male juveniles incarcerated in Indonesia than female juveniles, this present study chose to examine MJOs.

This study aimed to examine the (1) psychological distress that the Indonesian MJO population experiences and (2) identify the risk factors that influence psychological distress among Indonesian MJOs.

Methods

An anonymous cross-sectional survey was conducted on 1324 MJOs aged 12-17 and incarcerated at 28 LPKAs across 33 provinces in Indonesia. The outcome variable of this study was a categorical scale with dichotomous variables; namely abnormal and normal.[26] Therefore, the present study used this proportion to calculate the ideal sample size using a formula that existing studies had used to calculate the prevalence rate.[27-28] Only one domestic study has examined the prevalence of psychological distress using the SDQ. The study, which was conducted at a Class II LPKA in Bandung, Indonesia, reported a prevalence rate of 14.2 % when the cut-off was ≥ 20. A further 10 % of the sample size was added in anticipation of incomplete questionnaires or refusals to participate.[29] As such, the participants of this present study numbered 206.

Stratified random sampling was used as the examined population comprised several strata or subgroups and separate samples had already been obtained from each subgroup. Probability proportional to size (PPS) sampling was used to determine the ideal sample size for each province. The inclusion criteria were incarcerated MJOs aged 12-17 at the time of the survey and had been incarcerated for at least a night while the exclusion criteria were MJOs who did not agree to participate, those with severe disabilities that affect their communication; such as slurred or unclear speech, non-verbal and impaired hearing.

Socio-demographic data; such as age, education level, religion, marital status, employment pre-incarceration, living arrangement, length of incar-

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ceration, type of offense, history of misbehaviour pre-incarceration, parents' marital status and psychological trauma symptoms was collected. The SDQ was used to determine the extent of the participants' psychological distress.

All the socio-demographic variables of the participants were categorised. The age was grouped into early adolescence (12-15) and middle adolescence (16-17). The education was grouped into low (no schooling/had dropped out of school or only completed primary education) or high (completed secondary education). The religion was grouped into Muslim or non-Muslim while the marital status was grouped into single or married. The employment pre-incarceration was grouped into possessed a permanent job, worked as a day labourer or unemployed while the living arrangement was grouped into alone, with spouse without children and with family.

The length of incarceration was grouped into ≤ 1 year and > 1 year while the type of offense was grouped into violent (sexual crimes, murder, robbery, persecution and assault) and non-violent (property offenses, public order offenses, drug offenses, possession of offensive weapons and others). The history of misbehaviour pre-incarceration was grouped into having three misbehaviours (smoking, prior gang involvement, alcohol use or drug use), having two misbehaviours (thieving and smoking) and only one misbehaviour (thieving, smoking, prior gang involvement, alcohol use, or drug use). Lastly, the parents' marital status was grouped into divorced/widowed or married while the psychological trauma symptoms were grouped into no and yes.[30]

The global psycho-trauma screen (GPS) was used to measure the psychological trauma symptoms of the participants as it is a screening instrument that is designed to identify reactions to severe stressors or potentially traumatic events. It was translated to Indonesian by Indira Primasari, with a reliability of 0.83, sensitivity of 0.83 and specificity of 0.711. It comprised 17 symptomatic questions and five risk/protective factor questions, each answered by a Yes or No and one functioning item. Each item was given two response options and scored. More specifically, items 1-21 were scored No = 0 and Yes = 1 while Item 22 were scored No = 1 and Yes = 0. The GPS score is the sum of Items 1 to 17 and Item 18.[31] A GPS symptom score of ≥ 8 indicates the presence of psychological trauma symptoms while a score of < 8 indicates no psychological trauma symptoms.[32, 33]

As the SDQ was a tool for screening psychological distress among juveniles, it was used to examine the incarcerated MJOs.[34] The SDQ is Indonesia's standard mental health screening.[35] It has a reliability of 0.73, sensitivity of 0.67 and a specificity of 0.68. The SDQ comprised 25 items divided across five subscales; namely, 1) emotional symptoms (five items), 2) conduct problems (five items), 3) hyperactivity/inattention (five items), 4) peer relationship problems (five items) and 5) pro-social behaviours (five items); with three response options; namely, not true = 0, somewhat true = 1 and certainly true = 2. It took 10-20 minutes to administer test and its subscale scores range from 0-10.

Data collection

Once ethical approval had been obtained from the Indonesian National Research and Innovation Agency and the Ministry of Laws and Rights, the permission letter was sent to the 28 LPKAs. The primary researcher then explained the data collection process to the LPKAs' staff and shared the data that the 28 research assistant had already collected. The research assistants comprised 17 undergraduate degree students and 11 bachelor's degree students living near each of the 28 LPKAs. Therefore, the total of research assistants was 28 people. Via a Zoom™ meeting, the primary researcher explained the research protocols for data collection to the research assistants to protect the participants and to strengthen the validity and reliability of the collected data. A data collection practice test was also given.

Each participant had to verbally consent to participate in the present study before they were administered the questionnaire by a research assistant in a counselling room at the facility in which the participant was incarcerated. Each completed questionnaire was labelled with a code number and the participant's age instead of their name to ensure anonymity. The participants and the research assistant were strictly prohibited from taking photo and videos during the session. If a participant was illiterate, the research assistant read the questionnaire to the participant. The participants were required to deposit their questionnaires in the research assistant's box at the front of the counselling room within 30 minutes. The research assistants were unaware of the re-

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sponses in the questionnaires. The research assistants then mailed the questionnaires to the researchers *via* post office document delivery between October to November 2022. Therefore, only the researchers were aware of the responses in the questionnaires.

Data analysis

The means and frequencies of all the examined variables were calculated using descriptive statistics. The mean scores and prevalence of the SDQ outcomes was estimated. Binary logistic regression analyses were applied in three steps to analyse the correlations between the risk factors and the outcomes. A concurrent test was first designed to detect if a risk factor affected psychological distress. An individual test or single independent variable was then used to determine if a risk factor significantly affected psychological distress. Last, the odds ratios were calculated to determine the direction and magnitude of the risk factor.[36] This was repeated for all the risk factors examined. IBM's® Statistical Package for the Social Sciences (SPSS) 26, which was under the university's license, was used to analyse the collected data.

Results

More than 70 % of the participants were aged 16-17, Muslims, single and lived with their families with married parents. Even though most of the participants were unemployed (68.9 %), the participants had completed secondary school (68.9 %) and been incarcerated for < 1 year (84.0 %). Most of them were violent offenders (84.0 %) with only one history of misbehaviour pre-incarceration (79.6 %). Also, more than half of them exhibited symptoms of psychological trauma (Table 1).

Psychological distress

Over half of the MJOs experienced abnormal peer relationship problems and conduct problems. Emotional problems and hyperactivity were reported by 46.6 and 11.2 % of participants, respectively. Furthermore, 97.6 % of the MJOs exhibited normal pro-social behaviours, followed by 2.4 % abnormal. Therefore, the total difficulty score of the MJOs was abnormal (47.6 %) (Table 2).

Table 1: The sociodemographic characteristics of the male juvenile offenders (MJOs) (n = 206)

Variables	Categories	N	%
Age (Years)	12-15	37	18.0
	16-17	169	82.0
Education	No schooling/had dropped out of school/only completed primary education	64	31.1
	Completed secondary education	142	68.9
Religion	Muslim	185	89.8
	Non-Muslim	21	10.2
Marital status	Single	204	99.0
	Married	2	1.0
Employment pre-incarceration	Possessed a permanent job	9	4.4
	Worked as a day labourer	55	26.7
	Unemployed	142	68.9
Living arrangement	Alone	19	9.2
	With spouse sans children	1	0.5
	With family	186	90.3
Length of incarceration (years)	≤ 1	173	84.0
	> 1	33	16.0
Type of offense	**Violent**	**173**	**84.0**
	Sexual crimes	90	43.7
	Murder	32	15.5
	Robbery	25	12.1
	Persecution	18	8.7
	Assault	8	3.9
	Non-violent	**33**	**16.0**
	Property offenses	6	2.9
	Public order offenses	6	2.9
	Drug offenses	12	5.8
	Possession of offensive weapons	3	1.5
	Others	6	2.9
History of misbehaviour pre-incarceration	**Having three misbehaviours** (smoking, prior gang involvement, alcohol use, or drug use)	**42**	**20.4**
	Having two misbehaviours (thieving and smoking)	**0**	**0.0**
	Only one misbehaviour	**164**	**79.6**
	Thieving	15	7.3
	Smoking	110	53.4
	Prior gang involvement	26	12.6
	Alcohol and/or drug use	13	6.3
Parents' marital status	Divorced/widowed	61	29.6
	Married	145	70.4
Psychological trauma symptoms	No	92	44.7
	Yes	114	55.3

Risk factors of psychological distress

Backward (conditional) binary logistic regression analysed significant variables (Table 3). The final model revealed that the prevalence odds ratio (POR) of education level was 0.357 (95 % CI: 0.174-0.733) and 0.098 for psychological trauma symptoms (95 % CI: 0.050-0.193), which correlat-

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Table 2: The psychological distress of the male juvenile offenders (MJOs) (n = 206)

Domains		M	SD	N	%
Emotional problems		4.49	2.807	206	100.0
	Normal			110	53.0
	Abnormal			96	47.0
Conduct problems		3.73	2.207	206	100.0
	Normal			100	49.0
	Abnormal			106	52.0
Hyperactivity		4.02	2.002	206	100.0
	Normal			183	89.0
	Abnormal			23	11.0
Peer relationship problems		4.16	1.791	206	100.0
	Normal			73	35.0
	Abnormal			133	65.0
Pro-social behaviours		8.15	1.839	206	100.0
	Normal			201	98.0
	Abnormal			5	2.0
Total difficulty score		16.40	5.947	206	100.0
	Normal			108	52.0
	Abnormal			98	48.0

M: mean; SD: standard deviation; N: number of respondents; %: percentage of total number of respondents;

ed with psychological distress among incarcerated MJOs. The Nagelkerke R^2 was 0.375, which indicates that the ability of the independent variable to explain the dependent variable was 37.5 %, meaning that 62.5 % of other factors outside the model describe the dependent variable.

Table 3: The result of the binary logistic regression of the risk factors for emotional and mental problems (n = 206)

Risk factors	B	p-value	POR	95 % CI
Educational level	-1.030	0.005	0.357	0.174 - 0.733
Traumatic events	-2.321	< 0.001	0.098	0.050 - 0.193
Constant	1.586			

Nagelkerke pseudo-R^2: 0.375; POR: prevalence odds ratio; CI: confidence interval;

Each variable had a significant and partial effect on psychological distress. Education level had a significant and partial impact on incidences of emotional and mental problems while psychological trauma symptoms had a significant and partial impact the incidences of psychological distress. The POR indicated the magnitude of these influences. Participants with no schooling, had dropped out of school, or only completed primary education are 0.357 times more likely to experience psychological distress than their peers who completed their secondary education. Furthermore, MJOs with psychological trauma symptoms were 0.098 times more likely to experience psychological distress than their peers without psychological trauma symptoms. As the B value was negative, education level and psychological trauma symptoms inversely correlate with incidences of psychological distress. Therefore, the higher the level of education, the lower the likelihood of experiencing psychological distress. Also, the fewer the psychological trauma symptoms, the lower the likelihood of experiencing psychological distress.

Discussion

The socio-demographic characteristics of the incarcerated MJOs were similar to that of ordinary adolescents in Indonesia.[37] More specifically, most of them further their education, were single, unemployed, lived with family and their parents were married. However, they commit acts of violence as well as exhibit psychological trauma symptoms and had misbehaviours.

The findings indicate that almost half of the MJOs had abnormal emotional problems and that more than half of them had abnormal conduct and peer relationship problems. This present study's SDQ's total difficulty score was higher than that of the only other Indonesian study as the latter was conducted at only one LPKA (Sungai Raya Special Child Development Institution) and its sample size was small (27 participants). However, the abnormal emotional problem finding of this present study is similar to that of a study on Brazilian MJOs, which reported that more than a half of Brazilian MJOs have difficulties related to emotional and mental health.[38] This could be because MJOs are in a transitional period, during which adolescents experience emotional, behavioural and social changes.[39] Therefore, when MJOs conduct criminal acts, they lack psychological maturity and rational thinking.[40] They may also have emotional, conduct and peer relationship problems.

Present study found that low levels of education strongly correlate with incidences of psychological distress. Studies from Malaysia, the United Kingdom, Chile, Kenya and Cambodia, have, similarly, concluded that MJOs with low levels of education; such as primary education only, no schooling, did not complete schooling, dropped out of school, or did not pursue a secondary education; had higher and more severe incidences psychological distress. Education is essential for adolescents as adolescents with low levels of education cannot make logical judgments as they cannot efficiently utilise their cognitive skills.[41] It is neces-

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sary to improve their cognitive abilities to enable them to think logically and critically and understand emotions that affect their mental health.[42] Therefore, low levels of education significantly affect incidences of psychological distress.

Present study also found that psychological trauma symptoms correlate with incidences of psychological distress. An extant study, similarly, reported that incidences of psychological trauma pre-incarceration; such as a history of child, sexual, emotional and physical abuse as well as exposure to bullying, neglect and homelessness; correlate with higher incidences of psychological distress.[43] Traumatic events can significantly alter the emotions, thoughts and behaviours of individuals, leading to high-risk mental health challenges in later life.[44] As adolescence is a transition period, MJOs have unstable feelings and cannot control their emotions, thoughts and behaviours, which affect their coping mechanisms. As such, adolescents cannot cope with internal or external stressors due to their inability psychologically and cognitively. Therefore, MJOs with fewer psychological trauma symptoms have fewer incidences of psychological distress.

To the best of authors' knowledge, only one 2018 study has examined the prevalence of psychological distress at an LPKA in Indonesia. It then went on to examine the risk factors of psychological distress among incarcerated Indonesian MJOs. Therefore, the findings from the 206 MJOs incarcerated at 28 LPKAs may represent problems that can be generalised to the rest of the country. However, as this present study solely focused on MJOs, the collected data cannot be evaluated from a gender perspective as female juvenile offenders were excluded as there were too few of them. Lastly, as this present study was a cross-sectional, it could not determine if the MJOs developed symptoms of psychological distress pre-incarceration or due to a chronic event that occurred during incarceration.

Future studies may consider developing appropriate interventions that help juveniles, especially incarcerated MJOs, manage and overcome psychological distress. Furthermore, the government, especially the Ministry of Law and Rights, should help every juvenile offender to continue their education and implement policy campaigns that address symptoms of psychological distress among juveniles. Apart from that, healthcare providers could promote the importance of educational attainment to ordinary adolescents. They may also recruit the help of the family if an adolescent has problems in school and communicate with teenagers to prevent them from committing acts of juvenile delinquency and avoid incarceration. Healthcare providers may also prevent adolescents from developing symptoms of psychological distress by giving them mental health education and counselling or therapy. Meanwhile, healthcare providers could encourage incarcerated juveniles to further their education. They may also screen them for symptoms of psychological distress and recommend trauma-specific counselling for specific events; such as PTSD, disturbances in self-organisation (DSO), anxiety, depression and insomnia.

Conclusion

The psychological distress of Indonesian MJOs is a public health concern that warrants comprehensive mental health care to be integrated into the present healthcare system for incarcerated juveniles. Furthermore, education level and symptoms of psychological distress must be examined when routine mental health examinations are conducted followed by the implementation of appropriate treatments. Government and healthcare providers should make an effort to implement support, education and psychological health management in juvenile facilities.

Ethics

The study was approved by The Khon Kaen University Ethics Committee For Human Research, decision No HE652141, dated 16 August 2022. The study was organised and implemented based on the Belmont report and GCP in social and behavioural research.

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Conflicts of interest

The authors declare that there is no conflict of interest.

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Data access

The data that support the findings of this study are available from the corresponding author upon reasonable individual request.

Author ORCID numbers

Prasetyo Aji Nugroho (PAN):
0000-0002-7770-5194
Somporn Rungreangkulkij (SR):
0000-0001-9307-9237
Suriadi Jais (SJ):
0000-0001-5794-7826

Author contributions

Conceptualisation: PAN, SJ
Methodology: PAN, SR
Validation: PAN, SR
Formal analysis: PAN
Investigation: PAN
Resources: PAN, SR
Data curation: PAN, SR
Writing - original draft: PAN, SJ
Writing - review and editing: PAN, SJ
Supervision: SR
Project administration: PAN
Funding acquisition: SR

References

1. Australian Bureau of Statistics (ABS). Recorded Crime - Offenders: 2021-22 financial year [Internet]. 2023 February [Cited: 8-Feb-2023]. Available at: https://www.abs.gov.au/statistics/people/crime-and-justice/recorded-crime-offenders/2021-22.
2. Rochmawati DH. [The relationship between self-concept and the ability to the meaning of life ability to establish the meaning of life in incarcerated juvenile in Semarang Class 1 Prison] JKS. 2014 Nov 1;9(3):197-204. doi: 10.20884/1.jks.2014.9.3.614. Indonesian.
3. Hilman DP, Indrawati ES. [The experience of being a juvenile incarcerated in Semarang Class I Prison]. Jurnal Empati. 2018 Mar 5;7(3):189-203. Indonesian.
4. Development Services Group. Intersection between mental health and the juvenile justice system. Off Juv Justice Delinq Prev. [Internet]. 2017 July [Cited: 12-Mar-2021];1-13. Available at: https://www.ojjdp.gov/mpg/litreviews/Intersection-Mental-Health-Juvenile-Justice.pdf.
5. Bartram M, Chodos H. Changing directions, changing lives: The mental health strategy for Canada: calling for critical deliberation on implementation. Can J Comm Mental Health. 2013;32(4):1-8. Doi:10.7870/cjcmh-2013-001.
6. Åkesdotter C, Kenttä G, Eloranta S, Franck J. The prevalence of mental health problems in elite athletes. J Sci Med Sport. 2019 Nov 2;23(4):329-35. doi: 10.1016/j.jsams.2019.10.022.
7. Haavik L, Joa I, Hatloy K, Stain HJ, Langeveld J. Help seeking for mental health problems in an adolescent population: the effect of gender. J Ment Health. 2017 July 18;28(5):467-74. doi: 10.1080/09638237.2017.1340630.
8. Kuranga AT, Yussuf AD. Psychiatric morbidity amongst adolescents in a Nigerian juvenile correctional facility. S Afr J Psychiatr. 2021 Aug 16;27(0):1590. doi: 10.4102/sajpsychiatry.v27i0.1590.
9. Kim Ji, Kim B, Kim BN, Hong SB, Lee DW, Chung JY, et al. Prevalence of psychiatric disorders, comorbidity patterns, and repeat offending among male juvenile detainees in South Korea: a cross-sectional study. Child Adolesc Psychiatry Ment Health. 2017 Jan 18;11(6):1-9. doi: 10.1186/s13034-017-0143-x.
10. Okwara LV. Prevalence of psychiatric morbidity among juvenile offenders committed to Borstal institutions in Kenya. Doctoral dissertation, University of Nairobi, 2013.
11. Westberg KH, Nyholm M, Nygren JM, Svedberg P. Mental health problems among young people—a scoping review of help-seeking. International journal of environmental research and public health. Int J Environ Res Public Health. 2022 Jan 27;19(3):1430. doi: 10.3390/ijerph19031430.

CONTENT

12. Murphy M, Fonagy P. Mental health problems in children and young people. Annual report of the chief medical officer 2012:1-3. [Internet]. [Cited: 8-Feb-2023]. Available at: https://assets.publishing.service.gov.uk/media/5a7c4971e5274a1b00422bf9/33571_2901304_CMO_Chapter_10.pdf.
13. Borschmann R, Janca E, Carter A, Willoughby M, Hughes N, Snow K, et al. The health of adolescents in detention: a global scoping review. Lancet Publ Health. 2020 Jan 6;5(2):E114-26. doi: 10.1016/S2468-2667(19)30217-8.
14. Kinner S, Degenhardt L, Coffey C, Sawyer SM, Hearps SSJC, Patton GC. Complex health needs in the youth justice system: a survey of community-based and custodial offenders. J Adolesc Health. 2014 May;54(5):521-6. doi: 10.1016/j.jadohealth.2013.10.003.
15. Casswell M, French P, Rogers A. Distress, defiance or adaptation? A review paper of at-risk mental health states in young offenders. Early Interv Psychiatry. 2012 Feb 5;6(3):219-28. doi: 10.1111/j.1751-7893.2012.00344.x.
16. Houchins DE, Jimenez E, Langley N, Plescow K, Henrich CC. Predictors of self- determination and mental health symptoms among youth in juvenile justice facilities. Behavioral Disorders 2021 Mar 13;46(3):138-48. doi:10.1177/0198742920911182.
17. Pat P, Richter-Sundberg L, Jegannathan B, Edin K, San Sebastian M. Mental health problems and suicidal expressions among young male prisoners in Cambodia: a cross- sectional study. Glob Health Action. 2021 Oct 13;14(1):1985229. doi: 10.1080/16549716.2021.1985229.
18. Tanzania D, Mbelwa S. Mental disorders and associated factors among adolescents in juvenile detention, Dar es Salaam, Tanzania. Open J Nurs. 2017 Sep 9;7(9):993–1020. doi: 10.4236/ojn.2017.79073.
19. Atilola O, Ola B, Abiri G, et al. Status of mental-health services for adolescents with psychiatric morbidity in youth correctional institutions in Lagos. J Child Adol Ment Health. 2017 May 29; (1):63-83. doi: 10.2989/17280583.2017.1321550.
20. Yusuf AD, Ajiboye PO, Issa BA, Buhari OI. Prevalence and risk factors for psychiatric disorders among youths in a Borstal Institution in Nigeria. J Pak Psychiatr Soc. 2011;8(1):22.
21. Bryant A, Guy J, Holmes J, Astle D, Baker K, Gathercole S, et al. The strengths and difficulties questionnaire predicts concurrent mental health difficulties in a transdiagnostic sample of struggling learners. Front Psychol. 2020 Nov 12;11:587821. doi: 10.3389/fpsyg.2020.587821.
22. Vishwakarma G. Sample size and power calculation. In: Nursing Research in 21st Century. Darya Ganj, Delhi: CBS Publishers & Distributors 2017; pp. 1-21.
23. Aida SA, Aili HH, Manveen KS, Salwina WI, Subash KP, Ng CG, et al. Prevalence of psychiatric disorders among juvenile offenders in Malaysian prisons and association with socio-demographic and personal factors. Int J Prison Health. 2014 Jun 10;10(2):132-43. doi: 10.1108/IJPH-06-2013-0029.
24. Gaete J, Labbé N, Del Villar P, Allende C, Araya R, Valenzuela E. Mental health and associated factors among young offenders in Chile: a cross-sectional study. Crim Behav Ment Health. 2017 Apr 12;28(1):13-27. doi: 10.1002/cbm.2029.
25. Pourhoseingholi MA, Vahedi M, Rahimzadeh M. Sample size calculation in medical studies. Gastroenterol Hepatol Bed Bench. 2013 Winter;6(1):14-7. PMID: 24834239.
26. Gray JR, Grove SK, Sutherland S. Burns and Grove's the practice of nursing research: appraisal, synthesis, and generation of evidence. Amsterdam, Netherlands: Elsevier; 2017.
27. Colarusso CA. Adolescence (ages 12–20). In: Colarusso CA, editor. Child and adult development: a psychoanalytic introduction for clinicians. Berlin/Heidelberg, Germany: Springer;1992; p. 91-105. doi: 10.1007/978-1-4757-9673-5_7.
28. Lyu SY, Chi YC, Farabee D, Tsai LT, Lee MB, Lo FE, et al. Psychological distress in an incarcerated juvenile population. J Formos Med Assoc. 2014 May 1;114(11):1076-81. doi: 10.1016/j.jfma.2014.03.011.
29. Dyer F, Gregory L. Mental health difficulties in the youth justice population: learning from the first six months of the IVY project. Proceedings of the Centre for Youth & Criminal Justice; 2014; Glasgow, Scotland. Scotland: Centre for Youth & Criminal Justice; 2014. pp. 14.
30. Agustine EM, Sutini T, Mardhiyah A. [The screening of juvenile behaviour in incarcerated juvenile Class II]. Jurnal Keperawatan Komprehensif. 2018 Feb 12;40(1):32-40. Indonesian. doi: https://journal.stikep-ppnijabar.ac.id/index.php/jkk/article/view/96.
31. Olff M, Bakker A, Frewen P, Aakvaag H, Ajdukovic D, Brewer D, et al. Screening for consequences of trauma–an update on the global collaboration on traumatic stress. Eur J Psychotraumatol. 2020 Apr 27;11(1):1752504. doi: 10.1080/20008198.2020.1752504.
32. Frewen P, McPhail I, Schnyder U, Oe M, Olff M. Global Psychotrauma Screen (GPS): psychometric properties in two Internet-based studies. Eur J Psychotraumatol. 2021 Jun 25;12(1):1881725. doi: 10.1080/20008198.2021.1881725.
33. Primasari I, Qing Y, Coimbra BM, Hovnanyan A, Grace E, Williamson RE, et al. Mental health responses to COVID-19 around the world. Eur J Psychotraumatol. 2021 Jun 30;12(1):1929754. doi: 10.1080/20008198.2021.1929754.
34. Goodman A, Goodman R. Strengths and difficulties questionnaire as a dimensional measure of child mental health. J Am Acad Child Adolesc Psychiatry. 2009 Apr;48(4):400-3. doi: 10.1097/CHI.0b013e3181985068.
35. Matthew MA. [Analysis of the development of inmates with mental disorders in the implementation of the correctional inmates development program (case study in a class 1A correctional institution in Bandar Lampung]. Digital Repository Unila@Universitas Lampung. 2023 Mar. Indonesian. Available from: https://digilib.unila.ac.id/70271/.
36. Alabshar N, Giyarsih SR, Pitoyo AJ. Factors influencing the prosperity of migrants in Indonesia. Solid State Technology. 2020;63(3):4358-72.
37. Dhamayanti M, Peryoga SU, Firmansyah MR. Emotional mental problems among adolescents: urban and semi-urban settings. Althea Med J. 2018 June;5(2):77-81. doi: 10.15850/amj.v5n2.1416.

CONTENT

38. Keliat BA, Wiyono AP, Susanti H. Case management of mental disorders: CMHN (intermediate course). Jakarta: EGC; 2011. p. 35.
39. Icenogle G, Steinberg L, Duell N, Chein J, Chang L, Chaudhary N, et al. Adolescents' cognitive capacity reaches adult levels prior to their psychosocial maturity: evidence for a "maturity gap" in a multinational, cross-sectional sample. Law Hum Behav. 2019 Feb;43(1):69-85. doi: 10.1037/lhb0000315.
40. Dahmann SC. How does education improve cognitive skills? instructional time versus timing of instruction. Labour Econ. 2017;47:35-47. doi: 10.1016/j.labeco.2017.04.008.
41. Lovett A, Kwon HR, Kidia K, Machando D, Crooks M, Fricchione G, et al. Mental health of people detained within the justice system in Africa: systematic review and meta-analysis. Int J Ment Health Syst. 2019 May 6;13:31. doi: 10.1186/s13033-019-0273-z.
42. Scott MJ. CBT for common trauma responses. New York: Sage Publications Ltd; 2012.
43. Pessoa MD, Matsukura TS, Cid MF, Minatel MM. Young offenders in Brazil: mental health and factors of risk and protection. J Hum Growth Devel. 2015 Oct 20; 25(2):162-9. doi: 10.7322/JHGD.102999.
44. Bailen NH, Green LM, Thompson RJ. Understanding emotion in adolescents: a review of emotional frequency, intensity, instability, and clarity. Emotion Review. 2018 Aug 2;11(1):63-73. doi: 10.1177/1754073918768878.

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ORIGINAL ARTICLE

Inhibitory Effect of Selenium Nanoparticles on the Biofilm Formation of Multidrug-Resistant *Acinetobacter Baumannii*

Aleksandra Šmitran,[1] Bojana Luković,[2] Ljiljana Božić,[1] Bojan Golić,[3] Ina Gajić[4]

1. Department of Microbiology and Immunology, Faculty of Medicine, University of Banja Luka, Banja Luka, The Republic of Srpska, Bosnia and Herzegovina.
2. Academy of Applied Studies Belgrade, College of Health Sciences, Belgrade, Serbia.
3. PI Veterinary Institute of the Republic of Srpska "Dr Vaso Butozan", Banja Luka, The Republic of Srpska, Bosnia and Herzegovina.
4. Institute of Microbiology and Immunology, Faculty of Medicine, University of Belgrade, Belgrade, Serbia.

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Corresponding author:
ALEKSANDRA ŠMITRAN
M: +387 65 977 518
E: aleksandra.smitran@med.unibl.org

Abstract

Background/Aim: Treatment of infections caused by biofilm-producing multidrug-resistant (MDR) pathogens represents a huge global problem due to primary antimicrobial multi-resistance enhanced by reduced penetration of antibiotics in the biofilm-embedded bacteria. The aim of this study was to determine the capacity of biofilm production among MDR *Acinetobacter baumannii* (*A. baumannii*) isolates obtained from different clinical specimens and to evaluate the inhibitory effect of selenium nanoparticles (SeNPs) coated with cationic polymer cetyltrimethylammonium bromide (CTAB) on the biofilm formation.

Methods: Antimicrobial effect of antibiotics (meropenem, imipenem, gentamicin, amikacin, ciprofloxacin, levofloxacin and trimethoprim-sulfamethoxazole) was determined by disk-diffusion assay, while sensitivity to colistin was determined with E test. All 60 isolates were tested on biofilm production in microtiter plates with crystal violet dye. Minimal biofilm inhibitory concentration (MBIC) of SeNPs was tested in order to prevent biofilm formation in microtiter plates.

Results: All tested clinical isolates were classified as MDR (n = 60) and extensively drug-resistant (XDR, n = 60). Out of the total 60 isolates, 55 isolates (92 %) showed the ability for biofilm formation, with the majority of them classified as strong (42 %) and moderate (42 %) biofilm producers. MBIC values of SeNPs for 55 biofilm-producing isolates ranged from 0.07 to 1.25 mg/mL. Strong biofilm producers had statistically higher MBIC (0.15 mg/mL) in correlation to other biofilm-producing isolates (0.07 mg/mL). There was no correlation between invasiveness of isolates with biofilm production and MBIC values.

Conclusion: Presented results are very promising and interesting especially in nanotechnology and medical fields, while SeNPs with the addition of cationic surfactant inhibit biofilm formation of MDR *A. baumannii* clinical isolates.

Key words: *Acinetobacter baumannii*; Selenium nanoparticles; Biofilm.

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Introduction

Acinetobacter baumannii (*A. baumannii*) is one of the most common human pathogens, associated predominantly with nosocomial infections such as ventilator-associated pneumonia and sepsis, urinary tract and skin and soft tissue infections, especially among critically ill patients

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in intensive care units.[1] *A. baumannii* belongs to the ESKAPE group of microorganisms (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, A. baumannii, Pseudomonas aeruginosa, and Enterobacter spp*), which are globally notorious for human health due to their ability to acquire diverse resistance genes rapidly. Carbapenems and colistin are the most commonly used antibiotics for the treatment of multi-resistant bacterial isolates. Unfortunately, resistance to carbapenems is now significantly widespread, because of the genome plasticity of this bacterium, which easily acquires resistance genes, including genes for carbapenemases production. The high prevalence of carbapenem-resistant *A. baumannii* (CRAB) isolates carrying bla_{OXA-23}-like and bla_{OXA-24}-like genes has emerged as a serious problem in healthcare settings in Serbia.[2, 3] Also, bacteria can become resistant to other classes of antibiotics by regulation of antibiotic transportation through bacterial membranes (eg reduced expression of porins or enhanced activation of efflux pump), alteration of the target site (eg ribosomal protection protein) and enzymatic modifications and inactivation of antimicrobial substance (eg aminoglycosidases).[1] During the COVID-19 pandemic, the importance of this pathogen was extremely emphasised, because it was determined that co-infection between the SARS-CoV2 virus and this bacterium was very often associated with a fatal outcome.[4, 5] Most of the patients who died were in intensive care units with respiratory failure and were treated with the invasive mechanical ventilation. *A. baumannii* is capable of forming biofilm on artificial substrates, such as respiratory ventilators or vascular and urinary catheters. It is an excellent biofilm producer and biofilm production represents an additional factor contributing to the development of antimicrobial resistance. Taking all these facts together, it is obvious that the World Health Organization ranked multidrug-resistant (MDR) *A. baumannii* as the number one priority microorganism for antimicrobial substance research and development.

Nanoparticles (NPs) are one of the most promising antibacterial agents. Due to their small size and high surface-to-volume ratio, NPs have physical and chemical properties that differ from their bulk material. Selenium is an essential mineral and micronutrient that is well known for its anticancer and antimicrobial activity, as well as being an important substance for improving reproductive capabilities.[6] Selenium nanoparticles (SeNPs), compared with selenium inorganic and organic compounds, emerged as a promising agent for antimicrobial and biomedical uses due to their low toxicity, degradability and high bioavailability.[7] When it comes to NPs, their ability for aggregation is quite challenging. In order to avoid this unfavourable occurrence, one uses surfactant-coated NPs methodology. Essentially, NPs are surrounded and covered by the surfactant which allows NPs to be more stable.

The aim of this study was to determine the capacity for biofilm production among MDR *A. baumannii* isolates obtained from different clinical specimens and to evaluate the antimicrobial effect of SeNPs coated with cationic polymer cetyltrimethylammonium bromide (CTAB) against planktonic isolates and biofilm formation of clinical isolates of MDR *A. baumannii*.

Methods

Bacterial isolates, species identification and antimicrobial resistance

The present experimental study included 60 non-redundant randomly selected MDR *A. baumannii* isolates from the bacterial collection of the Institute of Microbiology and Immunology in Belgrade. The isolates were recovered from patients admitted to hospitals in Serbia during January-June 2018. Identification of the *A calcoaceticus–baumannii* complex (Acb complex) was done using conventional bacteriological techniques employed in clinical microbiology. Species identification of isolates as *A. baumannii* was confirmed by Vitek 2 System (*Biomerieux*, France). Invasive isolates were those collected from normally sterile body sites (such as blood, peritoneal fluids and cerebrospinal fluids). Non-invasive isolates were those obtained from skin and soft tissue, urine and respiratory tract.

According to the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST), the antimicrobial susceptibility of *A. baumannii* to meropenem, imipenem, gentamicin, amikacin, ciprofloxacin, levofloxacin and trimethoprim-sulfamethoxazole was determined by disk-diffusion assay (*Bio-Rad*, UK).[8] ComASP Colistin (*Liofilchem*, Italy) was used to determine the minimum inhibitory concentrations (MICs) for colistin, in accordance with EU-

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CAST recommendations.[8] Based on antimicrobial resistance, all *A. baumannii* isolates were classified as follows: (i) MDR- resistant to at least one agent in three or more antimicrobial categories, (ii) extensively drug-resistant (XDR)- resistant to at least one agent in all, but two or fewer antimicrobial categories and (iii) pandrug-resistant (PDR)- resistant to all agents in all antimicrobial categories tested.[9]

Biofilm production assay

Quantification of biofilm production was done following the protocol by Stepanovic et al.[10] After the 37 °C overnight incubation in Trypticase Soy broth (TSB, *Biorad*, UK), strains were diluted in fresh TSB to achieve a final concentration of 10^6 CFU/mL. Aliquots of *A. baumannii* suspension (100 µL) were transported to each well of the 96-well microtiter plate and were incubated for 24 h at 37 °C. Microtiter plates were aspirated and washed three times with sterile phosphate-buffered saline (PBS). The plates were dyed with 100 µL of 2 % (w/v) crystal violet for 15 min, washed three times and dried overnight at room temperature. Thereafter, 100 µL of glacial acetic acid at 33 % (v/v) was used to dissolve the dye that had been bound to the biofilm matrix. Using an automated microtiter plate reader, the optical density (OD) of each well was determined spectrophotometrically at 570 nm (ICN Flow Titertek Multiskan Plus Reader, *Meckenheim*, Germany). TSB was the only suspension in the negative control wells. *A. baumannii* ATCC 19606 was used as positive control. Three standard deviations more than the mean OD of the negative control were designated as the cut-off OD (ODc).

The results were evaluated as follows:
OD ≤ ODc non-biofilm producers,
ODc < OD ≤ (2 × ODc) = weak biofilm producers,
(2 × ODc) < OD ≤ (4 × ODc) = moderate biofilm producers and
OD > (4 × ODc) = strong biofilm producers.

SeNPs synthesis

Five hundred mg of sodium selenite ($Na_2S_2O_3$) was dissolved in 50 mL of double distilled water, then it was sonicated for 10 min. Along with this solution, the CTAB solution was also prepared. Two hundred fifty mg of CTAB was dissolved in 50 mL of double distilled water and sonicated for 10 min. Afterward, both solutions were mixed and stirred for 10 min on a magnetic stirrer. After the addition of 1500 mg of ascorbic acid, the stirring was continued for 4 h and the temperature was set up to 80 °C. Finally, 6.25 mL of double distilled water and 1.876 mL of hydrazine were added. After 2 h, the solution was kept for 4 days at room temperature and it was filtered and washed with double distilled water and dried in an oven for 1 h at 50 °C. A similar procedure was reported previously.[11, 12]

Characterisation of SeNPs

Characterisation of SeNPs was performed using scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDX) technique. Images were produced by TM3030 SEM Hitachi Japan SEM.

Testing of inhibitory effect of SeNPs on the biofilm formation of *A. baumannii* isolates

The minimum biofilm inhibitory concentration (MBIC) of SeNPs was analysed following the protocol of Bagheri-Josheghani et al.[13] Aliquots of 100 µL of bacterial isolates (in a final concentration of 10^6 CFU/mL) in double-strength MH broth were incubated with 100 µL of SeNPs (in final concentrations ranging from 1.25-0.0015 mg/mL). After overnight incubation at 37 ^{0}C, microtiter plates were treated as described above in section 2.2.

Statistical analyses

SPSS version 20.0 (*SPSS Inc*, Chicago, IL, USA) was used for statistical analysis. Statistical comparison of multiple groups on the occasion of deviation of normal data distribution was done using the Kruskal-Wallis rank sum test. To compare variances without normal distribution between the two groups, the Mann-Whitney U test was used. Fisher's exact test was used for comparison of the frequency of occurrence of the analysed categorical variables. A p value less than 0.05 was considered to be significant.

Results

Bacterial isolates and antimicrobial testing

All 60 isolates of *Acb complex* were identified as *A. baumannii.* Bacterial isolates were recovered from blood samples (18), cerebrospinal fluid (1), peritoneal fluid (1), lower respiratory specimens (20), urine (6) and from the wound and soft tissue specimens (14). Bacterial isolates were obtained from 19 female and 41 male patients. The median age of patients was 67.3 years, with a range of 18-88 years.

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All isolates were resistant to carbapenems (imipenem, meropenem), while other antibiotics showed also high resistance rate: ciprofloxacin (93 %), levofloxacin (93 %), amikacin (95 %), gentamicin (98 %) and trimethoprim-sulfamethoxazole (93 %). All isolates were sensitive to colistin. According to antimicrobial resistance, all isolates were classified as MDR (n = 60) and XDR (n = 60) isolates. None PDR isolate was detected.

Biofilm production

Out of a total of 60 tested isolates, the majority of isolates were strong (n = 25, 42 %) and moderate (n = 25, 42 %) biofilm producers. Only 5 (8 %) isolates were weak producers, while 5 (8 %) isolates were not capable of biofilm production. Among non-biofilm producing isolates, 2 isolates were obtained from blood and 3 isolates were obtained from lower respiratory samples. Average biofilm mass according to O.D. for strong, moderate and weak biofilm producers were 0.720, 0.300 and 0.166, respectively. When the capacity of isolates to form biofilm with invasiveness of isolates or specimen type was compared, there was no statistical significance in both correlations ($p > 0.05$). There was no statistical correlation between antimicrobial resistance and biofilm production ($p > 0.05$). The distribution of biofilm production abilities of the tested isolates obtained from various clinical samples is displayed in Figure 1.

SeNPs characterisation

Figure 2A presents the SEM micrographs of the synthesised SeNPs which showed well-defined spherical morphology. The EDX spectrum (Figure 2B) contains a pronounced Se peak, along with the nitrogen (N), carbon (C) and oxygen (O) peaks. The last three originate from the cationic surfactant CTAB. Its role is to prevent agglomeration of SeNPs, as well as to make energetic SeNPs more stable.

Inhibitory effect of SeNPs on biofilm formation of MDR *A. baumannii*

SeNPs exhibited an inhibitory effect on biofilm formation against 55 biofilm-forming isolates. One isolate from the wound swab had MBIC value of 1.25 mg/mL, while 4 isolates (2 respiratory, 1 urinary and 1 blood sample) had MBIC value of 0.625 mg/mL. All other 50 isolates have MBIC values of 0.3 mg/mL or lower. The median MBIC value for all 55 biofilm-producing isolates was 0.15 mg/mL, whereas $MBIC_{50}$ and $MBIC_{90}$ were 0.07 and 0.3, respectively. When SeNPs MBIC values with the invasiveness of isolates was compared, there was no statistically significant difference among tested isolates ($p > 0.05$). On the other hand, strong biofilm producers had higher MBIC in correlation to other isolates ($p < 0.001$), as represented in Table 1. There was no statistical correlation between antimicrobial resistance to antibiotics and MBIC SeNPs values ($p > 0.05$).

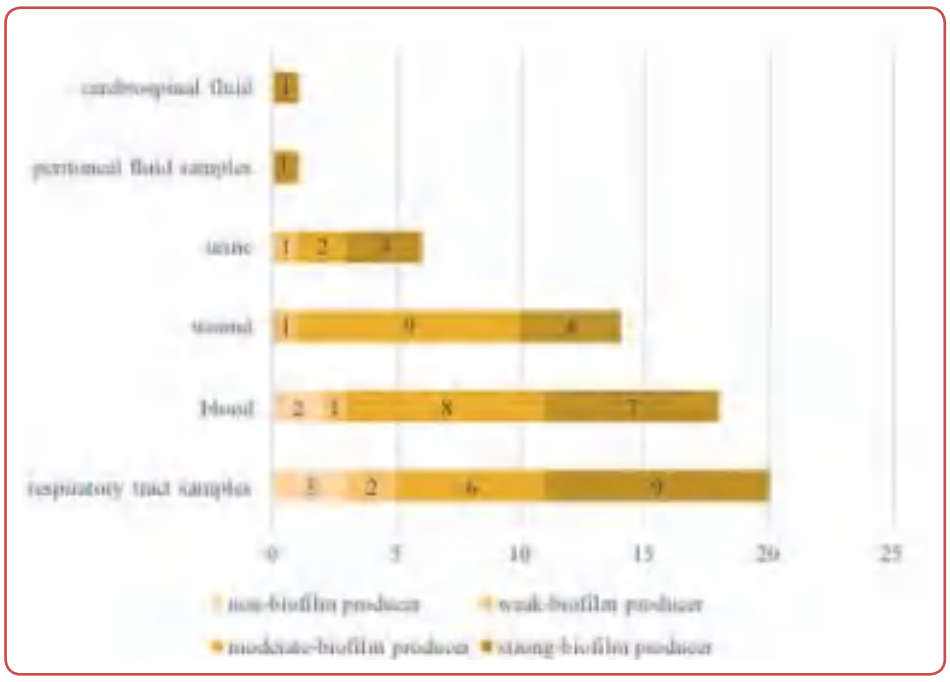

Figure 1: Biofilm formation abilities of 60 multidrug-resistant (MDR) isolates of Acinetobacter baumannii obtained from various clinical specimens

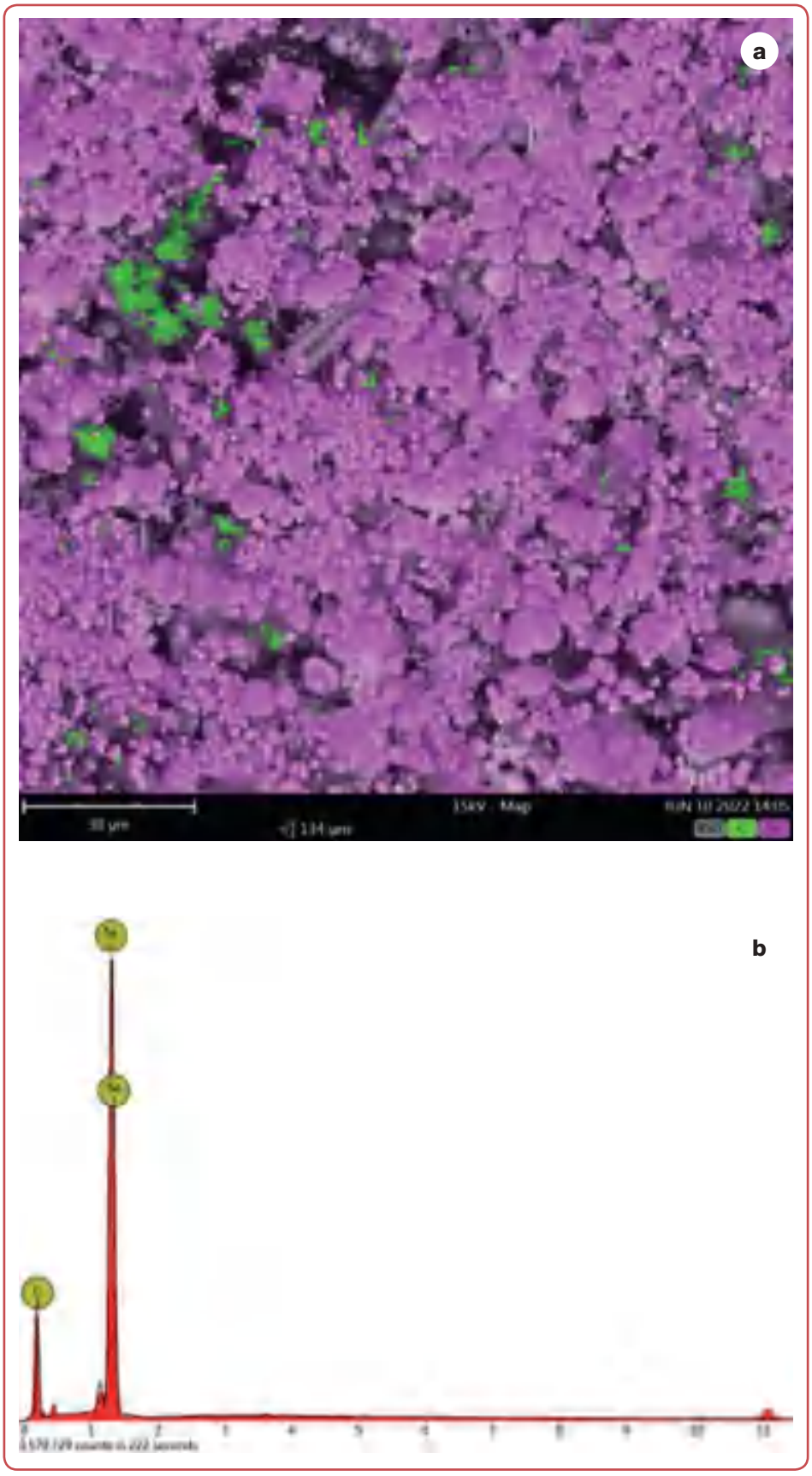

Figure 2: Characterisation of selenium nanoparticles (SeNPs) using scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDX) technique

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Table 1: Median minimal biofilm inhibitory concentration (MBICs) values of selenium nanoparticles (SeNPs) among non-producers, weak, moderate and strong biofilm producers

MBIC (mg/mL)	Non-producers (N = 5)	Weak producers (N = 5)	Moderate producers (N = 25)	Strong producers (N = 25)
Median (range)	0.00 (0.00-0.00)	0.07 (0.03-0.15)	0.07 (0.007-1.25)	0.15 (0.03-0.625)

Discussion

The emergence of MDR and XDR *A. baumannii* is becoming a critical health problem worldwide. Additionally, biofilm production of MDR and XDR *A. baumannii* isolates contributes both to antimicrobial resistance and to bacterial endurance in the hospital environment, leading to the increased prevalence of nosocomial infections.[14, 15] In presented study, all *A. baumannii* isolates were MDR and XDR, sensitive only to colistin, a last-resort antibiotic, which is now a first choice for the treatment of resistant isolates. Fortunately, resistance to colistin is still low and uncommon, but is worrisome and needs to be observed. Although presented resistance to other tested antibiotics is extremely high, similar frequencies of MDR and XDR isolates have been detected globally.[16, 17] Also, it is important to notice that all isolates were resistant to carbapenems, which, unfortunately, is becoming a common finding both in our region and globally.[18, 19]

Given that *A. baumannii* is most frequently associated with nosocomial infections, eg ventilator associated-pneumonia, sepsis, urinary, skin and soft tissue infections, the majority of presented isolates were recovered from lower respiratory samples (20/60, 33 %), blood (18/60, 30 %) and swabs obtained from skin and soft tissue infection (14/60, 23 %), as expected.[20] *A. baumannii* has excellent capacity to form biofilm in comparison to other bacteria, so it is not surprising that almost all of isolates (55/60, 92 %) were biofilm producers with an equal and significant percentage of moderate (25/55, 45 %) and strong (25/55, 45 %) biofilm producers. In the present study, equal numbers of non-invasive and invasive isolates were non-biofilm and strong biofilm producers, respectively, without a clear statistical correlation between bacterial invasiveness and capacity for biofilm production. This could be explained by the shedding of the exopolysaccharide biofilm envelope, enabling planktonic bacteria to be released from the biofilm community and enter the bloodstream causing bacteraemia. Although invasive bacteria are isolated from the blood, no one can claim with certainty and without additional sub-molecular testing that they do not come from the biofilms in tissue or on indwelling devices.[21] Also, the treatment of infections caused by biofilm-producing MDR *A. baumannii* isolates represents a huge challenge, while these isolates could be resistant to all antibiotics, regardless of the results of the antibiogram. This resistance stems from reduced or disabled penetration of antibiotics into the biofilm-embedded bacteria. Presented isolates represent a great example of a problematic situation for our healthcare system, similar to the results of Zeighami et al.[16] Better biofilm production among MDR *A. baumannii* isolates is explained by several mechanisms: lipid A modification, overexpression of efflux pumps and exposure to subminimal antibiotic concentration which creates positive feedback that allows a switch from planktonic to sessile growth in biofilm.[22]

Infections caused by biofilm-producing MDR *A. baumannii* are very demanding for treatment and there are worldwide efforts for the development of new promising and effective antibacterial substances. According to available literature data, this study represents one of the scarce investigations about the antimicrobial activity of SeNPs against MDR *A. baumannii* isolates. The results obtained in this study are very encouraging, because SeNPs prevented biofilm formation. Strong biofilm producers showed significantly higher MBIC values in relation to moderate and weak biofilm-producing isolates, as expected. This results, as well as the results of other authors, showed that SeNPs have great potential as medical devices coatings, because they efficiently prevented biofilm formation at low concentrations.[23-25] Hoseini Bafghi et al demonstrated that SeNPs reduced antifungal resistance due to diminished expression of resistance- related genes in resistant fungal isolates.[26] Biogenic SeNPs are capable of both the prevention of biofilm formation and degradation of mature biofilm matrix by degradation of the bacterial cell membrane and bacterial envelope called glycocalyx in biofilm producing isolates of *P. aeruginosa, S. aureus* and *S. Typhi*.[27] There are no precise data about mecha-

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nism of action of SeNPs against biofilm producing *A. baumannii* isolates and according to available literature, it can only be assumed that SeNPs act on quorum sensing molecules, preventing the onset of biofilm formation by the switch interruption from individual planktonic isolates into collective, biofilm-associated existence.[28] The main goal of this work was to examine the possible antibiofilm effect of modified SeNPs. Due to technical limitations, authors' were not able to perform a complete characterisation of the NPs or determine the precise mechanism of action of these NPs during the process of biofilm formation. In future experimental work, it would be very valuable to obtain precise information about these two unanswered questions to highlight the importance of presented modification method for SeNPs synthesis.

Conclusion

In the present study, it was demonstrated that modified SeNPs successfully prevented biofilm formation in MDR *A. baumannii* isolates. Although stronger biofilm producers required higher concentrations of SeNPs, results suggest that SeNPs coated with CTAB should be considered as a potential coating for indwelling medical devices and further development in pharmaceutical nanotechnology.

Ethics

The study was approved by the Ethical Committee of the Medical Faculty, University of Belgrade (permission No 1550/II-4, dated 21 February 2019). The written informed consent was obtained from all patients.

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Conflicts of interest

The authors declare that there is no conflict of interest.

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Data access

The data that support the findings of this study are available from the corresponding author upon reasonable individual request.

Author ORCID numbers

Aleksandra Šmitran (AŠ):
0000-0002-6039-5978
Bojana Luković (BL):
0000-0003-2830-2572
Ljiljana Božić (LJB):
0000-0003-1564-2391
Bojan Golić (BG):
0000-0001-5726-4507
Ina Gajić (IG):
0000-0002-1578-2776

Author contributions

Conceptualisation: AŠ, LB
Methodology: AŠ, LB
Software, Validation, formal analysis: LJB
Investigation: AŠ, BL, IG
Resources: BG
Data curation: BG
Writing-original draft: AŠ, BG, LJB
Writing-review and editing: IG, BL
Visualisation: LJB, BG, IG
Supervision: IG
Project administration: LJB
Funding acquisition: IG, BG.

CONTENT

References

1. Kyriakidis I, Vasileiou E, Pana ZD, Tragiannidis A. Acinetobacter baumannii antibiotic resistance mechanisms. Pathogens. 2021 Mar 19;10(3):373. doi: 10.3390/pathogens10030373.
2. Gajic I, Jovicevic M, Milic M, Kekic D, Opavski N, Zrnic Z, et al. Clinical and molecular characteristics of OXA-72-producing Acinetobacter baumannii ST636 outbreak at a neonatal intensive care unit in Serbia. J Hosp Infect. 2021 Jun;112:54-60. doi: 10.1016/j.jhin.2021.02.023
3. Lukovic B, Gajic I, Dimkic I, Kekic D, Zornic S, Pozder T, et al. The first nationwide multicenter study of Acinetobacter baumannii recovered in Serbia: emergence of OXA-72, OXA-23 and NDM-1-producing isolates. Antimicrob Resist Infect Control. 2020 Jul 6;9(1):101. doi: 10.1186/s13756-020-00769-8.
4. Sharifipour E, Shams S, Esmkhani M, Khodadadi J, Fotouhi-Ardakani R, Koohpaei A, et al. Evaluation of bacterial co-infections of the respiratory tract in COVID-19 patients admitted to ICU. BMC Infect Dis. 2020 Sep 1; 20:646. doi: 10.1186/s12879-020-05374-z.
5. Russo A, Gavaruzzi F, Ceccarelli G, Borrazzo C, Oliva A, Alessandri F, et al. Multidrug-resistant Acinetobacter baumannii infections in COVID-19 patients hospitalized in intensive care unit. Infection. 2020 Feb;50(1):83-92. doi: 10.1007/s15010-021-01643-4.
6. Bafghi MH, Darroudi M, Zargar M, Zarrinfar H, Nazari, R. Biosynthesis of Selenium nanoparticles by Aspergillus Flavus and Candida Albicans for antifungal applications. Micro Nano Lett. 2021;16:656–69. doi:10.1049/mna2.12096.
7. Johnson J, Shanmugam R, Lakshmi T. A review on plant-mediated selenium nanoparticles and its applications. J Popul Ther Clin Pharmacol. 2022 Jan 6;28(2):e29-e40. doi: 10.47750/jptcp.2022.870.
8. The European Committee on Antimicrobial Susceptibility Testing. [Internet]. Breakpoint tables for interpretation of MICs and zone diameters, Version 14.0. [Cited: 15-Jan-2024]. Available at: http://www.eucast.org.
9. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012 Mar;18(3):268-81. doi: 10.1111/j.1469-0691.2011.03570.x.
10. Stepanović S, Vuković D, Hola V, Di Bonaventura G, Djukić S, Cirković I, et al. Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. APMIS. 2007 Aug;115(8):891-9. doi: 10.1111/j.1600-0463.2007.apm_630.x.
11. Filipović N, Ušjak D, Milenković MT, Zheng K, Liverani L, Boccaccini AR, et al. Comparative study of the antimicrobial activity of selenium nanoparticles with different surface chemistry and structure. Front Bioeng Biotechnol. 2021 Jan 25;8:624621. doi: 10.3389/fbioe.2020.624621.
12. Ahmad W, Shams S, Ahmad A, Wei Y, Yuan Q, Ullah Khan A, et al. Synthesis of selenium–silver nanostructures with enhanced antibacterial, photocatalytic and antioxidant activities. Appl Nanosci. 2020;10(4):1191-204. doi: 10.1007/s13204-019-01213-z.
13. Bagheri-Josheghani S, Bakhshi B. Investigation of the antibacterial and antibiofilm activity of Selenium nanoparticles against Vibrio cholerae as a potent therapeutics. Can J Infect Dis Med Microbiol. 2022 Mar 23;2022:3432235. doi: 10.1155/2022/3432235.
14. Elkheloui R, Laktib A, Mimouni R, Aitalla A, Hassi M, Elboulani A, et al. Acinetobacter baumannii biofilm: intervening factors, persistence, drug resistance, and strategies of treatment. Mediterr J Infect Microb Antimicrob. 2020 Sep 10;9(7):1–12. doi: 10.4274/mjima.galenos.2020.2020.7.
15. Gedefie A, Demsis W, Ashagrie M, Kassa Y, Tesfaye M, Tilahun M, et al. Acinetobacter baumannii biofilm formation and its role in disease pathogenesis: a review. Infect Drug Resist. 2021 Sep 10;14:3711-3719. doi: 10.2147/IDR.S332051.
16. Zeighami H, Valadkhani F, Shapouri R, Samadi E, Haghi F. Virulence characteristics of multidrug resistant biofilm forming Acinetobacter baumannii isolated from intensive care unit patients. BMC Infect Dis. 2019 Jul 17;19(1):629. doi: 10.1186/s12879-019-4272-0.
17. Hu H, Lou Y, Feng H, Tao J, Shi W, Ni S, et al. Molecular characterization of carbapenem-resistant Acinetobacter baumannii isolates among intensive care unit patients and environment. Infect Drug Resist. 2022 Apr 13;15:1821-9. doi: 10.2147/IDR.S349895.
18. D'Onofrio V, Conzemius R, Varda-Brkić D, Bogdan M, Grisold A, Gyssens IC, et al. Epidemiology of colistin-resistant, carbapenemase-producing Enterobacteriaceae and Acinetobacter baumannii in Croatia. Infect Genet Evol. 2020 Jul;81:104263. doi: 10.1016/j.meegid.2020.104263.
19. Afhami S, Borumand MA, Bazzaz NE, Saffar H, Hadadi A, Nezhadet MJ, al. Antimicrobial resistance pattern of Acinetobacter; a multicenter study, comparing European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Clinical and Laboratory Standards Institute (CLSI); evaluation of susceptibility testing methods for polymyxin. Immunopath Persa. 2020;7(1):e04-e04. doi: 10.34172/ipp.2021.04.
20. Harding CM, Hennon SW, Feldman MF. Uncovering the mechanisms of Acinetobacter baumannii virulence. Nat Rev Microbiol. 2018 Feb;16(2):91-102. doi: 10.1038/nrmicro.2017.148.
21. Jain A, Agarwal A. Biofilm production, a marker of pathogenic potential of colonizing and commensal staphylococci. J Microbiol Methods. 2009 Jan;76(1):88-92. doi: 10.1016/j.mimet.2008.09.017.
22. Pompilio A, Scribano D, Sarshar M, Di Bonaventura G, Palamara AT, Ambrosi C. Gram-negative bacteria holding together in a biofilm: the Acinetobacter baumannii way. Microorganisms. 2021 Jun 22; 9(7):1353. doi: 10.3390/microorganisms9071353.
23. Smitran A, Lukovic B, Bozic L, Jelic D, Jovicevic M, Kabic J, et al. Carbapenem-resistant Acinetobacter baumannii: biofilm-associated genes, biofilm-eradication potential of disinfectants, and biofilm-inhibitory effects of Selenium nanoparticles. Microorganisms. 2023 Jan 10;11(1):171. doi: 10.3390/microorganisms11010171.

CONTENT

24. Tran PA, O'Brien-Simpson N, Palmer JA, Bock N, Reynolds EC, Webster TJ, et al. Selenium nanoparticles as anti-infective implant coatings for trauma orthopedics against methicillin-resistant Staphylococcus aureus and epidermidis: in vitro and in vivo assessment. Int J Nanomedicine. 2019 Jul 1;14:4613-24. doi: 10.2147/IJN.S197737.
25. Ramya S, Shanmugasundaram T, Balagurunathan R. Biomedical potential of Actinobacterially synthesized Selenium nanoparticles with special reference to anti- biofilm, anti-oxidant, wound healing, cytotoxic and anti-viral activities. J Trace Elem Med Biol. 2015 Oct;32:30–9. doi: 10.1016/j.jtemb.2015.05.005.
26. Hosseini Bafghi M, Zarrinfar H, Darroudi M, Zargar M, Nazari R. Green synthesis of selenium nanoparticles and evaluate their effect on the expression of ERG3, ERG11 and FKS1 antifungal resistance genes in Candida albicans and Candida glabrata. Lett Appl Microbiol. 2022 May;74(5):809-19. doi: 10.1111/lam.13667.
27. Ullah A, Mirani ZA, Binbin S, Wang F, Chan MWH, Aslam S, et al. An elucidative study of the anti-biofilm effect of selenium nanoparticles (SeNPs) on selected biofilm producing pathogenic bacteria: A disintegrating effect of SeNPs on bacteria. Process Biochem. 2023;126:98-107. doi:10.1016/j.procbio.2022.12.031.
28. Prateeksha, Singh BR, Shoeb M, Sharma S, Naqvi AH, Gupta VK, et al. Scaffold of Selenium nanovectors and honey phytochemicals for inhibition of Pseudomonas aeruginosa quorum sensing and biofilm formation. Front Cell Infect Microbiol. 2017 Mar 23;7:93. doi: 10.3389/fcimb.2017.00093.

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ORIGINAL ARTICLE

Comparison of Dexmedetomidine and Fentanyl as Adjuvants to Intrathecal Isobaric Levobupivacaine in Lower Segment Caesarean Section

Talha Rehman Zahid,[1] Arshad Hayat,[3] Rashk-e-Hinna,[2] Rubina Bashir,[4] Arshman Rauf Asghar,[3] Muhammad Afzal[5]

Abstract

Background/Aim: Caesarean section is one the most common surgeries encountered in the operating room worldwide in the younger demographic ages from 18-39 years of age. The objective of this study was to compare the efficacy of dexmedetomidine versus fentanyl as adjuvants to intrathecal levobupivacaine in the lower segment caesarean section.

Methods: This quasi-experimental study was carried out at the Anaesthesia Department, Combined Military Hospital, Rawalpindi, Punjab, Pakistan from July 2021 to July 2023. A total of 240 patients were studied. They were divided into the dexmedetomidine group (n = 120) and fentanyl group (n = 120) group. Patients in both groups received 2.5 mL of 0.5 % of isobaric levobupivacaine with the dexmedetomidine group receiving 5 mcg of the drug and the fentanyl group 25 mcg of fentanyl to a total volume of 3 mL. Primary variables measured were: time to complete sensory and motor block, total duration of the block, time to first rescue analgesia after block regression in the post anaesthesia care unit (PACU) and mean PACU stay. Secondary variables observed were hypotension, nausea, vomiting and shivering.

Results: The time on onset for a sensory block in the dexmedetomidine group was delayed compared to the fentanyl group (4.35 ± 0.14 min and 3.39 ± 0.11 min, respectively), ($p < 0.0001$). The duration of the block was longer for the dexmedetomidine group with a mean time of 327.26 ± 12.60 min versus 243.3 ± 22.75 min ($p < 0.0001$). When comparing the motor blockade, the time of onset to successfully reach Bromage score 3 was similarly delayed in the dexmedetomidine group with a mean time of onset of 3.33 ± 0.12 min versus 2.36 ± 0.09 min ($p < 0.0001$). A similar trend was seen in the duration of the block with a mean time of 262.17 ± 13.31 min versus 203.34 ± 1.47 min ($p < 0.0001$).

Conclusion: Dexmedetomidine offered advantages over fentanyl as an adjunct to levobupivacaine spinal anaesthesia with a longer block duration and less adverse effects profile. It is recommended to use dexmedetomidine due to its better safety profile, longer duration and better hemodynamic stability. Fentanyl should be reserved when the early onset of the block is required in emergency cases.

Key words: Levobupivacaine; Fentanyl; Dexmedetomidine; Spinal anaesthesia; Adjunct.

1. Department of Anaesthesia, Combined Military Hospital, Rawalpindi, Punjab, Pakistan.
2. Department of Gastroenterology, Pak Emirates Military Hospital, Rawalpindi, Punjab, Pakistan.
3. Department of Medicine, Combined Military Hospital, Lahore, Punjab, Pakistan.
4. Lahore Medical and Dental College, Lahore, Punjab, Pakistan.
5. Medicine Department, Margalla Institute of Health Sciences, Rawalpindi, Punjab, Pakistan.

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Corresponding author:
RASHK-E-HINNA
E: roshni3004@gmail.com

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Introduction

Caesarean section is one the most common surgeries encountered in the operating room worldwide in the younger demographic from 18-39 years of age.[1] With the increasing rates of caesarean section deliveries worldwide, local studies also confirm the increase in Pakistan over the last two decades from 3.2 % in the 1990s to 18-20 % by 2018-2019.[2] This translates to around 1 in 5 mothers being delivered through a caesarean section in the country. With such an increased burden, safety and success in the provision of anaesthesia becomes mandatory to ensure the health of the mother as well as the baby.

Spinal anaesthesia remains the modality of choice when compared to general anaesthesia in these patients.[3] With a better safety profile and the mother being awake, spinal anaesthesia provides better pain relief both pre and post-operatively reducing the need for intravenous (iv) medications for induction and analgesia resulting in a better anaesthesia profile.[4] However, even with all its advantages, the procedure is not without its deficiencies - hypotension, bradycardia[5] and a failure rate ranging from 1-17 %.[6] To improve upon the technique, various advances have been made to offer a better density of block, prolonged duration of action with decreased use of rescue analgesia with added benefits of sedation, anxiolysis and patient comfort.

Isobaric levobupivacaine (s-isomer of racemic bupivacaine)[7] is introduced in recent years as the choice drug for spinal anaesthesia in the obstetric age group due to its less neuro- and cardio- toxicity with an improved density of block and prolonged duration of action when compared to conventional racemic bupivacaine.[8] However, literature is scarce when combining it with adjuvants for better pain control, anxiolysis and prolongation of block time especially in presented demographic area where its use is still considered novel since its onset is slower than that of racemic bupivacaine. Due to its better adverse effect profile and longer duration of block, adjuvants should be sought to improve its onset time as well.[8, 9]

The two most common adjuvants used internationally in spinal anaesthesia have been the α-agonist dexmedetomidine and the opioid fentanyl. It can be hypothesised that compared to the opioid fentanyl, dexmedetomidine would provide effective and comparable analgaesia without the opioid-related adverse effects.

The objective of this study was to compare potential superiority and efficacy of dexmedetomidine versus fentanyl as adjuvants to intrathecal levobupivacaine in the lower segment caesarean section.

Methods

This quasi-experimental study was carried out at the Department of Anaesthesiology, Combined Military Hospital, Rawalpindi, Punjab, Pakistan from July 2021 to July 2023 after approval from the ethical review board. Two hundred forty patients requiring elective caesarean sections were included in the study after calculating the sample size using the WHO calculator keeping the confidence interval (CI) at 95 %, margin of error at 5 % and keeping the population proportion of caesarean sections in analysed local demographic area at 19 %.[2]

Inclusion criteria included all ASA-I and II (American Society of Anesthesiology) adult patients of 18-30 years of age with a weight between 50-90 kg presenting in the obstetric department for scheduled elective caesarean delivery under spinal anaesthesia. Non-inclusion criteria comprised of patients unwilling to spinal anaesthesia refused to be included in the study, patients with allergy to either fentanyl, levobupivacaine or dexmedetomidine, patients who underwent spontaneous labour and emergency lower (uterine) segment caesarean section (LSCS) before the operation, patients with failed spinal for the procedure and were given general anaesthesia and patients with known major respiratory or cardiovascular disease.

The patients were divided into the dexmedetomidine group (n = 120) and the fentanyl group (n = 120). The method of sampling was a non-probability consecutive type. This was a double-blind study and once the patients were divided into two groups, the anaesthetist on duty in the operating room unaware of the study protocol received sealed envelopes with the two adjuvant vials labelled 1 and 2 with the anaesthetist not knowing which vial had dexmedetomidine and

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which one had fentanyl. Both groups received 500 mL of normal saline in the patient holding bay 15 min before being shifted to the operating room. Standard monitoring including non-invasive blood pressure, heart rate, capnography and ECG in both groups.

Patients in both groups received 2.5 mL of 0.5 % of isobaric levobupivacaine with the dexmedetomidine group and fentanyl group receiving 5 mcg of the dexmedetomidine and 25 mcg of fentanyl to a total volume of 3 mL, respectively. The patient was injected the study solutions in both groups in the L2-L3 or L3-L4 space by a standard needle (*Quincke*) with free flow confirmed by the barbotage method and the spinal solution injection and patient was placed in the supine position with a wedge placed underneath.

Sensory blockade till the T6 dermatome level was confirmed by loss of sensation to cold ethyl chloride spray in the mid-line bilaterally and motor blockade with Bromage score[10] of 3 was considered as a successful block and the surgery was then continued. The total duration of the block was calculated once the sensory level was at S1 dermatome and Bromage score of 0. Bradycardia was defined as a heart rate of < 60 beats per minute[11] and hypotension as mean arterial pressure (MAP) < 50 mm Hg[12] and was treated with 5 mg ephedrine and 600 mcg of glycopyrrolate when needed.

Primary variables measured were: time to complete sensory and motor block with total duration of the block, time to first rescue analgesia after block regression in the post anaesthesia care unit (PACU) and mean PACU stay as secondary variables. Adverse effects observed were hypotension, nausea, vomiting and shivering. Demographic data were statistically described in terms of mean ± standard deviation (SD), frequencies and percentages when appropriate. A p-value of < 0.05 was considered statistically significant. All statistical calculations were performed using SPSS 26.0.

Results

A total of 240 patients were studied divided into the dexmedetomidine group (n = 120) and fentanyl group (n = 120) group. The mean age of patients in the dexmedetomidine group was 24.12 ± 2.22 years versus 24.20 ± 2.17 years in the fentanyl group. Both groups were comparable in age. The mean weight of patients in the groups was 75.31 ± 9.30 kg for dexmedetomidine and 75.80 ± 9.56 kg for the fentanyl group (Table 1).

Table 1: Age and height characteristics of patients in both groups (n = 240)

Variable	Dexmedetomidine group (n = 120)	Fentanyl group (n = 120)
Mean age (years)	24.1 ± 2.22	24.2 ± 2.17
Mean weight (kg)	75.3 ± 9.30	75.8 ± 9.56

When the primary outcome variables were seen, the time on onset for a sensory block in the dexmedetomidine group was delayed compared to the fentanyl group (4.35 ± 0.14 min and 3.39 ± 0.11 min, respectively), (p < 0.0001). The duration of the block was longer for the dexmedetomidine group with a mean time of 327.26 ± 12.60 min versus 243.3 ± 22.75 min (p < 0.0001). When comparing the motor blockade, the time of onset to successfully reach Bromage score 3 was similarly delayed in the dexmedetomidine group with a mean time of onset of 3.33 ± 0.12 min versus 2.36 ± 0.09 min (p < 0.0001). A similar trend was seen in the duration of the block with a mean time of 262.17 ± 13.31 min versus 203.34 ± 1.47 min (p < 0.0001) in the dexmedetomidine and fentanyl group, respectively (Table 2).

Time to first rescue analgesia after cessation of sensory block in both groups showed that the mean time for patients requiring iv analgesia was 274.7 ± 12.92 min in the dexmedetomidine group versus 243.49 ± 2.64 min in the fentanyl group (p < 0.0001). The mean length of PACU stay was comparable between both groups (p = 0.806).

When talking about the adverse effect profile between both the groups, the frequency of hypotension was 21 (17.5 %) patients in the dexmedetomidine group versus 28 (23.3 %) in the fentanyl group. Six (5.0 %) patients had nausea and vomiting in both the dexmedetomidine group and the fentanyl group, respectively. There was no incidence of shivering and respiratory depression in

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Table 2: Comparison of block onset, block regression and rescue analgesia (n = 240)

Variable	Dexmedetomidine group (n = 120)	Fentanyl group (n = 120)	p-value
Sensory block			
Mean time for onset of block (T6) (min)	4.35 ± 0.14	3.39 ± 0.11	< 0.0001
Mean time for block regression (S1) (min)	327.26 ± 12.60	243.3 ± 22.75	< 0.0001
Motor block			
Mean time for onset of block (Bromage: 3) (min)	3.33 ± 0.12	2.36 ± 0.09	< 0.0001
Mean time for block regression (Bromage: 0) (min)	262.17 ± 13.31	203.34 ± 11.47	< 0.0001
Mean time to first dose rescue analgesia (min)	274.7 ± 12.92	243.49 ± 2.64	< 0.0001
Mean PACU stay (h)	5.10 ± 0.25	5.11 ± 0.24	0.8060

PACU: post anaesthesia care unit; Bromage: Bromage score;

Table 3: Incidence of side effects between groups (n = 240)

Variable	Dexmedetomidine group (n = 120)	Fentanyl group (n = 120)
Hypotension	21 (17.5 %)	28 (23.3 %)
Nausea/vomiting	6 (5.0 %)	6 (5.0 %)
Shivering	0 (0.0 %)	9 (7.5 %)
Respiratory depression	0 (0.0 %)	14 (11.7 %)

the dexmedetomidine group, however, nine (7.5 %) of patients exhibited shivering and 14 (11.7 %) patients had an episode of respiratory depression after the procedure (Table 3).

Discussion

Even though many studies have been done on adjuvants and their role in spinal anaesthesia, the role of adjuvants in the new formulation of levobupivacaine has not been studied, especially in presented demographic area and setups. Since levobupivacaine is a relatively new formulation associated with less cardiotoxicity and prolonged duration of action, it is preferable over precious regimes due to good patient safety and satisfaction.

Dexmedetomidine is a selective 2 agonist[13] and its effects on the spinal cord *via* subarachnoid administration are explained by stimulation of α2 receptors at the *substantia gelatinosa* of the dorsal horn leading to inhibition of the release of substance P.[14] The spinal mechanism is principal for the analgesic effects of dexmedetomidine even though there is evidence for both supraspinal and peripheral sites of action. Fentanyl is a pure μ receptor agonist and exerts its effects by binding to opioid receptors at the spinal cord level as well as para-spinal when absorbed.[15] Studies carried out by Khosravi et al[16] and Davis et al[17] concluded that dexmedetomidine was a better alternative to fentanyl when it came to the duration of the blockade and haemodynamic stability. However, when talking about the onset of the block, fentanyl was better at the initial onset for both sensory and motor blockade. This was confirmed by studies carried out by Hamed et al.[18]

When talking about mean PACU stay due to pain, there was no difference in the length of stay, however, the patient required more rescue iv analgesia in the PACU in the fentanyl group. This was also observed in a study carried out by Sun et al.[19]

A study carried out by Liu et al[20] also confirmed that dexmedetomidine was better at preventing shivering than other adjuncts. This effect was seen in presented study where no patients exhibited the adverse effect when administered with the drug as an adjunct. When assessing the degree of respiratory depression, fentanyl was observed to cause more respiratory depression than its counterpart. This is closely related to its spinal depressing effects as discussed above.

The limitations are that the study is single-centre only. A multi-centre study would result in a wider demographic area with more confirmative results. This study doesn't consider high-risk ASA III and IV cases.

CONTENT

Conclusion

Dexmedetomidine offers advantages over fentanyl as an adjunct to spinal anaesthesia with longer block duration and less adverse effects profile. It is recommended to use dexmedetomidine its better safety profile, longer duration and better hemodynamic stability. Fentanyl should be reserved when the early onset of the block is required in emergency cases.

Ethics

This study was approved by the local Ethics Committee at the Combined Military Hospital, decision No 241, dated 15 June 2021.

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None.

Conflicts of interest

The authors declare that there is no conflict of interest.

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Data access

The data that support the findings of this study are available from the corresponding author upon reasonable individual request.

Author ORCID numbers

Talha Rehman Zahid (TRZ):
0009-0006-7595-3756
Rashk-e-Hinna (ReH):
0000-0003-0409-1061
Arshad Hayat (AH):
0000-0001-9020-3933
Rubina Bashir (RB):
0009-0003-8708-8710
Arshman Rauf Asghar (ARA):
0009-0006-0212-878X
Muhammad Afzal (MA):
0009-0009-2796-2844

Author contributions

Conceptualisation: TRZ, MA
Methodology: TRZ
Software: RB
Formal analysis: RB, ARA
Investigation: AH
Data curation: TRZ
Writing - original draft: RB
Writing - review and editing: ReH, ARA
Visualisation: TRZ
Supervision: MA
Project administration: AH

References

1. Fowler AJ, Abbott TEF, Prowle J, Pearse RM. Age of patients undergoing surgery. Br J Surg. 2019 Jul;106(8):1012-8. doi: 10.1002/bjs.11148.
2. Amjad A, Imran A, Shahram N, Zakar R, Usman A, Zakar MZ, et al. Trends of caesarean section deliveries in Pakistan: secondary data analysis from Demographic and Health Surveys, 1990-2018. BMC Pregnancy Childbirth. 2020 Dec 2;20(1):753. doi: 10.1186/s12884-020-03457-y.
3. Khan ZH, Eftekhar N, Barrak RS. General versus spinal anesthesia during caesarean section; a narrative review. Arch Anesth Crit Care. 2018;5(1):18-21. doi: 10.18502/aacc.v5i1.743.
4. Al-Husban N, Elmuhtaseb MS, Al-Husban H, Nabhan M, Abuhalaweh H, Alkhatib YM, et al. Anesthesia for cesarean section: retrospective comparative study. Int J Womens Health. 2021 Feb 2;13:141-52. doi: 10.2147/IJWH.S292434.

CONTENT

5. Heesen M, Hilber N, Rijs K, Rossaint R, Girard T, Mercier FJ, et al. A systematic review of phenylephrine vs. noradrenaline for the management of hypotension associated with neuraxial anaesthesia in women undergoing caesarean section. Anaesthesia. 2020 Jun;75(6):800-8. doi: 10.1111/anae.14976.
6. Colish J, Milne AD, Brousseau P, Uppal V. Factors associated with failure of spinal anesthetic: an 8-year retrospective analysis of patients undergoing elective hip and knee joint arthroplasty. Anesth Analg. 2020 Jan;130(1):e19-e22. doi: 10.1213/ANE.0000000000004304.
7. Metta R, Chakravarthy K, Hemnath Babu K, Jyotsna Rani P, Arun P. Comparison of isobaric levobupivacaine with hyperbaric bupivacaine in spinal anesthesia in patients undergoing lower abdominal surgeries. J Med Sci Clin Res. 2019;21(7):731-7. doi: 10.18535/jmscr/v7i12.128.
8. Rao DG, Anand S, Pasha N. Comparative study of isobaric levobupivacaine and hyperbaric bupivacaine for subarachnoid block in elective cesarean sections. Anaesth Pain Int Care. 2020;24(2):215-22. doi: 10.35975/apic.v24i2.1258.
9. Dahiya S, Tiwari T, Dhama V, Kumar S, Singh N, Singh S. Comparative study of isobaric levobupivacaine and hyperbaric bupivacaine for lower segment caesarean section under spinal anaesthesia in Northen India. Ann Anesth Crit Care. 2018 April; 3(1):e66749. doi: 10.5812/aacc.66749.
10. Sane S, Shokouhi S, Golabi P, Rezaeian M, Kazemi Haki B. The effect of dexmedetomidine in combination with bupivacaine on sensory and motor block time and pain score in supraclavicular block. Pain Res Manag. 2021 Apr 10;2021:8858312. doi: 10.1155/2021/8858312.
11. Sidhu S, Marine JE. Evaluating and managing bradycardia. Trends Cardiovasc Med. 2020 Jul;30(5):265-72. doi: 10.1016/j.tcm.2019.07.001.
12. Yu Q, Qi J, Wang Y. Intraoperative hypotension and neurological outcomes. Curr Opin Anaesthesiol. 2020 Oct;33(5):646-50. doi: 10.1097/ACO.0000000000000904.
13. Bharthi Sekar E, Vijayaraghavan U, Sadiqbasha AM. Effect of intravenous dexmedetomidine on spinal anesthesia. Cureus. 2021 Jun 17;13(6):e15708. doi: 10.7759/cureus.15708.
14. Bhirud PH, Chellam S, Mote MN, Toal PV. Effects of intravenous dexmedetomidine on spinal anesthesia and sedation - A comparison of two different maintenance infusions. J Anaesthesiol Clin Pharmacol. 2020 Jan-Mar;36(1):78-82. doi: 10.4103/joacp.JOACP_129_18.
15. Laboureyras E, Boujema MB, Mauborgne A, Simmers J, Pohl M, Simonnet G. Fentanyl-induced hyperalgesia and analgesic tolerance in male rats: common underlying mechanisms and prevention by a polyamine deficient diet. Neuropsychopharmacology. 2022 Jan;47(2):599-608. doi: 10.1038/s41386-021-01200-5.
16. Khosravi F, Sharifi M, Jarineshin H. Comparative study of fentanyl vs dexmedetomidine as adjuvants to intrathecal bupivacaine in cesarean section: a randomized, double-blind clinical trial. J Pain Res. 2020 Oct 7;13:2475-82. doi: 10.2147/JPR.S265161.
17. Davis PR, Sviggum HP, Delaney DJ, Arendt KW, Jacob AK, Sharpe EE. Intravenous dexmedetomidine as an adjunct to neuraxial anesthesia in cesarean delivery: a retrospective chart review. Anesthesiol Res Pract. 2021 Dec 27;2021:9887825. doi: 10.1155/2021/9887825.
18. Hamed MA, Ghaber S, Reda A. dexmedetomidine and fentanyl as an adjunct to bupivacaine 0.5% in supraclavicular nerve block: a randomized controlled study. Anesth Essays Res. 2018 Apr-Jun;12(2):475-9. doi: 10.4103/aer.AER_50_18.
19. Sun S, Wang J, Wang J, Wang F, Xia H, Yao S. Fetal and maternal responses to dexmedetomidine intrathecal application during cesarean section: a meta-analysis. Med Sci Monit. 2020 Jan 29;26:e918523. doi: 10.12659/MSM.918523.
20. Liu J, Wang Y, Ma W. Shivering prevention and treatment during cesarean delivery under neuraxial anesthesia: a systematic review. Minerva Anestesiol. 2018 Dec;84(12):1393-405. doi: 10.23736/S0375-9393.18.12478-3.

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REVIEW ARTICLE

Innovative Approaches for Onychomycosis Treatment: An Insight Into Natural Remedies and Novel Pharmaceutical Formulations

Hitesh Chopra,[1] Kavita Munjal,[2] Rajat Goyal,[3] Kashish Wilson,[3] Kuldeep Dhama[4]

1. Department of Biosciences, Saveetha School of Engineering, Saveetha Institute of Medical and Technical Sciences, Chennai, Tamil Nadu, India.
2. Amity Institute of Pharmacy, Amity University, Noida, Uttar Pradesh, India.
3. MM College of Pharmacy, Maharishi Markandeshwar (Deemed to be University), Mullana-Ambala, Haryana, India.
4. Division of Pathology, ICAR-Indian Veterinary Research Institute, Bareilly, Izatnagar, Uttar Pradesh, India.

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Corresponding author:
HITESH CHOPRA
E: chopraontheride@gmail.com

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Abstract

Onychomycosis, commonly referred to as nail fungus, is a persistent and difficult-to-treat condition that affects both toes and fingernails. Even though traditional treatments such as antifungal medications and topical ointments are effective in some cases, they are often associated with significant side effects and a high recurrence rate. There has been a growing interest in alternative and complementary treatments in recent years, including natural remedies and new pharmaceutical formulations, which are becoming increasingly popular. This review aims to explore the current state of knowledge surrounding onychomycosis treatment and its challenges, with a particular focus on the benefits and limitations of the current therapeutic options. Also, light is shed on the prospects available as treatment options.

Key words: Onychomycosis; Fungus; Formulations; Natural; Antifungal.

Introduction

Onychomycosis, often known as a fungal infection of the nail, accounts for over half of all nail illnesses. Its frequency is estimated to be around 5.5 %.[1] The elderly (those over the age of 65), those with diabetes, people with impaired immune systems (particularly those with HIV) and sportsmen are more at risk for developing onychomycosis.[2] Both environmental variables and autosomal inheritance (HLA-DR8) have a role in the prevalence of onychomycosis in families. The first toenail is the most common nail on which onychomycosis manifests, yet it can affect any nail. It can spread to the skin around it, although systemic effects are extremely rare. Toenail fungus infections are often minor and easy to treat. Nails infected with fungus may change colour, become thick, brittle, or crack.

Depending on the clinical subtype, many factors contribute to developing onychomycosis. The most frequent kind of onychomycosis, known as distal lateral subungual onychomycosis, involves the migration of fungus from the plantar surface to the nail bed through the hyponychium. Distal lateral subungual onychomycosis manifests itself clinically by inflammation of the nail apparatus.[3, 4] On the other hand, white superficial onychomycosis is a less common presentation produced by

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the invasion of the nail plate's surface.[5, 6] Fungi infiltrate the nail matrix through the proximal nail fold and populate the deep section of the proximal nail plate in the rare condition known as proximal subungual onychomycosis.[7, 8] "End-onyx" onychomycosis is a kind of distal lateral subungual onychomycosis in which the fungus infects the nail *via* the epidermis and enters the nail plate.[9, 10] Onychomycosis that affects the entire nail bed is called total dystrophic onychomycosis. Candida invasion of the nails is uncommon because the yeast requires a compromised immune response as a predisposing factor to get under the nail plate. *Candida* is only a secondary coloniser in individuals with chronic *paronychia* or onycholysis, although it is frequently isolated from the proximal nail fold or the subungual region of these patients.

Nail as a barrier to drug permeation

Mammal claws and nails are epidermal derivatives that serve as armour for the digits and toes and as implements and weapons. About 25 layers of dead, keratinised, flattened cells make up the nail plate, which is thin (0.25 mm – 0.6 mm for fingernails and up to 1.3 mm for toes), rigid, but somewhat elastic, transparent, convex in shape and protected by a nail bed.[11] Cell structures called desmosomes, randomly distributed on the lateral surfaces of plasma membranes and specialised for cell-to-cell adhesion are responsible for the strong bonds between these cells.[12] Compared to the epidermis, the permeability parameters of the human nail plate are substantially different because of the nail plate's unique physicochemical attributes.[13] Nail plate behaviour is comparable to that of a hydrogel with high ionic strength and the structure of human nails has been compared to a hydrophilic gel membrane, while the SC acts as a lipid barrier to the absorption of low molecular weight substances.[14] The nail, which has a lipid content of just 0.1 %, loses water faster than the lipid-rich epidermis around it. Hydrophobic chemicals have been proven to permeate into and through the nail despite its purported hydrophilic characteristics.[15] For instance, long-chain alcohols can penetrate the nail through a lipid pathway, as described by Walters and colleagues.[16]

Increasing penetration is crucial for the success of transungual medications. To do this, the nail plate might be damaged mechanically or chemically. Iontophoresis and drug formulations inside vehicles that provide high drug partition into the nail plate are two further options for increasing medication penetration into the intact nail plate.[17, 18]

Current therapeutic options for treatment

The severity, number of nails damaged and kind of onychomycosis present all play a role in determining the best course of treatment.[19, 20] Proximal subungual onychomycosis, as well as distal lateral subungual onychomycosis affecting the lunula area, invariably necessitate systemic therapy. It is possible to use topical medication to treat white superficial onychomycosis and distal lateral subungual onychomycosis that is confined to the distal nail. The success rate of therapy rises when systemic and local approaches are used together. Lasers and photodynamic therapy are promising future therapeutic options.[21, 22] Figure 1 describes the common ways for the treatment of nail psoriasis.

Topical medications should only be used when the affected area is less than half of the distal nail plate or when the patient cannot tolerate systemic therapy. Nail treatments like ciclopiroxolamine 8 % and efinaconazole 10 % are commercially available in the USA. Topical therapies often fall short when it comes to onychomycosis since they cannot get deep enough under the nail plate to do the trick. Both ciclopirox and amorolfine solutions are said to be able to permeate all layers of the nail but only work somewhat when applied alone.[23] They could help prevent relapse in individuals treated with systemic medicines or as an adjuvant to oral therapy. Efinaconazole and ciclopirox require daily application and are used for an extended period (48 weeks) to be effective.[24, 25] Toenail onychomycosis treatment has been effective with efinaconazole.[26] Efinaconazole was associated with considerably higher mycologic cure rates than the drug vehicle.[27, 28]

When used topically, tavaborole (aboron-containing antifungal) is effective against *Trichophy-*

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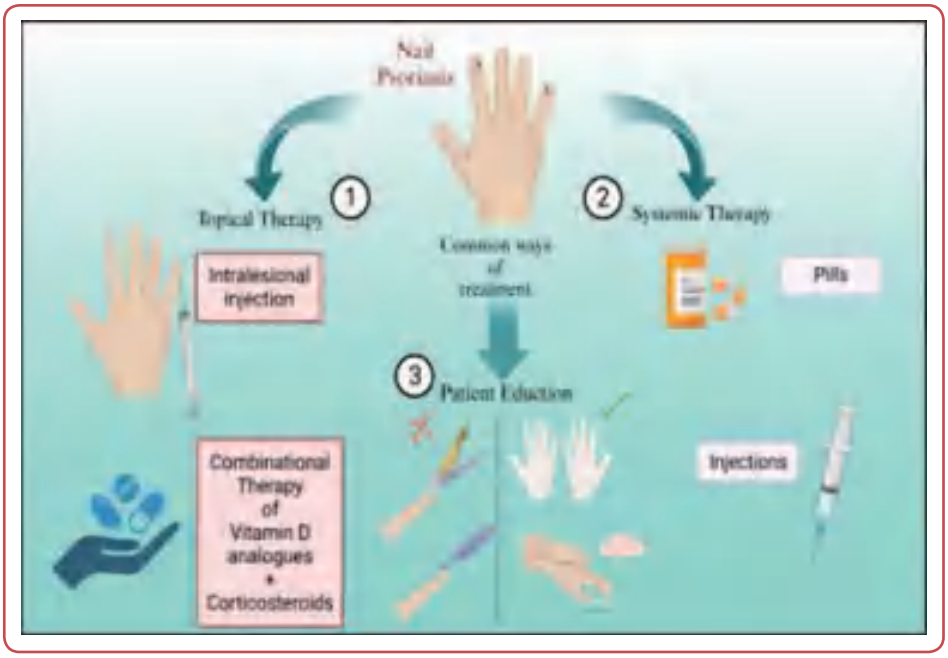

Figure 1: Common ways for the treatment of nail psoriasis

ton rubrum and *Trichophyton mentagrophytes*, the causative agents of toenail onychomycosis.[29] It was accepted after 1194 participants in two randomised, double-blind, placebo-controlled studies at many locations. Complete remission was observed in 6.5 % and 9.1 % of tavaborole-treated patients after 48 weeks of therapy, compared to 0.5 % and 1.5 %, respectively, of patients treated with the vehicle alone.[30] Effective treatment for onychomycosis has been found with fluconazole, 150–450 mg once weekly for adults; this may be especially helpful for patients with complex prescription regimens.[31, 32] However, contrasting once-weekly fluconazole with itraconazole and terbinafine has not shown comparable efficacy or cost-effectiveness.[33, 34]

Onychomycosis can be treated with griseofulvin. Low-quality evidence suggests that terbinafine is more effective than griseofulvin for these endpoints, while moderate-quality evidence suggests that azoles are just as effective as griseofulvin.[35] It is important to note that albaconazole, a broad range of oral antifungal medication, is not currently available on the market. Albaconazole may be a potential therapy option for onychomycosis since it was reported to be more effective than a placebo in a phase II randomised study for distal subungual onychomycosis.[36] As an example of a new generation (tetrazole antifungal) agent, VT-1161 inhibits lanosterol 14-alpha demethylase (CYP 51), which has the potential to be effective in the treatment of onychomycosis.[37]

Uncontrolled trials showing clinical improvement in a percentage of patients are largely the only evidence supporting the benefit of laser devices. Treatment with a dual-wavelength near-infrared diode laser for onychomycosis showed positive results in a small, randomised experiment.[38] Results from randomised controlled studies of Nd:YAG lasers for onychomycosis have been disappointing. After three months, there was no statistically significant difference between the groups in terms of the proportion of patients who had a mycologic clearance of all affected nails in a randomised trial in which 27 patients with onychomycosis involving 125 nails were randomly assigned to two treatments with 1064 nm Nd:YAG laser (17 patients) or no treatment (10 patients).[39, 40]

Several mechanisms of action have been proposed for laser therapy for onychomycosis. These include direct fungicidal effects, inhibition of fungus by laser-induced changes in the tissue environment and laser-induced immunologic effects.[41] Patients who do not respond to pharmacological treatment alone may have surgical excision of the nail (nail avulsion).[42] The use of iontophoretic drug delivery, in which a mild electrical current is applied to a topical medicament to increase its absorption, shows promise for enhancing the efficacy of topical antifungal therapy.[43, 44] Combination therapy appears to enhance the chance for new healthy nail development in a small, unblinded, randomised study comparing

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the treatment of toenail onychomycosis with topical terbinafine and iontophoresis with the sole topical terbinafine.[45]

Natural remedies

Natural products have been proven to be beneficial in various other diseases for ages. Due to the poor permeation characteristic of topical products and poor oral bioavailability of antifungal drugs, there has been a steep rise in interest in natural remedies for the treatment of onychomycosis. Based on data available through various sources the following products were found to possess anti-onychomycosis activity.

Tea tree oil

Before modern antimicrobials were developed, essential oils were often used. Tea tree oil has been shown to have beneficial effects against bacteria, fungi, viruses and inflammation.[46, 47] Tea tree oil has been found in many trials to have antifungal effects.[48] For the most part, studies including tea tree oil have concentrated on its potential to cure *Candida* infections.[49, 50] Tea tree oil has also been demonstrated to be an effective therapy for dermatitis, ringworm (*T equinum*) and stomatitis, according to studies.[51, 52]

Essential oils have also improved the skin's ability to absorb and hold onto medications.[53, 54] Furthermore, this suggests that tea tree oil may be useful as a delivery vehicle for other antifungal drugs. Tea tree oil has been proven in many tests when used with antifungal drugs to have a synergistic impact.[55] Tea tree oil is effective against *T rubrum*, the most prevalent cause of onychomycosis, in *in vitro* antifungal assays.[56] Misner discovered that a blend of essential oils applied topically to the feet and containing tea tree oil blocked the growth of aerobic bacteria, yeast and fungus on the foot when enclosed in shoes.[57] Tea tree oil and clotrimazole are both used to treat onychomycosis, but Buck and coworkers compared how well they work. Treatment with clotrimazole resulted in an 11 % cure rate, whereas treatment with tea tree oil resulted in an 18 % cure rate in this randomised, controlled study of 117 patients.[58]

Flores and colleagues recently assessed the antifungal effectiveness of tea tree oil in an onychomycosis model. Results showed that *T rubrum* growth was inhibited when the oil was encapsulated in nanocapsules and then suspended. A model-based study was performed and it was found that the diameters of the fungal colony were 2.88 ± 2.08 mm^2, 14.59 ± 2.01 mm^2, 40.98 ± 2.76 mm^2 and 38.72 ± 1.22 mm^2. Based on the data it can be said that oil in nanocapsules was found to be best for onychomycotitic treatment.[59]

Ageratina pichinchensis

Using ciclopirox (8 %) as a positive control, researchers created a standardised 10 % lacquer with a depigmented extract of the aerial portions of *A pichinchensis* and tested it in a randomised, double-blind clinical trial on patients with cutaneous mycosis with less than 50 % fungal infection. The therapeutic and mycological effectiveness of the group treated with the standardised extract was 71.1 % and 59.1 %, respectively, whereas the control group's results were 80.9 % and 63.8 %, respectively, with no adverse effects. Since encecalin was the most abundant component, the authors reasoned that it must be responsible for the desired pharmacological action.[60]

Romero-Cerecero et al prepared two types of lacquer using either 12.6 % or 16.8 % extracts and tested them agains*t T mentagrophytes*. Patients with mild and severe onychomycosis, defined as having between one and ten infected nails, were followed for six months while receiving one of these therapies. No fungi were detected in mycological microscopic tests and patients treated with lacquer (16.8 % extract) exhibited a statistically significant improvement over those treated with lacquer containing 12.6 % extract.[61]

Coniferous resin lacquer

Natural coniferous resin from the Norway spruce (*Picea abies*) is highly effective against all dermatophytes responsible for onychomycosis in humans.[62, 63] Sipponen et al conducted a study using natural coniferous resin to treat onychomycosis.[64] Thirty-seven people having a clinical diagnosis of onychomycosis were included in the research. During the study, all participants applied a topical resin lacquer treatment once daily. Nail samples were processed at the start and finish of the investigation using a mycological culture and a potassium hydroxide (KOH) stain. Twenty patients with positive mycological cultures or KOH stains for dermatophytes at study enrolment. Only 6 patients had positive results towards the end of the research. There was a clinical success

with resin lacquer therapy, as reported by 14 patients who followed all instructions. There is some proof that the natural coniferous resin used topically for onychomycosis works clinically, as shown by the findings. Although this is only preliminary observational research, it does provide some evidence that coniferous resin applied topically over an extended period (30 days) in the form of lacquer at a concentration of 30 % may aid in the healing of onychomycosis.[65, 66]

Novel treatment options

Emulsions

An emulsion is a mixture of two or more liquids normally immiscible (unmixable or unblendable) owing to liquid-liquid phase separation. Emulsions may be made using a variety of liquids, although water and oil are the most frequent. However, nanoemulsions are granted kinetic stability due to the lower attraction between the small-sized droplets, which prevents the droplets from gravitationally separating and aggregating.[67] In contrast to microemulsions, nanoemulsions are not sensitive to changes in physical and chemical conditions like temperature and pH. They can be prepared using a lesser quantity of surfactants. The optical quality and stability of a nanoemulsion are not the only concerns affected by the droplet size; the nanoemulsion's rheological and release behaviour are, too. Therefore, nanoemulsions are superior to microemulsions in several settings.[68]

An additional non-invasive local treatment for onychomycosis is photodynamic therapy (PDT). Photosensitiser drugs are used in PDT and these medications must be "photoactivated" by certain wavelengths of light. Also, PDT does not cause any systemic negative effects and may be used repeatedly without developing resistance in the fungus.[69] There have been many published clinical trials using PDT to treat onychomycosis. Aluminium chloride phthalocyanine nanoemulsions were used by Morgado et al for the treatment of onychomycosis. This combination of photosensitiser and nanoemulsion known as a third-generation photosensitiser. This generation of photosensitisers offers several benefits over earlier generations, including photoactivation at longer wavelengths, which makes it possible to treat deeper nail layers, ease of distribution throughout the nail and skin and absence of staining due to methylene blue.[70, 71]

Emulsions are often stabilised by surfactants.[72] Pickering emulsions are particle-stabilised emulsions that provide various benefits over traditional emulsions.[73, 74] Due to the increased adsorption energy of solid particles compared to surfactants at oil-water interfaces,[75] this adsorption can be deemed irreversible, allowing Pickering emulsions to be as stable as traditional emulsions. The size of emulsion droplets is also a crucial factor in developing and administering emulsion-based pharmaceuticals and medical treatments. On this basis, Horváth et al created tea tree essential oil-based Pickering emulsions.[76] *In vitro* studies showed that Pickering emulsion formulation showed better drug permeation in agar gel membranes. The Pickering emulsion with the smallest droplet size of about 1.85 µm could deliver 89.9 % of actives through the agar membrane.[77]

Agrawal et al formulated a microemulsion based on efinaconazole for the transungual route to treat onychomycosis. The microemulsion formulations of efinaconazole showed enhanced penetration compared to the reference formulation in *ex vivo* permeation and nail clipping experiments without noting any delay in drug release from the nail plate. Microemulsion formulations showed higher antifungal efficacy than the reference formulation in an *in vitro* examination of three fungal species (*Trichophytonrubrum, Trichophytonmentagrophytes* and yeast *Candida albicans*).[78]

Pal et al developed a microemulsion-containing gel using benzyl alcohol and isopropyl myristate was added as an oil, Pluronic F68 as a surfactant and ethanol as a co-surfactant, in double-distilled water and loading itraconazole as the model antifungal drug for the treatment of onychomycosis. Because of itraconazole hydrophobicity, it prefers to stay in an intermediate location, ie at the palisade layer inside the surfactant molecules of the microemulsion structure, rather than in the oil phase or the aqueous phase. Keratolytic ingredients such as salicylic acid, which regulates the formulation's pH and urea are thought to improve the nail's ability to retain water significantly.[79] Nails, it is thought, respond well to water as a plasticiser. The rate at which molecules can diffuse through the nail plate grows in tandem with the nail's moisture content. The formulation's pH, the molecular weight of the penetration molecule, etc are all crucial considerations.[80]

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Hydrogels

Hydrogels are a three-dimensional polymer matrix that swells when exposed to the water phase. They have been reported in various applications such as wound healing, drug delivery, imaging, etc.[81, 82] Kesharwani et al developed an itraconazole and difluorinated-curcumin-based nanoparticle entrapped hydrogel system to treat onychomycosis. The drugs were loaded into chitosan nanoparticles using the ionic gelation method and they were dispersed in Carbopol 940-based hydrogels. The hydrogels provide a good residence time in the fungal-affected region. As a result, this increases the permeation due to hydration, enhances drug delivery and reduces the burden of higher drug dosing. *In vitro* data showed a significant decrease in the colony-forming units compared to free drug molecules applied. This can be due to nano-sized formulation, which causes the slow release of drugs but at a steady rate from the polymer matrix.[83]

Amra et al developed a ketoconazole-based microemulsion loaded in hydrogel using nigella oil as a permeation enhancer and various polymers such as HPMC K100, HPMC K4M, HPMC K100M, Carbopol 971, Carbopol 974, Carbopol 980, xanthan gum, sodium alginate and sodium CMC.[84] The optimised formulation containing alginate and HPMC (1:1), was further evaluated for drug release study. A sustained-release formulation is favoured over the currently available cream for treating fungal infections since it requires fewer applications.[85]

Nanoparticles and nanocapsules

Gaballah et al developed nanocapsules to deliver ciclopirox using Poly lactide-co-glycolide *via* nanoprecipitation technique and to incorporate them into hydroxypropyl chitosan-based nail lacquer.[86] The placebo lacquer exhibited antifungal activity and a zone of inhibition at 15.80 ± 0.14 mm, while medicated nail lacquer showed a significantly higher zone of inhibition of a mean diameter of 62.60 ± 13.32 mm.[87]

Nail polishes with water-soluble film-forming agents solve the issues with water-insoluble nail varnishes by ensuring strong adhesion to the nail and facilitating the partition and/or release of the active substance to the nail. Penetration enhancers containing vesicles (PEVs) have been reported to have a good permeation effect on the skin barrier. PEVs are quite similar to liposomes but are distinguished by possessing many permeation enhancers, such as oleic acid, labrasol and transcutol. Bseiso et al developed PEVs of sertaconazole skin fungal disease treatment.[88] The same authors later developed PEVs, including nail permeation enhancers such as N-acetyl-L-cysteine, thioglycolic acid, thiourea and ethanol and sertaconazole as a model drug for the onychomycosis treatment. Microbial testing showed a zone of inhibition for control formulation of 5.3 ± 0.58 mm due to the presence of permeation enhancers such as cysteine and its derivative. The formulation showed (20.9 ± 0.25 mm) a significant increase in the zone of inhibition compared to the model (11.6 ± 0.44 mm). This was primarily due to the synergistic effect of sertaconazole and permeation enhancers.[89]

Vesicular drug delivery systems or carriers

Researchers proposed several liposomal-based formulations for treating onychomycosis, including those loaded with terbinafine as a model drug. Phospholipon 90 G and Lipoid S 100, as phospholipids for the liposomal layer and pullulan and Eudragit L100, as plasticisers for the liposomes, were used in the preparation of the newly acquired film formulations. The liposomes were dispersed in a gel to facilitate their use on fingernails. The proportion of medication released *in vitro* from liposome-loaded films ranged from 46.0 to 71.6 %. Nail plate accumulation of the various film formulations was within the therapeutic range, measuring between 8.17 to 31.16. As observed previously, all nail thicknesses dropped after the experiments due to the experimental settings. The transonychial water loss (TOWL) of nails was measured to demonstrate the nail plate's efflux of water. The nail plate expands when submerged in water because it acts like a hydrophilic gel membrane. Swelling causes the intercellular spaces between keratin structures to enlarge, allowing more water to permeate through them. The swelling was present throughout the experiment, the TOWL of the nails rose in each casen.[90] Similar results were reported by Shah et al, they also formulated the based liposomes of terbinafine HCl using a quality by design (QbD) approach and reported similar outcomes.[91]

Novel and investigational treatments for onychomycosis

ME1111

Meiji Seika Pharma Co, Ltd (Tokyo, Japan) recently discovered ME1111 [2-(3,5-dimethyl-1H-pyrazol-1-yl)-5-methylphenol], a new agent with potent *in vitro* antifungal activity against dermatophytes like *T rubrum* and *T mentagrophytes*, which are common etiologic agents of onychomycosis.[92] It has been suggested that a compound's molecular weight plays a crucial role in its capacity to penetrate nails.[93] ME1111's molecular weight of 202.25 makes it easily able to pierce human nails. The prior *in vitro* experiments showed that ME1111 penetrates nails more deeply than ciclopirox (the active ingredient in *Penlac* nail lacquer).[94, 95] There was no safety problems found in GLP-compliant general toxicity studies, including ME1111. These investigations included repeated-dose toxicity, safety pharmacology and genotoxicity. It was found that the succinate dehydrogenase (complex II) of the mitochondrial electron transport system is the molecular target of the new antifungal drug ME1111.[96, 97] Missense mutations in the genes encoding SdhB, SdhC and SdhD were found in ME1111-resistant *T mentagrophytes* mutants produced *in vitro.* Cross-resistance to carboxin and boscalid, which have been reported to bind to the ubiquinone-binding region surrounded by SdhB, SdhC and SdhD, was observed for most ME1111-resistant mutants. These data point to ME1111's binding site being the same as or very close to, the ubiquinone-binding site.[98]

NP213

NP213 (*Novetexatine*) is a topical antifungal medication for onychomycosis. NP213 is a cyclic antimicrobial peptide that is synthetic, water-soluble and very efficient in penetrating human nails.[99] NP213 was inspired by HDPs (host-defence peptides). The skin and nails are the primary sites of HDP expression and production[100] and they play a crucial role in the innate immune response to infection.[101, 102] At concentrations of 4-7 ppm, NP213 is quickly fungicidal in a water-based topical formulation and it has shown superior effectiveness to known antifungal drugs under *in vitro* circumstances mimicking those of the human nail. Unlike the comparative topical onychomycosis treatments ciclopirox and amorolfine, NP213 efficiently eliminated distinct strains of *T rubrum* from diseased nails in *ex vivo* human nails after just 28 days of daily administration. Unlike other topical onychomycosis therapeutics, NP213 can effectively penetrate the human nail *via* transungual and subungual routes thanks to its water-based film-forming vehicle, which eliminates the need for penetration enhancers,[103, 104] optical brighteners[105, 106] and the use of organic solvents.[107, 108]

Clinical trials

An extensive literature search has revealed the completion of 31 interventional clinical trials related to onychomycosis. The findings of these trials provide valuable insight into the current state of knowledge surrounding the treatment of onychomycosis. To make this information accessible and easy to understand, the details of these trials have been summarised and presented in Table 1.

Table 1: Summary of interventional clinical trials on onychomycosis

NCT number	Interventions	Study size	Phase
NCT01278394	Drug: AN2690 solution, 5.0 %	29	Phase 2
NCT00791219	Drug: SUBA-itraconazole, itraconazole, placebo	175	Phase 2
NCT01851590	Device: resin lacquer Drug: amorolfine, terbinafine	129	Phase 4
NCT03168841	Drug: Efinaconazole topical	40	Phase 3

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NCT02812771	Drug: Efinaconazole	62	Phase 4
NCT02588599	Device: Erchonia LUNULA	54	Not applicable
NCT00730405	Drug: albaconazole 100 mg, albaconazole 200 mg, albaconazole 400 mg, placebo 400 mg	584	Phase 2
NCT01666002	Device: laser treatment (pulsed Nd:YAG 1064 nm laser)	27	Not applicable
NCT02267356	Drug: VT-1161, placebo	259	Phase 2
NCT00871728	Drug: itraconazole	132	Phase 4
NCT00356915	Drug: itraconazole 100 mg capsules, itraconazole 200 mg tablets, placebo tablets	1381	Phase 3
NCT03110029	Drug: Efinaconazole 10 % topical application solution [JUBLIA] Other: application of nail polish	13	Phase 4
NCT03216200	Device: plasma treatment	5	Not applicable
NCT00459537	Drug: terbinafine hydrogen chloride, amorolfine nail lacquer	1029	Phase 3
NCT01302119	Drug: AN2690 topical solution, 5 %, solution vehicle	604	Phase 3
NCT01270971	Drug: AN2690 topical solution, 5 %, solution vehicle	594	Phase 3
NCT00491764	Drug: SCH 56592, terbinafine, placebo	218	Phase 2
NCT03072550	Device: RenewalNail™ plasma treatment system	26	Not applicable
NCT00935649	Device: PinPointeFootLaser	134	Phase 2/3
NCT02933879	Drug: NVXT topical, placebo (vehicle) topical	184	Phase 2
NCT02798380	Drug: HTS-519 insert	30	Phase 2
NCT02242019	Device: erchonia LUNULA	109	Not applicable
NCT01534689	Device: erchonia FX-405™ laser	105	Not applicable
NCT00443898	Drug: terbinafine, placebo	518	Phase 3
NCT00443820	Drug: terbinafine, placebo	526	Phase 3
NCT03405818	Drug: tavaborole 5 % topical solution	55	Phase 4
NCT03098615	Drug: Jublia (efinaconazole 10 % topical solution)	19	Phase 4
NCT02343627	Drug: NVXT solution, vehicle of the test product	47	Phase 2
NCT02679911	Drug: loceryl NL, ciclopirox NL	20	Phase 4
NCT02714504	Drug: voriconazole or posaconazole	239	Not applicable
NCT02321098	Drug: loceryl NL + cosmetic varnish, loceryl NL 12 weeks, loceryl NL 15 months	50	Phase 4

Conclusion

Nail fungus is a complicated and long-lasting medical illness and this study has summarised the current therapy options for onychomycosis. Although antifungal drugs and topical ointments are the mainstays of traditional therapy, they can have certain side effects. There has to be a change in treatment approaches because of the serious side effects and high recurrence rates of these current options. As part of a larger movement in medicine towards more integrative and patient-centred methods, they include novel pharmaceutical formulations and natural cures. The rising desire for therapies that are effective, safe and tolerated over the long term is reflected in the popularity of these options. Additional clinical studies and research are needed to confirm the effectiveness of alternative therapies, but some show promise. There is hope for the future of onychomycosis therapy in the scientific community. New insights into the pathophysiology of the illness, together with developments in me-

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dicinal research and pharmaceutical development, have the potential to completely alter current approaches to therapy. Reducing the burden of side effects and recurrence, personalised medicine and targeted treatments have the potential to deliver more effective and individualised treatment alternatives. To sum up, onychomycosis therapy has come a long way, but there's still a long way to go. The existing limits must be overcame *via* ongoing research and innovation so that patients may have access to safer, more effective and more personalised treatment alternatives in the future. Both academics and healthcare providers face new obstacles in the treatment of onychomycosis, but patients with this persistent and agonising ailment have reason to be hopeful about the future.

Ethics

This study was a secondary analysis based on the currently existing data and did not directly involve with human participants or experimental animals. Therefore, the ethics approval was not required in this paper

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Conflicts of interest

The authors declare that there is no conflict of interest.

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Data access

The data that support the findings of this study are available from the corresponding author upon reasonable individual request.

Author ORCID numbers

Hitesh Chopra (HC):
0000-0001-8867-7603
Kavita Munjal (KM):
0000-0001-9694-8151
Rajat Goyal (RG):
0000-0001-5702-0932
Kashish Wilson (KW):
0000-0002-4784-6383
Kuldeep Dhama (KD):
0000-0001-7469-4752

Author contributions

Conceptualisation: HC
Formal analysis: HC, KM
Resources: RG, KW
Writing - original draft: HC, KM, RG, KW
Writing - review and editing: KD

References

1. Piraccini BM, Alessandrini A. Onychomycosis: a review. J Fungi. 2015 Mar 27;1(1):30-43. doi: 10.3390/jof1010030.
2. Thomas J, Jacobson GA, Narkowicz CK, Peterson GM, Burnet H, Sharpe C. Toenail onychomycosis: an important global disease burden. J Clin Pharm Ther. 2010 Oct;35(5):497-519. doi: 10.1111/j.1365-2710.2009.01107.x.
3. Vanam HP, Rao PN, Mohanram K, Yegneswaran PP, Rudramurthy SP. Distal lateral subungual onychomycosis owing to Tritirachium oryzae: a bystander or invader?. Mycopathologia. 2018 Apr;183(2):459-63. doi: 10.1007/s11046-017-0226-5.
4. Kaynak E, Göktay F, Güneş P, Sayman E, Turan D, Baygül A, et al. The role of dermoscopy in the diagnosis of distal lateral subungual onychomycosis. Arch Dermatol Res. 2018 Jan;310:57-69. doi: 10.1007/s00403-017-1796-2.

CONTENT

5. Baran R, Hay R, Perrin C. Superficial white onychomycosis revisited. J Eur Acad Dermatol Venereol. 2004 Sep;18(5):569-71. doi: 10.1111/j.1468-3083.2004.01015.x.
6. Piraccini BM, Tosti A. White superficial onychomycosis: epidemiological, clinical, and pathological study of 79 patients. Arch Dermatol. 2004 Jun 1;140(6):696-701. doi: 10.1001/archderm.140.6.696.
7. Phaitoonwattanakij S, Leeyaphan C, Bunyaratavej S, Chinhiran K. Trichophyton erinacei onychomycosis: The first to evidence a proximal subungual onychomycosis pattern. Case Rep Dermatol. 2019 Sep 2;11(2):198-203. doi: 10.1159/000501424.
8. Borgohain P, Barua P, Dutta PJ, Shaw D, Rudramurthy SM. Onychomycosis associated with superficial skin infection due to Aspergillus sydowii in an immunocompromised patient. Mycopathologia. 2019 Oct;184(5):683-9. doi: 10.1007/s11046-019-00383-2.
9. Tosti A, Baran R, Piraccini BM, Fanti PA. Endonyx onychomycosis: a new modality of nail invasion by dermatophytes. Acta Derm Venereol. 1999 Jan 1;79(1):52-3. doi: 10.1080/000155599750011714.
10. Souza LW, Souza SV, Botelho AC. Endonyx toenail onychomycosis caused by Trichophyton rubrum: treatment with photodynamic therapy based on methylene blue dye. An Bras Dermatol. 2013 Nov;88:1019-21. doi: 10.1590/abd1806-4841.20132180.
11. Bhattacharjee B, Dey N, Barman D, Karmakar A, Ahmed N. Understanding the drug delivery through nails: a comprehensive review. J Drug Deliv Ther. 2021 Jul 15;11(4):116-31. doi: 10.22270/jddt.v11i4.4941.
12. Kowalczyk AP, Green KJ. Structure, function, and regulation of desmosomes. Prog Mol Biol Transl Sci. 2013 Jan 1;116:95-118. doi: 10.1016/B978-0-12-394311-8.00005-4.
13. Kobayashi Y, Komatsu T, Sumi M, Numajiri S, Miyamoto M, Kobayashi D, et al. In vitro permeation of several drugs through the human nail plate: relationship between physicochemical properties and nail permeability of drugs. Eur J Pharm Sci. 2004 Mar 1;21(4):471-7. doi: 10.1016/j.ejps.2003.11.008.
14. Baswan S, Kasting GB, Li SK, Wickett R, Adams B, Eurich S, et al. Understanding the formidable nail barrier: A review of the nail microstructure, composition and diseases. Mycoses. 2017 May;60(5):284-95. doi: 10.1111/myc.12592.
15. McAuley WJ, Jones SA, Traynor MJ, Guesné S, Murdan S, Brown MB. An investigation of how fungal infection influences drug penetration through onychomycosis patient's nail plates. Eur J Pharm Biopharm. 2016 May 1;102:178-84. doi: 10.1016/j.ejpb.2016.03.008.
16. Walters KA, Flynn GL, Marvel JR. Physicochemical characterization of the human nail: solvent effects on the permeation of homologous alcohols. J Phar Pharmacol. 1985 Nov;37(11):771-5. doi: 10.1111/j.2042-7158.1985.tb04966.x.
17. Chen K, Puri V, Michniak-Kohn B. Iontophoresis to overcome the challenge of nail permeation: considerations and optimizations for successful ungual drug delivery. AAPS journal. 2021 Jan;23:1-5. doi: 10.1208/s12248-020-00552-y.
18. Amichai B, Nitzan B, Mosckovitz R, Shemer A. Iontophoretic delivery of terbinafine in onychomycosis: a preliminary study. Br J Dermatol. 2010 Jan 1;162(1):46-50. doi: 10.1111/j.1365-2133.2009.09414.x.
19. Lipner SR, Scher RK. Onychomycosis: Treatment and prevention of recurrence. J Am Acad Dermatol. 2019 Apr 1;80(4):853-67. doi: 10.1016/j.jaad.2018.05.1260.
20. Gupta AK, Mays RR, Versteeg SG, Shear NH, Friedlander SF. Onychomycosis in children: Safety and efficacy of antifungal agents. Pediatr Dermatol. 2018 Sep;35(5):552-9. doi: 10.1111/pde.13561.
21. Manevitch Z, Lev D, Hochberg M, Palhan M, Lewis A, Enk CD. Direct antifungal effect of femtosecond laser on Trichophyton rubrum onychomycosis. JPPA. 2010 Mar;86(2):476-9. doi: 10.1111/j.1751-1097.2009.00672.x.
22. Gupta AK, Drummond-Main C, Cooper EA, Brintnell W, Piraccini BM, Tosti A. Systematic review of nondermatophyte mold onychomycosis: diagnosis, clinical types, epidemiology, and treatment. JAAD. 2012 Mar 1;66(3):494-502. doi: 10.1016/j.jaad.2011.02.038.
23. Ameen M, Lear JT, Madan V, Mohd Mustapa MF, Richardson M, Hughes JR, et al. British Association of Dermatologists' guidelines for the management of onychomycosis 2014. BJD. 2014 Nov 1;171(5):937-58. doi: 10.1111/bjd.13358.
24. Piraccini BM, Iorizzo M, Lencastre A, Nenoff P, Rigopoulos D. Ciclopirox hydroxypropyl chitosan (HPCH) nail lacquer: a review of its use in onychomycosis. J Dermatol Treat. 2020 Oct;10:917-29. doi: 10.1007/s13555-020-00420-9.
25. Aslam R, Hussain T, Yousaf AM, Ghori MU, Khan IU, Rizvi SA, et al. Onychomycosis: current understanding and strategies for enhancing drug delivery into human nail tissue. Curr. Drug Abuse Rev. 2021 Mar 1;13(1):25-35. doi: 10.2174/2589977512666200731171505.
26. Cook-Bolden FE, Lin T. Efinaconazole solution 10% for treatment of toenail onychomycosis in Latino patients. Cutis. 2017 Apr 1;99(4):286-9. PMID: 28492600.
27. FDA OKs First Topical Triazole Antifungal for Onychomycosis [Internet]. [Cited: 26-Jan-2023]. Available from: https://www.medscape.com/viewarticle/826480.
28. Elewski BE, Rich P, Pollak R, Pariser DM, Watanabe S, Senda H, et al. Efinaconazole 10% solution in the treatment of toenail onychomycosis: two phase III multicenter, randomized, double-blind studies. JAAD. 2013 Apr 1;68(4):600-8. doi: 10.1016/j.jaad.2012.10.013.
29. Gupta AK, Hall S, Zane LT, Lipner SR, Rich P. Evaluation of the efficacy and safety of tavaborole topical solution, 5%, in the treatment of onychomycosis of the toenail in adults: a pooled analysis of an 8-week, post-study follow-up from two randomized phase 3 studies. J Dermatol Treat. 2018 Jan 2;29(1):44-8. doi: 10.1080/09546634.2017.1329510.
30. Elewski B, Zane L, Rich P, Aly R, Gonzalez Soto R. Pivotal phase III safety and efficacy results of tavaborole (AN2690), a novel boron-based molecule for the topical treatment of toenail onychomycosis. JAAD. 2014 May;70(5):AB91-AB91. doi: 10.1016/j.jaad.2014.01.379.
31. Piraccini BM, Sisti A, Tosti A. Long-term follow-up of toenail onychomycosis caused by dermatophytes after successful treatment with systemic antifungal agents. JAAD. 2010 Mar 1;62(3):411-4. doi: 10.1016/j.jaad.2009.04.062.
32. American Academy of Dermatology | Choosing Wisely [Internet]. [Cited: 26-Jan-2023]. Availablefrom: https://www.choosingwisely.org/societies/american-academy-of-dermatology/.

CONTENT

33. Tosti A, Piraccini BM, Lorenzi S. Onychomycosis caused by nondermatophytic molds: clinical features and response to treatment of 59 cases. JAAD. 2000 Feb 1;42(2):217-24. doi: 10.1016/S0190-9622(00)90129-4.
34. Tosti A, Piraccini BM, Lorenzi S, Iorizzo M. Treatment of nondermatophyte mold and Candida onychomycosis. Dermatol Clin. 2003 Jul 1;21(3):491-7. doi: 10.1016/S0733-8635(03)00036-6.
35. Kreijkamp-Kaspers S, Hawke K, Guo L, Kerin G, Bell-Syer SE, Magin P, et al. Oral antifungal medication for toenail onychomycosis. Cochrane Database Syst Rev. 2017 Jul 14;7(7):CD010031. doi: 10.1002/14651858.CD010031.pub2.
36. Sigurgeirsson B, van Rossem K, Malahias S, Raterink K. A phase II, randomized, double-blind, placebo-controlled, parallel group, dose-ranging study to investigate the efficacy and safety of 4 dose regimens of oral albaconazole in patients with distal subungual onychomycosis. JAAD. 2013 Sep 1;69(3):416-25. doi: 10.1016/j.jaad.2013.03.021.
37. Gupta AK, Studholme C. Novel investigational therapies for onychomycosis: an update. EOIDER. 2016 Mar 3;25(3):297-305. doi: 10.1517/13543784.2016.1142529.
38. Landsman AS, Robbins AH, Angelini PF, Wu CC, Cook J, Oster M, et al. Treatment of mild, moderate, and severe onychomycosis using 870-and 930-nm light exposure. JAMA. 2010 May 1;100(3):166-77. doi: 10.7547/1000166.
39. Hollmig ST, Rahman Z, Henderson MT, Rotatori RM, Gladstone H, Tang JY. Lack of efficacy with 1064-nm neodymium: yttrium-aluminum-garnet laser for the treatment of onychomycosis: a randomized, controlled trial. J Am Acad Dermatol. 2014 May 1;70(5):911-7. doi: 10.1016/j.jaad.2013.12.024.
40. Karsai S, Jäger M, Oesterhelt A, Weiss C, Schneider SW, Jünger M, et al. Treating onychomycosis with the short-pulsed 1064-nm-Nd: YAG laser: results of a prospective randomized controlled trial. J Eur Acad Dermatol Venereol. 2017 Jan;31(1):175-80. doi: 10.1111/jdv.13798.
41. Gupta AK, Paquet M, Simpson FC. Therapies for the treatment of onychomycosis. Clin Dermatol. 2013 Sep 1;31(5):544-54.https://doi.org/10.1016/j.clindermatol.2013.06.011.
42. Amichai B, Nitzan B, Mosckovitz R, Shemer A. Iontophoretic delivery of terbinafine in onychomycosis: a preliminary study. Br J Dermatol. 2010 Jan 1;162(1):46-50. doi: 10.1111/j.1365-2133.2009.09414.x.
43. Amichai B, Mosckovitz R, Trau H, Sholto O, Ben-Yaakov S, Royz M, et al. Iontophoretic terbinafine HCL 1.0% delivery across porcine and human nails. Mycopathologia. 2010 May;169:343-9. doi: 10.1007/s11046-009-9265-x.
44. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev. 1999 Oct 1;12(4):564-82. doi: 10.1128/cmr.12.4.564.
45. Low WL, Kenward K, Britland ST, Amin MC, Martin C. Essential oils and metal ions as alternative antimicrobial agents: a focus on tea tree oil and silver. Int Wound J. 2017 Apr;14(2):369-84. doi: 10.1111/iwj.12611.
46. Tea tree oil for onychomycosis: does it work? [Internet]. [Cited: 27-Jan-2023]. Available from: https://www.hmpgloballearningnetwork.com/site/podiatry/blogged/tea-tree-oil-onychomycosis-does-it-work.
47. Hammer K, Carson CF, Riley TV. Antifungal activity of the components of Melaleuca alternifolia (tea tree) oil. J Appl Microbiol. 2003 Oct 1;95(4):853-60. doi: 10.1046/j.1365-2672.2003.02059.x.
48. Ramage G, Milligan S, Lappin DF, Sherry L, Sweeney P, Williams C, et al. Antifungal, cytotoxic, and immunomodulatory properties of tea tree oil and its derivative components: potential role in management of oral candidosis in cancer patients. Front Microbiol. 2012 Jun 18;3:220. doi: 10.3389/fmicb.2012.00220.
49. Mondello F, De Bernardis F, Girolamo A, Salvatore G, Cassone A. In vitro and in vivo activity of tea tree oil against azole-susceptible and-resistant human pathogenic yeasts. J Antimicrob Chemother. 2003 May 1;51(5):1223-9. doi: 10.1093/jac/dkg202.
50. Wallengren J. Tea tree oil attenuates experimental contact dermatitis. Arch Dermatol Res. 2011 Jul;303(5):333-8. doi: 10.1007/s00403-010-1083-y.
51. Fang JY, Leu YL, Hwang TL, Cheng HC. Essential oils from sweet basil (Ocimum basilicum) as novel enhancers to accelerate transdermal drug delivery. Biol Pharm Bull. 2004;27(11):1819-25. doi: 10.1248/bpb.27.1819.
52. Biruss B, Kählig H, Valenta C. Evaluation of an eucalyptus oil containing topical drug delivery system for selected steroid hormones. Int J Pharm. 2007 Jan 10;328(2):142-51. doi: 10.1016/j.ijpharm.2006.08.003.
53. Rosato A, Vitali C, Gallo D, Balenzano L, Mallamaci R. The inhibition of Candida species by selected essential oils and their synergism with amphotericin B. Phytomedicine. 2008 Aug 1;15(8):635-8. doi: 10.1016/j.phymed.2008.05.001.
54. Barchiesi F, Silvestri C, Arzeni D, Ganzetti G, Castelletti S, Simonetti O, C, et al. In vitro susceptibility of dermatophytes to conventional and alternative antifungal agents. Med Mycol. 2009 May 1;47(3):321-6. doi: 10.1080/13693780802641920.
55. Misner BD. A novel aromatic oil compound inhibits microbial overgrowth on feet: a case study. JISSN. 2007 Dec;4:1-4. doi: 10.1186/1550-2783-4-3.
56. Buck DS, Nidorf DM, Addino JG. Comparison of two topical preparations for the treatment of onychomycosis: Melaleuca alternifolia (tea tree) oil and clotrimazole. JFP 1994 Jun 1;38(6):601-6. PMID:8195735.
57. Flores FC, De Lima JA, Ribeiro RF, Alves SH, Rolim CM, Beck RC, et al. Antifungal activity of nanocapsule suspensions containing tea tree oil on the growth of Trichophyton rubrum. Mycopathologia. 2013 Apr;175:281-6. doi: 10.1007/s11046-013-9622-7.
58. Romero-Cerecero O, Zamilpa A, Jiménez-Ferrer JE, Rojas-Bribiesca G, Román-Ramos R, Tortoriello J. Double-blind clinical trial for evaluating the effectiveness and tolerability of Ageratina pichinchensis extract on patients with mild to moderate onychomycosis. A comparative study with ciclopirox. Planta Med. 2008 Oct;74(12):1430-5. doi: 10.1055/s-0028-1088372.
59. Romero-Cerecero O, Román-Ramos R, Zamilpa A, Jiménez-Ferrer JE, Rojas-Bribiesca G, Tortoriello J. Clinical trial to compare the effectiveness of two concentrations of the Ageratina pichinchensis extract in the topical treatment of onychomycosis. J Ethnopharmacol. 2009 Oct 29;126(1):74-8. doi: 10.1016/j.jep.2009.08.007.
60. Sipponen A, Peltola R, Jokinen JJ, Laitinen K, Lohi J,

CONTENT

Rautio M, et al. Effects of Norway spruce (Picea abies) resin on cell wall and cell membrane of Staphylococcus aureus. Ultrastr Pathol. 2009 Jan 1;33(3):128-35. doi: 10.1080/01913120902889138.
61. Rautio M, Sipponen A, Lohi J, Lounatmaa K, Koukila-Kähkölä P, Laitinen K. In vitro fungistatic effects of natural coniferous resin from Norway spruce (Picea abies). Eur J Clin Microbiol. 2012 Aug;31:1783-9. doi: 10.1007/s10096-011-1502-9.
62. Rautio M, Sipponen A, Peltola R, Lohi J, Jokinen JJ, Papp A, et al. Antibacterial effects of home-made resin salve from Norway spruce (Picea abies). Apmis. 2007 Apr;115(4):335-40. doi: 10.1111/j.1600-0463.2007.apm_548.x.
63. Sipponen A, Laitinen K. Antimicrobial properties of natural coniferous rosin in the European Pharmacopoeia challenge test. Apmis. 2011 Oct;119(10):720-4. doi: 10.1111/j.1600-0463.2011.02791.x.
64. Sipponen P, Sipponen A, Lohi J, Soini M, Tapanainen R, Jokinen JJ. Natural coniferous resin lacquer in treatment of toenail onychomycosis: an observational study. Mycoses. 2013 May;56(3):289-96. doi: 10.1111/myc.12019.
65. Malay DS, Yi S, Borowsky P, Downey MS, Mlodzienski AJ. Efficacy of debridement alone versus debridement combined with topical antifungal nail lacquer for the treatment of pedal onychomycosis: a randomized, controlled trial. JFAS. 2009 May 1;48(3):294-308. doi: 10.1053/j.jfas.2008.12.012.
66. Shemer A, Nathansohn N, Trau H, Amichai B, Grunwald MH. Ciclopirox nail lacquer for the treatment of onychomycosis: an open non-comparative study. J Dermatol. 2010 Feb;37(2):137-9. doi: 10.1111/j.1346-8138.2009.00773.x.
67. Kracht T, Müller-Goymann CC. Antifungal efficacy of liquid poloxamer 407-based emulsions loaded with sertaconazole nitrate. Int J Pharm. 2020 Jul 30;585:119400. doi: 10.1016/j.ijpharm.2020.119400.
68. Choi FD, Juhasz ML, Mesinkovska NA. Topical ketoconazole: a systematic review of current dermatological applications and future developments. J Dermatol Treat. 2019 Feb 14. doi: 10.1080/09546634.2019.1573309.
69. Silva AP, Chiandrone DJ, Tinta JW, Kurachi C, Inada NM, Bagnato VS. Development and comparison of two devices for treatment of onychomycosis by photodynamic therapy. J Biomed Opt. 2015 Jun 1;20(6):061109. doi: 10.1117/1.JBO.20.6.061109.
70. Morgado LF, Trávolo AR, Muehlmann LA, Narcizo PS, Nunes RB, Pereira PA, et al. Photodynamic therapy treatment of onychomycosis with Aluminium-Phthalocyanine Chloride nanoemulsions: A proof of concept clinical trial. J Photochem Photobiol Biol. 2017 Aug 1;173:266-70. doi: 10.1016/j.jphotobiol.2017.06.010.
71. Senge MO. mTHPC–A drug on its way from second to third generation photosensitizer?. PDPDT. 2012 Jun 1;9(2):170-9. doi: 10.1016/j.pdpdt.2011.10.001.
72. Pape WJ, Pfannenbecker U, Hoppe U. Validation of the red blood cell test system as in vitro assay for the rapid screening of irritation potential of surfactants. Mol Toxicol. 1987 Sep 1;1(4):525-36. PMID: 3509700.
73. Yang Y, Fang Z, Chen X, Zhang W, Xie Y, Chen Y, et al. An overview of Pickering emulsions: solid-particle materials, classification, morphology, and applications. Front Pharmacol. 2017 May 23;8:235054. doi: 10.3389/fphar.2017.00287.
74. Chevalier Y, Bolzinger MA. Emulsions stabilized with solid nanoparticles: Pickering emulsions. Colloids Surf A Physicochem Eng. 2013 Dec 20;439:23-34. doi: 10.1016/j.colsurfa.2013.02.054.
75. Binks BP. Particles as surfactants—similarities and differences. COCIS. 2002 Mar 1;7(1-2):21-41. doi: 10.1016/S1359-0294(02)00008-0.
76. Vörös-Horváth B, Das S, Salem A, Nagy S, Böszörményi A, Kőszegi T, et al. Formulation of tioconazole and melaleuca alternifolia essential oil pickering emulsions for onychomycosis topical treatment. Molecules. 2020 Nov 26;25(23):5544. doi: 10.3390/molecules25235544.
77. Agrawal V, Patel R, Patel M, Thanki K, Mishra S. Design and evaluation of microemulsion-based efinaconazole formulations for targeted treatment of onychomycosis through transungual route: Ex vivo and nail clipping studies. Colloid Surf B. 2021 May 1;201:111652. doi: 10.1016/j.colsurfb.2021.111652.
78. Pal P, Thakur RS, Ray S, Mazumder B. Design and development of a safer non-invasive transungual drug delivery system for topical treatment of onychomycosis. Drug Dev Ind Pharm.2015 Jul 3;41(7):1095-9. doi: 10.3109/03639045.2014.931966.
79. Souza AM, Ribeiro RC, Pinheiro GK, Pinheiro FI, Oliveira WN, Souza LB, et al. Polishing the therapy of onychomycosis induced by Candida spp.: amphotericin B–loaded nail lacquer. Pharmaceutics. 2021 May 24;13(6):784. doi: 10.3390/pharmaceutics13060784.
80. Al-Obaidi H, Petraityte I, Hibbard T, Majumder M, Kalgudi R, Zariwala MG. Antifungal nanosuspensions with surfactants and silver for the treatment of onychomycosis. Eur J Pharm Biopharm. 2022 Oct 1;179:194-205. doi: 10.1016/j.ejpb.2022.09.004.
81. Chopra H, Kumar S, Singh I. Bioadhesive hydrogels and their applications. Bioadhes Drug Deliv. 2020 Jun 1:147-70. doi: 10.1002/9781119640240.ch6.
82. Chopra H, Bibi S, Kumar S, Khan MS, Kumar P, Singh I. Preparation and evaluation of chitosan/PVA based hydrogel films loaded with honey for wound healing application. Gels. 2022 Feb 11;8(2):111. doi: 10.3390/gels8020111.
83. Kesharwani P, Fatima M, Singh V, Sheikh A, Almalki WH, Gajbhiye V, et al. Itraconazole and difluorinated-curcumin containing chitosan nanoparticle loaded hydrogel for amelioration of onychomycosis. Biomimetics. 2022 Nov 21;7(4):206. doi: 10.3390/biomimetics7040206.
84. Amra K, Momin M. Formulation evaluation of ketoconazole microemulsion-loaded hydrogel with nigella oil as a penetration enhancer. J Cosmet Dermatol. 2019 Dec;18(6):1742-50. doi: 10.1111/jocd.12945.
85. Puri V, Froelich A, Shah P, Pringle S, Chen K, Michniak-Kohn B. Quality by design guided development of polymeric nanospheres of terbinafine hydrochloride for topical treatment of onychomycosis using a nano-gel formulation. Pharmaceutics. 2022 Oct 12;14(10):2170. doi: 10.3390/pharmaceutics14102170.
86. Gaballah EY, Borg TM, Mohamed EA. Hydroxypropyl chitosan nail lacquer of ciclopirox-PLGA nanocapsules for augmented in vitro nail plate absorption and onychomycosis treatment. J Drug Deliv. 2022 Dec 31;29(1):3304-16. doi: 10.1080/10717544.2022.2144543.
87. Flores FC, Rosso RS, Cruz L, Beck RC, Silva CB. An innovative polysaccharide nanobased nail formulation

CONTENT

for improvement of onychomycosis treatment. Eur J Pharm Sci. 2017 Mar 30;100:56-63. doi: 10.1016/j.ejps.2016.12.043.
88. Bsieso EA, Nasr M, Moftah NH, Sammour OA, Abd El Gawad NA. Could nanovesicles containing a penetration enhancer clinically improve the therapeutic outcome in skin fungal diseases?. Nanomedicine. 2015 Jul;10(13):2017-31. doi: 10.2217/nnm.15.49.
89. Bseiso EA, Nasr M, Sammour OA, Abd El Gawad NA. Novel nail penetration enhancer containing vesicles "nPEVs" for treatment of onychomycosis. J Drug Deliv. 2016 Oct 12;23(8):2813-9. doi: 10.3109/10717544.2015.1099059.
90. Tuncay Tanrıverdi S, Hilmioğlu Polat S, Yeşim Metin D, Kandiloğlu G, Özer Ö. Terbinafine hydrochloride loaded liposome film formulation for treatment of onychomycosis: in vitro and in vivo evaluation. J Liposome Res. 2016 Apr 2;26(2):163-73. doi: 10.3109/08982104.2015.1067892.
91. Shah VH, Jobanputra A. Enhanced ungual permeation of terbinafine HCl delivered through liposome-loaded nail lacquer formulation optimized by QbD approach. AAPS Pharm Sci Tech. 2018 Jan;19(1):213-24. doi: 10.1208/s12249-017-0831-0.
92. Ghannoum M, Isham N, Long L. In vitro antifungal activity of ME1111, a new topical agent for onychomycosis, against clinical isolates of dermatophytes. AAC. 2015 Sep;59(9):5154-8. doi: 10.1128/aac.0092-15.
93. Murdan S. Drug delivery to the nail following topical application. Int J Pharm 2002 Apr 2;236(1-2):1-26. doi: 10.1016/S0378-5173(01)00989-9.
94. Tabata Y, Takei-Masuda N, Kubota N, Takahata S, Ohyama M, Kaneda K, et al. Characterization of antifungal activity and nail penetration of ME1111, a new antifungal agent for topical treatment of onychomycosis. Antimicrob Agents Chemother. 2016 Feb;60(2):1035-9. doi: 10.1128/aac.01739-15.
95. Satyavert, Gupta S, Choudhury H, Jacob S, Nair AB, Dhanawat M, et al. Pharmacokinetics and tissue distribution of hydrazinocurcumin in rats. Pharmacol Rep. 2021 Dec;73:1734-43. doi: 10.1007/s43440-021-00312-5.
96. Avenot HF, Sellam A, Karaoglanidis G, Michailides TJ. Characterization of mutations in the iron-sulphur subunit of succinate dehydrogenase correlating with boscalid resistance in Alternaria alternata from California pistachio Phytopathol. 2008 Jun;98(6):736-42. doi: 10.1094/PHYTO-98-6-0736.
97. Shima Y, Ito Y, Kaneko S, Hatabayashi H, Watanabe Y, Adachi Y, et al. Identification of three mutant loci conferring carboxin-resistance and development of a novel transformation system in Aspergillus oryzae. Fungal Genet Biol. 2009 Jan 1;46(1):67-76. doi: 10.1016/j.fgb.2008.10.005.
98. Sun F, Huo X, Zhai Y, Wang A, Xu J, Su D, Bartlam M, Rao Z. Crystal structure of mitochondrial respiratory membrane protein complex II. Cell. 2005 Jul 1;121(7):1043-57. doi: 10.1016/j.cell.2005.05.025.
99. Mercer DK, Stewart CS, Miller L, Robertson J, Duncan VM, O'Neil DA. Improved methods for assessing therapeutic potential of antifungal agents against dermatophytes and their application in the development of NP213, a novel onychomycosis therapy candidate. AAC. 2019 May;63(5):10-128. doi: 10.1128/aac.02117-18.
100. Hancock RE, Haney EF, Gill EE. The immunology of host defence peptides: beyond antimicrobial activity. Nat Rev Immunol.2016 May;16(5):321-34. doi: 10.1038/nri.2016.29.
101. Haney EF, Straus SK, Hancock RE. Reassessing the host defense peptide landscape. Front Chem. 2019 Feb 4;7:435645. doi: 10.3389/fchem.2019.00043.
102. Munjal K, Sharma S, Sharma S, Kumar D, Choudhary A, Berwal R, et al. Comparison of serum 25-hydroxyvitamin D levels after a single oral dose of vitamin D3 formulations in mild vitamin D3 deficiency. J Pharmacol Pharmacother. 2021 Dec;12(4):163-7. doi: 10.4103/jpp.jpp_105.
103. Cutrín-Gómez E, Anguiano-Igea S, Delgado-Charro MB, Gómez-Amoza JL, Otero-Espinar FJ. Effect of penetration enhancers on drug nail permeability from cyclodextrin/poloxamer-soluble polypseudorotaxane-based nail lacquers. Pharmaceutics. 2018 Dec 13;10(4):273. doi: 10.3390/pharmaceutics10040273.
104. Elsayed MM. Development of topical therapeutics for management of onychomycosis and other nail disorders: a pharmaceutical perspective. JCR. 2015 Feb 10;199:132-44. doi: 10.1016/j.jconrel.2014.11.017.
105. Brasch J, Kreiselmaier I, Christophers E. Inhibition of dermatophytes by optical brighteners. Mycoses. 2003 Mar 4;46(3-4):120-5. doi: 10.1046/j.1439-0507.2003.00857.x.
106. Kingsbury JM, Heitman J, Pinnell SR. Calcofluor white combination antifungal treatments for Trichophyton rubrum and Candida albicans. PLoS One. 2012 Jul 6;7(7):e39405. doi: 10.1371/journal.pone.0039405.
107. Malik J, Munjal K, Deshmukh R. Attenuating effect of standardized lyophilized Cinnamomum zeylanicum bark extract against streptozotocin-induced experimental dementia of Alzheimer's type. J Basic Clin Physiol Pharmacol. 2015 May 1;26(3):275-85. doi: 10.1515/jbcpp-2014-0012.
108. Hafeez F, Hui X, Chiang A, Hornby S, Maibach H. Transungual delivery of ketoconazole using novel lacquer formulation. Int J Pharm. 2013 Nov 18;456(2):357-61. doi: 10.1016/j.ijpharm.2013.08.082.

CONTENT

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CURRENT TOPIC

Blockchain Technology in Pharmaceutical Industry: A Review of Recent Research Articles on *PubMed*

Varun Pareek,[1] Divya Saran,[2] Lokendra Sharma,[1] Punam Jakhar,[1] Susheel Kumar[1]

1. Department of Pharmacology, RUHS College of Medical Sciences, Jaipur, India.
2. Department of Pharmacology, Mahatma Gandhi Medical College and Hospital, Jaipur, India.

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Corresponding author:
SUSHEEL KUMAR
E: susheelpn72@gmail.com

Abstract

Blockchain technology has emerged as a formidable force ready to transform the pharmaceutical business. This study investigated the integration of smart contracts and decentralised apps as potential future possibilities, emphasising their ability to automate crucial operations and strengthen pharmaceutical product integrity, based on the recently published articles in *PubMed* between 2015 to 2023 with “pharmacology” and “blockchain” as search keywords. Recent study backed up the idea that blockchain can improve openness, security and efficiency in the industry. According to research, it has the ability to speed up regulatory approvals while also considerably reducing the risk of counterfeit medications penetrating the supply chain. Furthermore, the ability of blockchain to disrupt existing intermediaries and enable disintermediation may result in a more streamlined and efficient industry. While there are implementation obstacles, the benefits of this technology in medicines are significant. Embracing blockchain promises a future of increased security, transparency and patient-centricity, ultimately changing healthcare. This article explored blockchain application in the pharmaceutical sector with innovations like *Medledger* and chaincodes, addressing drug tracing and supply chain security. It presents a structure for a private network using *Hyperledger Fabric*, showcasing blockchain's potential to enhance transparency, security and efficiency beyond traditional areas.

Key words: Blockchain technology; Pharmaceutical industry; Smart contracts; Decentralised applications (dApps); Drug supply chain.

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Introduction

Blockchain technology is a decentralised, impermeable digital ledger system. It is made up of a number of interconnected blocks, each containing a number of transactions. Since data cannot be modified after it has been recorded in a block, transparency and security are ensured. Blockchain is maintained by a network of computers as opposed to a single authority because it is distributed. In addition to enabling cryptocurrencies like *Bitcoin*, this technology offers a wide range of applications in fields outside of finance, including voting, supply chain management and healthcare. It offers trust, immutability and accountability in data management, making it a disruptive force in industries seeking transparency, traceability and increased efficiency.

Blockchain technology has a lot of potential ap-

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plications in the pharmaceutical sector, such as improving transparency, security and traceability across the whole supply chain. However, there are some challenges to overcome, such as regulatory constraints, interoperability issues and the need for sector-wide standards. However, as technology advances, it may totally alter how pharmaceuticals are manufactured, distributed and treated, which would benefit all stakeholders and increase patient safety.

Blockchain technology has the potential to revolutionise several industries, including pharmaceuticals. Blockchain technology has the potential to have a significant impact on the pharmaceutical industry.

This study investigated the integration of smart contracts and decentralised apps as potential future possibilities, emphasising their ability to automate crucial operations and strengthen pharmaceutical product integrity, based on the recently published articles in PubMed between 2015 to 2023 with “pharmacology” and “blockchain” as search keywords.

Increased transparency in the pharmaceutical industry through blockchain technology

Transparency is a cornerstone of trust and integrity in any industry, but it is especially important in the pharmaceutical industry, since the quality and legitimacy of medicines directly touch human lives. Blockchain technology offers a game-changing alternative for increasing openness throughout the pharmaceutical supply chain.

As blockchain technology advances and more firms realise its promise, the manufacture, distribution and consumption of medications may be transformed, benefiting patients and stakeholders across the sector. Although blockchain technology has many benefits for the pharmaceutical industry, there are still obstacles to application, such as interoperability issues, legal constraints and the need for broad industry standards.

The foundation of transparency: distributed ledger system

The distributed ledger system[1] is at the heart of blockchain's contribution to transparency. A blockchain ledger is distributed over numerous nodes or computers, as opposed to traditional centralised databases, which are vulnerable to manipulation or have single points of failure. A network of participants verifies and records each transaction or record, making it extremely difficult to alter or tamper with data without the network's consensus.

This distributed ledger technology ensures that every stage of the supply chain, from the initial acquisition of raw materials to the final delivery of medicines to patients, is properly recorded and made accessible to all important players in the pharmaceutical business.

This indicates that manufacturers, regulators, distributors, healthcare providers and even patients can track the journey of a pharmaceutical product with complete confidence in the accuracy and integrity of the information.

Tracking and verifying the supply chain

The ability of blockchain to offer end-to-end traceability is one of the most significant applications of blockchain in the pharmaceutical sector. Each batch of medication can be branded with a digital signature that records critical information such as the origin of raw materials, the production process, quality control checks and distribution paths using unique identifiers and smart contracts. This data is added to the blockchain in a sequential and unchangeable manner.

As a result, each stakeholder in the pharmaceutical supply chain can now obtain a detailed record of a product's journey. A pharmacist, for example, can scan a QR code on pharmaceutical packaging to instantaneously retrieve a complete history of the drug's creation and distribution. This transparency not only strengthens confidence in the authenticity of the product but also allows for quick action in the event of a recall or quality concerns.

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Strengthening regulatory compliance

The pharmaceutical sector relies heavily on regulatory compliance.[2] To assure the safety and efficacy of their products, businesses must follow a plethora of regulations and standards. Blockchain technology has the potential to help streamline and improve compliance processes.

Smart contracts, a blockchain feature, can automate compliance and regulatory processes. When certain circumstances are met, these contracts carry out predetermined activities. For example, if a batch of medication fulfils all quality control norms, the smart contract can immediately activate distribution clearance.

This automation not only decreases the administrative load for pharmaceutical businesses, but it also reduces the possibility of human error or supervision. Furthermore, it gives authorities real-time access to compliance data, allowing for more efficient oversight and faster response to deviations from industry standards.

Facilitating trust in clinical trials

Clinical trials are the foundation of drug development, providing the evidence required to show the safety and efficacy of a new medicine. Transparency and data integrity in clinical trials, on the other hand, have been issues of concern. Blockchain technology has the ability to completely transform this crucial stage of drug development.

Every step of a clinical trial can be recorded in a secure and transparent manner using blockchain. This includes recruiting participants, collecting data and analysing it. The immutability of blockchain means that trial data is unmodified and can be independently validated, building trust in the results' integrity (Figure 1).

Furthermore, blockchain can facilitate safe data sharing and collaboration among trial stakeholders such as researchers, ethics committees and regulatory authorities. This not only speed up the trial process but also makes sure that the highest ethical and scientific standards are a guarantee.

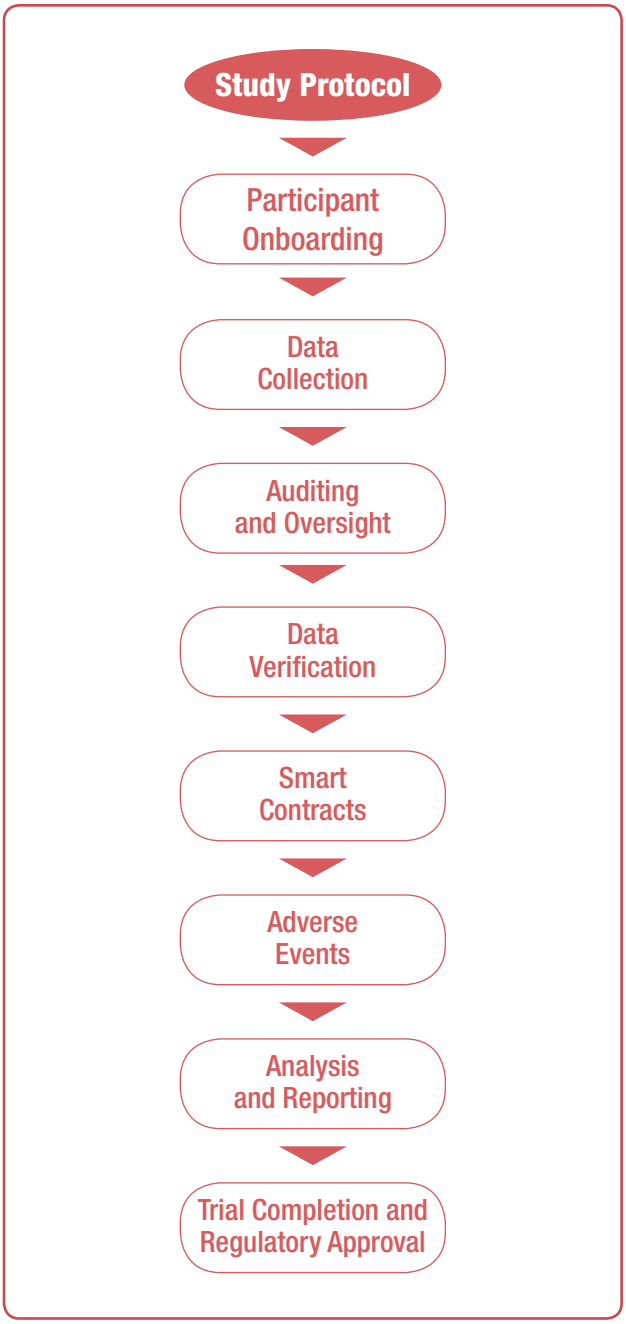

Figure 1: Flowchart for clinical trials security and transparency using blockchain

Enhanced security through blockchain technology

In an era of increased digitisation and interconnection, security has become a top priority, particularly in businesses where lives are on the line, such as medicines. Blockchain technology takes a fresh approach to data protection by employing cryptographic techniques and a distributed ledger system (Figure 2).

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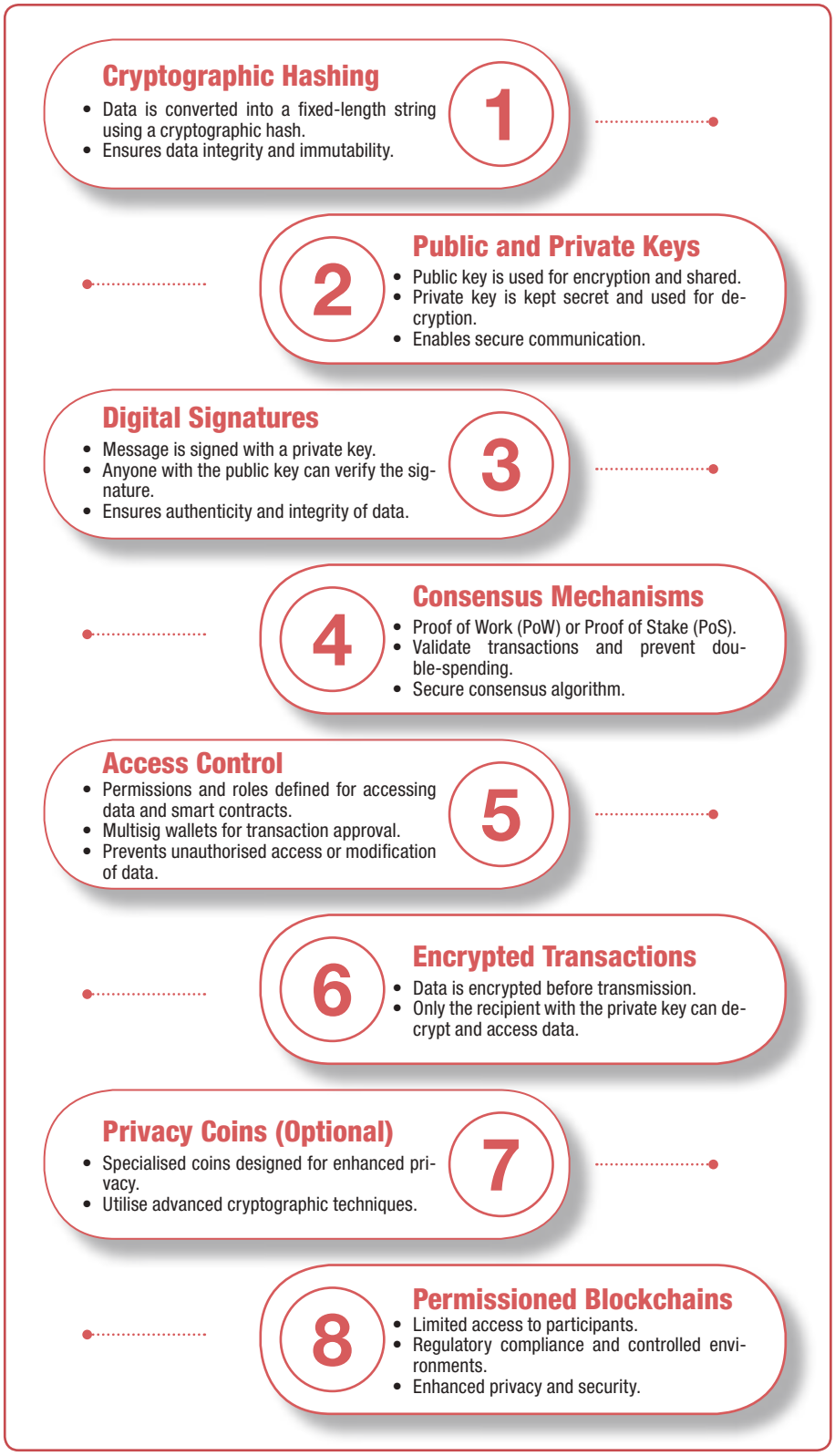

Figure 2: A flowchart about how security and privacy is ensured on blockchain

Cryptographic techniques: the backbone of security

The effectiveness of cryptographic algorithms underpins blockchain security.[3] When data is recorded on a blockchain, it is encrypted and linked to previous blocks using a complicated mathematical method. This technique generates a chain of blocks, each with an unique identity, making data modification or tampering extremely difficult.

A bad actor would need to not only change the data in a single block, but also recalculate the cryptographic puzzle for all following blocks in the chain, to change information on a blockchain. This would necessitate processing power well beyond the capabilities of even the most sophisticated supercomputers. Furthermore, since blockchain is decentralised, obtaining a consensus among all stakeholders in the network is necessary for any changes to be made. This cements the confidence that the integrity of data remains intact and protected.

Countering counterfeit drugs and ensuring patient safety

Counterfeit medications are a major hazard to worldwide public health. They not only endanger patients, but they also undermine trust in the pharmaceutical sector. The transparency aspect of blockchain acts as a major deterrent to the development and distribution of counterfeit medications.

Blockchain ensures that every step in the production and distribution process is recorded and verifiable by enabling end-to-end traceability. This means that a medication's legitimacy may be verified at any stage along the supply chain. If a questionable batch is discovered, stakeholders can immediately track out its source, allowing for targeted recalls and investigations.

Furthermore, by including unique IDs and digital signatures, counterfeit pharmaceuticals can be discovered quickly. These identifiers function as a digital fingerprint, enabling for instant authentication of a product's legitimacy.[4] This not only protects patients, but also builds the reputation of pharmaceutical firms committed to delivering high-quality, authentic pharmaceuticals.

Pharmaceutical firms can ensure the legitimacy and quality of their medicines by protecting the entire supply chain on a blockchain, from raw material acquisition through ultimate distribution. Once a batch of medication is stored on the blockchain, it is nearly impossible to alter or replace it with counterfeit pharmaceuticals without being detected. This sophisticated security mechanism not only protects patients from potentially hazardous pharmaceuticals, but it also protects pharmaceutical businesses' reputation.

Improved efficiency with smart contracts

Efficiency is important in any industry, but it is especially important in the pharmaceutical industry, where quick reaction times and adherence to regulatory schedules are vital. Smart contracts (Figure 3), a game-changing tool for increasing efficiency[5] are introduced by blockchain.

Automating predefined actions

Smart contracts are contracts that execute themselves based on established criteria and situations. These contracts are defined in code and execute automatically when certain criteria are met. Companies may automate a wide range of procedures in the pharmaceutical supply chain by adding smart contracts, from payments and quality checks to regulatory compliance.

For instance, if a batch of medication passes all quality control checks, the smart contract can immediately pay the supplier. This eliminates the need for manual intervention, lowering administrative costs and the possibility of human error. Furthermore, smart contracts enable stakeholders to follow progress and respond quickly to any deviations by providing real-time visibility into the state of multiple operations.

Challenges and limitations of implementing blockchain technology in the industry

The use of blockchain technology into the pharmaceutical sector holds enormous promise in terms of better transparency, enhanced security and increased efficiency. This transformational potential, however, is not without its problems

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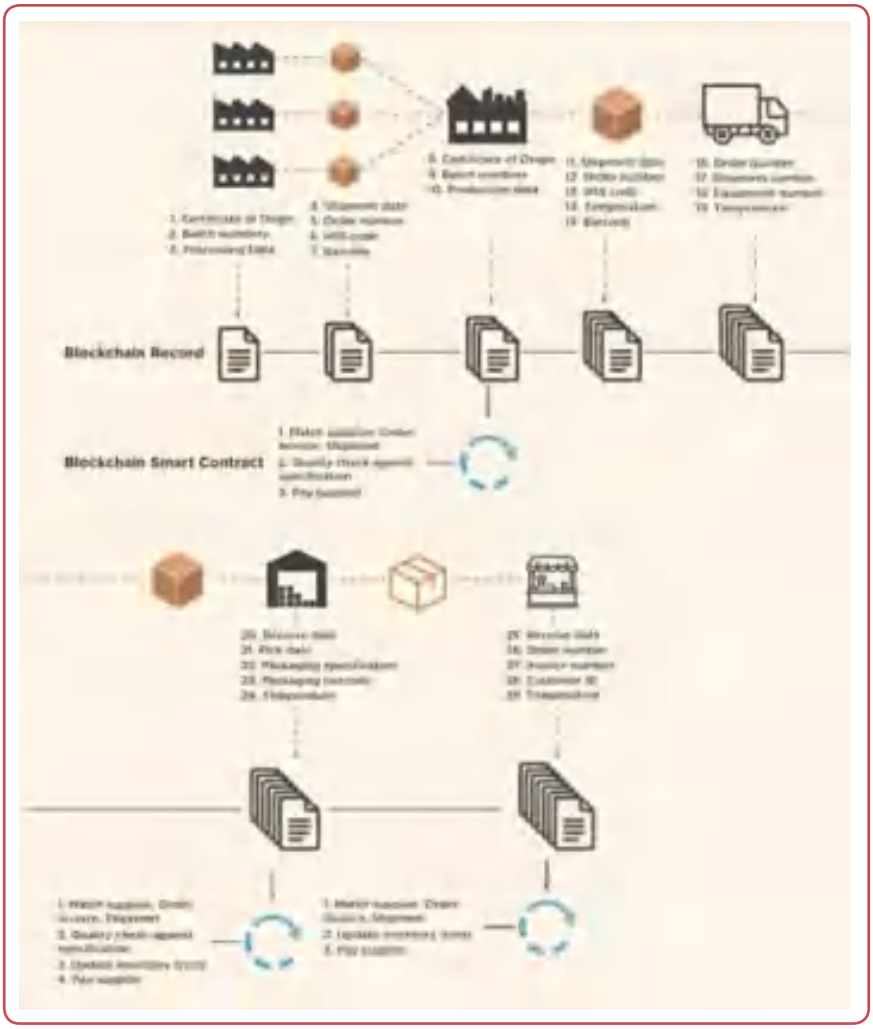

Figure 3: Smart contract workflow

and constraints: the need for standards, data privacy concerns, regulatory constraints. Understanding and addressing these difficulties is critical to realising blockchain's full promise for the industry's and ultimately, patients' well-being.

Need for standardisation

The requirement for standardisation is one of the key hurdles in using blockchain technology in the pharmaceutical sector. The sector operates in a complex ecosystem that includes multiple players like as manufacturers, distributors, regulators and healthcare providers. Each of these entities may have its own set of systems, processes and protocols. A standardised approach that enables interoperability and easy communication between these numerous participants is required for blockchain to be properly implemented.[6]

The full benefits of blockchain, such as end-to-end traceability and real-time information sharing, may remain elusive in the absence of a uniform framework. Furthermore, the lack of standards may result in fragmented and disjointed blockchain deployments, limiting the technology's scalability and overall influence.

Furthermore, standardisation necessitates collaborative efforts from industry leaders, regulatory organisations and technological specialists. Collaboration and consensus-building among stakeholders are essential for developing a coherent framework that can be universally implemented throughout the pharmaceutical supply chain.

Data privacy concerns

Data privacy is a top priority in any company, but it is especially important in healthcare and pharmaceuticals, where sensitive patient information is at stake. The intrinsic openness and immutability of blockchain may collide with stringent data privacy requirements, such as the Health Insurance Portability and Accountability Act (HIPAA) in the United States.[7]

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While blockchain provides strong security through cryptographic techniques, maintaining patient data confidentiality while reaping the benefits of the technology is a tricky balancing. Some of these concerns can be addressed by permissioned blockchains, which limit access to approved parties. Even with permissioned blockchains, however, ensuring compliance with existing data protection rules and regulations is vital. To address data privacy issues, pharmaceutical businesses and technology specialists must collaborate to create new solutions that protect patient information while leveraging blockchain's disruptive capabilities.

Regulatory barriers

The deployment of blockchain technology in the pharmaceutical industry faces major regulatory challenges. The sector is under to rigorous scrutiny from regulatory organisations around the world, which attempt to assure pharmaceutical safety, efficacy and quality. Integrating a new technology such as blockchain necessitates conforming to and, in certain circumstances, altering current legislation to fit this creative approach.

Regulatory authorities must establish clear rules and guidelines for blockchain implementation. This involves difficulties like record-keeping, compliance and auditability. Regulatory compliance will necessitate open communication between industry stakeholders, technology specialists and regulatory bodies.

Navigating the complex environment of foreign legislation adds another element of complication. Pharmaceutical firms operating across borders must deal with a variety of regulatory constraints, necessitating a global effort to develop a uniform approach to blockchain adoption.

Steinwantder et al[8] emphasise the vital necessity of data integrity in the pharmaceutical business, particularly in the context of process validation. To ensure patient safety and the profitability of industrial enterprises, process validation relies significantly on accurate and trustworthy data. Regulatory authorities, such as the FDA, have issued new standards for handling data in the pharmaceutical industry in response to previous breaches in data integrity.

The authors suggest a technological method for improving data integrity that does not rely on trusted third parties or centralised systems. They employ a strategy that combines traditional software development tools with a new smart contract created on the *Ethereum* network. The case study showed how this approach may efficiently detect data manipulation or result backdating and how regulatory agencies can completely audit the entire data flow from the regulatory report back to the original raw data.

The outcomes of this contribution provide a potential roadmap for the creation of production-ready solutions, such as versioned database systems that interface seamlessly with distributed ledgers. This improvement is projected to improve the dependability of pharmaceutical manufacturing data, protecting both industrial businesses' intellectual property.

In layman's words, this emphasises the need of reliable data in the pharmaceutical sector. It presents a technological solution based on blockchain, which is well-known for its role in cryptocurrencies such as *Bitcoin*. The authors present a method to secure data integrity without depending on traditional middlemen by merging blockchain with existing computing tools. This breakthrough has the potential to significantly improve the reliability of data used in pharmaceutical manufacture, benefiting both firms and patients.

A study by Jia et al[9] addresses long-standing issues in the pharmaceutical sector with medical data sharing, anti-tampering methods and data leak prevention. The issue develops when patients are referred to several hospitals and are unable to provide a thorough medical history due to information exchange limits between healthcare facilities. Instead, for transmitting partial medical information, dependence on easily misplaced paper data such as medical records and test sheets becomes necessary. This raises the possibility of authenticity and impartiality difficulties in medical disagreements.

To address these issues, the article suggests creating a consortium medical blockchain system. This system is built on a Byzantine Fault Tolerance algorithm,[10] which ensures that numerous nodes store and share medical information collectively. This method provides a strong barrier against medical data modification and leakage. The stated issues in medical data administration can be efficiently solved by deploying this solution. Furthermore, when compared to existing

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medical blockchain systems, the proposed system has advantages and a broader applicability.

Peng et al[11] address a major issue affecting vaccine production, specifically the necessity for strict oversight to assure the safety and efficacy of vaccines, which are predominantly delivered to young children with vulnerable immune systems. Currently, vaccine production oversight is deemed insufficient because manufacturers have complete control over production data. When vaccines are ready for distribution, these documents are only sent to regulatory agencies for evaluation, leaving possibility for forgeries and modifications.

The authors propose a unique approach based on a two-tiered blockchain architecture[12] to address these inadequacies in centralised management. The first tier includes confidential data specific to vaccine manufacturing companies, such as production records and hash values. The following tier includes public data, such as manufacturing record hashes and vaccine information. This system incentivises vaccine companies to report production records as soon as possible without worry of jeopardising their privacy. Furthermore, because of the blockchain's tamper-proof characteristics and timestamps, it discourages organisations from tampering with or falsifying records.

The authors propose a consensus approach for multi-node collaboration to improve time efficiency. The principal supervising node is in charge of sorting services and guaranteeing the blockchain replica's accuracy. Ordinary supervisory nodes can temporarily substitute the primary node and help with data recovery in the event of data loss. Furthermore, review nodes are responsible for giving complete and accurate blockchain copies to other nodes, avoiding the time and resource waste that is frequent in traditional blockchain systems.

In addition to addressing concerns about time efficiency, the authors offer a mechanism for reducing vaccine data redundancy within the blockchain. To evaluate if a block can be pruned, the technique uses timestamps and vaccination validity periods. This determination is also aided by information exchange with vaccine institutions. These ingenious methods collectively facilitate efficient spatiotemporal supervision of vaccine manufacturing.

This approach is deemed innovative given the scarcity of research in the subject of vaccine production monitoring. The suggested blockchain-based system has the potential to transform how vaccine production is monitored, ensuring increased safety and reliability in this essential area of healthcare.

Tseng et al[13] introduce the concept of using blockchain, which is known for its trust-building characteristics, outside of the *Fintech* sector. The *Gcoin* blockchain is proposed as the foundation for transparent drug transaction data flow. This method intends to shift the medication supply chain's regulatory model away from inspection and examination and toward a more complete surveillance net model.

Under this new approach, every organisation in the medication supply chain can actively participate, working together to prevent counterfeit drugs and protect public health, particularly patient health. This novel use of blockchain technology has great promise for improving transparency and security in the pharmaceutical business.

The research by Mackey et al[14] demonstrates blockchain technology's potential to revolutionise healthcare by providing a secure and transparent digital ledger system for data management. It highlights that the applicability of blockchain goes well beyond cryptocurrency, particularly in the context of healthcare. Various healthcare stakeholders are currently investigating how blockchain might streamline operations, reduce costs, improve patient outcomes, ensure compliance and increase the usage of healthcare-related data.

However, the authors emphasise the significance of building blockchain solutions with a thorough understanding of the actual needs of healthcare, taking into account consumer, patient, provider and regulatory viewpoints. Addressing the particular issues that healthcare faces in comparison to other sectors of the economy is critical. The authors suggest the concept of a "fit-for-purpose" health blockchain, which refers to a blockchain system that is specifically designed to satisfy the unique needs and demands of the healthcare business.

The paper brings together a broad collection of professionals who are actively involved in the design, development and implementation of

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blockchain solutions in healthcare to share insights into this topic. Their diverse perspectives and expertise contribute to a thorough grasp of how blockchain might be used to generate good change in the healthcare sector.

Mackey et al[15] analyse Japan's changing demographic landscape, which is marked by an aging population and dropping birth rates. This demographic shift poses significant problems to Japan's internationally regarded universal health coverage (UHC) system. A surge in national public health expenditures, increased demand for healthcare services, an urgent need for elder and long-term care, a scarcity of healthcare experts and disparities in healthcare accessibility between rural and urban areas are among the predicted concerns.

Blockchain technology has emerged as a potential answer to some of these issues. The authors underline, however, that for blockchain to be effective in Japan, it must be envisioned, constructed and deployed in a way that conforms with the country's centralised UHC-focused public health system.

The technology should also be flexible enough to accommodate Japan's particular national health and innovation regulations, which include a regulatory sandbox system. Lessons learned from blockchain adoption in the commercial sector and in other nations should also be incorporated into the deployment strategy. The position addresses both the possible benefits and drawbacks of implementing blockchain technology in Japan's healthcare system. It emphasises the importance of blockchain solutions being carefully localised and integrated to ensure they suit Japan's specific healthcare landscape and legislation.

Potential future directions

Smart contracts

Smart contracts are a significant innovation in blockchain technology that has far-reaching consequences for the pharmaceutical business. These self-executing contracts can automate crucial procedures because they are governed by preset code. They can, for example, supervise regulatory compliance checks to ensure that each procedure corresponds to set standards. This automation speeds up procedures, increasing efficiency and lowering the likelihood of human error.

Furthermore, smart contracts may supervise quality assurance procedures, ensuring that products fulfil stringent quality standards before moving further down the supply chain. This automation ensures that every product meets the highest quality standards, enhancing patient safety. Furthermore, smart contracts can make payments between stakeholders more seamless and secure. This not only simplifies financial transactions but also reduces potential conflicts, given the conditions of the contract are coded and automatically executed upon meeting the criteria.

Decentralised applications (dApps)

Another intriguing path for the pharmaceutical business is decentralised applications, or dApps. These blockchain-based applications work without the need for a central authority, giving a level of transparency and security unrivalled by traditional systems.

dApps can promote secure and transparent communication between stakeholders in the pharmaceutical industry. They can, for example, provide smooth communication among producers, distributors and regulatory organisations. These interactions may be recorded and validated in real time using blockchain, ensuring the validity and integrity of medicinal medicines throughout their entire lifecycle.

dApps' openness and security have the ability to transform supply chain processes. They create a trust layer between stakeholders, reducing the possibility of counterfeit medications and ensuring that every product meets the highest quality and safety standards.

Potential future implications for the industry: disrupting traditional intermediaries

Impact on intermediaries

Blockchain's distributed ledger architecture has the potential to reshape the roles of traditional

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pharmaceutical intermediaries. Intermediaries have historically played an important role in checking and confirming transactions, assuring compliance and validating data integrity. However, with blockchain, the necessity for third-party interference may be greatly reduced.

The immutability and transparency of blockchain records establish trust in transactions and data. This means that stakeholders can directly check information on the blockchain, removing the need for intermediaries. This transformation could have far-reaching consequences for how pharmaceutical transactions are performed. It has the ability to reduce costs, boost operational efficiency and reduce potential points of failure or inefficiency associated with traditional intermediaries. Furthermore, enhanced transparency can boost stakeholder trust, eventually enhancing patient safety and the integrity of pharmaceutical operations.

Potential for disintermediation

The decentralised structure of blockchain allows for direct peer-to-peer contacts, which has the potential to reshape numerous areas of the pharmaceutical value chain, particularly the distribution process. Stakeholders can follow the complete route of pharmaceutical products from production to delivery by employing blockchain's immutable ledger. This openness not only protects the authenticity and integrity of medications, but it also fosters trust among participants. As a result, certain intermediaries that normally oversee or support these transactions may be avoided.

Reduced reliance on middlemen may result in a more nimble and efficient supply chain. However, while disintermediation can provide significant benefits, it can also provide obstacles. Established intermediaries may need to adapt or find new roles as the ecosystem evolves. Furthermore, considerable thought and regulation will be required to ensure that the shift to a more decentralised ecosystem takes place in a controlled and secure manner, eventually enhancing patient safety and the integrity of the pharmaceutical sector.

Finally, the incorporation of smart contracts and decentralised applications in the pharmaceutical business has the potential to completely transform operations. Smart contracts streamline operations and reduce the chance of errors by automating important processes ranging from regulatory compliance checks to quality assurance procedures. Decentralised apps encourage transparent interactions among stakeholders, ensuring authenticity and integrity throughout the lifecycle of a product.

Furthermore, the impact of blockchain on pharmaceutical intermediaries could lead to a more efficient supply chain, with transactions validated directly on the blockchain. Disintermediation, particularly in the distribution process, has the ability to reduce the risk of counterfeit pharmaceuticals. However, in order to preserve patient safety and industry integrity, this change must be properly managed. By embracing these technology innovations, the pharmaceutical business will usher in a new era of efficiency, transparency and eventually, patient well-being. *Medledger*, clinical research, patient data management and supply chain management are some of real example of blockchain technology implementation in the pharmaceutical industry.

MedLedger

Uddin et al[16] conducted study on the vital issue of counterfeit pharmaceuticals, which constitute a substantial danger to the pharmaceutical industry, particularly in developing nations. According to the World Health Organization (WHO), around 10 % of pharmaceuticals produced in these locations are counterfeit, putting human lives in danger. The growth of online and Internet-based pharmacies has challenged medicine supply chain security even further.

To address this, the study recommends the *Medledger* system, which is powered by blockchain technology and uses the *Hyperledger Fabric* platform with smart contracts known as "chaincodes". This technology creates a safe and efficient framework for performing transactions inside a private and permissioned distributed network of pharmaceutical stakeholders. It significantly decreases dependency on centralised authorities and middlemen, hence improving operating efficiency and safety. It also reduces the possibility of data tampering within the *Medledger*.

Chaincodes[17] manage and organise interactions among actors in the drug supply chain ecosystem, which are represented by sequence diagrams. The system keeps a complete and immutable record of all activities, events and transactions on the *Medledger* blockchain. It also incorporates

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peer-to-peer decentralised file systems like as IPFS, Swarm and filecoin[18] to improve transparency and traceability. While the research is encouraging, it does admit some implementation issues related to the *Hyperledger Fabric* architecture. The paper continues by suggesting future research topics and open problems that could help to develop medication traceability solutions further. Overall, the planned *Medledger* system is an important step toward securing pharmaceutical supply chains and protecting public health. Some of the world's major pharmaceutical corporations, including *Pfizer* and *Roche* are now using the platform.

Clinical research

Blockchain technology is being utilised to increase clinical trial transparency and integrity. For example, the US National Library of Medicine's *ClinicalTrials.gov*[19] website is investigating the use of blockchain technology to increase the accuracy and completeness of trial data.

Another example is the Clinical Research Blockchain Platform, which *IBM* and *Boehringer Ingelheim* are developing.[20] This platform promises to improve clinical trial data security and privacy while simultaneously making it easier for researchers to access and evaluate the data.

Patient data management

Blockchain technology is being used to improve patient data management in the healthcare business. *EncrypGen's*[21] is one example. Patients can use this platform to securely store and share their genomic data with researchers and healthcare practitioners. Another example is the *Patientory* platform,[22] which is being utilised to improve patient data sharing and management among healthcare providers.

Supply chain management

IBM Blockchain for Pharmaceuticals. IBM has collaborated with pharmaceutical companies such as *Pfizer* and *Merck* to develop blockchain-based supply chain management solutions. These platforms improve the transparency and traceability of the pharmaceutical supply chain, minimising the risk of counterfeit pharmaceuticals and increasing the chain's overall effectiveness.[20, 23]

Chronicled. Chronicled, a blockchain company, has developed a platform that employs blockchain technology to provide pharmaceutical companies with end-to-end supply chain visibility. It enables firms to monitor and verify products at every stage of the supply chain, from manufacturing through distribution to final consumers.[17, 24] This helps to reduce the risk of acquiring counterfeit pharmaceuticals while also enhancing supply chain processes.

Conclusion

Blockchain technology has the potential to transform the pharmaceutical sector by providing solutions that improve transparency, security and efficiency. The integration of smart contracts and decentralised apps holds great promise for automating crucial operations and maintaining pharmaceutical product integrity. Furthermore, the ability of blockchain to disrupt existing middlemen and encourage disintermediation may result in a more streamlined and efficient industry. Recent research articles highlight the substantial progress made in utilising blockchain's potential for the benefit of the pharmaceutical industry. As the industry evolves, adopting blockchain technology will be critical in crafting a more secure, transparent and patient-centric future.

Critical processes in pharmaceutical operations can be automated through the integration of smart contracts and decentralised apps, resulting in enhanced efficiency and integrity. The potential for blockchain to disintermediate existing intermediaries and disrupt the sector could transform the industry, enabling a more streamlined and secure supply chain.

Recent research studies, such as those discussed in this article, give solid proof of blockchain's disruptive impact on the pharmaceutical industry. Several studies show how blockchain improves transparency, security and traceability, solving critical issues like counterfeit pharmaceuticals and data integrity. As the industry evolves, adopting blockchain technology will be critical in crafting a more secure, transparent and patient-centric future. However, navigating implementation obstacles and legal issues is critical to ensuring a smooth transition to a blockchain-powered pharmaceutical industry. By doing so, the industry will be able to realise the full potential

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of this breakthrough technology, eventually benefiting patients, stakeholders and the general public.

The review article is extremely important in today's climate because of its extensive examination of blockchain technology's impact on the pharmaceutical business. It addresses major concerns in global healthcare, such as counterfeit medications, supply chain integrity and regulatory compliance. The paper provides a transformational approach to addressing these difficulties by suggesting creative solutions such as smart contracts and decentralised applications. This review article delivers relevant insights and ideas that can potentially change the industry and defend public health in an era when trust, openness and efficiency are paramount in pharmaceutical operations.

Ethics

This study was a secondary analysis based on the currently existing dataset from the *PubMed* and did not directly involve with human participants or experimental animals. Therefore, the ethics approval was not required in this paper.

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Conflicts of interest

The authors declare that there is no conflict of interest.

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Data access

The data that support the findings of this study are available from the corresponding author upon reasonable individual request.

Author ORCID numbers

Varun Pareek (VP):
0000-0001-5517-1411
Divya Saran (DS):
0000-0001-5506-2856
Lokendra Sharma (LS):
0000-0001-8949-9079
Punam Jakhar (PJ):
0000-0002-5050-8473
Susheel Kumar (SK):
0000-0003-3560-718X

Author contributions

Conceptualisation: VP
Methodology: VP, SK
Writing – original draft: VP, DS
Writing - review and editing: VP, DS, PJ
Project administration: VP, LS
Supervision: VP, SK

References

1. Kuo TT, Kim HE, Ohno-Machado L. Blockchain distributed ledger technologies for biomedical and health care applications. J Am Med Inform Assoc. 2017 Nov 1;24(6):1211-20. doi: 10.1093/jamia/ocx068.
2. Hasselgren A, Wan PK, Horn M, Kralevska K, Gligoroski D, Faxvaag A. GDPR compliance for blockchain applications in healthcare. arXiv preprint arXiv:2009.12913. 2020 Sep 27. doi:10.48550/arXiv.2009.12913.
3. Alruwaili FF. Artificial intelligence and multi agent based distributed ledger system for better privacy and security of electronic healthcare records. PeerJ Comp Sci. 2020 Nov 30;6:e323. doi: 10.7717/peerj-cs.323.
4. Naughton BD. Medicine authentication technology: a quantitative study of incorrect quarantine, average response times and offline issues in a hospital setting. BMJ Open. 2019 Feb 1;9(2):e026619. doi: 10.1136/bmjopen-2018-026619
5. Kiester L, Turp C. Artificial intelligence behind the scenes: PubMed's Best Match algorithm. J Med Libr Assoc. 2022 Jan 1;110(1):15-22. doi: 10.5195/jmla.2022.1236.

CONTENT

6. Wu L, Ji C, Lu H, Hong X, Liu S, Zhang Y, et al. Standardization of medical service indicators: A useful technique for hospital administration. Plos One. 2018 Nov 28;13(11):e0207214. doi: 10.1371/journal.pone.0207214.
7. Moore W, Frye S. Review of HIPAA, part 1: history, protected health information, and privacy and security rules. J Nucl Med Technol. 2019 Dec;47(4):269-72. doi: 10.2967/jnmt.119.227819.
8. Steinwandter V, Herwig C. Provable data integrity in the pharmaceutical industry based on version control systems and the blockchain. PDA J Pharm Sci Technol. 2019;73(4):373-90. doi: 10.5731/pdajpst.2018.009407.
9. Jia Q. Research on medical system based on blockchain technology. Medicine (Baltimore). 2021 Apr 23;100(16):e25625. doi: 10.1097/MD.0000000000025625.
10. Wang Y, Zhong M, Cheng T. Research on PBFT consensus algorithm for grouping based on feature trust. Sci Rep. 2022 Jul 22;12(1):12515. doi: 10.1038/s41598-022-15282-8.
11. Peng S, Hu X, Zhang J, Xie X, Long C, Tian Z, et al. An efficient double-layer blockchain method for vaccine production supervision. IEEE Trans Nanobioscience. 2020 Jul;19(3):579-87. doi: 10.1109/TNB.2020.2999637.
12. Lee HA, Kung HH, Udayasankaran JG, Kijsanayotin B, B Marcelo A, Chao LR, et al. An architecture and management platform for blockchain-based personal health record exchange: development and usability study. J Med Internet Res. 2020 Jun 9;22(6):e16748. doi: 10.2196/16748.
13. Tseng JH, Liao YC, Chong B, Liao SW. Governance on the drug supply chain via Gcoin blockchain. Int J Environ Res Public Health. 2018 May 23;15(6):1055. doi: 10.3390/ijerph15061055.
14. Mackey TK, Kuo TT, Gummadi B, Clauson KA, Church G, Grishin D, et al. 'Fit-for-purpose?' - challenges and opportunities for applications of blockchain technology in the future of healthcare. BMC Med. 2019 Mar 27;17(1):68. doi: 10.1186/s12916-019-1296-7.
15. Mackey T, Bekki H, Matsuzaki T, Mizushima H. Examining the potential of blockchain technology to meet the needs of 21st-century Japanese health care: viewpoint on use cases and policy. J Med Internet Res. 2020 Jan 9;22(1):e13649. doi:10.2196/13649.
16. Uddin M. Blockchain Medledger: Hyperledger fabric enabled drug traceability system for counterfeit drugs in pharmaceutical industry. Int J Pharm. 2021 Mar 15;597:120235. doi:10.1016/j.ijpharm.2021.120235.
17. Xiao Y, Xu B, Jiang W, Wu Y. The HealthChain blockchain for electronic health records: development study. J Med Internet Res. 2021 Jan 22;23(1):e13556. doi: 10.2196/13556.
18. Nair R, Zafrullah SN, Vinayasree P, Singh P, Zahra MM, Sharma T, et al. Blockchain-based decentralized cloud solutions for data transfer. Comput Intell Neurosci. 2022 May 30;2022:8209854. doi: 10.1155/2022/8209854.
19. Gopalakrishna P, Suresh SA blockchain framework for clinical trial data management. J Med Sys. 2019;43(9):1-10. doi: 10.1007/s10916-019-1426-4.
20. Mak BC, Addeman BT, Chen J, Papp KA, Gooderham MJ, Guenther LC, et al. Leveraging blockchain technology for informed consent process and patient engagement in a clinical trial pilot. Blockchain Healthc Today. 2021;4. doi: 10.30953/bhty.v4.182.
21. Gürsoy G, Brannon CM, Ni E, Wagner S, Khanna A, Gerstein M. Storing and analyzing a genome on a blockchain. Genome Biol. 2022 Jun 29;23(1):134. doi: 10.30953/bhty.v4.182.
22. Russo-Spena T, Mele C, Cavacece Y, Ebraico S, Dantas C, Roseiro P, et al. Enabling value co-creation in healthcare through blockchain technology. Int J Environ Res Public Health. 2022 Dec 21;20(1):67. doi:10.3390/ijerph20010067.
23. Dubovitskaya A, Novotny P, Xu Z, Wang F. Applications of blockchain technology for data-sharing in oncology: results from a systematic literature review. Oncology. 2020;98(6):403-11. doi:10.1159/000504325.
24. MediLedger-Blockchain in Life Sciences 2017 [Internet]. [Cited: 17-May-2022]. Available from: https://www.chronicled.com/.

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CURRENT TOPIC

Bibliometric Analysis of Triggers on Environmental Stress Among Medical and Health Sciences Students at the University

Wawan Kurniawan,[1] Satriya Pranata,[2] Aric Vranada,[2] Aat Agustini,[1] Lalu Muhammad Irham[3]

1. Faculty of Nursing, Imam Bonjol Majalengka Education Foundation University, West Java, Indonesia.
2. Department of Nursing, Faculty of Nursing and Health Sciences, Universitas Muhammadiyah Semarang, Semarang, Central Java, Indonesia.
3. Faculty of Pharmacy, Universitas Ahmad Dahlan, Yogyakarta, Indonesia.

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Corresponding author:
WAWAN KURNIAWAN
T: +62 8112434647
E: wawankurniawan.mjl@gmail.com

Abstract

Continuing to improve services to students while studying on campus, lecturers and all elements at the university need information about triggers of environmental stress among medical and health sciences students. Thus, the purpose of this study was to explore triggers of environmental stress among medical and health sciences students in the university through bibliometric analysis by analysing the network visualisation, overlay visualisation and density visualisation on the topic. Bibliometrics analysis was used in assessing related topics. The data sources were based on online searches *via https://app.dimensions.ai/*. Data was collected on 3 December 2023. The literature followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flowchart. Data were analysed using *VOSviewer* and further analysed and reviewed by co-occurrence and co-authors. Four major themes of environmental stress were found in this study: 1) The internal situation in the university; 2) External situations outside of the university; 3) Comfort situation during the study; 4) Academic process. Environmental stress factors related to internal situation in the academic situation were academic stress, air pollution, anxiety in the academic process, COVID-19, depression, fear, medical student, nursing student, physical activity, school regulation, smoking and uncertainty of study. From the visualisation and density visualisation, it can be seen that the academic process related to the duration of study was a trending theme discussed in several papers related to triggers of environmental stress among students in the university. All elements of the university especially policy makers should pay attention to this problem to reduce the risk of stress while students are studying.

Key words: Environmental stress; Students; Trigger; University; Medical Sciences; Health sciences.

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Introduction

A conducive university environment can improve student learning motivation so that it will have an impact on learning achievement.[1] A conducive university environment here includes good relations between fellow students and the relationship between students and lecturers.[2] On the other hand, an unhealthy environment will make students feel stressed and ultimately reduce the learning motivation and achievement of students.[3]

The university environment can be divided into

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two major parts: physical and non-physical.[4] The physical environment such as changes in temperature, both rising and falling temperatures affect work performance, but sometimes it also reduces work performance.[5] An increase in temperature to a certain limit causes arousal which stimulates work performance but after passing a certain threshold this increase in temperature begins to interfere with body temperature which results in disruption of work performance as well.[6] The literature generally shows that the factors that affect the physical work environment are: temperature, noise, lighting and air quality.[2]

The non-physical factor most often discussed and related to student learning motivation is the working relationship formed at the university between students and other students, students and campus staff or students and lecturers.[7] The study results show that the relationship is a critical strategic issue for organisations because the relationship between the employee can significantly impact morale, motivation and productivity.[8] Another theory says people work for money but for more than money.[9] Most students want to be respected as a student on campus and the working relationship between students and all elements of the campus is also a fundamental thing that needs attention.[10]

In continuing to improve services to students while studying on campus, lecturers and all elements at the university need information about triggers of environmental stress among students. The results of bibliometric analyses may guide the identification of all triggers of environmental stress among students in the university by determining the main research areas of existing publications in specific fields. Moreover, the bibliometric analysis enables researchers to easily obtain information about subjects of interest from among numerous and increasing number of published articles.[11] There is no bibliometric analysis on the publication of research topics triggers of environmental stress among students at the university. Thus, the purpose of the study was to explore triggers of environmental stress among students in the university through bibliometric analysis by analysing the network visualisation, overlay visualisation and density visualisation on the topic.

Methods

Bibliometrics analysis was more suitable for quantitatively analysing the distribution of research papers that discuss triggers of environmental stress among students in the university. Bibliometric analysis is essential in assessing related topics searched based on the repetition term received.[12] The data sources used in this study were based on online searches *via https://app.dimensions.ai/*. Data was collected on 3 December 2023. The literature search used the stages following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flowchart.[13]

Paper restricted in publications years 2015-2023, focuses on the field of health sciences, biomedical and clinical science, nursing, public health, psychology and human society. The article evaluated only article publication type. The book and chapter types were excluded.

Selecting data

The stages in PRISMA included identification, screening and including as shown in Figure 1. Stage 1 (identification) detected 158,764 records from *dimensions.ai*, taking into account, each of the main search terms 'trigger' AND 'environmental stress' AND 'student' AND 'university', article and proceeding document type and range period of publication from 2015 to 2023. In stage 2 (screening), the option "article title, abstract" was selected in the field of each search term, resulting in 152,982 articles being excluded. In phase 3 (included), the final sample yielded 5,782 articles. The detail of the process is shown in Figure 1.

Data analysis

Data were analysed using *VOSviewer*. *VOSviewer* is a computer program for creating and viewing bibliometric maps.[14] The type of analysis was selected to create a map based on text data. In this study, the analysis was reviewed by co-occurrence and co-authors.

a) Co-occurrence procedures

The procedure for co-occurrence analysis went through the following stages: The data source was selected, data were read from references manager files. Selected fields were chosen from

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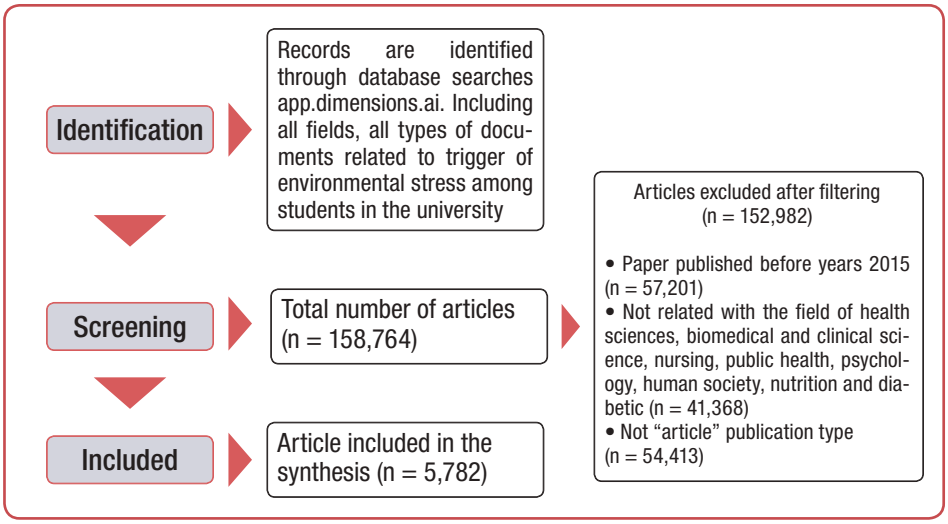

Figure 1: ***PRISMA flow chart***

PRISMA: preferred reporting items for systematic reviews and meta-analyses

which title and abstract fields were extracted. The counting method selected was full counting. The threshold selected was the minimum number of occurrences of a term was 10. Chosen several terms amounted to 133.

b) Co-authors procedures

The procedure for co-author analysis went through the following stages: The data type was chosen and a map based on bibliographic data was created. A co-authorship map based on bibliographic data was chosen. Chosen data source was: read data from reference manager files. Supported file type: *ris*. Chosen the type of analysis and counting method was: the type of analysis was co-authorship and the counting method was full counting. Chosen threshold was: the maximum number of documents of an author was 2. Of the 2,624 authors, 49 met the threshold. For each of the 49 authors, the total strength of the co-authorship links with other authors was calculated. The authors with the greatest total link strength were selected. The number of selected authors was 49.

Results

From the network visualisation Figure 2 and overlay visualisation Figure 3, it can be seen that academic issues, namely the duration of the study were a trending theme discussed in several papers related to triggers of environmental stress among students in the university.

Figure 2: ***Network visualisation***

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Figure 3: *Overlay visualisation*

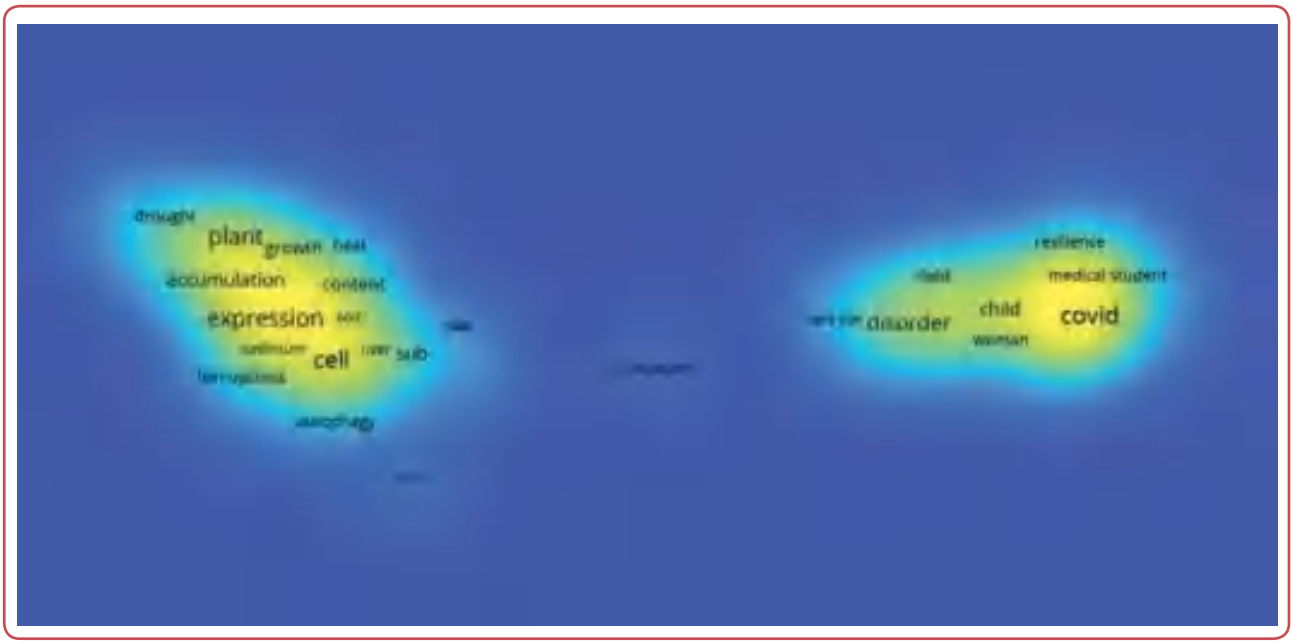

Figure 4: *Density visualisation*

Through the bibliometric analysis process, it can be concluded that the triggers of environmental stress among students in the university were grouped into four themes: internal situation in the university (academic stress, air pollution, anxiety in academic process, coronavirus disease, depression, fear, medical students, nursing student, physical activity, school regulation, smoking, uncertainty), external situation outside of university (oxidative stress), comfort situation during study (drought tolerance, low temperature) and academic process (duration).

Apart from that, from Figure 4 density visualisation, it can be seen that the academic process related to the duration of study was a trending theme discussed in several papers related to triggers of environmental stress among students in the university.

Discussion

The internal situation in the university

This study investigated several environmental stress factors related to internal situations in the academic situation such as academic stress, air pollution, anxiety in the academic process, coronavirus disease, depression, fear, medical students, nursing students, physical activity, school regulation, smoking and uncertainty.

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The educational journey for nursing, medical and health sciences-related students is punctuated by a university's internal situation of stress-inducing factors that extend beyond the conventional academic stressors.[15] The academic rigours inherent in the pursuit of a healthcare degree encapsulate a formidable array of demands, encompassing the mastery of a dense and complex body of knowledge, the acquisition of critical clinical skills and the execution of high-stakes practical assessments.[16] This intense academic workload often precipitates heightened levels of stress and anxiety, which can manifest in various forms, including performance anxiety, test anxiety and a pervasive fear regarding the ability to synthesise and apply knowledge in real-world clinical settings.[17] Such stress is frequently compounded by environmental and lifestyle factors present within the university setting, such as air pollution and smoking activity on campus. These elements, often overlooked, can further deteriorate the mental and physical well-being of students, undermining their academic endeavours and contributing to a cascade of health-related stress outcomes.[16, 17]

In recent times, the advent of the coronavirus disease (COVID-19) pandemic has introduced additional, unprecedented stressors into the academic milieu of healthcare students.[18, 19] The exigencies of the pandemic have led to a radical transformation in academic regulations and the structure of educational delivery, engendering an atmosphere fraught with uncertainty and apprehension.[20, 21] Students have been obliged to adapt to rapidly evolving teaching methods, often with diminished access to hands-on clinical experiences, which are vital to their education and future practice.[20] These pandemic-related academic disruptions are paralleled by the broader psychological impacts of the disease, including elevated concerns about personal and familial health risks, which have been shown to exacerbate feelings of depression and anxiety within this student population.[21] Furthermore, the pandemic has spotlighted the criticality of physical activity as a mitigative measure against stress, even as the constraints of study and infection control measures have made regular exercise more challenging. The cumulative effect of these stressors has culminated in a sense of graduate uncertainty, where students are left to ponder the stability and structure of their future roles in a healthcare system that has been deeply shaken by a global health crisis.[22, 23] Addressing these multifactorial stressors calls for a holistic and responsive approach from academic institutions, necessitating the provision of comprehensive mental health support, the adaptation of curriculum and assessment methods to accommodate the nuances of pandemic-era education and the fostering of environments conducive to physical and psychological wellness.[24, 25]

External situations outside of the university

Oxidative stress is discovered as an external situation which triggers environmental stress among nursing, medical and health-related students' university students in this study. Oxidative stress arises from a combination of environmental factors, such as air pollution and smoking and the chronic psychological stress associated with the demands of their studies.[26, 27] Intense academic workloads and high-stakes clinical environments may increase endogenous free radicals, leading to cellular damage.[28] This is compounded by lifestyle factors common among students, including poor sleep, diet and insufficient exercise, which can all contribute to oxidative imbalance. Although the direct link between academic and oxidative stress warrants further research, recognising and addressing these contributors is crucial for promoting long-term health in future healthcare professionals.[29, 30] Implementing stress reduction programs, nutritional guidance and healthy lifestyle promotion within academic curricula may help mitigate these risks.

Comfort situation during the study

The ambient conditions within educational settings, notably temperature, play a pivotal role in shaping the learning experience and comfort of nursing and medical students.[16] A suboptimal thermal environment, particularly one that is excessively cold, can precipitate a range of physiological and psychological stress responses that impede cognitive function and academic performance.[7] Cold classroom temperatures have been associated with decreased manual dexterity, reduced concentration and heightened perception of discomfort, which can challenge students' tolerance and adaptability, particularly during prolonged study periods.[31] This form of environmental stress can subtly yet significantly contribute to the overall stress burden experienced by these students, whose academic rigours already place substantial demands on their cognitive resources.[32] Recognising the

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impact of thermal stress on student well-being and learning efficacy, there is an emerging imperative for academic institutions to consider and regulate classroom temperatures to foster an environment conducive to comfort and optimal learning.[31] This consideration is particularly salient in the context of nursing and medical education, where the synthesis of complex information and the performance of precise clinical skills are paramount.[16, 17]

Academic process

The temporal dimension of academic processes, characterised by prolonged durations of study, is a significant source of stress for nursing, medical and health sciences-related students.[16, 17] The extensive periods required for mastering the expansive curricula of healthcare disciplines can lead to chronic stress, manifesting as both psychological strain and physical exhaustion. The relentless pressure to absorb voluminous medical knowledge within limited timeframes often necessitates extended study sessions, which can disrupt circadian rhythms, impair sleep quality and exacerbate cognitive overload.[33] This sustained intellectual exertion, without adequate rest, can diminish students' capacity for information retention and analytical thinking, critical faculties in the medical field. Furthermore, the constant state of academic engagement can preclude necessary leisure and social activities, contributing to a sense of isolation and burnout.[34] Acknowledging the deleterious effects of such prolonged academic engagement, it is incumbent upon educational institutions to implement pedagogical strategies that optimise learning while minimising stress.[16] Such strategies could include modular curricula, integrated learning approaches and sufficient rest periods, which collectively aim to enhance academic efficiency and preserve the mental and physical well-being of the students.

Acknowledgement

We thank any parties who contributed to this study.

Conflicts of interest

The authors declare that there is no conflict of interest.

Conclusion

Through the bibliometric analysis process, it can be concluded that the triggers of environmental stress among students in the university are grouped into four themes: internal situation in the university (academic stress, air pollution, anxiety in academic process, coronavirus disease, depression, fear, medical students, nursing student, physical activity, school regulation, smoking, uncertainty), external situation outside of university (oxidative stress), comfort situation during study (drought tolerance, low temperature) and academic process (duration). All elements of the university especially policy makers should pay attention to this problem to reduce the risk of stress while students are studying.

Ethics

This study was a secondary analysis based on the currently existing dataset from the *Dimensions*.ai and did not directly involve with human participants or experimental animals. Therefore, the ethics approval was not required in this paper.

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Data access

The data that support the findings of this study are available from the corresponding author upon reasonable individual request.

Author ORCID numbers

Wawan Kurniawan (WK):
0000-0002-2026-7912
Satriya Pranata (SP):
0000-0002-2026-8931

CONTENT

Aric Vranada (AV):
0000-0002-6158-8659
Lalu Muhammad Irham (LMI):
0000-0002-0091-4887
Aat Agustini (AA):
0009-0007-5835-8426

Author contributions

Conceptualisation: SP
Methodology: AV
Validation: WK
Formal analysis: LMI
Investigation: AA
Resources: SP
Data curation: SP
Writing - original draft: WK
Writing - review and editing: AV
Visualisation: LMI
Supervision: WK
Project administration: LMI
Funding acquisition: WK

References

1. Schunk DH. Learning theory: an educational perspective. 6th edition. Boston, MA: Pearson Education, Inc; 2012.
2. Flynn O, Fullen BM, Blake C. Migraine in university students: A systematic review and meta-analysis. Eur J Pain (United Kingdom). 2023;27(1):14-43. doi:10.1002/ejp.2047.
3. Córdova A, Caballero-García A, Drobnic F, Roche E, Noriega DC. Influence of stress and emotions in the learning process: the example of COVID-19 on university students: a narrative review. Healthcare. 2023;11(12):1787. doi:10.3390/healthcare11121787.
4. Tahmassian K, Moghadam NJ. Relationship between self-efficacy and symptoms of anxiety, depression, worry and social avoidance in a normal sample of students. Iran J Psychiatry Behav Sci. 2011;5(2):91-8. PMID: 24644452.
5. Garatti M, Costa R, Reghizzi SC, Rohou E. The impact of alias analysis on VLIW scheduling. In: Zima HP, Joe K, Sato M, Seo Y, Shimasaki M, Eds. High performance computing. ISHPC 2002. Lect Notes Comput Sci. 2002;2327:93-105. doi:10.1007/3-540-47847-7_10.
6. Gray K, Chapman W, Khan UR, Borda A, Budge M, Dutch M, et al. The rapid development of virtual care tools in response to COVID-19: case studies in three Australian health services. JMIR Form Res. 2022 Apr 6;6(4):e32619. doi: 10.2196/32619.
7. Attia M, Ibrahim FA, Elsady MA, Khorkhash MK, Rizk MA, Shah J, et al. Cognitive, emotional, physical, and behavioral stress-related symptoms and coping strategies among university students during the third wave of COVID-19 pandemic. Front Psychiatry. 2022;13:933981. doi:10.3389/fpsyt.2022.933981.
8. Parihar A, Devanathan N, Sepulveda MN, Sevilla-Martir JF. Financial impact of the Indiana University student-run free clinic. J Community Health. 2023 Oct;48(5):878-81. doi: 10.1007/s10900-023-01234-4.
9. Pranata S, Harriami R, Kurnia A, Wulandari H, Sepriani D, Bistara DN. Sociological public perception of the coronavirus disease 2019 situation in Indonesia: A phenomenological study. Iran J Psychiatry Clin Psychol. 2022;28(1):122-37. doi:10.32598/ijpcp.28.1.4082.1.
10. Hsieh M. A preliminary study of bullying on University of Nursing in Taiwan. Eur J Public Health. 2022;32(Suppl 3):ckac131.428. doi:10.1093/eurpub/ckac131.428.
11. Wu YY, Chou WH. A bibliometric analysis to identify research trends in intervention programs for smartphone addiction. Int J Environ Res Public Health. 2023;20(5):3840. doi:10.3390/ijerph20053840.
12. Pahwa B, Goyal S, Chaurasia B. Understanding anterior communicating artery aneurysms: A bibliometric analysis of top 100 most cited articles. J Cerebrovasc Endovasc Neurosurg. 2022 Dec;24(4):325-34. doi: 10.7461/jcen.2022.E2022.01.001.
13. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ. 2021 Mar 29;372:n71. doi: 10.1136/bmj.n71.
14. Van Eck NJ, Waltman L. Software survey: VOSviewer, a computer program for bibliometric mapping. Scientometrics. 2010;84(2):523-38. doi:10.1007/s11192-009-0146-3
15. Mulianda D, Rahmanti A, Margiyati M, Sari NW, Haksara E, Pranata S. Behavioral activation, mindfulness exercises, and loving-kindness meditation exercises as effective therapies to reduce stress among nursing students' during COVID-19 pandemic. Open Access Maced J Med Sci. 2022;10(G):228-32. doi:10.3889/oamjms.2022.8695.
16. Zheng YX, Jiao JR, Hao WN. Stress levels of nursing students: A systematic review and meta-analysis. Medicine (Baltimore). 2022 Sep 9;101(36):e30547. doi: 10.1097/MD.0000000000030547.
17. Abdulghani HM, AlKanhal AA, Mahmoud ES, Ponnamperuma GG, Alfaris EA. Stress and its effects on medical students: a cross-sectional study at a college of medicine in Saudi Arabia. J Health Popul Nutr. 2011;29(5):516-22. doi:10.3329/jhpn.v29i5.8906.
18. Tamulevicius N, Save R, Gandhi N, Lubiak S, Sharma S, Aguado Loi CX, et al. Perceived stress and impact on role functioning in university students with migraine-like headaches during COVID-19. Int J Environ Res Public Health. 2023 Apr 13;20(8):5499. doi: 10.3390/ijerph20085499.
19. Usher Am K, Jackson D, Massey D, Wynaden D, Grant J, West C, et al. The mental health impact of COVID-19 on pre-registration nursing students in Australia: Findings from a national cross-sectional study. J Adv Nurs. 2023;79(2):581-92. doi:10.1111/jan.15478.
20. Sa'diyah H, Rahma Pratiwi EY. The effect of online learning on students learning motivation. IJPSE Indones J Prim Sci Educ. 2021;2(1):11-8. doi:10.33752/ijpse.v2i1.2063.

CONTENT

21. Salari N, Hosseinian-Far A, Jalali R, Vaisi-Raygani A, Rasoulpoor S, Mohammadi M, et al. Prevalence of stress, anxiety, depression among the general population during the COVID-19 pandemic: a systematic review and meta-analysis. Global Health. 2020 Jul 6;16(1):57. doi: 10.1186/s12992-020-00589-w.
22. Bogaert B, Kozlakidis Z, Caboux E, Péron J, Saintingy P. What went right during the COVID crisis: The capabilities of local actors and lasting innovations in oncology care and research. PLOS Glob Public Heal. 2023;3(9):e0002366. doi:10.1371/journal.pgph.0002366.
23. Lyng HB, Ree E, Wibe T, Wiig S. Healthcare leaders' use of innovative solutions to ensure resilience in healthcare during the Covid-19 pandemic: a qualitative study in Norwegian nursing homes and home care services. BMC Health Serv Res. 2021;21(1):878. doi:10.1186/s12913-021-06923-1.
24. Boston-Fleischhauer C. The global pandemic. JONA J Nurs Adm. 2021;51(6):304-6. doi:10.1097/nna.0000000000001017.
25. Desmarais C, Roy M, Nguyen MT, Venkatesh V, Rousseau C. The unsanitary other and racism during the pandemic: analysis of purity discourses on social media in India, France and United States of America during the COVID-19 pandemic. Anthropol Med. 2023;30(1):31-47. doi:10.1080/13648470.2023.2180259.
26. Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, et al. Oxidative stress: harms and benefits for human health. Oxid Med Cell Longev. 2017;2017:8416763. doi: 10.1155/2017/8416763.
27. Sharifi-Rad M, Anil Kumar NV, Zucca P, Varoni EM, Dini L, Panzarini E, et al. Lifestyle, oxidative stress, and antioxidants: back and forth in the pathophysiology of chronic diseases. Front Physiol. 2020 Jul 2;11:694. doi: 10.3389/fphys.2020.00694.
28. Pisoschi AM, Pop A, Iordache F, Stanca L, Predoi G, Serban AI. Oxidative stress mitigation by antioxidants - An overview on their chemistry and influences on health status. Eur J Med Chem. 2021 Jan 1;209:112891. doi: 10.1016/j.ejmech.2020.112891.
29. Rani V, Deep G, Singh RK, Palle K, Yadav UC. Oxidative stress and metabolic disorders: Pathogenesis and therapeutic strategies. Life Sci. 2016 Mar 1;148:183-93. doi: 10.1016/j.lfs.2016.02.002.
30. Moshtagh M, Moodi M, Moezi SA, Sharifi F, Khazdair MR. Inflammatory and oxidative stress biomarkers in the elderly, the Birjand longitudinal aging study. Biomed Res Int. 2023;2023:4683542. doi:10.1155/2023/4683542.
31. Meece JL, Anderman EM, Anderman LH. Classroom goal structure, student motivation, and academic achievement. Annu Rev Psychol. 2006;57:487-503. doi: 10.1146/annurev.psych.56.091103.070258.
32. Troy D, Anderson J, Jessiman PE, Albers PN, Williams JG, Sheard S, et al. What is the impact of structural and cultural factors and interventions within educational settings on promoting positive mental health and preventing poor mental health: a systematic review. BMC Public Health. 2022 Mar 17;22(1):524. doi: 10.1186/s12889-022-12894-7.
33. Boskma ACP, Wolthuis FW, Roelofs PDDM, van Wijlen AT, van Schie JE, de Man-van Ginkel JM, et al. Experienced career perspectives of nursing students and their supervisors in learning departments: a qualitative study. BMC Nurs. 2023 Sep 27;22(1):332. doi: 10.1186/s12912-023-01479-3.
34. Virpi V, Anna-Maria T, Kristina M. Practical nursing students' learning and assessment during work-based placement: A qualitative study. Nurs Open. 2023;10(9):6150-64. doi:10.1002/nop2.1849.

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CURRENT TOPIC

Metformin and Vitamin B_{12} Deficiency – What is the Evidence?

Bruno Daniel Oliveira Peixoto,[1] Mariana Abreu Gonçalves,[2] Teresa Filipa Ramôa Gonçalves,[1] Aníbal Alberto Sá Martins[2]

Abstract

The widespread adoption of metformin as a primary therapeutic agent for type 2 diabetes has prompted inquiries into its potential impact on vitamin B_{12} (cobalamin) levels and subsequent deficiency. This study aimed to elucidate this complex relationship and enhance the care provided to patients undergoing metformin treatment. A comprehensive search of meta-analyses, systematic reviews, randomised controlled trials and guidelines published between January 2010 and September 2021 was conducted. MeSH terms 'metformin' and 'vitamin B_{12},' along with corresponding DeCS terms, guided the search. Varied recommendations from different scientific associations underscore the need for regular monitoring of vitamin B_{12} levels in patients undergoing long-term metformin therapy. Different durations of metformin exposure, spanning from 6 weeks to 48 months, were associated with decreased vitamin B_{12} concentrations. Observed decreases in B_{12} concentrations ranged from 7.7 to 65.8 pmol/L, with percentage reductions ranging from 6.3 % to over 35 %. The evidence highlights a dosage-dependent correlation between higher metformin doses and an increased prevalence of B_{12} deficiency. The results obtained highlight the association between metformin and B_{12} deficiency. The prevalence of B_{12} deficiency under metformin is of a greater magnitude than the one declared on the Summary of Product Characteristics approved by the medicine regulatory agencies. Thus, clinicians should be aware of this possible side effect when prescribing metformin, in order to prevent, monitor and treat if present.

Key words: Metformin; Vitamin B_{12}; Diabetes mellitus.

1. USF Nova Estação, ACeS Ave/Famalicão, Braga, Portugal.
2. USF Antonina, ACeS Ave/Famalicão, Braga, Portugal.

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Corresponding author:
BRUNO OLIVEIRA PEIXOTO
E: brunopeixxoto@hotmail.com

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Introduction

The World Health Organization (WHO) estimates that approximately 463 million adults had diabetes in 2019, a number which is expected to increase to 700 million by 2045. Type 2 diabetes, characterised by insulin resistance and impaired glucose regulation, is associated with family history, sedentary lifestyle and obesity.[1]

The diabetes treatment involves a multifactorial risk-reduction strategy, that includes pharmacological treatment. Metformin is generally one of the first-line therapy options due to its favourable safety profile, glucose-lowering efficacy and potential cardiovascular benefits. However, questions about its potential side effects have arisen, since emerging evidence suggests that metformin may interfere with the absorption of vitamin B_{12} (cobalamin) by affecting the gastrointestinal tract and intrinsic factor secretion, altering gut microbiota and increasing renal excretion of vitamin B_{12}.[2] This emphasises the need for monitoring of B_{12} levels in long-term metformin users due to potential deficiency-related complications.

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CONTENT

As vitamin B_{12} deficiency is associated with a spectrum of clinical manifestations, including anaemia, neuropathy and cognitive impairments, a comprehensive review of the current literature is imperative to elucidate the nature and extent of the association between metformin use and vitamin B_{12} deficiency.[3–6]

By synthesising available research findings, this review aimed to assess the current state of knowledge regarding the relationship between these two factors, thus informing clinical practice and guiding future research directions in the realm of diabetes management and patient well-being.

Methods

The authors used the PICO approach outlined by O'Connor et al to perform this evidence-based review, which acronym enables the review questioned to be performed in terms of the population (P), intervention (I), comparator (C) and outcome (O).

The population included adults of both sexes medicated with metformin, due to the diagnosis of diabetes, pre-diabetes or polycystic ovarian syndrome. The therapeutic intervention consisted of comparing the use of metformin with placebo, another drug or not taking medication. The primary outcome was vitamin B_{12} deficiency.

The authors excluded articles with paediatric populations, patients with prior gastrointestinal surgery, intrinsic factor deficiency, inflammatory bowel disease or celiac disease. The exclusion criteria also included duplicated articles, opinion articles and articles that were not consonant with the objective of the review. Thus, the Medical Subject Headings (MeSH) words selected from the Pubmed's MeSH Database were 'metformin' and 'vitamin B_{12}'. These MeSH words were used to search for synopses, guidelines, meta-analyses, systematic reviews and original papers, published between January 2010 and September 2021 in the databases *MEDLINE, National Guideline Clearinghouse, National Institute for Health Care and Excellence, Canadian Medical Association Practice Guidelines InfoBase, TRIP Database, the Cochrane Library, DARE, Bandolier* and *Index de Revistas Médicas Portuguesas* in English and Portuguese.

The strength of recommendation taxonomy (SORT) scale, from the American Academy of Family Physicians, was used to determine the level of evidence and strength of recommendation.

Results were standardised for comparison, converting time variables to months and vitamin B_{12} concentrations to pmol/L.

Results

The initial search identified a total of 85 results, of which 74 were obtained after removing duplicates. Of these, 46 were excluded after reading the title, 8 after reading the abstract and 3 after reading the full article. The results are summarised in Table 1.

The American Diabetes Association (ADA) and European Association for the Study of Diabetes (EASD) recommend routine vitamin B_{12} monitoring for long-term metformin users, without specifying exposure times or metformin doses. The Endocrine Society (ES), American Association of Clinical Endocrinology (AACE) and American College of Endocrinology (ACE) suggest assessing B_{12} levels for those with neuropathy symptoms.[2, 7–9]

The International Society of Nephrology (ISN) advises B_{12} evaluation after 4 years of metformin use or in high-risk individuals (eg, patients with malabsorption syndrome or reduced dietary intake [vegans]).[10] Furthermore, the Canadian Diabetes Association (CDA) suggests periodic B_{12} measurements in metformin users or individuals with signs or symptoms of deficiency (such as impaired proprioception or peripheral neuropathy).[11]

Correlation between metformin exposure time and decreased vitamin B_{12} concentration

Time

While the duration of the analysed studies varied, differences in B_{12} concentration were found after a minimum of 6 weeks of metformin exposure, with ranges from 14.89 ($p < 0.119$) to 19.7 pmol/L ($p = 0.004$).[12, 13]

Some studies identified variances in B_{12} concentrations after a 3-month period of metformin use

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Table 1: Summary of the information found in the systematic reviews and original studies

	Articles	N	Time (M)	Dose (mg)	% deficit	Δ B_{12} (pmol/L)	Δ B_{12} %	Risk	NNH	SORT	Observations
Systematic reviews	Niafar et al[19]	7,611	N/A	N/A	N/A	65.80 p < 0.00001*	N/A	OR: 2.45 p < 0.00001*	N/A	C	
	Yang et al[18]	5,500	36	N/A	N/A	63.70 p < 0.00001*	14.70 % p < 0.00001*	RR: 2.09 p < 0.00001*	N/A	C	Annual monitoring of vitamin B_{12} is recommended in patients receiving metformin.
	Chapman et al[22]	14,945	48	N/A	N/A	57.10 p < 0.001*	19 % p < 0.001*	N/A	N/A	C	It is prudent to monitor B12 in patients who are at increased risk of deficiency.
	Liu et al[6]	N/A	N/A	N/A	N/A	53.93 p = 0.0001*	N/A	N/A	N/A	C	
	Li et al[21]	218	N/A	N/A	N/A	24.70 p = 0.31	N/A	N/A	N/A	C	
Original studies	Aroda et al[24]	2,150	60.0 156.0	1,700	4.3 % vs 2.3 % p = 0.03* 7.4 % vs 5.4 % p = 0.13	N/A N/A	N/A N/A	OR: 1.13	N/A	3	
	Jager et al[26]	390	48.0	2,500	N/A	N/A	19 % p < 0.001*	1AR : 7.20 p = 0.004*	13.8	3	
	Lohmann et al[16]	500	6.0	1,700	N/A	51	6.30 %	N/A	N/A	3	Monitoring vitamin B_{12} on a regular basis may be prudent.
	Sahin et al[12]	165	1.5	1,700	N/A	14.89 p < 0.119	N/A	N/A	N/A	3	
	Mastroianni et al[25]	165	36.0	1,700	32 % p < 0.02*	N/A	N/A	N/A	N/A	3	Monitor at baseline and during treatment routinely.
	Leung et al[14]	20	3.0	N/A	N/A	N/A	6.30 % p = 0.04*	N/A	N/A	3	
	Griffin et al[20]	249	6.0	N/A	N/A	7.70	ND	N/A	N/A	3	
	Gatford et al[13]	180	1.5	≤ 2,500	N/A	19.70 p = 0.004*	ND	N/A	N/A	3	
	Hassan et al[15]	1,200	3.0	1,000	N/A	ND	35 % p < 0.01*	N/A	N/A	3	
	Hansen et al[17]	412	18.0	N/A	N/A	19.90 p < 0.01*	N/A	N/A	N/A	3	
	Kancherla et al[23]	16,945	6.0	≥ 500	7 % vs 3 % p < 0.0001*	N/A	N/A	N/A	N/A	3	Clinically based vitamin B_{12} monitoring should be promoted.

AR - Absolute risk; M - months; N/A - Not applicable; NNH - Number needed to harm; OR - Odds ratio; RR - Relative risk; SORT - Strength of recommendations taxonomy;

(6.3 %, p = 0.04, Leung et al) (35 %, p < 0.01, Hassan et al) and others found a similar variation after a 6-month period (6.3 %, Lohmann et al).[14–16] Furthermore, Hansen et al identified a 19.9 pmol/L variance (p < 0.01) after 18 months.[17]
In a longer approach (48 months), Hassan et al also found a 19 % (p < 0.01) variation in B_{12} concentration, similar to the systematic reviews of Yang et al at 36 months (14.7 %, p < 0.0001) and Chapman (19 %, p < 0.001) at 48 months.[15, 18]

B_{12} concentration variation

Serum B_{12} levels were assessed based on concentration or relative variation in the reviewed studies. Findings indicated a decrease in B_{12} concentration between 7.7 pmol/L (Griffin et al, Sahin et al) and 65.8 pmol/L (p < 0.0001, Niafar et al).[12, 19, 20] Original studies reported a variation from 7.7 pmol/L to 51 pmol/L while systematic reviews showed a variation from 24.7 pmol/L (p = 0.31, Li et al) to 65.8 pmol/L (Niafar et al).[12, 16, 19, 21]

In terms of percentage, reductions in B_{12} were noted from 35 % (p < 0.01, Hassan et al) to 6.3 % (Lohman et al and the Leung et al), with systematic reviews indicating reductions below 20 % (14.7, Yang et al and 19 %, Chapman et al).[18, 22]

B_{12} deficiency

More significant than changes in B_{12} concentration is the detection of deficiency, due to its potential health and quality of life implications.

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Kancherla et al found a statistically significant difference among groups, with a 7 % prevalence of B_{12} deficiency in 16,945 patients treated with metformin for 6 months at doses as low as 500 mg, compared to 3 % in the non-metformin group.[23]

Another study reported a 4.3 % prevalence of B_{12} deficiency after 60 months in patients treated with metformin at an average dose of 1700 mg per day, contrasting with 2.3 % in the non-metformin group ($p = 0.03$). After 156 months, the prevalence of B_{12} deficiency was 7.4 % in the metformin group versus 5.4 % in the non-metformin group ($p = 0.13$).[24] Mastroianni et al identified the highest prevalence of B_{12} deficiency (32 %, $p < 0.02$) in 165 patients receiving the same daily dose of metformin.[25]

B_{12} deficiency and its association with metformin dose

Metformin dose across studies ranged from 500 to 2500 mg per day. Kancherla et al found a 7 % prevalence of B_{12} deficiency among patients using at least 500mg of metformin daily for 6 months.[23] Other studies showed that daily use of metformin at doses greater to or exceeding 1700 mg were linked to vitamin B_{12} deficiency after 36 or 60 months (Mastroianni et al and Aroda et al, respectively).[24, 25]

Risk measurements: odds ratio, relative risk and absolute risk

Niafar et al observed a greater prevalence of B_{12} deficiency in the metformin group (OR = 2.45, $p < 0.0001$), while Aroda et al found a heightened risk (OR = 1.13).[19, 24]

Yang et al reported a significantly increased risk of vitamin B_{12} deficiency among metformin users (RR 2.09, $p < 0.0001$).[18] Jager et al demonstrated a 7.2 percentage point higher absolute risk of vitamin B_{12} deficiency ($p = 0.004$), with a number needed to harm of 13.8.[26]

Discussion

In presented study, a prevalence of vitamin B_{12} deficiency of 25.3 % among patients with type 2 diabetes receiving metformin therapy was identified. This high prevalence underscores the imperative need to promptly address this deficiency to alleviate potential symptoms and mitigate overall health repercussions. The observed prevalence aligns with findings from other studies, which report a range of B_{12} deficiency between 6 % and 30 %.

Kim et al identified a prevalence of 22.2 % in a study involving 1111 patients, while Aroda et al, in a prospective study with 1073 participants, reported a prevalence of 19.1 % after 5 years and 20.3 % after 13 years of metformin usage.[10, 11] Additionally, the National Health and Nutrition Examination Survey demonstrated that 41 % of B_{12} deficiency cases among individuals with diabetes were attributable to metformin use.[12] In a Korean study, the prevalence of vitamin B_{12} deficit was lower (9.5 %), emphasising the influence of population differences as a potential bias.

Regarding the duration of metformin use, some studies suggest a cutoff of 4 years for detecting B_{12} deficiency.[13] In presented study, patients with B_{12} deficiency were identified after just 1 year of metformin use. The mean duration under metformin for the B_{12} deficiency group was 5.33 years, consistent with previous data.

The dosage of metformin is also a significant consideration in various studies. For instance, Kim et al reported a decrease in vitamin B_{12} levels by 0.142 pg/mL with a 1 mg increase in metformin, while Beulens et al found a decrease of 0.042 pg/mL.[10, 14] In presented study, doses as low as 500 mg per day were associated with B_{12} deficiency and the majority of the deficiency group (55 %, n = 11) had prescribed doses exceeding 2000 mg/day, aligning with prior data.

In future studies, aim is to replicate these findings on a multicentre or national level to enhance the robustness of presented conclusions. Additional secondary outcomes, including folic acid levels, homocysteine levels, and methylmalonyl-CoA mutase, can also be explored to deepen understanding of B_{12} deficiency.

B_{12} deficiency presents with varied symptoms that may mislead doctors and patients, as neurologic symptoms (characterised by decreased position and vibratory sensation in the extremities accompanied by mild to moderate weakness and hyporeflexia, that may develop in a stocking-glove distribution). It can mimic the diabetic foot symptoms, leading to unnecessary therapy and investigation. Other symptoms such as irri-

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tability, depression, weight loss and poorly localised abdominal pain may occur, leading to poor quality of life.[15]

Correcting this disorder is simple, as various B_{12} supplement formulations are available and haematologic abnormalities are usually corrected within 6 weeks. However, doctors should be aware that neurologic symptoms may take much longer and may even become irreversible if they persist for months or years.[15]

Conclusion

The significance of presented results, revealing a 25.3 % prevalence of B_{12} deficiency in patients under metformin, emphasises the importance of physician awareness and proactive management of this side effect to minimise possible symptoms of the patients that may diminish their quality of life.

Etics

The study was approved by the Ethic Committee of the *Administração Regional de Saúde do Norte* (Northern Regional Health Administration), decision No CE/2024/1, dated 4 January 2024.

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Conflicts of interest

The authors declare that there is no conflict of interest.

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Data access

The data that support the findings of this study are available from the corresponding author upon reasonable individual request. Consent statement and permission obtained by the Technical Committee of USF Nova Estação.

Author ORCID numbers

Bruno Daniel Oliveira Peixoto (BDOP):
0000-0003-4694-1044
Mariana Abreu Gonçalves (MAG):
0000-0002-3236-367X
Ana Isabel Ferreira da Costa (AIFdC):
0000-0001-7234-2876
Teresa Filipa Ramôa Gonçalves (TFRG):
0009-0008-6848-1556

Author contributions

Conceptualisation: BDOP, MAG, AIFdC, TFRG
Methodology: BDOP, MAG, AIFdC, TFRG
Validation: BDOP, MAG
Formal analysis: BDOP, MAG, AIFdC, TFRG
Investigation: BDOP, MAG
Data curation: BDOP, MAG
Writing - original draft: BDOP, MAG
Writing - review and editing: BDOP, MAG, AIFdC, TFRG
Visualisation: BDOP, MAG
Supervision: BDOP, MAG, AIFdC, TFRG
Project administration: BDOP, MAG

References

1. World Health Organization. Diabetes- Key facts.[Internet]. [Cited: 20-Mar-2021]. Available at: https://www.who.int/news-room/fact-sheets/detail/diabetes/.
2. American Diabetes Association. Standards of medical care in diabetes-2020 abridged for primary care providers. Clin Diabetes. 2020 Jan;38(1):10-38. doi: 10.2337/cd20-as01.
3. Reinstatler L, Qi YP, Williamson RS, Garn JV, Oakley GP Jr. Association of biochemical B_{12} deficiency with metformin therapy and vitamin B_{12} supplements: the National Health and Nutrition Examination Survey, 1999-2006. Diabetes Care. 2012 Feb;35(2):327-33. doi: 10.2337/dc11-1582.

CONTENT

4. O'Leary F, Samman S. Vitamin B12 in health and disease. Nutrients. 2010 Mar;2(3):299-316. doi: 10.3390/nu2030299.
5. Tavares Bello C, Capitão RM, Sequeira Duarte J, Azinheira J, Vasconcelos C. [Vitamin B12 Deficiency in Type 2 Diabetes Mellitus]. Acta Med Port. 2017 Oct 31;30(10):719-26. Portuguese. doi: 10.20344/amp.8860..
6. Liu Q, Li S, Quan H, Li J. Vitamin B12 status in metformin treated patients: systematic review. PLoS One. 2014 Jun 24;9(6):e100379. doi: 10.1371/journal.pone.0100379.
7. Davies MJ, D'Alessio DA, Fradkin J, Kernan WN, Mathieu C, Mingrone G, et al. Management of hyperglycemia in type 2 diabetes, 2018. a consensus report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). Diabetes Care. 2018 Dec;41(12):2669-701. doi: 10.2337/dci18-0033.
8. LeRoith D, Biessels GJ, Braithwaite SS, Casanueva FF, Draznin B, Halter JB, et al. Treatment of diabetes in older adults: an endocrine society* clinical practice guideline. J Clin Endocrinol Metab. 2019 May 1;104(5):1520-74. doi: 10.1210/jc.2019-00198.
9. Garber AJ, Abrahamson MJ, Barzilay JI, Blonde L, Bloomgarden ZT, Bush MA, et al; American Association of Clinical Endocrinologists (AACE); American College of Endocrinology (ACE). Consensus statement by the American Association of Clinical Endocrinologists and American College of Endocrinology on the comprehensive type 2 diabetes management algorithm--2016 executive summary. Endocr Pract. 2016 Jan;22(1):84-113. doi: 10.4158/EP151126.
10. Kidney Disease: Improving Global Outcomes (KDIGO) Diabetes Work Group. KDIGO 2020 Clinical Practice Guideline for Diabetes Management in Chronic Kidney Disease. Kidney Int. 2020 Oct;98(4S):S1-S115. doi: 10.1016/j.kint.2020.06.019.
11. Diabetes Canada Clinical Practice Guidelines Expert Committee; Lipscombe L, Booth G, Butalia S, Dasgupta K, Eurich DT, Goldenberg R, et al. Pharmacologic glycemic management of type 2 diabetes in adults. Can J Diabetes. 2018 Apr;42 Suppl 1:S88-S103. doi: 10.1016/j.jcjd.2017.10.034.
12. Sahin M, Tutuncu NB, Ertugrul D, Tanaci N, Guvener ND. Effects of metformin or rosiglitazone on serum concentrations of homocysteine, folate, and vitamin B12 in patients with type 2 diabetes mellitus. J Diabetes Complications. 2007 Mar-Apr;21(2):118-23. doi: 10.1016/j.jdiacomp.2005.10.005.
13. Gatford KL, Houda CM, Lu ZX, Coat S, Baghurst PA, Owens JA, et al. Vitamin B12 and homocysteine status during pregnancy in the metformin in gestational diabetes trial: responses to maternal metformin compared with insulin treatment. Diabetes Obes Metab. 2013 Jul;15(7):660-7. doi: 10.1111/dom.12080.
14. Leung S, Mattman A, Snyder F, Kassam R, Meneilly G, Nexo E. Metformin induces reductions in plasma cobalamin and haptocorrin bound cobalamin levels in elderly diabetic patients. Clin Biochem. 2010 Jun;43(9):759-60. doi: 10.1016/j.clinbiochem.2010.02.011.
15. Hassan MH, Abd-Allah GM. Effects of metformin plus gliclazide versus metformin plus glimepiride on cardiovascular risk factors in patients with type 2 diabetes mellitus. Pak J Pharm Sci. 2015 Sep;28(5):1723-30. PMID: 26408873.
16. Lohmann AE, Liebman MF, Brien W, Parulekar WR, Gelmon KA, Shepherd LE, et al; From the CCTG, Alliance, SWOG, ECOG, NSABP Cooperative Groups. Effects of metformin versus placebo on vitamin B12 metabolism in non-diabetic breast cancer patients in CCTG MA.32. Breast Cancer Res Treat. 2017 Jul;164(2):371-8. doi: 10.1007/s10549-017-4265-x.
17. Hansen CS, Jensen JS, Ridderstråle M, Vistisen D, Jørgensen ME, Fleischer J. Vitamin B12 deficiency is associated with cardiovascular autonomic neuropathy in patients with type 2 diabetes. J Diabetes Complications. 2017 Jan;31(1):202-8. doi: 10.1016/j.jdiacomp.2016.08.025.
18. Yang W, Cai X, Wu H, Ji L. Associations between metformin use and vitamin B12 levels, anemia, and neuropathy in patients with diabetes: a meta-analysis. J Diabetes. 2019 Sep;11(9):729-43. doi: 10.1111/1753-0407.12900.
19. Niafar M, Hai F, Porhomayon J, Nader ND. The role of metformin on vitamin B12 deficiency: a meta-analysis review. Intern Emerg Med. 2015 Feb;10(1):93-102. doi: 10.1007/s11739-014-1157-5.
20. Griffin SJ, Bethel MA, Holman RR, Khunti K, Wareham N, Brierley G, et al. Metformin in non-diabetic hyperglycaemia: the GLINT feasibility RCT. Health Technol Assess. 2018 Apr;22(18):1-64. doi: 10.3310/hta22180.
21. Li X, Fang Z, Yang X, Pan H, Zhang C, Li X, et al. The effect of metformin on homocysteine levels in patients with polycystic ovary syndrome: A systematic review and meta-analysis. J Obstet Gynaecol Res. 2021 May;47(5):1804-16. doi: 10.1111/jog.14725.
22. Chapman LE, Darling AL, Brown JE. Association between metformin and vitamin B12 deficiency in patients with type 2 diabetes: A systematic review and meta-analysis. Diabetes Metab. 2016 Nov;42(5):316-27. doi: 10.1016/j.diabet.2016.03.008.
23. Kancherla V, Elliott JL Jr, Patel BB, Holland NW, Johnson TM 2nd, Khakharia A, et al. Long-term metformin therapy and monitoring for vitamin b12 deficiency among older veterans. J Am Geriatr Soc. 2017 May;65(5):1061-6. doi: 10.1111/jgs.14761.
24. Aroda VR, Edelstein SL, Goldberg RB, Knowler WC, Marcovina SM, Orchard TJ, et al; Diabetes Prevention Program Research Group. Long-term metformin use and vitamin b12 deficiency in the diabetes prevention program outcomes study. J Clin Endocrinol Metab. 2016 Apr;101(4):1754-61. doi: 10.1210/jc.2015-3754.
25. Mastroianni A, Ciniselli CM, Panella R, Macciotta A, Cavalleri A, Venturelli E, et al. Monitoring vitamin b12 in women treated with metformin for primary prevention of breast cancer and age-related chronic diseases. Nutrients. 2019 May 7;11(5):1020. doi: 10.3390/nu11051020.
26. de Jager J, Kooy A, Lehert P, Wulffelé MG, van der Kolk J, Bets D, et al. Long term treatment with metformin in patients with type 2 diabetes and risk of vitamin B-12 deficiency: randomised placebo controlled trial. BMJ. 2010 May 20;340:c2181. doi: 10.1136/bmj.c2181.

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PROFESSIONAL ARTICLE

Radiological Changes in Leprosy Patients With Disabilities and Deformities of Hands and Feet

Sunakshi Sharma,[1] Vikas Malgotra,[2] Mashkoor Wani[1]

1. Department of Dermatology, Government Medical College, Jammu, India.
2. Department of Dermatology, Government Medical College, Udhampur, India.

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Corresponding author:
VIKAS MALGOTRA
E: drvikas2911@gmail.com

Abstract

Background/Aim: Leprosy (Hansen's disease) affects the skin and nerves. Hands and feet are commonly involved in all types of leprosy often leading to deformities. This study explored radiological changes in leprosy patients with hand and foot disabilities/deformities.

Methods: Observational retrospective study was the chosen design. Study was conducted on 50 leprosy patients presenting at a tertiary care hospital with hand and foot disabilities during 2020-2022. Leprosy types were clinically diagnosed, confirmed by acid-fast bacilli staining. Bacteriological index (BI) and morphological index (MI) were calculated *via* Ridley's scale. Histopathological examination of the skin lesions was also conducted. Radiological exams, anteroposterior and lateral X-rays, identified specific/non-specific bone changes.

Results: Mean age was 38.8 years, bone changes identified at 40.3 years. Radiological changes were seen in 42.0 % patients (34 % patients had non-specific and 16 % had specific changes). These changes were seen more common and earlier in females as compared to males, the difference however was not significant (p = 0.6). Mean BI (2.8) and MI (32 %) of the patients with bone changes were slightly higher than those without bone changes but the differences were not statistically significant (2.2 and 27 %) (p = 0.2). Common specific changes were periostitis and subarticular erosion while osteopenia and phalangeal resorption were the most common non-specific changes.

Conclusion: Non-specific bone changes were more common than specific ones and in females as compared to males. Lepromatous leprosy (LL) was associated with maximum bone changes with varied involvement in other types of leprosy.

Key words: Leprosy bone changes X-ray; Bone changes in leprosy; Leprosy radiological changes; Leprosy specific and non-specific changes.

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Introduction

Leprosy, also known as Hansen's disease, is a chronic granulomatous infectious disease caused by *Mycobacterium leprae*. It primarily affects the skin and nerves, with nerve damage leading to sensory/motor impairments, deformities and disabilities leading to substantial social and economic consequences.

Systemic manifestations of leprosy have been well-documented in the literature. In addition

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to the skin and peripheral nerves, other tissues commonly affected by leprosy include bones, eyes, upper respiratory tract, kidneys, liver and testes. The occurrence of bone changes in leprosy has been reported to vary significantly in different studies. Hands and feet are commonly involved in all types of leprosy. According to a study on bone changes, approximately 25 % of untreated leprosy patients may develop deformities in their hands and feet, while about 80 % of joint lesions may involve metatarsophalangeal joints of the foot or the inter-phalangeal joints of the hands and feet.[1]

The main focus of this study was to analyse the radiological changes observed in the hands and feet of leprosy patients with deformities.

Methods

Retrospective observational study was performed. Fifty leprosy patients with disabilities of hands and feet who attended the leprosy clinic were included in this observational retrospective study conducted at the Department of Dermatology at the Tertiary Care Hospital, Jammu, India, over 24 months (2020-2022). All the patients had grade I disability with 12 patients additionally having grade II disability (deformity) of hands and feet. Ethical clearance for the study protocol was obtained from the institutional ethical committee and informed consent was taken from all the patients.

The provisional diagnoses of the patients were classified into different types of leprosy based on the clinical examination: lepromatous (LL), borderline lepromatous (BL), borderline (BB), borderline tuberculoid (BT), tuberculoid (TT) and pure neuritic leprosy (PNL). To confirm the type of leprosy, acid-fast bacilli (AFB) were demonstrated through slit skin smears. The bacteriological index (BI) and morphological index (MI) were calculated according to Ridley's scale. Moreover, a histopathological examination of the skin lesions in cases of lepromatous, borderline and tuberculoid leprosy was conducted.

In addition to clinical and histopathological assessments, radiological examinations were performed on the selected patients. X-rays, including antero-posterior (AP) and lateral of the patients' hands and feet, were thoroughly studied to identify any bone changes. These changes were further classified into specific and non-specific categories.

Statistical analysis was conducted on the collected data using appropriate methods. Descriptive data, including numbers and percentages, were analysed for all the categories examined in the study.

Results

Mean age of patients was 38.8 ± 11.6 years. Bone changes were seen at a mean age of 40.3 years. This study involved 35 males and 15 females with lepromatous leprosy (LL) accounting for the maximum number of patients (20) followed by BL (12), BT (11), BB (3) and PNL (4) (Figure 1). However, bone changes were seen in 53 % females and 37 % males. Bone changes were seen in females at a mean age of 38.6 ± 16.1 years and in males at 41.4 ± 8.9 years ($p = 0.6$). Mean BI (2.8) and MI (32 %) of the patients with bone changes were slightly higher than those without bone changes but the differences were not statistically significant (2.2 and 27 %) ($p = 0.2$).

Radiological changes were seen in 21 patients (42 %). Specific radiological changes were present in 8 patients (16 %) and non-specific changes were seen in 17 patients (34 %). Primary periostitis and subarticular erosion were the most common specific radiological finding present in 4 (8 %) and 3 (6 %) patients respectively (Table 1). Osteopenia and resorption of terminal phalanges were the most common non-specific findings in study seen in 5 (10 %) and 4 (8 %) patients, respectively.

The maximum number of patients with bone changes had LL (13 patients or 26 %). Other types of leprosy in patients having bone changes with different frequency were: BL - 5 (10 %), PNL - 2 (4 %), BB - 1 (2 %). Specific bony changes were seen in multibacillary patients, while the non-specific changes were seen in paucibacillary and multibacillary patients (Figure 2). Some examples of bone changes are shown in Figure 3 and Figure 4.

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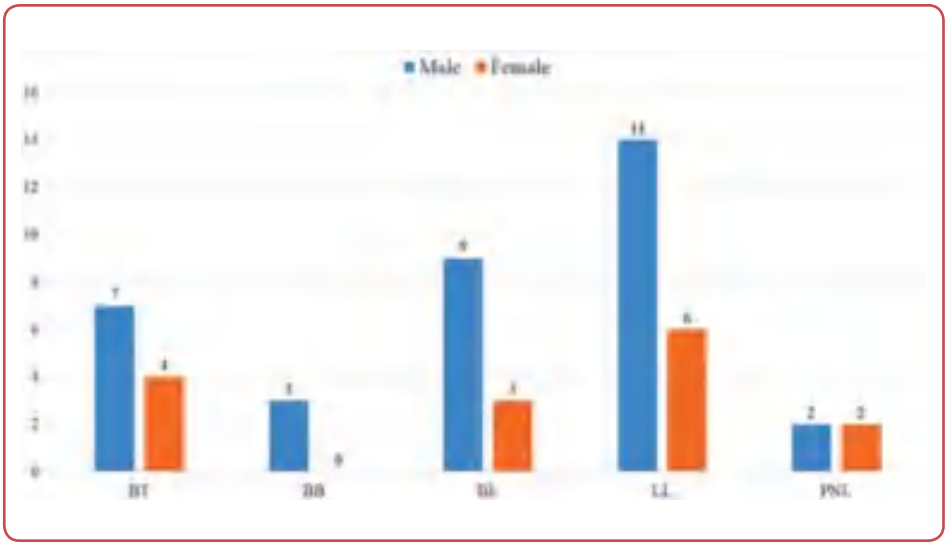

Figure 1: Gender distribution of different types of leprosy seen in the patients.

LL: lepromatous; BL: borderline lepromatous; BB: borderline; BT: borderline tuberculoid; TT: tuberculoid; PNL: pure neuritic leprosy;

Table 1: Presented bone deformities in patients with leprosy

Type	Deformity	Total	Hands	Feet	Hands and feet
Specific	Primary periostitis	4	2	1	1
	Subarticular erosion	3	3	-	-
	Honeycombing	2	-	-	2
	Sclerosis	2	-	2	-
	Areas of bone destruction	1	-	1	-
Non-specific	Osteopenia (periarticular/diffuse)	5	5	-	-
	Resorption of terminal phalanges	4	2	1	1
	Bone erosions	3	2	1	-
	Joint subluxation/ dislocation	3	3	-	-
	Joint contractures	3	-	3	-
	Soft tissue thickening	2	2	-	-
	Secondary periostitis	2	1	-	1
	Hitchhiker's deformity	1	1	-	-
	Joint destruction	1	1	-	-

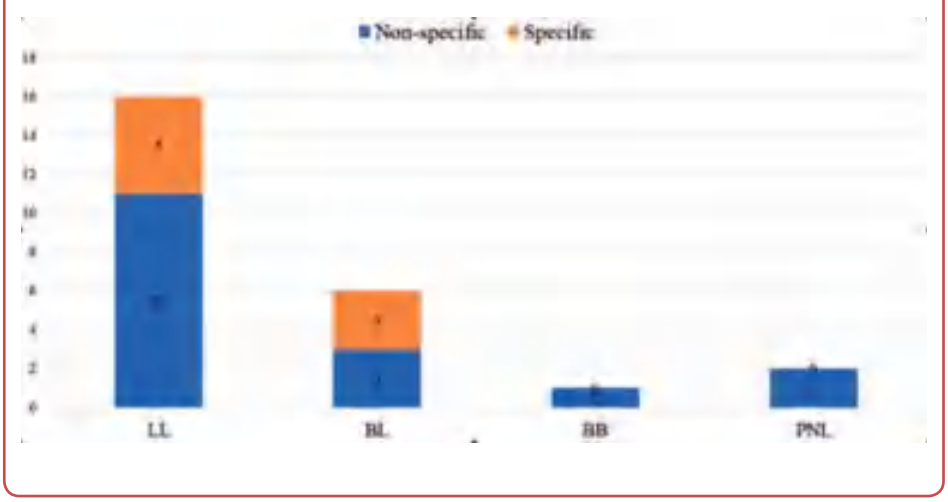

Figure 2: Types of bone changes seen in the studied patients

LL: lepromatous; BL: borderline lepromatous; BB: borderline; BT: borderline tuberculoid; TT: tuberculoid; PNL: pure neuritic leprosy;

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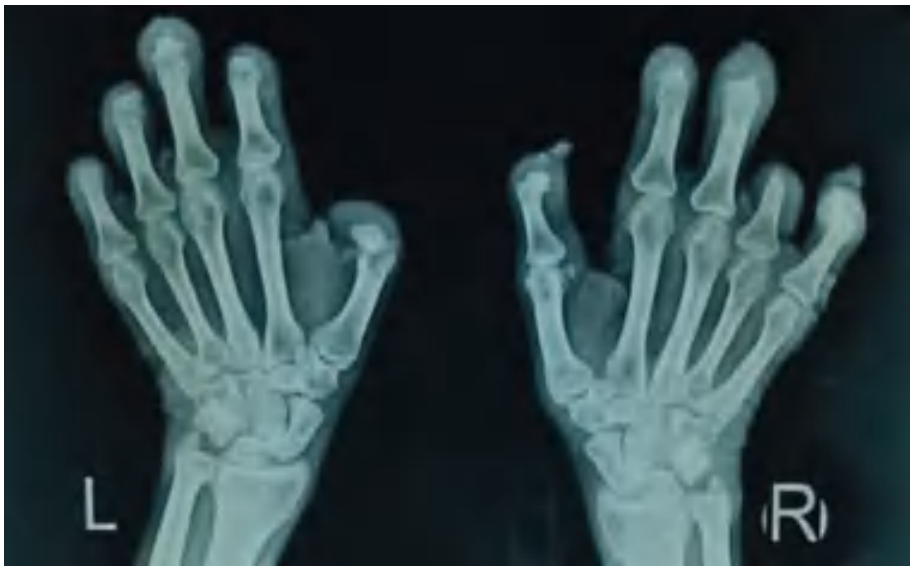

Figure 3: X-ray of hands and wrist joint (AP) of a patient with lepromatous leprosy (LL) showing: complete resorption of all terminal phalanges of both hands, partial resorption of all middle phalanges of both hands except the 4th digit of the right hand which shows complete resorption along with partial resorption of proximal phalanx. Soft tissue thickening also seen in digits of both hands with periarticular osteopenia.

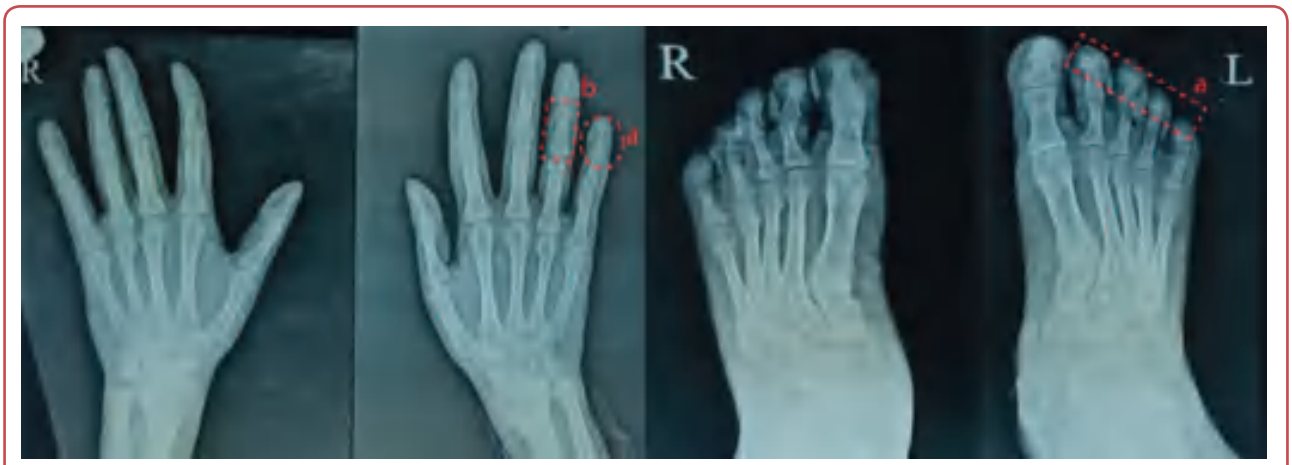

Figure 4: X-ray of the hands and feet (AP) showing: a) resorption of terminal phalanx of 5th digit with subarticular erosion of middle phalanx of left hand, resorption of most of the terminal phalanges of both feet; b) periarticular osteopenia seen in multiple interphalangeal joints of both feet; c) osteoporotic changes associated with cortical thinning seen in middle phalanges of 2nd, 3rd, 4th and 5th digits and metacarpals of left hand.

Discussion

This study aimed to analyse the radiological changes observed in the hands and feet of leprosy patients with deformities. The mean BI and MI of patients with bone changes were slightly higher than those without bone changes but the differences were not statistically significant. This suggests that the presence of bone changes were not strongly associated with bacterial or morphological indices. In addition, bone changes were seen more commonly and at an earlier mean age (38.6 years) in females as compared to males (41.4 years), the difference however was not significant.

CONTENT

Bone changes in leprosy may be specific- when they occur due to direct invasion by *Mycobacterium leprae* or non-specific when they occur indirectly due to sensory impairments, repeated trauma, trophic changes and restricted movements of muscles. Radiological changes were detected in 21 out of the 50 patients, resulting in an overall incidence of bone changes at 42.0 %, which was lower than reported in studies conducted by Choudhuri et al (87.3 %) and Thappa et al (82.9 %).[2, 3] The most common specific radiological finding was primary periostitis and subarticular erosion, whereas osteopenia (subperiosteal or diffuse) and resorption of terminal phalanges were the most common non-specific changes observed in presented study. Specific bony changes were seen in multibacillary patients, while the non-specific changes were seen in paucibacillary and multibacillary patients.

Specific bone changes in leprosy have been noted to be rare with an estimated prevalence of 3 % to 5 % among hospitalised patients.[4] However, an increased incidence of specific changes was noted (16 %) perhaps because this study was conducted on patients having disabilities/deformities. In addition, non-specific radiological changes were two times more common (34 %) than specific changes (16 %), the results being consistent with previously conducted studies.[2, 5, 6] Regarding the distribution of radiological changes across different types of leprosy, this study revealed interesting patterns. Lepromatous leprosy (LL) was associated with the highest number of patients (13 patients or 26 %) exhibiting radiological alterations. This aligns with previous studies that have consistently shown a higher prevalence of bone involvement and deformities in lepromatous leprosy.[7] Among the other types of leprosy, 3 patients (6 %) with borderline lepromatous (BL) leprosy exhibited specific changes, while 3 patients (6 %) displayed non-specific changes. There was no patient with a diagnosis of TT in presented study. In addition, patients with BT leprosy did not have any bone involvement. Moreover, LL and BL accounted for the highest percentage of patients (32 %) with bone changes. These findings are in agreement with the fact that bone involvement in paucibacillary leprosy (PB) is relatively uncommon and indicate the importance of considering leprosy type when evaluating bone pathology.

The observed mean age of patients in presented study was 38.8 ± 11.6 years and bone changes were seen at a mean age of 40.3 years. While age has been identified as a contributing factor to the development of deformities in leprosy patients, study did not specifically explore age-related differences in radiological changes. Further investigations with larger sample sizes and age-stratified analyses would provide more comprehensive insights into the relationship between age and radiological changes in leprosy patients.

Conclusion

This study revealed that while specific radiological changes were relatively rare, they occurred with a notable incidence in patients with disabilities/deformities, particularly in those with multibacillary forms of leprosy. Non-specific changes, on the other hand, were more prevalent and occurred in both paucibacillary and multibacillary patients, underlining the broader impact of the disease beyond the direct effects of the *Mycobacterium leprae* infection. It highlights slight differences in gender with a higher incidence noted in female patients, although this difference was not statistically significant. In conclusion, this study contributes to the understanding of the radiological manifestations of leprosy in affected patients and underscores the importance of comprehensive diagnostic evaluations in the management of this debilitating disease.

Ethics

The study was approved by the Institutional Review Board at GMC Jammu (decision No IEC/GMC/2020/247, dated 20 October 2020). Written informed consent was obtained before the study. The procedures adhered to the ethical guidelines of the Declaration of Helsinki.

Acknowledgement

None.

CONTENT

Conflicts of interest

The authors declare that there is no conflict of interest.

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Data access

The data that support the findings of this study are available from the corresponding author upon reasonable individual request.

Author ORCID numbers

Sunakshi Sharma (SM):
0000-0002-5387-3249
Vikas Malgotra (VM):
0000-0002-1839-2589
Mashkoor Wani (MW):
0009-0008-5091-8537

Author contributions

Conceptualisation: SM, MW
Methodology: SM, MW
Software: SM, VM
Validation: VM
Formal analysis: SM, VM
Investigation: SM, VM, MW
Resources: SM, VM
Data curation: SM, MW
Writing - original draft: SM, VM
Writing - review and editing: SM, VM, MW
Visualisation: SM, VM, MW
Supervision: SM, MW
Project administration: SM, VM

References

1. Ankad Balachandra S, Hombal A, Sudhakar R, Naidu Varna M. Radiological changes in the hands and feet of leprosy. J Clin Diagnostic Res. 2011;5(4):703-7.
2. Choudhuri H, Thappa DM, Kumar RH, Elangovan S. Bone changes in leprosy patients with disabilities/deformities (a clinico-radiological correlation). Indian J Lepr. 1999 Apr-Jun;71(2):203-15. PMID: 10506954.
3. Thappa DM, Sharma VK, Kaur S, Suri S. Radiological changes in hands and feet in disabled leprosy patients: a clinico-radiological correlation. Indian J Lepr. 1992 Jan-Mar;64(1):58-66. PMID: 1533411.
4. Moonot P, Ashwood N, Lockwood D. Orthopaedic complications of leprosy. J Bone Joint Surg Br. 2005;87-B(10):1328-32. doi:10.1302/0301-620X.87B10.16596.
5. Paterson DE, Rad M. Bone changes in leprosy, their incidence, progress, prevention and arrest. Int J Lepr. 1961 Oct-Dec;29:393-422. PMID: 14484319.
6. Mohammad W, Malhotra SK, Garg PK. Clinico-radiological correlation of bone changes in leprosy patients presenting with disabilities/deformities. Indian J Lepr. 2016 Apr;88(2):83-95. PMID: 29757540.
7. Reddy KB, Sreedevi L, Suryanarayana S. Study of radiological changes of bones in leprosy patients. Ann Int Med Den Res. 2017; 4:5–8. doi: 10.21276/aimdr.2018.4.1.dt2.

CONTENT

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CASE REPORT

Is Surgery of Atypical Carcinoid Possible During Active Pulmonary Tuberculosis?

Lora Novaković Lacković,[1, 2] Sonja Ukmar,[2] Mladen Duronjić,[2] Milica Srećić Tomić,[2] Marko Kantar[3]

Abstract

Atypical lung carcinoids are rare compared to other lung cancers and are classified as tumours of medium malignancy. Pulmonary tuberculosis is the second leading cause of death from infectious diseases. The paper presents the case of a 46-year-old female patient who was surgically treated during acute tuberculosis. The patient recovered and is under the supervision of a pulmonologist oncologist.

Key words: Cough; Carcionid tumour; Tuberculosis; Pulmonary.

1. Faculty of Medicine, University of Banja Luka, Banja Luka, The Republic of Srpska, Bosnia and Herzegovina.
2. Clinic for Pulmonary Diseases, University Clinical Centre of the Republic of Srpska, Banja Luka, The Republic of Srpska, Bosnia and Herzegovina.
3. Department of Thoracic Surgery, University Clinical Centre of the Republic of Srpska, Banja Luka, The Republic of Srpska, Bosnia and Herzegovina.

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Novaković Lacković L, Ukmar S, Duronjić M, Srećić Tomić M, Kantar M. Is surgery of atypical carcinoid possible during active pulmonary tuberculosis? Scr Med. 2024 May-Jun;55(3):391-5.

Corresponding author:
LORA NOVAKOVIĆ LACKOVIĆ
T: +387 51 343 209
E: lora.novakovic@kc-bl.com

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Introduction

Carcinoid tumours constitute a mere 2 % of the total incidence of pulmonary tumours, originating from Kulchitsky cells, which are stem cells located within the bronchial epithelium exhibiting neuroendocrine differentiation.[1] Atypical carcinoid (AC) tumours, with a frequency of merely 0.2 %, are less prevalent compared to their typical counterparts. These atypical variants are distinguished by an augmented mitotic count (exceeding 2 per 2 mm^2), presence of punctate necrosis and an enhanced propensity for metastatic spread.[2] Diagnostic confirmation of carcinoid tumours *via* immunohistochemical staining is achieved using markers such as chromogranin A, synaptophysin, CD56 and TTF-1.[3] Approximately 80 % of AC tumours exhibit a central location within the pulmonary architecture and demonstrate no association with smoking. Therapy of choice is surgical intervention, which typically involves complete resection, including lobectomy and segmentectomy.[4]

In the realm of infectious diseases, pulmonary tuberculosis (TB) ranks as the second cause of mortality after COVID-19, with a reported prevalence of 10.6 million individuals in 2022. This figure represents a 4.5 % increase since 2020, including a 3.5 % rise in new cases.[5] TB is prevalent in all countries and across all age groups. It is critical to emphasise that pulmonary TB can be effectively prevented through vaccination and successfully treated with antibiotic therapy.

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Case history

In November 2022, a 46-year-old female patient underwent a computed tomography scan of the chest. Patient had a persistent cough lasting approximately one year. This radiological examination described pathological alterations in middle and lower lobes of right lung: neoplastic lesion with spiculated margins, measuring 14 x 12 x 16 mm (Figure 1a, red arrow). This lesion, exhibiting pleural retraction, was predominantly localised within the inferior lobe. Additionally, a distinct solid lesion was detected in the right middle lobe, in close proximity to the pericardium. Characterised by adjacent adhesions, this lesion was diagnostically classified as post-inflammatory (Figure 1b, blue arrow). The female patient with a 20-year history of tobacco use had a multifaceted medical history, including bronchial asthma (diagnosed in 2017), ulcerative colitis (diagnosed in 2006), hypothyroidism (diagnosed in 2020) and sideropenic anaemia.

The current pharmacological regimen included inhaled corticosteroids combined with long-acting β-agonists (ICS + LABA), mesalazine in both tablet and suppository forms and systemic corticosteroids administered during exacerbations of ulcerative colitis. Additionally, the therapeutic protocol incorporated levothyroxine for thyroid insufficiency and iron supplements to address the anaemic condition. Clinically, the patient reported experiencing fatigue under conditions of heightened physical stress.

Pulmonary function assessment *via* spirometry

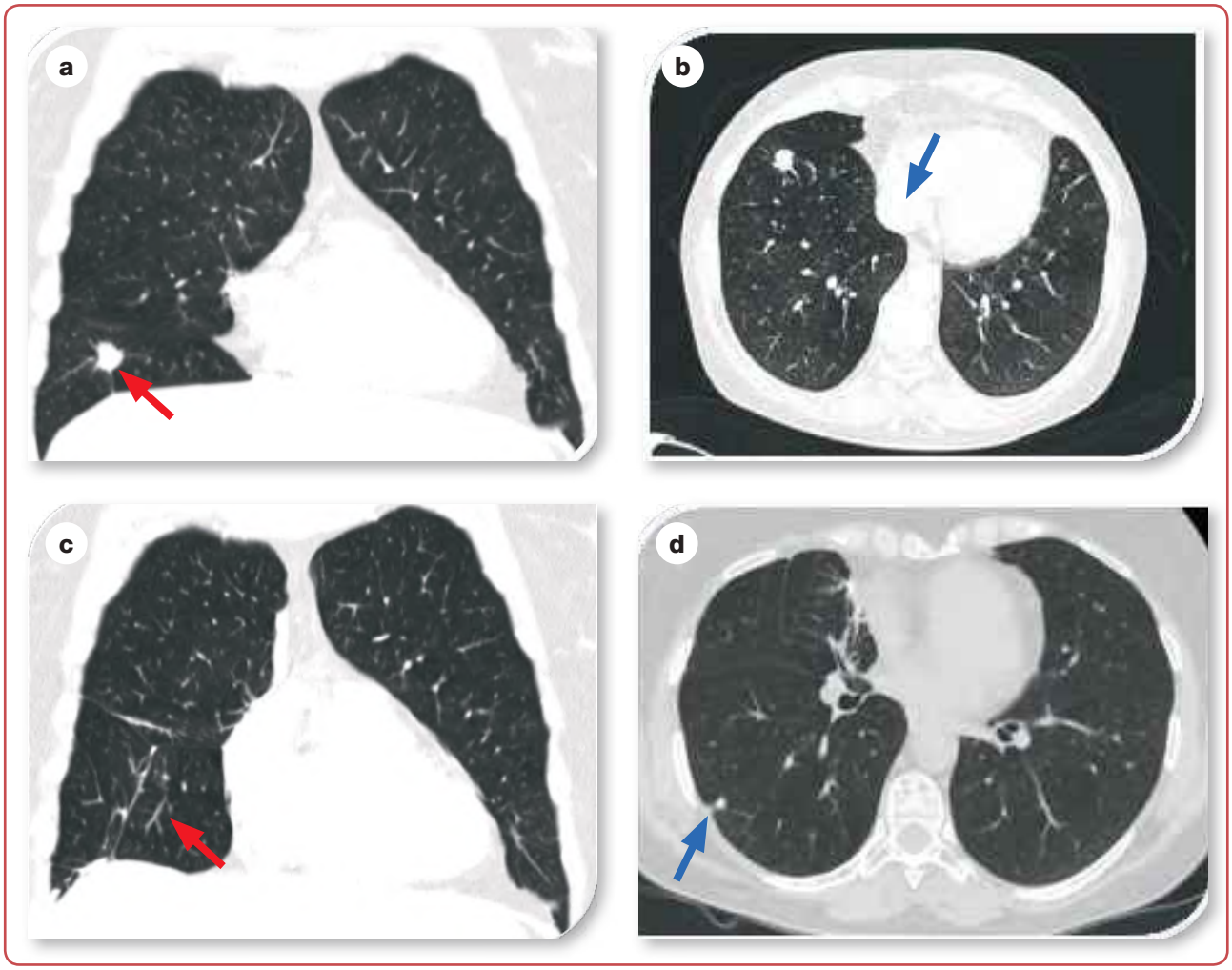

Figure 1: Contrast-enhanced CT image of the chest showing: a) tumour in the right lung's lower lobe with spiculated margins, measuring 14x12x16 mm (red arrow) that retracts the pleura; b) solid lesion in the right middle lobe, in close proximity to the pericardium with adjacent adhesions (blue arrow); c) fibrotic alterations in the right lower lobe after surgery (red arrow); d) subpleural nodule measuring less than one cm in segment S6 of the right lung after surgery (blue arrow).

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revealed a mild obstructive ventilatory impairment, quantified by the following parameters: vital capacity (VC) at 81 %, forced expiratory volume in the first second (FEV1) at 82 %, Tiffeneau-Pinelli Index at 58 % and maximal expiratory flow at 50 % of lung volume (MEF50) at 23 %. Upon bronchoscopic examination, normal endoscopic findings were observed, prompting the thoracic surgeon to recommend video-assisted thoracoscopic surgery (VATS) with intraoperative extemporaneous histopathological assessment. In November 2022, the patient underwent uniportal video-assisted thoracoscopic surgery (UVATS), encompassing an atypical resection of the right lung's lower lobe (histologically confirmed as benign ex tempore) and an atypical resection of the right lung's middle lobe.

Pathohistological analysis post-surgery characterised the lesion in the lower lobe as granulomatous inflammation accompanied by caseous necrosis. Microscopic examination revealed lung tissue interspersed with granulation tissue and granulomas, comprising epithelioid cells, lymphocytes and multinucleated giant cells of the Langhans type, along with foreign body-type giant cells. The lesion in the middle lobe was defined as an AC tumour, staged pT1aNxL+V0PN0. Immunohistochemical staining yielded positive results for synaptophysin, chromogranin and TTF-1, along with moderate positivity for CK7 and Ki-67, the latter exhibiting nuclear positivity in approximately 5 % of cells and was negative for p40. Following the establishment of malignancy and the acquisition of definitive pathohistological evidence, the thoracic surgical team recommended conducting a lobectomy of the middle lobe coupled with lymphadenectomy.

However, the preoperative strategy was modified due to pathohistological confirmation of TB. This necessitated the initiation of anti-TB therapy, spanning a duration of 6-8 weeks, prior to the surgical intervention. The 4-drug regimen in tablet form (isoniazid, rifampicin, ethambutol and pyrazinamide), commenced in February 2023. After completing seven weeks of anti-TB treatment, a mediastinal lobectomy of the middle lobe, along with hilar and mediastinal lymphadenectomy, was performed in March 2023. Subsequent pathohistological analysis substantiated the diagnosis of an AC tumour, with no evidence of lymphatic metastasis (classified as pT2aN0). Post-operatively, the regimen of anti-TB therapy, comprising isoniazid and rifampicin, was sustained until August 2023. A follow-up chest computed tomography scan conducted in September 2023 revealed the absence of oncological or TB recurrence. This imaging study described fibrotic alterations in the right lower lobe (Figure 1c, red arrow), alongside the identification of a subpleural nodule measuring less than one centimetre in segment S6 of the right lung (Figure 1d, blue arrow). Additionally, a standard positron emission tomography-computed tomography (PET-CT) scan performed within the same month did not depicted any metabolic activity within the S6 nodule on the right side. Continuous surveillance of the patient's condition remains an ongoing process.

Discussion

The cough reflex predominantly represents the primary symptomatic manifestation of pulmonary pathologies. A cough that persists more than three weeks may be indicative of an array of alternative health complications. The potential for concurrent lung diseases to be obscured warrants a heightened level of clinical vigilance. The main challenge encountered in the management of chronic cough lies within the precise etiological identification, as accurate diagnosis is imperative for the cessation of symptoms through targeted therapeutic interventions.

Within the categorisation of neuroendocrine neoplasms, AC are classified as neoplasms exhibiting intermediate malignant potential. These neoplasms are comparatively rare, constituting approximately 20 % of pulmonary carcinoids. Pulmonary carcinoids themselves account for a mere 1 % of all neoplastic formations within the United States.[6] Typically, these tumours are diminutive in size, with a central pulmonary localisation evident in 80 % of cases.[7] It is imperative to note that all pulmonary carcinoids are inherently malignant, possessing metastatic capabilities.[8] From a diagnostic perspective, the identification of AC tumours presents significant challenges, frequently due to their clinical presentation mimicking bronchial asthma, a phenomenon extensively documented in the scientific literature.[9, 10] In the present case, the radiological manifestation of pulmonary TB deviates from the conventional pattern. Ordinarily, the primary site of TB is in the apical regions of the lungs. However, the detection of TB in the lower lobe, especially when

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considering its radiographic profile, presented an unusual clinical scenario. Notably, it has been observed that in patients with comorbidity such as diabetes mellitus, TB frequently exhibits atypical radiographic characteristics. This variant, often impacting the middle and lower zones of the pulmonary field, is referred to as basal or lower lobe TB and may extend to hilar, parahilar and perihilar regions. Such atypical presentations underscore the complexity of TB pathology, particularly in the context of concurrent endocrine disorders.[11]

The concomitant diagnosis of pulmonary TB in patients slated for surgical intervention significantly increases both intraoperative and postoperative risks, predominantly attributed to the potential for dissemination and clinical exacerbation of TB. Optimal management often necessitates deferral of surgical procedures until the completion of anti-TB treatment. In scenarios where individuals with active TB require emergent surgical intervention, preoperative initiation of anti-TB therapy is imperative.[12] In such clinical cases, a carefully designed and long-term TB treatment strategy is needed. This approach require close cooperation between the surgical team and pulmonologists.[13] It merits attention that a considerable number of cases documenting the concurrent occurrence of asthma and TB have been registered globally, with numerous scholarly inquiries endeavouring to elucidate their interrelation. Asthmatic patients exhibit an elevated susceptibility to pulmonary infections, attributable to the asthma-induced immune response, which culminates in hypertrophy and inflammatory cell infiltration of the respiratory tract mucosa. This pathological state fosters increased reactivity and bacterial colonisation.[14]

Historical records tracing the simultaneous presentation of TB and lung cancer date back to the 19th century. The correlation between TB and lung cancer has been extensively scrutinised in various scientific studies, yet the determination of whether this association is merely coincidental or possesses an etiological underpinning remains ambiguous.[15] The literature reveals a paucity of research exploring the triad of lung diseases – asthma, AC and TB. The predominant factors contributing to this scarcity are likely rooted in a generalised lack of clinical suspicion regarding disease coexistence, limitations in diagnostic acumen, or constraints in available diagnostic modalities.[16]

Conclusion

This clinical case exemplifies the phenomenon of a rare pathology being obscured by a more prevalent condition. It underscores the imperative in all instances of chronic cough, irrespective of pre-existing diagnoses, to rigorously pursue an expansive differential diagnosis. Such approach is crucial in unveiling potentially underlying rare pathologies that might otherwise remain undetected in the shadow of more common diseases.

Ethics

Access to her medical records was approved in writing by the patient and approved by the Ethics Committee of the University Clinical Centre of the Republic of Srpska (decision No 01-19-78-2/24, dated 20 March 2024).

Acknowledgement

None.

Conflicts of interest

The author declares that there is no conflict of interest.

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Data access

The data that support the findings of this study are available from the corresponding author upon reasonable individual request.

CONTENT

Author ORCID numbers

Lora Novaković Lacković (LNL):
0000-0002-7588-1477
Sonja Ukmar (SU):
0009-0006-4233-2590
Mladen Duronjić (MD):
0009-0005-6686-0525
Milica Srećić Tomić (MST):
0009-0000-9857-1124
Marko Kantar (MK):
0009-0005-9405-9162

Author contributions

Conceptualisation: LNL
Resources: MD, MK
Writing – original draft: SU
Writing – review and editing: LNL
Visualisation: MK, LNL
Supervision: MST
Project administration: LNL

References

1. Garg R, Kumar R, Singh P, Kshetrimayum S. Atypical carcinoid tumor of the lung: A rare entity. Lung India. 2019 May-Jun;36(3):236-8. doi: 10.4103/lungindia.lungindia_111_16.
2. Arrigoni MG, Woolner LB, Bernatz PE. Atypical carcinoid tumors of the lung. J Thorac Cardiovasc Surg. 1972;64(3):413–21. PMID: 5054879.
3. Rekhtman N. Neuroendocrine tumors of the lung: an update. Arch Pathol Lab Med. 2010 Nov;134(11):1628-38. doi: 10.5858/2009-0583-RAR.1.
4. Caplin ME, Baudin E, Ferolla P, Filosso P, Garcia-Yuste M, Lim E, et al, ENETS consensus conference participants. Pulmonary neuroendocrine (carcinoid) tumors: European Neuroendocrine Tumor Society expert consensus and recommendations for best practice for typical and atypical pulmonary carcinoids. Ann Oncol. 2015 Aug;26(8):1604-20. doi:10.1093/annonc/mdv041.
5. World Health Organization. Global Tuberculosis Report 2023 [Internet]. [Accessed: 7-Nov-2023]. Available from: www.who.int/publications/news-room/fact-sheet/detail/tuberculosis.
6. Nenekidis I, Stathopoulos GT, Anagnostakou V, Kokkori A, Dedeilias P, Kokotsakis J, et al. Atypical pulmonary carcinoid tumour in a 28-year-old nonsmoker with Prader–Willi syndrome. Eur Respir J. 2011;38(5):1230-3. doi:10.1183/09031936.00034711.
7. Gosain R, Mukherjee S, Yendamuri SS, Iyer R. Management of typical and atypical pulmonary carcinoids based on different established guidelines. Cancers. 2018;10(12):510. doi:10.3390/cancers10120510.
8. Rosado de Christenson ML, Abbott GF, Kirejczyk WM, Galvin JR, Travis WD. Thoracic carcinoids: radiologic-pathologic correlation. Radiographics. 1999 May-Jun;19(3):707-36. doi:10.1148/radiographics.19.3.g99ma11707.
9. Turrin M, Pontoriero FM, Fiorentù G, Grisostomi G, Zampieri F, Savoia F, et al. Tracheal atypical solitary carcinoid in a so called "difficult asthma": a diagnostic challenge. Monaldi Arch Chest Dis. 2023 Aug 2. doi: 10.4081/monaldi.2023.2586.
10. Rajendran V, Iqbal, Kumar V. Atypical bronchial carcinoid masquerading as bronchial asthma. J Assoc Physicians India. 2015 Nov;63(11):69-70. PMID: 29900716.
11. Bambulović Petrović R, Stojanović M, Mavija Z, Tomanić M, Milekić J. [Comparison of characteristics of pulmonary tuberculosis in patients with and without diabetes]. Biomed Istraž. 2017;8(2):105-12. doi:10.7251/BII1702105B. Serbian.
12. Dara M, Sotgiu G, Zaleskis R, Migliori GB. Untreatable tuberculosis: is surgery the answer? Eur Respir J. 2015;45:577–82. doi:10.1183/09031936.00229514.
13. Harris HW. The evaluation and preparation for surgery of patients with pulmonary tuberculosis. Am J Cardiol. 1963 Sep;12:327-9. doi: 10.1016/0002-9149(63)90221-2.
14. Juhn YJ. Risks for infection in patients with asthma (or other atopic conditions): is asthma more than a chronic airway disease? J Allergy Clin Immunol. 2014;134:247–57.e3. doi:10.1016/j.jaci.2014.04.024.
15. Srećić M, Latinčić M, Lacković LN. [Association between tuberculosis and lung cancer – our results]. Respiratio. 2018;8(1-2):62-5. doi:10.26601/rsp.aprs.18.8. Serbian.
16. Orakwe OI, Ukekwe FI, Okwulehie V, Iloanusi N, Aghaji M. Bronchial carcinoid tumors: A rare malignant tumor. Niger J Clin Pract. 2015 Sep-Oct;18(5):698-701. doi: 10.4103/1119-3077.158985.

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LETTER TO THE EDITOR

Data on Smoking Across Three Generations Increase Both Smoking Prevention and Cessation

Rajko Igić[1, 2, 3]

1. Department of Anesthesiology and Pain Management, John Stroger Hospital, Chicago, Illinois, USA.
2. Department of Pharmacology, Toxicology and Clinical Pharmacology, Faculty of Medicine, University of Banja Luka, Banja Luka, The Republic of Srpska, Bosnia and Herzegovina.
3. Medical Centre Sombor, Sombor, Serbia.

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Corresponding author:
RAJKO IGIĆ
E: igicrajko@gmail.com

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Smoking combustible tobacco is a leading cause for morbidity and mortality of cancer globally.[1] However, two-thirds of lung cancer deaths due to smoking could be prevented through effective tobacco-control policies, regulations and other measures. Therefore, its elimination is a very important public health priority. It is important to establish the prevalence of smoking in each community before an antismoking campaign. The aim of this article was to point out a role of smoking data on intergenerational characteristics of smoking.

It is well known that there is an association between parent (G2) and child (G3) smoking behaviour and a connection between grandparent (G1) and G3 smoking that takes place through the G2.[2] The transmission of smoking behaviour from one generation to the next indicates that it is worth paying attention to family effect when it comes to tobacco prevention; such influence takes place not only through genetics, but also by environmental influences. That is why it is important to establish environmental circumstances when investigating intergenerational influences, eg level of education, occupation, attitude toward women, urbanisation and economic status of family. Change in the number of women smokers may arise due to arrival of the women's liberation movement, increased employment and divorces. The generation of men that went through the war or forced labour often increases incidence of smoking.

In such study, it is possible to include young adult volunteers, eg high school or college students, factory workers or any group of local participants as a convenience sampling.[3] The participants should be asked if they, their parents and grandparents are/were non-smokers or current smokers/ever smokers, the age of each member, their level of education. It is worth including a question on the smoking status of siblings and best friends of the G3 person. The questionnaire can be distributed *via* the Internet (eg *Google Module*) or by the classic way. The results obtained can be displayed graphically and textually.

The data obtained, together with comments written by an experienced medical doctor on antismoking activity, should be presented *via* the public media to the community or wider area,

Figure 1: Elements of teachable moments for combustible tobacco or nicotine smokers

COPD: chronic obstructive pulmonary disease; CAD: coronary artery disease; CHF: congestive heart failure. ¶: Intergenerational data may also contribute to prevention of tobacco use.

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including other teachable elements.[4, 5] When smokers and non-smokers realise what factors contributed to smoking, this knowledge increases motivation for quitting or smoking prevention (Figure 1). Studies on intergenerational characteristics of smoking reveal genetic and environmental influences on smoking in the area, as well.

Ethics

No ethics approval was required, since the form of this article is a letter to the editor, expressing author's personal views.

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Conflicts of interest

The author declares that there is no conflict of interest.

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Data access

The data that support the findings of this study are available from the corresponding author upon reasonable individual request.

Author ORCID number

Rajko Igić (RI): 0000-0001-9084-3391

Author contributions

Conceptualisation: RI
Data curation: RI
Writing - original draft: RI
Writing - review and editing: RI
Visualisation: RI
Supervision: RI

References

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics 2021. CA Cancer J Clin. 2021;71(1):7-33. doi: 10.3322/caac.21654.
2. Vandewater EA, Park SE, Carey FR, Wilkinson AV. Intergenerational transfer of smoking across three generations and forty-five years. Nicotine Tob Res. 2014;16(1):11-7. doi: 10.1093/ntr/ntt112.
3. Stratton SJ. Population research: convenience sampling strategies. Prehosp Disaster Med. 2021;36(4):373-4. doi: 10.1017/S1049023X21000649.
4. Stojaković N, Jonjev Ž, Igić R. Smoking abstinence in patients scheduled for elective surgery. Scr Med. 2013;44:97-99. doi: 10.7251/SMD1302097S.
5. Domić A, Tomasović I, Bernaciak P, Voronov G, Igić R, Škrbić R. Role of physicians in eliminating risk of cancer caused by combustible tobacco smoke. Med Pregl. 2022;75:363-9. doi: 10.2298/MPNS2212363D.

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Instructions to authors

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Scripta Medica publishes editorials, original articles, review articles, current topics, special articles, history of medicine articles, professional articles, case reports, images in clinical medicine, letters to the editor and *in memoriam* notes.
Scripta Medica publishes papers in English only that were not published before, nor submitted to any other journals, in the order determined by the Editor-in-Chief. Each submitted manuscript is checked by the plagiarism-detecting software *iThenticate*.

General instructions

1. Manuscripts should be submitted in the *doc or *docx format (Microsoft Word), using the Times New Roman font. The text should be single spaced in 12 point. The main heading should be 14 point **bold**. Subheadings should be 12 point **bold**. Tables must be in 10 point, single spaced; headings within tables should be in 10 point **bold**; the main table heading should be in 12 point **bold**; legends should be single spaced in 10 point. Figures should be made using standard graphic formats (*pdf, *ai, *cdr). Photographs should be delivered in *jpg format, 1:1, 300 dpi or higher resolution, as separate files.

2. Language. *Scripta Medica* uses the British spelling of the English orthography. For details please consult the University of Oxford Style Guide. American spelling should be limited to the official titles of the US institutions and original US references only.

3. Drugs and chemicals should be indicated by international non-proprietary names (INN) or generic names. Instruments, apparatus or other devices should be indicated by trade names, with the producer's name and place of production indicated in brackets.

4. Numbers in text and tables should be provided if expressed as %; means should be accompanied by standard deviations (SDs) and medians by interquartile range (IQR). In text, use the following rule: spell out numbers up to nine and then use numerical designation for 10 and above.

5. All images must have minimum resolution of 300 dpi. The main figure heading should be in 10 point **bold**; legends should be single spaced in 10 point.

6. References should be indicated in the text sequentially in the Vancouver numbering style, as superscripted numbers after any punctuation mark.

7. Units of measurement, length, height, weight and volume are to be expressed in metric units (eg, metre - m, kilogram - kg, litre -L) or subunits. Temperature should be in degrees Celsius (°C); quantities of substances are given in moles (mol) and blood pressure is expressed as millimetres of mercury (mm Hg). All values of haematological, clinical and biochemical measurements use the metric system according to the International System of Units (SI units).

9. Abbreviations may be used for very long names, including those of chemical compounds. The full name should be given when first mentioned in the text unless it is a standard unit of measurement. If abbreviations are to be used in the Abstract, each should be explained when first mentioned in the text. Well-known abbreviations, such as DNA, AIDS, HIV, ADP, ATP etc, do not need to be introduced by the full name. Titles should include abbreviations only when the abbreviation is universally accepted.

10. Ethics. Consent statement and permission obtained by the institutional ethics committee (IEC). A cover letter should state that written informed consent was obtained from all subjects (patients and volunteers) included in the study and that the study was approved by the IEC. The name of the issuing ethics committee should be clearly disclosed, as well as its decision number and date of its issuing.

11. Acknowledgement. A statement listing all persons and institutions that has assisted the authors in performing the research in question.

12. Conflict of interest. A statement listing all cases of non-financial involvement of any author that could be perceived to be potentially damaging to the objectivity of the research project.

13. Financial disclosure. A disclosure state-

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ment declaring any potential conflict of interest must be signed by each author. (See the policy statement on conflict of interest issued by the World Association of Medical Editors, WAME, www.wame.org or ICMJE uniform disclosure form for potential conflicts of interest, www.icmje.org.) This disclosure includes all affiliations or financial involvement (eg, employment, consulting fee or honorarium, gifts, stock ownership or options, travel/accommodations expenses, grants or patents received or pending and royalties) with any organisation having a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This information will be held in confidence while the paper is under review. If the manuscript is accepted for publication, the editors will discuss with the author how such information is communicated to the reader in the section "Conflicts of interest."

14. Data access. Statement on where and how the original data used to prepare the manuscript can be obtained.

15. Author ORCID numbers. After authors' names their initials should be listed and then the 16-digit ORCID numbers.

16. Author contributions. To qualify for authorship, one must made substantial intellectual contributions to the study on which the article is based (see Policy Statements - Authorship at www.wame.com). The author should participate at least in one of these three categories:

a. research question, conception and design, data acquisition and analysis,

b. statistical analysis, interpretation of data, provision of funding, technical or material support, overall supervision of the project.

c. drafting or critical revision of the manuscript.

In some research projects experts (such as biostatisticians or epidemiologists) may participate and although they may not be equally familiar with all aspects of the work (for example, some clinical variables or laboratory measurements), they may be qualified as the authors. A statement acknowledging contribution to the manuscript should be signed by all the authors. It will be published in the section "Author contributions." The corresponding author is responsible for the integrity of the work as a whole. It is dishonest to omit mentioning the investigator who had important engagement with some aspects of the work.

A pre-defined list of possible author contributions is given below. For each author one or more of the following 14 potential functions should be selected:

- Conceptualisation
- Methodology
- Software
- Validation
- Formal analysis
- Investigation
- Resources
- Data curation
- Writing - original draft
- Writing - review and editing
- Visualisation
- Supervision
- Project administration
- Funding acquisition.

After each of the applicable function, a colon (:) should be written, followed by the initials of the authors pertaining to this particular contribution (eg, Conceptualisation: AB, CD, Supervision: AB, XY, VW, etc).

17. Authorship statement. It should include the following:

a. A statement that the manuscript has been read and approved by all authors.

b. A statement that the authors confirm the sequence of their names in the order that will be reproduced in the article (if accepted for publication), listed after consecutive Arabic numbers.

c. The name of the corresponding author and a statement that they empower him/her to sign all the requested statements and to communicate with the Editorial Office on their behalf.

This statement should be signed by all the authors.

18. Cover letter. The letter accompanying the submission should include the following:

a. A statement that the submitted text is the result of the original work of the authors.

b. A statement that the paper has not been previously published, nor is it concurrently submitted to any other journal.

d. Assertion that written acknowledgements, consent statements and/or permission by the institutional ethics committee were obtained. The name of the ethics committee, the decision number and date should be included.

e. A brief description of the study, with accent on its novelty and why the authors believe that it should be published in *Scripta Medica*.

This letter should be signed by corresponding author.

The majority of these instructions are in accordance with "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" (www.icmje.org).

19. Submission of manuscripts. Manuscripts

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and all enclosures (cover letter, authorship statement and financial disclosures) should be uploaded via the journal official website http://scriptamedica.com/submit-a-manuscript/.

20. Editorial process. Manuscripts deemed suitable for publication by in-house assessment will be reviewed by at least two outside experts. Contributors are encouraged to provide names of two or more qualified reviewers with experience in the subject of the submitted manuscript, but this is not mandatory. Galley proofs of the accepted articles will be sent to the corresponding author, who should reply within 48 h and either approve the galley proof for publication or mark the necessary corrections. The entire process, from the initial submission of the manuscript to the final review, including the sending and receiving of page proofs, will be completed online, vis the editorial platform SCIndeks.

21. Review procedure. Manuscripts suitable for peer review will be sent to at least two international reviewers, based on the topic of the manuscript and the potential reviewers' expertise. Reviewers will be allotted seven days to decide whether they will accept the invitation to review a manuscript or not, plus another seven days to write a review. In case of some delay, this may be extended for another seven days. In case that even then a review is not received, new reviewers will be invited instead. Some manuscripts may be accepted without revision, but if revision is required, the corresponding author must address each question, criticism and suggestion made by the reviewers or editor. He/she has 10 days to respond following the receipt of the reviewers' remarks. These topics should be addressed in a letter to the editor along with a revised manuscript. All the changes made to the text should be marked with a different colour, so that they can be tracked. Following receipt of a galley proof, authors are allotted a 48-h period to either approve it for publication or to suggest some changes.

The acceptance rate for *Scripta Medica* is around 40 %.

22. For further information, please contact the journal manager at the following address: slavica.serdar-janjus@med.unibl.org. For this purpose, the journal website http://scriptamedica.com/contact/ may also be consulted.

Specific instructions for a manuscript

Title page. The title page of the manuscript contains the title of the article, the full name of each author (without titles) and the departments and institutions of the author(s) in the order they are listed. The title page must also include the name of the corresponding author, (along with address, phone and e-mail address) to which the work should be attributed. The word count should be indicated, as well. Original articles may have up to 2.500 words, excluding abstract and references.
The title should identify the main topic or the message of the paper. The standard title of a research paper is a phrase (rarely a sentence) that identifies the topic of the paper; it should be concise and precise, informative and descriptive.
The title of a descriptive paper should include the necessary description, function, purpose, animal species or population. When a method is described, the title should indicate whether it is new or improved.

Abstract and key words. Structured abstract should be included in papers reporting original research. Abstracts are limited to 300 words in four labelled paragraphs: Background/Aim, Methods, Results and Conclusion. The abstract should state concisely the objectives of the study, methods used, results obtained and adequately answer the research question. The abstract should provide pertinent information when read alone. Abstracts should not contain any citing of references. Below the abstract, authors should provide 3-5 key words, according to the terms from the Medical Subject Headings – MeSH (www.nlm.nih.gov/mesh).

Introduction. Generally, this section provides the motivation for the paper (ie, what is missing or unknown in the research literature at this time), an overview of the scientific theory or conceptual models on which the research was based and the purpose of the study and why it is important. Cite only relevant references.

Methods. This section accurately describes the procedures used to carry out the study; it should be complete enough to permit others to replicate the study. Describe the methodological design, subjects, data sources, data collection methods and any statistical and analytical procedures. These five parts may not be needed in all papers. Short papers may include these details in different paragraphs, but titled subsections may be used in longer papers. The Methods section should describe how the research was structured, how subjects or groups of subjects (defined by sex, age and other characteristics) and how the subjects were chosen and assigned to

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these groups. Identify all drugs and chemicals by generic names, exact drug dosages and routes of administration. Variability should be expressed in terms of means and standard deviations (SD). A p-value can be used to disprove the null hypothesis, but the authors should also give an estimate of the power of the study and state the exact tests used for statistical analysis.

Results. This section presents findings in logical sequence using the text, tables and figures. This section should show how the results of the study answer the research question. This may be shortest part of the entire paper. Details may be presented concisely in one or more tables or figures. Do not repeat the data presented in tables or figures in the text. Emphasise or summarise only important observations and how these answer the question posed in the introduction.

Tables. Each table with its legends, should be self-explanatory and numbered in Arabic numerals in order of their mentioning in the text. The title should be typed above the table and any explanatory text, including definitions of abbreviations, is placed below the table.

Figures. All figures (photographs, graphs or schemes) should be numbered with Arabic numerals in the order of their mentioning in the text. All lettering should be dark against a white background and of sufficient size to be legible when reduced for publication. Do not send original artwork, X-ray films or ECG tracings but rather photographs of such material. Images need to be at least 300 DPI (JPG or TIF files). Figure legends should be typed double-spaced on a separate page with Arabic numerals corresponding to the figure. All symbols, arrows, numbers or letters should be explained in the legend. An internal scale should appear on photomicrographs and methods of staining should be described in the legend.

Discussion. Discussion should be always written as a separate chapter and not together with the Results. Briefly state the principal finding that relates to the purpose or research question posed in the Introduction and follow the interpretation of the results obtained. Compare your findings with work reported previously by others. Discuss the implications of your findings and their limitations with respect to the methods used.

Conclusion. The conclusion should be brief and clear, answering the aim of the research and should not consist of repetition of the main results. Avoid unqualified statements and conclusions not completely supported by your data.

References. The reference list is the responsibility of the authors. List all the papers or other sources cited in describing previous or related research. Cite references in the text sequentially in the Vancouver numbering style, as superscripted number after any punctuation mark. For example: ...as reported by Vulić and colleagues.[12] When two references are cited, they should be separated by comma, with a space. Three or more consecutive references are given as a range (eg, ...as was published earlier.[12-14]). References in tables and figures should be in numerical order according to where the item is cited in the text. For citations according to the Vancouver style, see *Uniform Requirements for Manuscripts Submitted to Biomedical Journals*; this source gives the rules and formats established by the International Committee of Medical Journal Editors (www.icmje.org). The standardised abbreviations of the titles of scientific journals cited should be used. If there are six authors or fewer, list all six by last name, space, initials, comma. If there are seven or more, list the first three in the same way, followed by et al. For a book, list the editors and the publisher, the city of publication, and year of publication. For a chapter or section of a book, give the authors and title of the section, and the page numbers. For online material, please cite the URL and the date you accessed the website. Online journal articles can be cited using the doi number. Do not quote references within the Abstract and Conclusion section. All titles of cited manuscripts should be in English (the name of the original language should appear in brackets). Every effort should be done to add the doi number after the reference; if not available, PMID number should be listed. See examples below that conform to the Uniform Requirements for Manuscripts Submitted to Biomedical Journals:

- Miller AL, Evanson NK, Taylor JM. Use of donepezil for neurocognitive recovery after brain injury in adult and pediatric populations: a scoping review. Neural Regen Res. 2024 Aug 1;19(8):1686-95. doi: 10.4103/1673-5374.389628.
- International Committee of Medical Journal Editors (ICMJE). International Committee of Medical Journal Editors (ICMJE). Uniform Requirements for Manuscripts Submitted to

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Biomedical Journals: writing and editing for biomedical publication. Haematologica. 2004 Mar;89(3):264. PMID: 15020262.

- Hull J, Forton J, Thompson A. Paediatric respiratory medicine. Oxford: Oxford University Press; 2015.
- Bydder S. Liver metastases. In: Lutz S, Chow E, Hoskin P, editors. Radiation oncology in palliative cancer care. Chichester (UK): John Wiley & Sons, Ltd.; 2013. p. 283-298.
- Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic program- ming. In: Foster JA, Lutton E, Miller J, Ryan C, Tet- tamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer; 2002. p. 182-91.
- Polgreen PM, Diekema DJ, Vandeberg J, Wiblin RT, Chen YY, David S, et al. Risk factors for groin wound infection after femoral artery catheterization: a case-control study. Infect Control Hosp Epidemiol [Internet]. 2006 Jan [cited 5 Jan 2007];27(1):34-7. Available from: http://www.journals.uchicago.edu/ ICHE/ journal/ issues/v27n1/2004069/2004069. web.pdf.

Review article

Review articles are written by individuals who have studied a particular subject or area extensively and who are considered experts. Narrative reviews and Systematic reviews should be identified as such in their titles. For these reviews, the word count should not exceed 2.500 words, excluding references and abstract. The manuscript may have up to 4 tables or figures and as many as 50 references. Any deviation from these limitations may be approved at the discretion of the Editor-in-Chief.

Current topic

Current topics are actually mini-reviews. They also review a certain topic, but less extensively and by quoting a considerably smaller number of original publications.

Special article

Special articles of 1500 words or less may be devoted to any medical problem, historic perspective, education, demography or contemporary issues. Up to 15 references may be cited and the piece may contain 2 tables or figures. An unstructured abstract in English (150 words or less) should accompany a specific article. Financial disclosure should be presented.

History of medicine

History of medicine articles deal with some historic aspects of medical research and practice. They should be considered as a type of a special article.

Case report

Case reports are most likely to be published if they describe any of the following: an unreported drug side effects (adverse or beneficial), drug interactions; a new, unexpected, or unusual manifestation of a disease; previously unsuspected causal association between two diseases; presentations, diagnosis and/or management of new and emerging diseases; an unexpected association between diseases or symptoms; an unexpected event in the course of observing or treating a patient, findings that shed new light on the possible pathogenesis of a disease or an adverse effect; a previously unknown disease. *Scripta Medica* does not publish instructive case reports, that is, presentations that make important teaching point of what is already well known but often forgotten.

Case reports (no longer than 750 words) should include the following: title, introduction, case history (including up to three figures) and discussion, references (up to six) and an unstructured abstract in English. The abstract may be a single paragraph containing no more than 100 words and followed by key words. Title should facilitate retrieval with electronic searching. Case presentation should include the history, examination and investigations adequately, description of treatments, all available therapeutic options that have been considered and outcomes related to treatments. Discussion includes the following: statement an unusual diagnosis, prognosis, therapy; report of a literature review of other similar cases; explain rationale for reporting the case; what is unusual about the case; could things be done differently in a similar case. There should also be a short conclusion.

Case reports may have as many as five authors. A very short case, about a particular disease can be submitted as a Letter to the Editor. Consent for publication must be obtained from the patients involved; if this is not possible, permission from a close relative or a legal guardian must be obtained before submission. Only non-identifiable data should be included, thus protecting the identity of the patient.

In a cover letter authors should indicate how the case report contributes to the medical literature. Submissions that do not include this information

will be returned to authors prior to peer review. For all case reports, informed written consent is required; the cover letter should state that consent was obtained. Authorship statement and financial disclosure should be presented.

Images in clinical medicine
The editors will consider original, clear and interesting images that depict new or "classic" clinical pictures submitted along with a descriptive paragraph of up to 200 words. The report may include two authors and three references. The authors must obtain a signed, informed consent from the patient or from a close relative or a legal guardian. The cover letter from the corresponding author should state that written consent was obtained.

Letter to the editor
If the letter refers to a recent journal article, it should not exceed 250 words, excluding references. All letters should be brief and to the point with no more than five reference citations. Figures or tables are not permitted in this format. Financial disclosure should be presented.

Editorial
Editorials are solicited by the editor to provide perspective on articles published in the journal and/or to express the general policies or opinions of the Editorial Board. Editor-in-Chief may invite a respectable scholar to write an editorial on a certain topic.

Submission of papers

Manuscripts, tables and figures should be submitted via the official journal website http://scriptamedica.com/submit-a-manuscript/, whenever it is possible, **all in one file. To assist the reviewing process, besides this full-text file, additional files should be uploaded, too:**

(1) Authorship statement, signed by all the authors (see above for details)
(2) Cover letter, signed by the corresponding author (see above for details)
(3) Title page, containing the manuscript title, full names and surnames (in this order!) and affiliations of the authors and the following seven statements/assertions:
- Ethics (number and date of issuing of the ethics committee – state its name – clearance or an explanation why it was waived of not sought at all)
- Acknowledgement
- Funding
- Conflict of interest
- Data access
- Author ORCID numbers
- Author contributions

All these data should be a part of the Title page, since they could reveal the identity of the authors.
(4) Body text, containing the title only and the rest of the manuscript with tables and figures (only this file is to be sent to reviewers in a double-blind manner) and
(5) Filled out WAME Manuscript Checklist (and MS Word form can be downloaded from the journal website)

Signed Authorship statement and Cover letter should be scanned and submitted electronically together with previous materials.

To minimise delays, we advise that you prepare signed copies of all statements before submitting the manuscript. In case of the incompleteness of the submission, the corresponding author will have to upload the missing documents within 48 h. Failure to comply to this deadline will result in the rejection of the submitted manuscript, based on its incompleteness. In such a case, the authors can start a new submission.