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ASSOCIATION OF MEDICAL DOCTORS OF THE REPUBLIC OF SRPSKA,
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54

Q2

Original Articles

Geotropism and Oncogenic Potential of HPV Infections in Cohort Study Populations in Vojvodina, North Region of Serbia

Comparison of Stone Scoring Systems as Predictive Tools for Percutaneous Nephrolithotomy Outcome in Kidneys with Anatomical Abnormalities: A Retrospective Study

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Association of Systemic Diseases with Chronic Pruritus

Prevalence of Nutraceutical Use in Younger Population of North India and the Association Between Gender and Community in Its Usage – Cross-Sectional Study

The Prevalence of *VKORC1* Alleles in the Population of the Republic of Srpska, Bosnia and Herzegovina

Review Article

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CONTENT

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SCRIPTA MEDICA 2023 | 54 | Q2

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CONTENT

Original Articles

Geotropism and Oncogenic Potential of HPV Infections in Cohort Study Populations in Vojvodina, North Region of Serbia 115-123
Aljoša Mandić,Nataša Nikolić,Slobodan Maričić,Bojana Gutić,Nemanja Stevanović,Branka Bašica

Comparison of Stone Scoring Systems as Predictive Tools for Percutaneous Nephrolithotomy Outcome in Kidneys with Anatomical Abnormalities: A Retrospective Study 125-131
Gökhan Çil, Mehmet Yılmaz, Yusuf Şahin, Ahmet Yaser Müslümanoğlu

Role of Cryopreserved Placenta Extract in Prevention and Treatment of Paracetamol -Induced Hepatotoxicity in Rats 133-139
Illia V Koshurba, Mykola O Chyzh, Fedir V Hladkykh, Roman R Komorovsky, Mykhailo M Marchenko

Spontaneous Closure of Isolated Ventricular Septal Defect in the First Year 141-147
Jelica Predojević Samardžić, Nina Marić, Olivera Ljuboja

Association of Cardiovascular and Metabolic Diseases with Risk of Dementia in the Urban Population of North India 149-155
Ram B Singh, Agnieszka Wilczynska, Jan Fedacko,Rie Horiuchi, Toru Takahashi, Ghizal Fatima, MA Manal Ismail, Aminat Magomedova, Arsha Moshiri, Mahmood Moshiri

Left Gastric Artery Variants: A Cadaveric, Postmortem and Radiological Investigation 157-161
Thanuja Ande, Thanuja Kumari Makani, Kavya Nannam, Subhadra Devi Velichety, Jyothi Ashok Kumar

Association of Systemic Diseases with Chronic Pruritus 163-167
Sanja Jovičić, Jagoda Balaban, Vesna Gajanin

Prevalence of Nutraceutical Use in Younger Population of North India and the Association Between Gender and Community in Its Usage – Cross-Sectional Study 169-173
Divya Saran, Alka Bansal, Ashish Agrawal, Lokendra Sharma, Smita Jain, Punam Jakhar

The Prevalence of *VKORC1* Alleles in the Population of the Republic of Srpska, Bosnia and Herzegovina 175-179
Vanja Vidović, Jelena Bećarević, Žana Radić Savić, Aljoša Marić, Stojko Vidović, Irina Milovac, Nela Maksimović

Review Article

Regulatory Role of Some Protein Kinases in Signal Transduction Pathways in Heart Health and Disease 181-195
Mohamad Nusier, Vijayan Elimban, Jaykishan Prasad, Anureet K Shah, Naranjan S Dhalla

Current Topics

Development of Critical Care Medicine in Post-War Republic of Srpska - Banja Luka Region 197-200
Peđa Kovačević

Professional Article

Risky Behaviour Among Adolescents
Alen Greš, Dijana Staver, Branislav Šakić, Ljubomir Radovančević 201-206

Case Reports

Role of the Double Muscle Gastrocnemius-Soleus Flap in Soft Tissue Defect Reconstruction of the Leg in Children: Case Series 207-211
Suryakanth Kalluraya, Shershah Fakruddin Kammar, Adiveppa Hosangadi, Akash Kumar

Emerging Non-Pharmacological Refractory Intervention for Pain Relief in Fibromyalgia: A Case Report 213-216
Saif Al-Zoubi, Alameen Alsabbah, Maggie Wassouf, Asmaa Al-Mnayyis

Images in Medicine

A Quadricuspid Aortic Valve Combined with Coronary Artery Disease 217-218
Živojin S Jonjev, Novica Kalinić

Instructions to Authors ..... *i-ii*

Mandić et al. Scr Med 2023 Jun;54(2):115-23. DOI:10.5937/scriptamed54-43352
ORIGINAL ARTICLE

Geotropism and Oncogenic Potential of HPV Infections in Cohort Study Populations in Vojvodina, North Region of Serbia

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Abstract

Background/Aim: Geotropism of the human papillomavirus (HPV) represents the heterogeneous distribution of different genotypes worldwide. Aim of this study was to evaluate the prevalence of the HPV infection in women from Vojvodina, Serbia, according to cytological status and pathological changes of cervix - dysplasia and cancer.

Methods: The research was conducted as a retrospective study at the Oncology Institute of Vojvodina and the Institute of Public Health of Vojvodina (IPHV). Data from the medical records of female patients treated for cervical intraepithelial neoplasia or cervical cancer at the Department of Gynaecology, Clinic for Surgical Oncology, Oncology Institute of Vojvodina in Sremska Kamenica in the period from 2016 to 2021 were used, as well as the laboratory findings of the IPHV for a group of patients with normal cytological results of the Papanikolau (PAPA) smear.

Results: A total of 731 women, from 20 to 82 years of age, with different cytological results were enrolled. 567 samples were classified as NILM, while 164 samples belong to a group of abnormal histopathology (LSIL/HSIL/cervical cancer). The HPV genotyping assay was performed using the EUROArray HPV test to detect 30 HPV genotypes. In the overall number with normal cytological findings, HPV infection was verified in 242 (42.7 %) patients, of which 135 (55.8 %) were verified with high risk HPV, while 76 (31.4 %) were verified with a mixed group of HPV (Low risk/High risk HPV). Most prevalent genotypes were HPV 16, 31, 53, 51 and 18 in NILM cytological status. In the samples with the abnormal histopathology, the most prevalent genotypes were HPV 16, 33, 31 and 56, while 18 and 39 were equally verified. Genotype 16 was the most prevalent in the examined sample, with a higher prevalence in higher-grade histopathological findings: 18.8 % in LSIL, 31.9 % in HSIL and 75.0 % in cervical cancer samples. Infection with multiple associated genotypes of HPV was not correlated with histopathology. By comparing histopathological diagnosis and age, older patients had higher-grade lesions.

Conclusion: Based on the estimated oncogenic potential of HPV genotypes as well as their prevalence in presented sample, it can be concluded that the nine-valent HPV vaccine for genotypes 6, 11, 16, 18, 31, 33, 45, 52 and 58 would have the potential to prevent HPV infection and the incidence of precancerous lesions and cervical cancer in about 85 % of women. Observing trends in the prevalence of HPV, especially HR HPV genotypes, can be important in the further strategy of applying secondary and primary prevention, as well as the application of HPV detection as part of co-testing or considering the introduction of HPV testing in the initial screening program.

Key words: HPV infections; Geotropism; Precancerous lesions; Prevention.

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CONTENT

Introduction

Geotropism of the human papillomavirus (HPV) represents the heterogeneous distribution of different genotypes worldwide. The aetiology of intraepithelial lesions and carcinoma of the cervix is, in most cases, of an infectious nature and the hypothesis about the role of the human papillomavirus in the pathogenesis of these lesions is well known.[1] HPV infection is the most common sexually transmitted disease today, the transmission of which requires contact with the genital skin, mucous membrane or bodily fluids of the partner.[2] The highest prevalence of this infection was observed in women in their early twenties. Spontaneous elimination of the virus within the first two years occurs in 90 % of women under the age of 30, while the remaining 10 % have the possibility of developing condyloma, dysplasia and even invasive cervical cancer, depending on the virus genotype.[1] The infection develops very quickly after sexual intercourse with a carrier of the HPV, but the progression of the infection to cervical intraepithelial neoplasia (CIN) and cancer takes years. Genital HPV infection is multifocal, often involving several organs of the lower reproductive tract. Any neoplasia associated with HPV infection increases the risk of neoplasia in other places, such as the vulva, vagina and anal region.[2] According to current estimates in Serbia, 1327 women get cervical cancer every year and 551 die from cervical cancer. Cervical cancer is the 4th most common cancer among women in Serbia and the second most common cancer among women between the ages of 15 and 44.[3, 4] Modern approaches to comprehensive screening and primary prevention of cervical cancer with the prophylactic HPV vaccine led to a global call by the World Health Organization (WHO) to initiate the idea of eradicating this disease by 2030.[5] The aim of this study was to examine the prevalence and distribution of different HPV genotypes in a cohort of female patients with normal cytological findings, as well as the oncogenic potential of HPV infection genotypes in a group of patients with histopathologically verified dysplasia and cervical cancer in Vojvodina, the northern region of Serbia.

Methods

The research was conducted as a retrospective study at the Oncology Institute of Vojvodina (IOV) and the Institute of Public Health of Vojvodina (IPHV). It used data from the medical records of female patients treated for cervical intraepithelial neoplasia or cervical cancer at the Department of Gynaecology, Clinic for Surgical Oncology, Oncology Institute of Vojvodina in Sremska Kamenica in the period from 2016 to 2021, as well as the laboratory findings of the IPHV for a group of patients with normal cytological results of the Papanikolau (PAPA) smear. The source of the material was the archival material of the IOV and IPHV obtained from the medical documentation on the histopathological material from operation and cytological findings, the identified genotypes of the HPV and the age of the patients. The use of medical documentation was approved by the competent Ethics Committee.

Inclusion criteria:
- group of female patients with regular cytological PAPA test and verified HPV typing of cervical smear;
- female patients older than 18 years with histopathological verified cervical intraepithelial neoplasia or cervical cancer;
- female patients with cervical smear HPV typing.

Non-inclusion criteria:
- female patients with histopathologically verified cervical intraepithelial neoplasia or cervical cancer in whom HPV typing was not performed;
- female patients with other malignant diseases or immunocompromised diseases;
- female patients with recurrent cervical dysplasia;
- pregnant women.

By analysing the documentation of IOV and IPHV and applying the criteria for sample selection, 164 patients with pathological changes on the cervix and 567 patients with a regular cytological smear were included in further data processing. Cytology findings that were collected and included in the analysis were obtained by conventional PAPA smear.

The histopathological data that were collected resulted from the analysis of the tissue obtained by biopsy of the cervix under the control of the colposcope from the fields that showed the highest degree of abnormality or by one of the excision methods on the cervix. The material obtained in this way was placed in 4 % formalin and further sent for standard processing in the pathological histological laboratory.

CONTENT

Genotyping of HPV DNA in cervical swab samples for all group was performed using a qualitative amplification and hybridisation test. Viral DNA extraction was performed using the commercial SaMag STD DNA Extraction Kit, using the SaMag-12 Automatic Nucleic Acids Extraction System (*Sacace Biotechnologies*, Como, Italy) in accordance with the manufacturer's instructions. In each EUROArray HPV test 5 µL of the extracted DNA was used. If amplification was not performed on the same day as extraction, the processed samples were stored at 2–8 °C for a maximum of five days or frozen at – 80 °C for longer periods. The HPV genotyping assay was performed using the EUROArray HPV test (*EUROIMMUN*, Luebeck, Germany) according to the manufacturer's instructions. The test uses panel of specific primers and probes, to detect 30 HPV genotypes in single reaction, among them, 18 high-risk HPVs (HR HPV) genotypes (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82) and 12 low-risk HPVs (LR HPVs) (6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, 89). Amplification and hybridisation steps allowed identification of both the target viral genetic material (E6/E7 genes) and the human *HSP90* gene used as endogenous controls for valid sample extraction and amplification.

The obtained results of genotyping were considered valid if the test results of all controls were satisfactory according to the manufacturer's instructions.

Statistical analyses

Qualitative data were described using frequencies and percentages. Quantitative data were described using range (minimum and maximum), mean and standard deviation. Differences in mean values of continuous variables were analysed by Student's t-test or ANOVA test for more than two samples and the difference between non-continuous variables was analysed by χ^2 test, using the software package JASP (University of Amsterdam, Amsterdam, the Netherlands). The p-value < 0.05 was used as the cut-off for statistical significance.

Results

The study included 567 patients with a normal cytological smear according to Bethesda classification (negative for intraepithelial lesion or malignancy, NILM) in which the presence of HPV was examined (Table 1).

In the overall number the presence of HPV infection was verified in 242 (42.7 %) female patients, of which 135 (55.8 %) were verified with high-risk (HR) HPV, while 76 (31.4 %) were verified with a mixed group of HPV (low-risk (LR)/HR HPV).

Based on gene typing in the mentioned group, the distribution of HPV genotypes is shown in Figure 1.

Based on the results obtained in the group of women with the negative cytological results, NILM, HPV genotype 16 (39.6 %) was the most represented, followed by genotype 31 (20.0 %), 51 (10.0 %) of highly oncogenic, while genotype 18 ranks fourth in the high-risk group with 5.2 %. Of the HPV genotypes with low oncogenic potency, HPV genotype 6 was verified in 96.7 %.

In patients with histopathological verification, a total of 164 patients were treated. The average age of the patients was 40.5 ± 12.5 years. The oldest patient was 82 and the youngest was 20 (Table 2).

A total of 49 (29.9 %) patients with low-grade squamous intraepithelial lesion (LSIL), 90 (54.9 %) with high grade squamous intraepithelial lesion (HSIL) and 25 (15.3 %) with cervical cancer were included in the analysis.

In patients with histopathological changes on the cervix (LSIL/HSIL/cancer), HPV infection was verified in 125 (76.2 %) patients, while in 39 (23.8 %), HPV was negative (Figure 2).

Table 1: Distribution of human papillomavirus (HPV) infection with normal cytological findings

Cytology	HPV negative	HPV positive	HR HPV positive	LR HPV positive	HR+LR HPV positive	Total
NILM	325 (57.3 %)	242 (42.7 %)	135 (55.8 %)	31 (12.8 %)	76 (31.4 %)	567 (100.0%)

HR HPV – High-risk HPV; LR HPV – Low-risk HPV; NILM – Negative for intraepithelial lesion or malignancy.

CONTENT

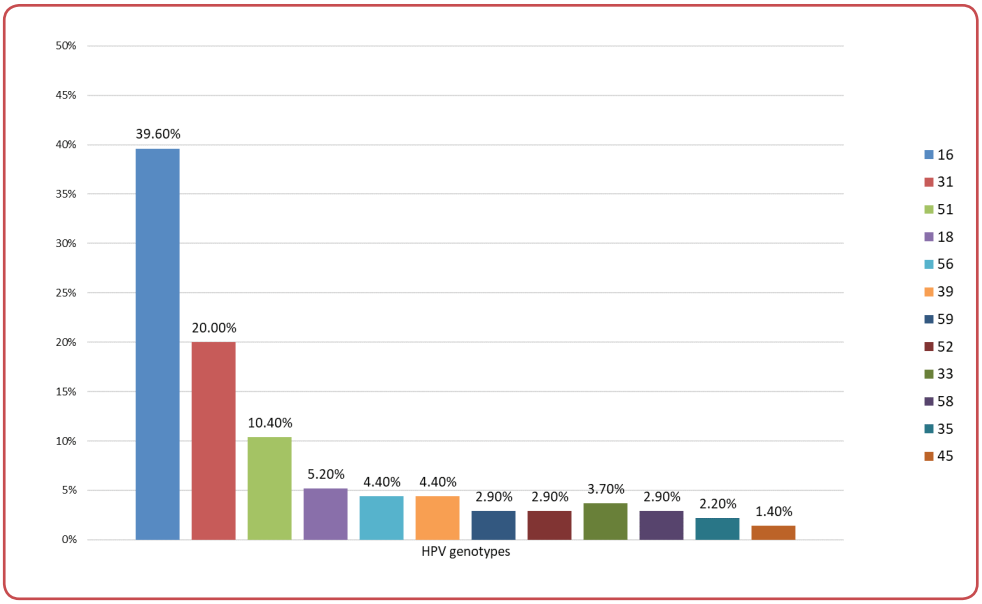

Figure 1: Distribution of human papillomavirus (HPV) genotypes in female patients with NILM cytological findings

IARC – International Agency for Research on Cancer (HPV classification); HR HPV – high risk human papillomavirus (genotype: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59); LR HPV – low risk human papillomavirus (genotype: 6, 11); NILM – Negative for intraepithelial lesion or malignancy;

Table 2: General characteristics of the examined group

Histopathology	N	%
LSIL	49	29.87
HSIL	90	54.87
Cervical cancer	25	15.26
Overall	164	100.00
Age: mean ± SD (range)	40.5 ± 12.5 (20 – 82 years)	

LSIL – Low-grade squamous intraepithelial lesion; HSIL -High grade squamous intraepithelial lesion;

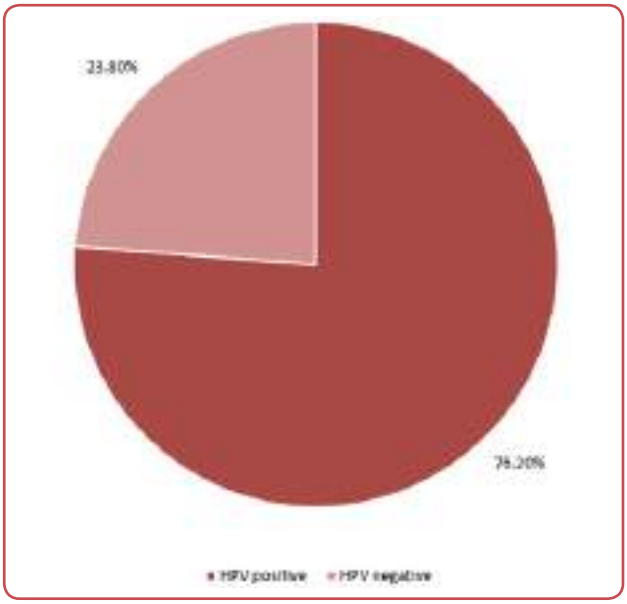

Figure 2: Prevalence of human papillomavirus (HPV) infection in LSIL/HSIL/cervical cancer samples

LSIL – Low-grade squamous intraepithelial lesion; HSIL -High grade squamous intraepithelial lesion;

In patients with LSIL changes, 32 (65.3 %) were positive and 17 (34.7 %) were negative for HPV infection. In patients with HSIL, 69 (76.7 %) were positive and 21 (23.3 %) were negative for HPV infection. In patients with cervical cancer, a total of 24 (96.0 %) were positive and (4.0 %) were negative for HPV infection (Figure 3).

In patients with LSIL/HSIL/cancer of the cervix concerning the distribution of HPV genotypes, the most represented verified HPV was genotype 16 (36.8 %), 33 (7.2 %), 31 (5.6 %) and genotype 56 (4.8 %). Multiple HPV infection with several genotypes was verified in 29.6 % patients (Figure 4).

Observing of HPV genotypes concerning the histopathological findings in patients with LSIL changes, the majority of patients with multiple HPV infections were verified 40.6 %, HPV genotype 16 was confirmed in 18.8 %, 31 in 9.45 % and genotypes 39 and 51 in 6.2 %, respectively. In female patients with HSIL changes, multiple HPV infections and HPV genotype 16 were equally verified; 31.9 % of the other genotypes, HPV genotype 33 prevailed; 11.6 % and genotype 56; 7.2 %. In patients with cervical cancer, HPV genotype 16 was the most prevalent (75.0 %), while the next was genotype 18 (8.3 %) and genotypes 45 and 35 (4.2 %). Multiple HPV infection was present in 8.3 % patients with cervical cancer (Table 3).

CONTENT

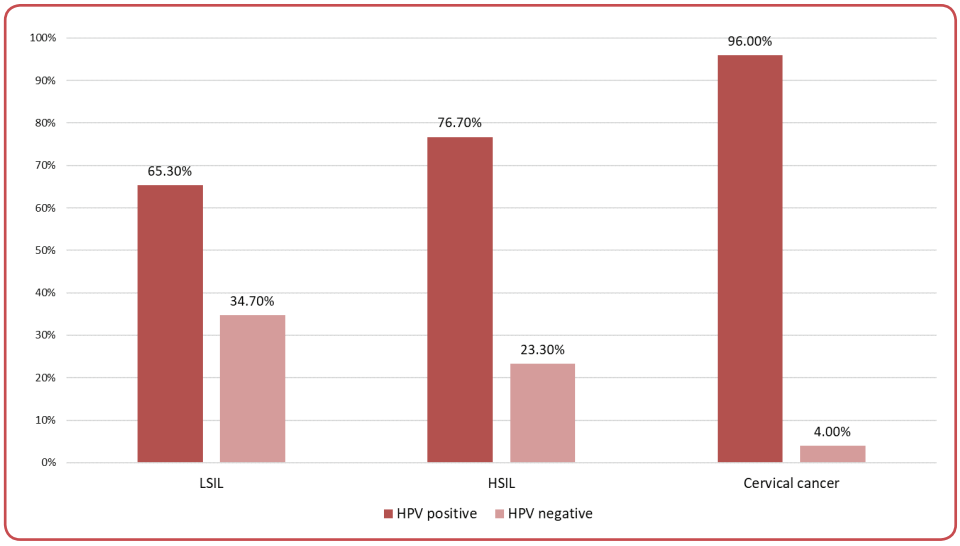

Figure 3: Prevalence of human papillomavirus (HPV) infection obtained by cytological swabs in histopathology confirmed diagnosis (LSIL/HSIL/cancer) of the cervix

LSIL – Low-grade squamous intraepithelial lesion; HSIL -High grade squamous intraepithelial lesion;

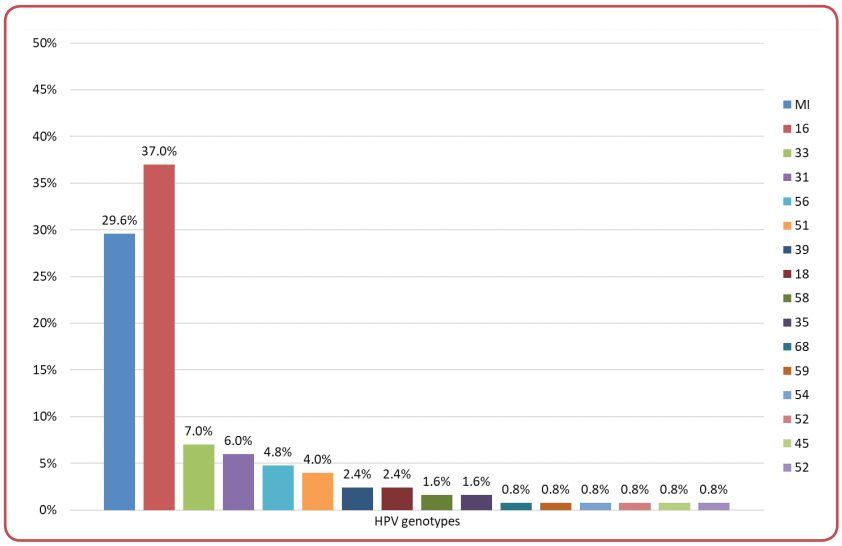

Figure 4: Prevalence of human papillomavirus (HPV) infection obtained by cytological swabs in histopathology confirmed diagnosis (LSIL/HSIL/cancer) of the cervix

LSIL – Low-grade squamous intraepithelial lesion; HSIL - High grade squamous intraepithelial lesion; MI – multiple infections; IARC – International Agency for Research on Cancer (HPV classification): HR HPV – high risk human papillomavirus (genotype: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59); LR HPV – low risk human papillomavirus (genotype: 6, 11);

Using the χ^2 test, a statistically significant difference (χ^2 = 18.024, df = 2; p < 0.001) was established in the prevalence of HPV genotype 16 between patients with histopathological findings of LSIL, HSIL and cancer. This highly oncogenic genotype of the virus is significantly more often present in histopathological findings of a higher degree, that is, in HSIL and cervical cancer. Using the same methods of statistical processing, no statistically significant difference was found in the prevalence of multiple associated genotypes of HPV between patients with histopathological findings of LSIL, HSIL and cancer (χ^2 = 4.371, df = 2; p = 0.112). Data processing and the Student's t-test did not establish a statistically significant difference (p = 0.914) between years of life and the presence of highly oncogenic HPV 16. Using the same methods, it was determined that there was a statistically significant difference (p < 0.001) in the presence of multiple associated genotypes of HPV in different age groups of patients. In younger female patients, more combined genotypes of the HPV were isolated more often than in older patients (Figure 5).

CONTENT

Table 3: Prevalence of human papillomavirus (HPV) genotypes obtained by cytological swabs in histopathology confirmed (LSIL/HSIL/cancer) of the cervix

HPV genotype	Pathological cervical cytology					
	LSIL		HSIL		Cervical cancer	
	N	%	N	%	N	%
Multiple infection	13	40.62	22	31.88	2	8.33
16	6	18.75	22	31.88	18	75.00
31	3	9.35	4	5.80	0	0.00
39	2	6.25	1	1.45	0	0.00
51	2	6.25	3	4.35	0	0.00
18	1	3.13	0	0.00	2	8.33
33	1	3.13	8	11.59	0	0.00
56	1	3.13	5	7.25	0	0.00
58	1	3.13	1	1.45	0	0.00
59	1	3.13	0	0.00	0	0.00
68	1	3.13	0	0.00	0	0.00
35	0	0.00	1	1.45	1	4.17
45	0	0.00	0	0.00	1	4.17
52	0	0.00	1	1.45	0	0.00
54	0	0.00	1	1.45	0	0.00
Total	**32**	**100.00**	**69**	**100.00**	**24**	**100.00**

LSIL – Low-grade squamous intraepithelial lesion; HSIL -High grade squamous intraepithelial lesion;

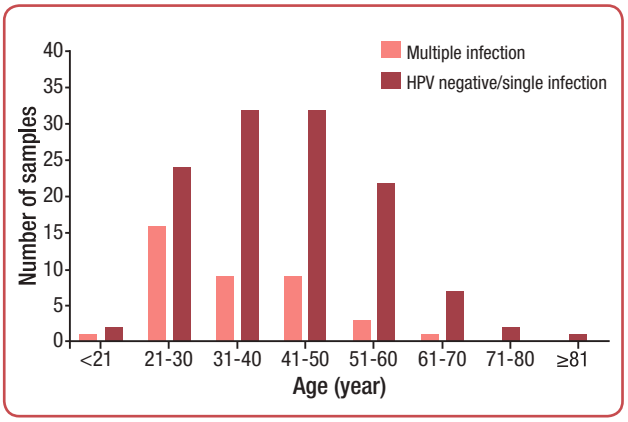

Figure 5: Frequency of multiple human papillomavirus (HPV) infection according to age

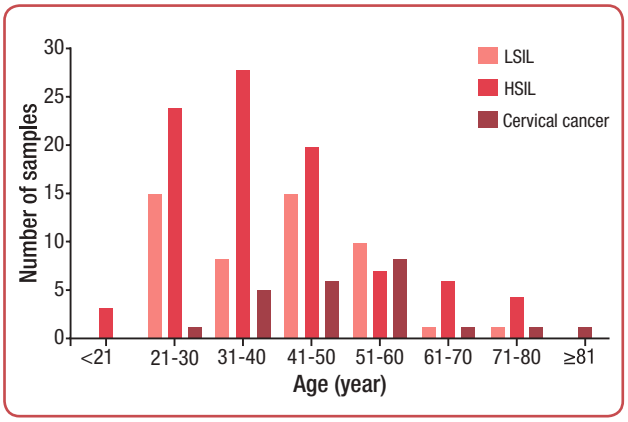

Figure 6: Correlation of age and histopathological findings

LSIL – Low-grade squamous intraepithelial lesion; HSIL -High grade squamous intraepithelial lesion;

Using the ANOVA test, statistical significance was confirmed in the existence of a significant correlation ($p < 0.001$) between age and histopathological findings. Older patients had lesions of a higher degree (Figure 6).

Discussion

Geotropism of HPV represents the heterogeneous distribution of different genotypes worldwide. Positive for HPV in patients with normal cervical findings range from 12-49 %.[6, 7] Worldwide, HPV 16 is the most common genotype of HR HPV found in 60 % of cervical cancer cases. In comparison, HPV genotype 18 is found in about 10 %, genotypes 45 and 31 in 4 % each and genotypes 33, 52 and 58 each in another 2 %.[10] HPV 16 and 18 genotypes are also associated with about 25 % of LSIL and 50 % to 60 % of HSIL.[6] The HPV genotype 16 is represented by 32.3 % in South Asia, 28.9 % in Southern Europe, 24.4 % in Western Europe, 24.3 % in North America and 12 % in Africa.[8] However, a low prevalence was recorded in the Middle East or in Qatar, where an HPV prevalence of 6.1 % was presented in the general population with normal or pathological cytology, especially HPV genotype 81.[9] Considering the presence of certain genotypes of HPV in the region of Vojvodina, the HPV genotype 16 (39.6 %) predominates in ex-

amined group with normal cytological findings, followed by genotype 31 (20.0 %), 51 (10.4 %) of the highly oncogenic ones, while genotype 18 was on fourth place in the high-risk group with 5.2 %. HPV 6 as the low-risk was detected in 96.7 %. It is important to note the presence of HPV genotype 56 (4.4 %) were seen, which warrants further monitoring of this type in terms of their oncogenetic since it is not among vaccine genotypes. In the study of Kaliterna et al, in Split and Dalmatia, Republic of Croatia, out of the total number of women tested, 200 (35 %) were positive for HR HPV. Polymerase chain reaction (PCR)-based assays were used for HR HPV genotyping in positive specimens. The following frequency was observed: HPV 16 in 10.0 %, HPV 18 in 6.1 %, HPV 31 in 2.6 %, HPV 33 in 1.9 %, HPV 52 in 1.4 %, HPV 59 in 0.7 %, HPV 45 in 0.4 % of samples.[10] In the study by Milutin-Gasperov et al, it was determined that the most frequent genotype was HPV 16, with a frequency of 15.9 %, including single and multiple infections. It was followed by HPV genotypes: 31, 6/11, 33, 18, 52, 45 and 58 with 8.7 %, 7.1 %, 4.5 %, 3.8 %, 2.3 %, 1.8 % and 1.1 %, respectively.[11]

In monitoring the oncopotentiality of HPV genotypes, this study included 164 female patients, average age 40.5 ± 12.5 years, with a histopathologically verified diagnosis (dysplasia or cervical cancer). Observing the prevalence of high-risk genotypes of the HPV in certain groups of histopathological findings, it can be concluded that genotype 16 was the most prevalent in the examined sample, with a higher prevalence in higher-grade pathohistological findings. Namely, of all patients with LSIL, this high-risk genotype was identified in 18.8 %, HSIL in 31.9 % and in cervical cancer, it was detected in even 75.0 %. The results match the literature data. It is characteristic that in total cohort, in contrast to literature data, HPV genotype 18 was not so represented, only 2.4 %. In the de Sanjos study, HPV genotypes 16 and 18 were present in 71 % of cancers.[12, 13] Martins et al obtained similar results in their study conducted in São Paulo between 2009 and 2011, analysing the data of 665 female patients. In their examined sample, HPV 16 was also the most prevalent high-risk genotype, with a prevalence of 38.1 % among HSIL findings and 66.7 % among invasive cervical cancer findings.[14] A meta-analysis of 85 studies including 10,058 patients with cervical cancer, also confirmed the predominant prevalence of HPV genotype 16 in squamous histological genotypes of cervical cancer ranging from 46 % in Asia to 63 % in North America. Another common genotype was HPV genotype 18, with about 10 % – 14 % in squamous cell carcinoma.[7] In presented cohort, HPV 16 and 18 were also prevalent in the group with cervical cancer, 75.0 % and 8.3 %, respectively. It should be noted that the subgroup with cervical cancer in this study was too small to determine the presence of other genotypes of viruses. Still, HPV 16 is predominant in the Vojvodina region as well. Based on the before mentioned meta-analysis, which included 133 studies and a total of 14,595 patients, the combination of HPV 16 and 18 was verified in 74 % – 77 % of squamous cell carcinomas in Europe and North America and 65 % – 70 % in Africa, Asia and South/Central America.[2, 7] Using the same methods of statistical processing (χ^2 test), no statistically significant difference was found in the prevalence of multiple associated genotypes of HPV between patients with pathohistological findings of LSIL, HSIL and cancer ($p = 0.112$). Schmitt et al also concluded that infection with multiple virus genotypes does not have a higher prevalence among high-grade cytological findings than low-grade ones.[15] Also, infections with multiple genotypes of viruses are more prevalent in the younger population and milder lesions, 40.62 % in LSIL vs 8.33 in carcinoma in presented material. Similar results were shown in the study by Milosevic et al, where the prevalence of multiple HPV infection in younger patients under the age of 35 was 43 % and NILM cytological findings were 30 % vs 13.9 % in HSIL-a cytological findings.[16]

The above data support the hypothesis that these are more transient infections that resolve spontaneously in most cases and that the oncogenesis initiates mainly one virus genotype. In the examined sample, a statistically significant difference between the age and the presence of several associated genotypes of the HPV was determined. In younger female patients, more combined genotypes of the HPV were isolated more often than in older patients. In their work, De Vuyst et al interpreted the results of the eighteen most extensive studies on the prevalence of the HPV in the countries of Northern and Western Europe. Each study showed the highest prevalence of high-risk genotypes among young women in the age group between 25 and 30 years, with a decline in the frequency of this infection after that.[17] Presented results showed no significant correlation between HPV 16 prevalence and age. By comparing patients' histopathological diagnosis and age,

it was observed that older female patients had higher-grade lesions. Kamineni et al came to the same results. They observed that the largest number of female patients with invasive cervical cancer were in the over 65 age category.[18] Observing the representation of HPV genotypes in the examined population of Vojvodina with regular cytological findings, the predominant genotypes of high-risk genotypes were genotypes 16 (27.3 %), 31 (16.5 %), 51 (11.2 %), genotype 53 (11.6 %) and only in fifth place was HPV genotype 18 (8.3 %). Concerning the oncogenic potential represented in the examined group with pathological changes on the cervix in Vojvodina, the prevalence of HPV genotype 16 prevails in HSIL changes and cancer. The obtained results correspond to literature data from several regions.[7, 15, 19, 20] Although the distribution of other genotypes is similar to other study, observing the relationships of geotropism and oncopotential in precancerous lesions in the examined cohort, it should be emphasised that the prevalence of HPV genotypes 31 (16.5 %) is higher in the population with regular findings, while in HSIL changes genotype 33 and 56 prevail, 11.6 % and 7.25 %, respectively. The obtained results contribute to understanding the transition of the HPV, but also the oncogenic potential of specific genotypes that are not included in the prophylactic vaccine, such as HPV genotype 56, which was verified in HSIL lesions (7.25 %) in this study and is similar to its prevalence in a healthy population.

In presented cohort, in patients with pathological changes on the cervix (LSIL/HSIL/cervical cancer), HPV infection was not verified by the available method in 39 (21.78 %) patients and HPV negativity decreased with the severity of the lesion, LSIL (34.69 %), HSIL (23.33 %) and in cervical cancer (4.00 %).

According to worldwide data, it is estimated that 5.5 % – 11 % of cervical cancers are HPV negative.[21-23] A decrease in HPV negative cases was observed in a meta-analysis of 243 studies that included 30,848 female patients with invasive cancer. The incidence of HPV positive cases ranged from 1990–1999, 2000–2005 and 2006–2010: 85.9 %, 87.9 % and 92.9 %, respectively. This trend is explained by better and more sensitive methods of detecting HPV infection and better classification of non-cervical cancers.[24] The cause of this group can be seen through the prism of false negative results and inadequate cancer classification.[25] Observing the histological genotype, real negative HPV cancers are often adenocarcinomas of unclear aetiology. For cervical adenocarcinoma, the HPV negative finding ranges from 15 % – 38 %.[13, 24, 26] Petry et al confirmed that about 68 % of HPV negative tumours were incorrectly diagnosed as primary cervical cancer.[27] However, in a study that used next-generation sequencing (NGS), HPV negative cervical cancer was around 5 % and a rare number of histopathological genotypes of cervical cancer that are HPV negative were verified. Still, they should be taken into account.[28-31]

Conclusion

In cervical intraepithelial neoplasia and cervical cancer, HPV 16 had the highest oncogenic potential, along with genotypes 33, 31, 18 and 56. Genotype 16 was most often associated with pathohistological changes on the cervix. Infection with multiple associated genotypes of the HPV was not correlated with histopathology. Observing trends in the prevalence of HPV, especially HR HPV genotypes, can be important in the further strategy of applying secondary and primary prevention, as well as the application of HPV detection as part of co-testing or considering the introduction of HPV testing in the initial screening program.

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None.

Conflict of interest

None.

CONTENT

References

1. Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 1999 Sep;189(1):12-9.
2. Chan CK, Aimagambetova G, Ukybassova T, Kongrtay K, Azizan A. Human papillomavirus infection and cervical cancer: epidemiology, screening, and vaccination-review of current perspectives. J Oncol 2019 Oct 10;2019:3257939. doi: 10.1155/2019/3257939.
3. Bruni L, Albero G, Serrano,B, Mena M, Gómez D, Muñoz J, et al [Internet]. ICO/IARC Information Centre on HPV and Cancer (HPVInformation Centre). Human papillomavirus and related diseases in Serbia. Summary Report 10 December 2018. [Cited: 1-Jan-2020]. Available at: https://hpvcentre.net/statistics/reports/SRB.pdf.
4. The Seventy-third World Health Assembly [Internet]. Global strategy to accelerate the elimination of cervical cancer as a public health problem and its associated goals and targets for the period 2020–2030. [Cited: 1-Jan-2021]. Available at: https://www.who.int/publications/i/item/9789240014107.
5. International Agency for Research on Cancer (IARC). IARC handbook of cancer prevention. cervical cancer screening, Vol.10. Lyon, France: IARC Press; 2005.
6. Beyazit F, Sılan F, Gencer M, Aydin B, Paksoy B, Unsal MA, et al. The prevelance of human papillomavirus (HPV) genotypes detected by PCR in women with normal and abnormal cervico-vaginal cytology. Ginekol Pol 2018;89(2):62-7.
7. Bruni L, Diaz M, Castellsagué X, Ferrer E, Bosch FX, de Sanjosé S. Cervical human papillomavirus prevalence in 5 continents: meta-analysis of 1 million women with normal cytological findings. J Infect Dis 2010 Dec 15;202(12):1789-99.
8. de Sanjosé S, Diaz M, Castellsagué X, Clifford G, Bruni L, Muñoz N, et al. Worldwide prevalence and genotype distribution of cervical human papillomavirus DNA in women with normal cytology: a meta-analysis. Lancet Infect Dis 2007 Jul;7(7):453-9.
9. Bansal D, Elmi AA, Skariah S, Haddad P, Abu-Raddad LJ, Al Hamadi AH, et al. Molecular epidemiology and genotype distribution of Human Papillomavirus (HPV) among Arab women in the State of Qatar. J Transl Med 2014 Nov 26;12:300. doi: 10.1186/s12967-014-0300-4.
10. Kaliterna V, Andelinović S, Pejković L, Hofman ID. Human papillomavirus DNA typing in the cervical specimens among women of Split and Dalmatian County. Coll Antropol 2007 Apr;31 Suppl 2:79-82.
11. Milutin-Gašperov N, Sabol I, Halec G, Matovina M, Grce M. High-risk HPV among Croatian women, Coll Antropol 2007 Apr;31 Suppl 2:89–96.
12. Haręża DA, Wilczyński JR, Paradowska E. Human papillomaviruses as infectious agents in gynecological cancers. Oncogenic properties of viral proteins. Int J Mol Sci 2022 Feb 5;23(3):1818. doi: 10.3390/ijms23031818.
13. de Sanjose S, Quint WG, Alemany L, Geraets DT, Klaustermeier JE, Lloveras B, et al; Retrospective International Survey and HPV Time Trends Study Group. Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. Lancet Oncol 2010 Nov;11(11):1048-56.
14. Martins TR, Mendes de Oliveira C, Rosa LR, de Campos Centrone C, Rodrigues CLR, Villa LL, et al. HPV genotype distribution in Brazilian women with and without cervical lesions: correlation to cytological data. Virol J 2016 Aug 12;13:138. doi: 10.1186/s12985-016-0594-3.
15. Schmitt M, Depuydt C, Benoy I, Bogers J, Antoine J, Arbyn M, et al; VALGENT Study Group. Multiple human papillomavirus infections with high viral loads are associated with cervical lesions but do not differentiate grades of cervical abnormalities. J Clin Microbiol 2013 May;51(5):1458-64.
16. Milosevic V, Mandic A, Kovacevic G, Natasa N, Petrovic V [Internet]. Prevalence of HPV genotypes among women in Vojvodina - Distribution of HPV among healthy population EUROGIN 2019, Monaco 4-7.12.2019. [Cited: 1-Jan-2021]. Available at: https://www.eurogin.com/content/dam/markets/aest/eurogin/pdfs/2023/EUROGIN2023_Abstracts_FreeCommunicationsSessions.pdf.
17. De Vuyst H, Clifford G, Li N, Franceschi S. HPV infection in Europe. Eur J Cancer 2009 Oct;45(15):2632-9.
18. Kamineni A, Weinmann S, Shy KK, Glass AG, Weiss NS. Efficacy of screening in preventing cervical cancer among older women. Cancer Causes Control 2013 Sep;24(9):1653-60.
19. Skinner SR, Wheeler CM, Romanowski B, Castellsagué X, Lazcano-Ponce E, Del Rosario-Raymundo MR, et al; VIVIANE Study Group. Progression of HPV infection to detectable cervical lesions or clearance in adult women: Analysis of the control arm of the VIVIANE study. Int J Cancer 2016 May 15;138(10):2428-38.
20. Mirabello L, Clarke MA, Nelson CW, Dean M, Wentzensen N, Yeager M, et al. The intersection of HPV epidemiology, genomics and mechanistic studies of HPV-mediated carcinogenesis. Viruses 2018 Feb 13;10(2):80. doi: 10.3390/v10020080.
21. Arezzo F, Cormio G, Loizzi V, Cazzato G, Cataldo V, Lombardi C, et al. HPV-negative cervical cancer: a narrative review. Diagnostics (Basel) 2021 May 26;11(6):952. doi: 10.3390/diagnostics11060952.
22. Blatt AJ, Kennedy R, Luff RD, Austin RM, Rabin DS. Comparison of cervical cancer screening results among 256,648 women in multiple clinical practices Cancer Cytopathol 2015;123(5):282–8.
23. Guan P, Howell-Jones R, Li N, Bruni L, de Sanjosé S, Franceschi S, et al. Human papillomavirus genotypes in 115,789 HPV-positive women: a meta-analysis from cervical infection to cancer. Int J Cancer 2012;131(10):2349–59.
24. Li N, Franceschi S, Howell-Jones R, Snijders PJF, Clifford GM. Human papillomavirus genotype distribution in 30,848 invasive cervical cancers worldwide: Variation by geographical region, histological genotype and year of publication. Int J Cancer 2011;128(4):927–35.
25. Xing B, Guo J, Sheng Y, Wu G, Zhao Y. Human papillomavirus-negative cervical cancer: a comprehensive review. Front Oncol 2021 Feb 17;10:606335. doi: 10.3389/fonc.2020.606335.
26. Holl K, Nowakowski AM, Powell N, McCluggage WG, Pirog EC, Collas DeSouza S, et al. Human papillomavirus prevalence and genotype-distribution in cervical glandular neoplasias: Results from a European multinational epidemiological study. Int J Cancer 2015;137(12):2858–68.
27. Petry KU, Liebrich C, Luyten A, Zander M, Iftner T. Surgical staging identified false HPV-negative cases in a large series of invasive cervical cancers. Papillomavirus Res 2017;4:85–9.
28. Cancer Genome Atlas Research Network; Albert Einstein College of Medicine; Analytical Biological Services; Barretos Cancer Hospital; Baylor College of Medicine; Beckman Research Institute of City of Hope; et al. Integrated genomic and molecular characterization of cervical cancer. Nature 2017 Mar 16;543(7645):378-84.
29. Pirog EC, Lloveras B, Molijn A, Tous S, Guimerà N, Alejo M, et al. HPV prevalence and genotypes in different histological subgenotypes of cervical adenocarcinoma, a worldwide analysis of 760 cases. Modern Pathol 2014;27(12):1559–67.
30. Pirog EC, Park KJ, Kiyokawa T, Zhang X, Chen W, Jenkins D, et al. Gastric-genotype adenocarcinoma of the cervix: tumor with wide range of histologic appearances. Adv Anat Pathol 2019;26(1):1–12.
31. Stolnicu S, Barsan I, Hoang L, Patel P, Terinte C, Pesci A, et al. International Endocervical Adenocarcinoma Criteria and Classification (IECC): A new pathogenetic classification for invasive adenocarcinomas of the endocervix. Am J Surg Pathol 2018;42(2):214–26.

CONTENT

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ORIGINAL ARTICLE

Comparison of Stone Scoring Systems as Predictive Tools for Percutaneous Nephrolithotomy Outcome in Kidneys with Anatomical Abnormalities: A Retrospective Study

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Abstract

Background/Aim: European urology guidelines recommend percutaneous nephrolithotomy (PNL) as a treatment modality to remove complex kidney stones over 2 cm in size. Aim of this study was to compare stone scoring systems in predicting stone-free status and complications rate after percutaneous nephrolithotomy (PNL) in abnormal kidneys.

Methods: Retrospective analysis of data from 94 patients with anatomical abnormalities who underwent PNL for the kidney stones in the Clinic between January 2017 and January 2022 was performed. Sixty-four patients with renal anomalies who underwent PNL were included in the study. Guy, S.T.O.N.E. and CROES nephrolitometry scores were evaluated for each patient by the same researcher using non-contrast computed tomography. The modified Clavien grading system was used to evaluate complications.

Results: The mean age and body mass index (BMI) of the patients were 46 ± 11.7 and 28 ± 6 kg/m^2, respectively. There was no differences between the groups in terms of operative parameters, renal anomaly categorisation and complications. Compared with the residual stone group, GSS (2.49 vs 3.03; p = 0.001) and S.T.O.N.E. scores (7.26 vs 8.38; p = 0.021) in the stone free group were statistically significantly lower, while the CROES score was lower in the group with residual stones (172 vs 245; p < 0.001). In the Chi-square analysis performed between Clavien complication rating and stone scoring systems, no success was found in predicting the presence of complications in any scoring system.

Conclusion: Although nomograms were successful in predicting postoperative stone-free status (SFS) after PNL in abnormal kidneys, they may not predict postoperative complications.

Key words: Renal anomaly; Percutaneous nephrolithotomy; Guy's score; S.T.O.N.E. score; CROES nomogram.

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Introduction

European urology guidelines recommend percutaneous nephrolithotomy (PNL) as a treatment modality to remove complex kidney stones > 2 cm size.[1] PNL can be applied in different cases from normal to abnormal kidneys. This procedure may become more difficult due to different kidney locations, anatomically abnormal calyxes, abnormal relationships with neighbouring organs and difficulties in the movements of endoscopic instruments.[2] Renal anatomy, stone burden, stone localisation, stone size and density, skeletal anomalies, comorbidities and surgeon experience affect the success of PNL.[3] Different scoring systems have been designed to evaluate

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CONTENT

complications and the stone free rate (SFR) that may develop after PNL, as well as to inform patients about possible outcomes before the operation. Guy's stone score (GSS), S.T.O.N.E. (stone size (S), tract length (T), obstruction (O), number of involved calyces (N) and essence or stone density (E)) nephrolithometry score and CROES (Clinical Research Office of the Endourological Society) nephrolithometry nomogram are the most widely used scoring systems.[4-6] There is no consensus on the best scoring system for kidneys with renal anomalies. Aim of this study was to compare the value of renal scoring nomograms in predicting PNL outcomes in kidneys with renal anomaly.

Methods

This study was approved by the local Ethics Committee of the Hospital (15 November 2022, Decision No 189) and complied with the principles of the Declaration of Helsinki. All patients provided written informed consent preoperatively.

The data of 94 patients with abnormal kidneys among 494 patients who underwent PNL between January 2017 and January 2022 were retrospectively analysed. Patients under 18 years of age (n = 11), patients with preoperatively ureteral stent or nephrostomy catheter inserted (n = 9), who underwent miniaturised percutaneous surgery (n = 6) and patients whose preoperative computed tomography (CT) images could not be accessed (n = 4) were excluded from the study (Figure 1).

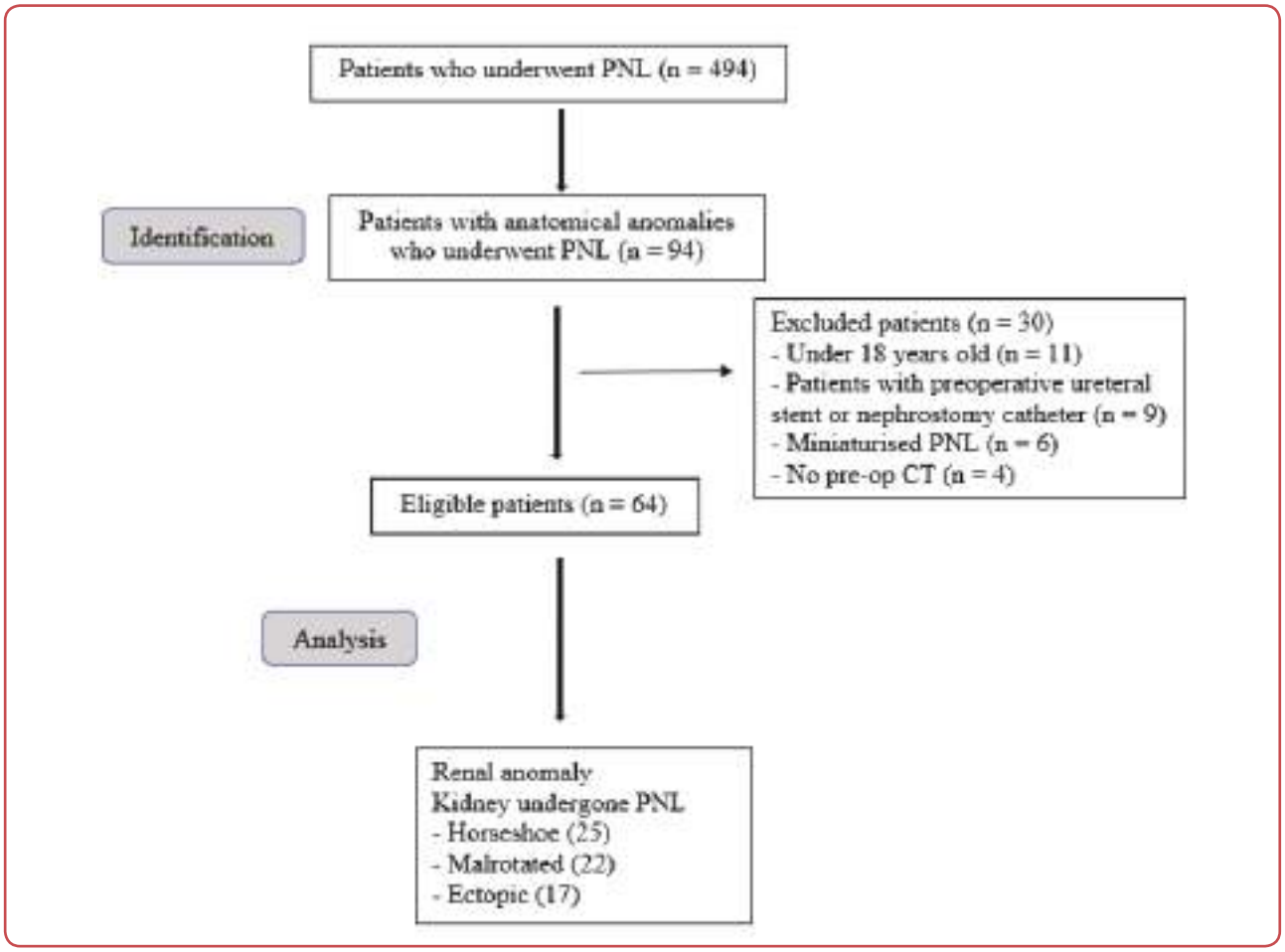

Figure 1: Patient selection and study design flowchart

PNL: percutaneous nephrolithotomy; CT: computed tomography;

CONTENT

Finally 64 patients were included in the study. Patients were divided into the two groups considering to their stone-free status (SFS). Group 1 consisted of 35 stone-free patients, while Group 2 consisted of 29 patients with residual stones. The groups were compared according to their demographic features (age, sex, body mass index - BMI), stone characteristics (stone load, location), anatomical abnormality type and operative parameters (nephrostomy length of stay, location and success, operation time and complications). Clavien grading system was used to evaluate postoperative complications.[7]

Measurements and patient grading

Low-dose non-contrast CT and/or urography, stone size (in mm^2 multiplied by the two longest dimensions), stone density (Hounsfield Unit), renal calyx anatomy in terms of skin-to-stone distance (mm) provided the most appropriate percutaneous access site estimation was performed. GSS, S.T.O.N.E. and CROES scoring systems were calculated by endourologists who mostly perform stone surgery in the daily practice of the Urology Clinic (Bağcılar Training and Research Hospital, İstanbul, Turkey). Patients with anatomical abnormalities for GSS were classified as 2nd, 3rd or 4th grade according to the number and location of stones. S.T.O.N.E. load, tract length, obstruction, relevant calyx number and stone density were noted while nephrolitometry score was used. When calculating the CROES score, the average annual case volume was accepted as 200 for the Clinic.

Surgical technique

The PNL procedure started with the placement of 5F open-ended Hydrophilic Ureteral Catheters (*Plasti-med*) retrograde into the involved kidney under general anaesthesia and 3rd generation cephalosporin prophylaxis and continued with the prone position. With an initial puncture needle (18 G/20 mm/2 parts, *Plasti-med*) retrograde contrast was given under fluoroscopy and posterior calyx access was achieved. Renal access was performed by entering a nephrostomy balloon dilator (*Nephroflex*) after an *Amplatz* dilator up to 12 French (F) and a 24 F nephroscope (*Alken-Hohenfellner, Karl Storz, Germany*) through a 30 F *Amplatz* sheath. Fragmentation of stones was performed using pneumatic and ultrasonic lithotripters. After endoscopic and fluoroscopic stone-freeness was achieved, a 14 F *Malecot* nephrostomy set was placed in the kidney. On the first postoperative day, both stone-free and pleural injury control were performed with direct urinary system radiography (CUB) and chest radiography in intercostal accesses. The discharge of the patients was carried out according to the dryness of the tract after the removal of the nephrostomy catheter. Stone-free control was achieved with CUB and urinary ultrasonography in the first month postoperatively and low-dose CT at the third month. Absence of residual fragments or < 4 mm fragments were considered stone free.

Statistical analysis

Statistical Package for Social Sciences software was used for statistical analysis of study data (IBM SPSS Version 22.0). The Chi-square test or Fisher's exact test was used for categorical variables, while continuous variables were compared with the independent sample t-test and One-way analysis of variance (ANOVA) test. Receiver operating characteristic (ROC) curves were generated to assess the predictive role of stone scoring systems and other significant variables on postoperative SFS. Logistic or linear regression analyses were performed to determine the possible relationship of stone scoring systems, BMI, stone burden and skin to stone distance (SSD) parameters with SFS. $P < 0.05$ was noted as statistically significant.

Results

The mean age and BMI of the patients included in the study were 46 ± 11.7 and 28 ± 6 kg/m^2, respectively and BMI was statistically significantly higher in Group 1 ($p = 0.002$). While 19 out of 64 patients were female, stone-free was achieved in 73 % of them ($p = 0.042$). The mean SSD (mm) was higher in Group 1 and the number of kidneys with staghorn stones was higher in Group 2 (98.54 mm ± 22.88, $p = 0.001$ and 7 (10.9 %) / $p = 0.013$, respectively). Table 1 shows patient demographics, stone characteristics and operative parameters. Compared with the Group 2, GSS (2.49 vs 3.03; $p = 0.001$) and S.T.O.N.E. scores (7.26 vs 8.38; $p = 0.021$) in the Group 1 were lower, while the CROES score was lower in the Group 2 (172 vs 245; $p < 0.001$).

The ROC curves of 3 scoring systems, SSD, stone burden and BMI on prediction of SF status are shown in Figure 2. According to the ROC analy-

Table 1: Patient demographics, stone characteristics and operative parameters

Parameters	Total	SFS: Yes	SFS: No	p-value
Age (years)	46 (11.70)	46.09 (10.08)	45.90 (13.72)	0.930
BMI (kg/m²)	28 (60)	30 (6.00)	26 (4.00)	**0.002**
Male	45 (70.30)	21 (32.81)	24 (37.50)	**0.042** *
Female	19 (29.70)	14 (21.88)	5 (7.81)	
The Guy's stone score	2.73 (0.64)	2.49 (0.61)	3.03 (0.56)	**0.001**
The S.T.O.N.E. nephrolithometry score	7.77 (1.64)	7.26 (1.06)	8.38 (1.99)	**0.021**
CROES nomogram score	211.92 (54.37)	245 (48.14)	172 (28.62)	**< 0.001**
Stone burden (mm²)	460.8 (379.80)	313.2 (212.23)	638.92 (458.02)	**< 0.001**
HU	980 (278.75)	978.26 (276.93)	981.97 (285.83)	0.989
SSD (mm)	90.5 (25.66)	98.54 (22.88)	80.79 (25.83)	**0.001**
Staghorn stones	8 (12.50)	1 (1.56)	7 (10.94)	**0.013** ¥
Stone side (right/left)	26 (40.6) / 38	14 (21.88) / 21 (32.81)	12 (18.75) / 17 (26.66)	0.556 *
Number of hydronephrosis	(59.40)	17 (26.66)	16 (25.00)	0.392 *
Renal abnormality	33 (51.60)			0.848
Horseshoe kidney	25 (39.10)	14 (21.90)	11 (17.19)	
Malrotated kidney	22 (34.40)	12 (18.80)	10 (15.63)	
Pelvic kidney	17 (26.60)	9 (14.10)	8 (12.50)	
OTT (min)	101.33 (33.64)	94.43 (27.30)	109.66 (38.86)	0.098
Time with nephrostomy tube (day)	2.17 (1.79)	1.8 (1.23)	2.62 (2.24)	0.088
EBL (gr/dL)	1.94 (1.24)	1.78 (1.35)	2.13 (1.07)	0.069
Complications	24 (37.50)	11 (17.20)	13 (20.30)	0.200 *

**Pearson Chi-square; ¥: Fisher's Exact Test; Values are presented as N (%) or mean (SD); BMI: Body mass index; EBL: Estimated blood loss; HU: Hounsfield Unit; OTT: Operation table time; SSD: Skin to stone distance; SFS: Stone free status;*

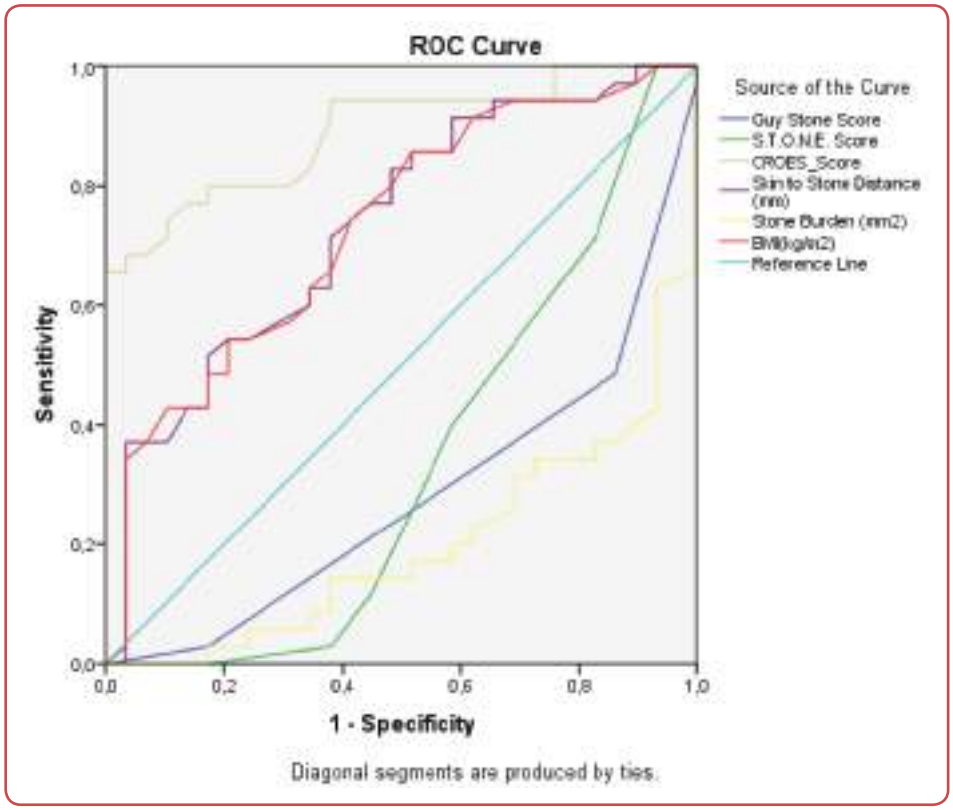

Figure 2: The ROC curves of 3 scoring systems, SSD, stone burden and BMI on prediction of stone-free status (SFS)

BMI: Body mass index; SSD: Skin to stone distance; SFS: Stone free status;

CONTENT

Table 2: ROC curves of variables with significant association with stone-free status (SFS)

Variable(s)	Area	p-value	95 % Confidence interval
Guy stone score	0.280	0.003	0.155 - 0.405
S.T.O.N.E. score	0.335	0.024	0.195 - 0.476
CROES score	**0.891**	**0.000**	0.812 - 0.969
SSD (mm)	0.733	0.001	0.610 - 0.856
Stone burden (mm^2)	**0.197**	**0.000**	0.091 - 0.303
BMI (kg/m^2)	0.731	0.002	0.608 - 0.854

BMI: Body mass index; SFS: Stone free status; SDD: Skin to stone distance;

Table 3: Clavien complication rating according to stone scoring systems

Scoring System	Clavien grading system					Total	P-value
	Grade 1	Grade 2	Grade 3a	Grade 3b	Grade 4a		
The Guy's scoring system							
2	1	2	1	1	0	5	0.404
3	8	4	4	0	1	17	
4	2	0	0	0	0	2	
The S.T.O.N.E. scoring system							
5-6	2	2	0	0	0	4	0.763
7-8	5	2	4	1	0	12	
9-13	4	2	1	0	1	8	
CROES score							
< 130	2	0	1	0	0	3	0.844
130-169	2	1	0	0	0	3	
170-219	4	3	2	0	1	10	
> 219	3	2	2	1	0	8	

Table 4: Logistic regression analyses of BMI, SSD, stone burden and nephrolithometry scoring systems on stone free status (SFS)

	Stone free status		
Variables	**OR**	**95 % CI**	**p-value**
The S.T.O.N.E. nephrolithometry score	1.222	0.589 - 2.534	0.590
CROES nomogram score	1.055	1.019 - 1.092	**0.002**
Guy's stone score	1.167	0.170 - 8.011	0.875
Stone burden (mm^2)	1	0.996 - 1.004	0.980
BMI (kg/m^2)	0.979	0.524 - 1.829	0.948
SSD (mm)	1.019	0.895 - 1.161	0.772

BMI: Body mass index, SSD: Skin to stone distance; OR: odds ratio; CI: confidence interval;

sis, although all parameters were significant for stone-free, the highest area under the curve (AUC) and significance were observed in the CROES and stone burden parameters (AUC = 0.891 and 0.197, respectively, $p < 0.001$). Parameters showing statistical significance according to ROC analysis and AUC values were summarised in Table 2.

In the Chi-square analysis performed between Clavien complication rating and stone scoring systems, no success was found in predicting the presence of complications in any scoring system (Table 3).

Logistic regression analysis showed that only CROES scoring systems were significantly associated with stone-free status (OR: 1.055, [95 % CI 1.019–1.092]; $p < 0.002$) (Table 4).

Discussion

In presented study, aim was to compare the GSS, S.T.O.N.E. and CROES nomograms in predicting PNL outcomes in abnormal kidneys. It was found that although these nomograms were successful in predicting postoperative SFS, they did not have predictive value in determining the postoperative complications of PNL. In this regard novel scoring systems may come in mind in predicting outcomes of PNL in abnormal kidneys.

Cağlayan et al evaluated 120 paediatric cases who had undergone PNL surgery, compared CROES with GSS and found that only the CROES scoring system was significant in demonstrating SFS. However, both scoring systems did not predict complications after PNL.[8] Karsiyakali et al evaluated 81 patients who underwent Retrograde Intrarenal Surgery and found that S.T.O.N.E. and CROES scoring systems were significant in showing post-op SFS, but GSS was not, but they did not compare the scoring systems in terms of complications.[9] Consistent with these studies, in presented study it was found that CROES, GSS and S.T.O.N.E. scoring systems could predict SFS after PNL. Similar to the study of Caglayan et al, it was found that these scoring systems cannot predict postoperative complications. Recently, Kocaaslan et al demonstrated that only CROES nomogram may predict the SFS of PNL patients with abnormal kidneys.[10] Yarimoglu et al conducted a study on the evaluation of complications with renal scoring systems in the preoperative period in 160 patients who had undergone PNL for staghorn stones and they found that scoring system did not show postoperative complications.[3] All these studies demonstrated that GSS, S.T.O.N.E. or CROES nomograms may predict postoperative SFS and complications of standard PNL performed in the normal kidneys. But while these nomograms were able to predict SFS, they were found to be unsuccessful in predicting post-operative complications for PNL surgeries performed in rare patient groups such as paediatric patients, staghorn kidney stones and abnormal kidneys. The CROES nomogram was found to be more significant in predicting SFS than other nomograms, including the clinical annual number of cases parameter.

It is very important to accurately predict the results and complications of PNL such as bleeding that will require transfusion, injuries to the colon or pleura, postoperative fever, sepsis and inform the patient about these possible complications. None of the nomograms were categorically associated with postoperative complications. There is necessity for new nomograms that include more specific criteria (kidney anomaly, surgeon case volume and stone surgery history, etc) that may predict complications.

Limitations of the study

The study had several limitations. Firstly, the retrospective data of the single centre were used in the study which may have led to biased selection. Secondly, the sample size in the study was small.

Conclusion

Standard PNL surgery may be more difficult for patients with renal anomaly. Aim was to compare the effects of scoring nomograms in kidney anomalies. Although CROES, Guy and S.T.O.N.E. scoring systems were able to detect stone-free status in the postoperative period, they may not detect complications in the preoperative period.

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Conflict of interest

None.

References

1. Zeng G, Cai C, Duan X, Xu X, Mao H, Li X, et al. Mini percutaneous nephrolithotomy is a noninferior modality to standard percutaneous nephrolithotomy for the management of 20-40mm renal calculi: a multicenter randomized controlled trial. Eur Urol 2021 Jan;79(1):114-21.

CONTENT

2. Ganpule AP, Desai MR. Urolithiasis in kidneys with abnormal lie, rotation or form. Curr Opin Urol 2011 Mar;21(2):145-53.
3. Yarimoglu S, Bozkurt IH, Aydogdu O, Yonguc T, Sefik E, Topcu YK, et al. External validation and comparison of the scoring systems (S.T.O.N.E, GUY, CROES, S-ReSC) for predicting percutaneous nephrolithotomy outcomes for staghorn stones: A single center experience with 160 cases. Kaohsiung J Med Sci 2017 Oct;33(10):516-22.
4. Al Adl AM, Mohey A, Abdel Aal A, AbuElnasr HAF, El Karamany T, Noureldin YA. Percutaneous nephrolithotomy outcomes based on S.T.O.N.E., GUY, CROES, and S-ReSC scoring systems: the first prospective study. J Endourol 2020 Dec;34(12):1223-8.
5. Kumar U, Tomar V, Yadav SS, Priyadarshi S, Vyas N, Agarwal N, et al. STONE score versus Guy's Stone Score-prospective comparative evaluation for success rate and complications in percutaneous nephrolithotomy. Urol Ann 2018;10:76-81.
6. Ketsuwan C, Kijvikai K, Kongchareonsombat W, Sangkum P, Rongthong S, Leenanupunth C. A comprehensive comparison of Guy's stone score, CROES nomogram, S.T.O.N. E. nephrolithometry, and the Seoul renal stone complexity scoring system in predicting perioperative outcomes after percutaneous nephrolithotomy. J Med Assoc Thai 2020;103:762–6.
7. de la Rosette JJ, Opondo D, Daels FP, Giusti G, Serrano A, Kandasami SV, et al; CROES PCNL Study Group. Categorisation of complications and validation of the Clavien score for percutaneous nephrolithotomy. Eur Urol 2012 Aug;62(2):246-55.
8. Caglayan V, Onen E, Avci S, Sambel M, Kilic M, Oner S. Comparison of Guy's Stone Score and clinical research of the endourological society nomogram for predicting surgical outcomes after pediatric percutaneous nephrolithotomy: a single center study. Minerva Urol Nefrol 2019 Dec;71(6):619-26.
9. Karsiyakali N, Karabay E, Erkan E, Kadihasanoglu M. Evaluation of nephrolithometric scoring systems to predict outcomes of retrograde intrarenal surgery. Urol J 2020 Jun 23;17(4):352-7.
10. Kocaaslan R, Tepeler A, Buldu I, Tosun M, Utangac MM, Karakan T, et al. Do the urolithiasis scoring systems predict the success of percutaneous nephrolithotomy in cases with anatomical abnormalities? Urolithiasis 2017 Jun;45(3):305-10.

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ORIGINAL ARTICLE

Role of Cryopreserved Placenta Extract in Prevention and Treatment of Paracetamol-Induced Hepatotoxicity in Rats

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Abstract

Background/Aim: Drug-induced liver injury is one of the major causes of acute liver failure. Under current circumstances of the pandemic of COVID-19, the use of paracetamol which has a proven hepatotoxic effect has increased. This prompts the search for novel agents with hepatoprotective properties. The purpose of this article was to evaluate the hepatoprotective activity of cryoextract of the placenta (CEP) on the model of paracetamol-induced hepatitis.
Methods: The study was performed on 28 male rats. Acute drug liver damage was modelled by intragastric administration of paracetamol twice at a dose of 1250 mg/kg.
Results: The development of paracetamol-induced hepatitis in rats was accompanied by a 71.3 % increase ($p < 0.001$) in the content of active products of thiobarbituric acid (TBA-AP) in liver homogenates as compared with intact animals. Besides, there was a 2.1-fold ($p < 0.001$) increase of ALT activity, a 58.8 % increase ($p < 0.001$) of AST activity and a 4.2-fold ($p < 0.001$) increase of the concentration of total bilirubin as compared with intact rats. The use of cryopreserved placenta extract showed significant hepatoprotection in a rat model of paracetamol-induced hepatitis. This was demonstrated by a 2.3-fold ($p < 0.01$) increase of the antioxidant-prooxidant index, a significant ($p < 0.001$) decrease of activity of ALT (by 44.0 %) and AST (by 29.6 %), as well as by a decrease of direct bilirubin level by 52.5 % ($p < 0.001$) in animals treated with CEP as compared with rats without treatment.
Conclusion: The development of acute paracetamol-induced hepatitis in rats was associated with activation of lipid peroxidation processes in liver tissues, while CEP showed marked hepatoprotective activity in paracetamol-induced hepatitis in rats.

Key words: Cryopreserved placenta extract; Paracetamol; Liver injury; Hepatoprotection.

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Introduction

Drug-induced liver injury (DILI) is one of the most common side effects of many medications. It is primarily related to the physiological role of the liver in the elimination of xenobiotics, as well as to the spread use of generic drugs which are not always of sufficient quality.[1, 2] DILI accounts for about 10 % of the total number of patients with liver disease. The global prevalence of DILI ranges from 2.4 cases per 100,000 population per year in Great Britain to 34.2 cases per 100,000 population per year in Spain.[3, 4] The relevance of the DILI problem is confirmed by the functioning

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of networks focusing their activity on control and prevention of development of drug-associated liver injuries, eg the database "Drug Induced Liver Injury Network" in the system of the Food and Drug Administration (USA), the system "LiverTox (USA), the "HepaTox" system (China), etc.[5-7]

Metabolic transformation and subsequent conjugation and excretion of hydrophilic products of drug biotransformation with bile and urine take place in liver. Among the drugs that most frequently cause the development of DILI, non-steroidal anti-inflammatory drugs (39 %) rank first, followed by antibacterial drugs (29 %), immunosuppressant (9 %) and antiplatelet drugs (7 %), antidiabetic drugs (4 %) and others (21 %).[8] Taking paracetamol, troglitazone, valproate, antibiotics and anticancer drugs was the most frequently associated with death in patients with DILI.[9-11]

Paracetamol or acetaminophen is the most widely used over-the-counter analgesic-antipyretic in the world.[12, 13] The relevance of the use of this drug has increased especially in the context of the pandemic of the new viral infection COVID-19. It is also worth noting that paracetamol is often used as a component of combined painkillers and anti-inflammatory drugs – [paracetamol + ibuprofen], [paracetamol + diclofenac sodium], [paracetamol + metamizole sodium], etc. The ability of paracetamol to covalently bind to mitochondrial proteins of hepatocytes and a number of enzymes (glutamine synthetase, glutamine dehydrogenase, carbonic anhydrase III, glutamate dehydrogenase, glycine-N-methyltransferase) is the basis of its hepatotoxic effect.[12, 13]

To date, according to the State Register of Medicinal Products of Ukraine, more than 100 drugs with hepatoprotective activity are registered on the pharmaceutical market of Ukraine, but none of them can fully satisfy the clinicians' needs. Authors' attention was drawn to the national-produced drug "Cryocell – placenta cryoextract" as a potential biotechnological agent with a hepatoprotective effect. Placenta cryoextract (CEP) was obtained by scientists of the Institute of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine.[14-16] In previous studies, it was established that the therapeutic and preventive administration of CEP normalised metabolic processes in the liver and restored its functional state due to antioxidant and membrane-stabilising effects, which weakened the cytolytic syndrome caused by the administration of D-galactosamine and restored the protein-synthesising function of the liver.[17-19] In addition, it was shown that CEP has an energy-stabilising effect on hepatocytes of rats with simulated tetrachloromethane liver damage.[20-22]

The aim of the present study was to analyse the hepatoprotective activity of placenta cryoextract on the model of paracetamol-induced hepatitis.

Methods

The study was performed on 28 male rats weighing 200–220 g which were divided into 4 groups[21]:

I – intact rats (n = 7);
II – rats (n = 7) with paracetamol-induced hepatitis (control group);
III – rats (n = 7) with paracetamol-induced hepatitis, which were injected intramuscularly (im) cryoextract of the placenta (CEP) in a dose of 0.16 mL/kg 5 times;[24, 25]
IV – rats (n = 7) with paracetamol-induced hepatitis, which were administered a derivative of the amino acid L-cysteine – acetylcysteine (ACC) intraperitoneally (ip) in a dose of 150 mg/kg.[21, 26]

Acute medical damage to the liver was simulated by intragastric (ig) administration of paracetamol at a dose of 1250 mg/kg once a day for 2 consecutive days.[21] CEP and ACC were administered 60 minutes after each administration of paracetamol (2 administrations) and further for 3 consecutive days after simulating hepatitis (a total of 5 administrations). The animals were withdrawn from the experiment 72 hours after the second injection of paracetamol and sacrificed under general anaesthesia.

Biochemical research methodology

The research material was whole blood and rat liver homogenates. To obtain the liver homogenate, the liver was perfused with a cold (+4 °C) isotonic 1.15 % KCl solution and homogenised at 3000 rpm (teflon/glass) in a buffer solution at a ratio of 1:10 (weight/volume: 250 mg + 2.25 mL of 1.15 % KCl solution), obtaining a 10.0 % homogenate.

Content of active products of thiobarbituric acid (TBA-AP) in liver homogenates was determined

spectrophotometrically according to Asakawa et al at the wavelength of λ = 535 nm.[27] The molar extinction coefficient of the red-coloured complex, which is 1.56 × 10^5 mol^{-1}/cm^{-1} expressed in μmol/kg tissue, was considered for further analysis. Catalase activity in liver homogenates was determined spectrophotometrically by light absorption at a wavelength of λ = 410 nm and expressed as mcat/kg of tissue.[28] Antioxidant-prooxidant index (API) was calculated according to the formula: API = (Catalase activity × 100) / TBA-AP content.[28]

Alanine aminotransferase (ALT) activity and aspartate aminotransferase (AST) activity in peripheral blood was determined spectrophotometrically according to the method of Reitman and Frankel.[30] De Ritis ratio was calculated as the AST to ALT ratio.[28] Concentration of bilirubin in peripheral blood was determined spectrophotometrically by the reaction of diazophenylsulphonic acid with direct bilirubin.[29, 31]

Statistical analysis

Differences between groups were assessed with the use of parametric or nonparametric criteria, as applicable. Normally distributed independent values were compared with the paired t-test, while other than normally distributed values were compared with a Mann-Whitney test. A p-value of < 0.05 was considered statistically significant. The averages of the normally distributed values are reported as mean ± standard error of the mean or mean (95 % confidence interval). The averages of other than normally distributed values are reported as medians [interquartile ranges].[32]

Bioethical compliance

All experimental studies on laboratory animals were performed in accordance with the requirements of Good Laboratory Practice and in compliance with the basic provisions of the Council of Europe Convention on the Protection of Vertebrate Animals Used in Experiments and Other Scientific Purposes of 18 March 1986, European Parliament and Council Directive 2010/63/EU of 22 September 2010 on the protection of animals. The comprehensive research program was considered and approved by the Committee on Bioethics at the Institute of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine (Protocol No 5 of 22 November 2022).

Results

A significant increase ($p < 0.001$) in the content of TBA-AP in liver homogenates by 71.3 % compared to the values of intact rats was noted (Table 1). ACC administration resulted in decrease of TBA-AP level by 18.6 % ($p = 0.04$). Administration of CEP resulted in almost complete recovery of the TBA-AP level in liver homogenates to the level of 9.3 ± 1.48 μmol/kg of tissue (as compared with 9.4 ± 0.68 μmol/kg of tissue in intact animals).

A significant decrease of catalase activity by 35.3 % ($p < 0.01$) as compared to that of intact rats (2.2 ± 0.24 mcat/kg of tissue) was noted. In CEP administration, this index increased by 18.2 % ($p = 0.03$) and in ACC administration, it increased by 59.1 % ($p < 0.01$) as compared with rats with paracetamol-induced hepatitis. A significant decrease in the value of the API by 62.2 % was noted ($p < 0.001$). The use of CEP, as well as of ACC, resulted in a significant 2.3-fold and 1.9-fold increase ($p < 0.01$) of API, respectively (Table 1).

In acute paracetamol-induced hepatitis in rats, there was a statistically significant ($p < 0.001$) 2.1-fold increase ALT activity, as well as an increase ($p < 0.001$) of AST activity by 58.8 % as compared with indices of intact rats. A disproportionate increase in the activity of aminotransferases led to a statistically significant ($p = 0.02$) decrease of the De Ritis ratio by 26.7 % as compared with the values in intact animals (Table 2).

The use of ACC led to a uniform decrease in the activity of ALT and AST in the peripheral blood of rats with paracetamol-induced hepatitis by 28.0 % ($p < 0.001$) and 25.9 % ($p < 0.01$), respectively, as compared to the parameters of animals without treatment. However, when the imbalance in the activity of aminotransferases caused by liver damage persisted, the De Ritis ratio was 26.7 % lower ($p = 0.03$) as compared with the untreated animals (Table 2). A significant ($p < 0.001$) decrease in the activity of ALT (by 44.0 %) and AST (by 29.6 %) was noted in group III when CEP was used, as compared to the respective indices of animals of the control group. In contrast to ACC (group IV), the use of CEP (group III) was associated with a statistically significant ($p = 0.01$) increase of De Ritis ratio (by 27.3 %).

In paracetamol-induced hepatitis in rats a statistically significant ($p < 0.001$) 4.2-fold increase of

CONTENT

the concentration of total bilirubin was observed as compared with intact rats (Table 3). Indirect bilirubin increased 5.2-fold, while direct bilirubin increased by 2.7 times compared to the values of intact rats and was, respectively, 14.1 ± 0.6 μmol/L compared with 47.0 ± 1.8 μmol/L. The level of direct bilirubin significantly ($p < 0.001$) decreased both with the use of CEP (by 52.5 %) and with the use of ACC (by 55.3 %).

Table 1: The effect of CEP on the biochemical lipid peroxidation and antioxidant system in tissue homogenates in paracetamol-induced hepatitis in rats (M ± m (95 % CI), n = 28)

The studied index, units of measurement	Experimental group			
	Group I Intact rats	Group II Paracetamol hepatitis	Group III Paracetamol + CEP	Group IV Paracetamol + ACC
n	7	7	7	7
TBA-AP, μmol/kg tissue	9.4 ± 0.7 (8.1 – 10.8)	16.1 ± 1.0 (14.1 – 18.2) $p_{1-2} < 0.001$	9.3 ± 1.5 (6.4 – 12.2) $p_{1-3} = 0.90$ $p_{2-3} < 0.01$	13.1 ± 0.8 (11.5 – 14.7) $p_{1-4} < 0.01$ $p_{2-4} = 0.04$ $p_{3-4} = 0.04$
Catalase, mcat/kg of tissue	3.4 ± 0.2 (3.0 – 3.9)	2.2 ± 0.2 (1.7 – 2.7) $p_{1-2} < 0.01$	2.6 ± 0.2 (2.2 – 3.1) $p_{1-3} = 0.03$ $p_{2-3} = 0.20$	3.5 ± 0.3 (2.9 – 4.1) $p_{1-4} = 0.90$ $p_{2-4} < 0.01$ $p_{3-4} = 0.03$
Antioxidant-prooxidant index	38.1 ± 4.5 (29.3 – 46.9)	14.4 ± 2.4 (9.7 – 19.2) $p_{1-2} < 0.001$	32.6 ± 5.4 (22.0 – 43.2) $p_{1-3} = 0.50$ $p_{2-3} < 0.01$	27.5 ± 2.8 (21.9 – 33.0) $p_{1-4} = 0.07$ $p_{2-4} < 0.01$ $p_{3-4} = 0.40$

Indices $_{1, 2, 3, 4}$ indicate the number of the group whose characteristics were compared; n – number of rats; CI: confidence interval; ACC – amino acid L-cysteine – acetylcysteine; CEP – cryoextract of the placenta; TBA-AP – active products of thiobarbituric acid;

Table 2: The effect of CEP on the activity of aminotransferases in the peripheral blood in rats with paracetamol hepatitis (M ± m (95 % CI), n = 28)

The studied index, units of measurement	Experimental group			
	Group I Intact rats	Group II Paracetamol hepatitis	Group III Paracetamol + CEP	Group IV Paracetamol + ACC
n	7	7	7	7
ALT, μmol/(mL×h)	1.2 ± 0.1 (1.0 – 1.4)	2.5 ± 0.1 (2.3 – 2.7) $p_{1-2} < 0.001$	1.4 ± 0.1 (1.2 – 1.6) $p_{1-3} = 0.15$ $p_{2-3} < 0.001$	1.8 ± 0.1 (1.7 – 1.9) $p_{1-4} < 0.001$ $p_{2-4} < 0.001$ $p_{3-4} < 0.01$
AST, μmol/(mL×h)	1.7 ± 0.1 (1.5 – 1.9)	2.7 ± 0.13 (2.4 – 2.9) $p_{1-2} < 0.001$	1.9 ± 0.1 (1.7 – 2.1) $p_{1-3} = 0.24$ $p_{2-3} < 0.001$	2.0 ± 0.2 (1.6 – 2.3) $p_{1-4} = 0.29$ $p_{2-4} < 0.01$ $p_{3-4} = 0.79$
De Ritis ratio (AST/ALT)	1.5 ± 0.2 (1.2 – 1.8)	1.1 ± 0.1 (1.0 – 1.2) $p_{1-2} = 0.02$	1.4 ± 0.1 (1.2 – 1.5) $p_{1-3} = 0.41$ $p_{2-3} = 0.01$	1.1 ± 0.1 (0.9 – 1.3) $P_{1-4} = 0.03$ $P_{2-4} = 0.89$ $P_{3-4} = 0.03$

Indices $_{1, 2, 3, 4}$ indicate the number of the group whose characteristics were compared; p_{2-1} is the level of statistical significance of difference between the groups; n – number of rats; CI: confidence interval; ACC – amino acid L-cysteine – acetylcysteine; CEP – cryoextract of the placenta; ALT – Alanine aminotransferase; AST – Aspartate aminotransferase;

Table 3: Effect of CEP on the concentration of bilirubin in the peripheral blood of rats against the background of paracetamol hepatitis (M ± m (95 % CI), n = 28)

The studied index, units of measurement	Experimental group			
	Group I Intact rats	Group II Paracetamol hepatitis	Group III Paracetamol + CEP	Group IV Paracetamol + ACC
n	7	7	7	7
Total bilirubin, μmol/L	14.4 ± 0.8 (12.8 – 16.1)	61.1 ± 2.2 (56.8 – 65.5) $p_{1-2} < 0.001$	50.1 ± 0.8 (48.6 – 51.7) $p_{1-3} < 0.01$ $p_{2-3} < 0.001$	27.7 ± 1.2 (25.3 – 30.1) $p_{1-4} < 0.001$ $p_{2-4} < 0.001$ $p_{3-4} < 0.001$
Direct bilirubin, μmol/L	5.3 ± 0.4 (4.6 – 12.0)	14.1 ± 0.6 (12.9 – 15.4) $p_{1-2} < 0.001$	6.7 ± 0.4 (5.9 – 7.5) $p_{1-3} = 0.02$ $p_{2-3} < 0.001$	6.3 ± 0.4 (5.5 – 7.1) $p_{1-4} = 0.10$ $p_{2-4} < 0.001$ $p_{3-4} = 0.48$
Indirect bilirubin, μmol/L	9.1 ± 0.7 (7.8 – 10.5)	47.0 ± 1.8 (43.5 – 50.5) $p_{1-2} < 0.001$	43.4 ± 1.0 (41.6 – 45.3) p1-3 < 0.001 p2-3 = 0.11	21.4 ± 1.00 (19.5 – 23.4) p1-4 < 0.001 p2-4 < 0.001 p3-4 < 0.001

Indices $_{1, 2, 3, 4}$ indicate the number of the group whose characteristics were compared; n – number of rats; CI: confidence interval; ACC – amino acid L-cysteine – acetylcysteine; CEP – cryoextract of the placenta;

Discussion

Presented study has shown that simulation of paracetamol-induced hepatitis in rats was accompanied by activation of lipid peroxidation processes in liver tissues. This was demonstrated by a statistically significant increase in the content of TBA-AP in liver homogenates by 71.3 % compared to the values of intact rats.

ACC administration resulted in decrease of TBA-AP level by 18.6 %. Administration of CEP resulted in almost complete recovery of the TBA-AP level in liver homogenates. The assessment of the level of catalase (reflecting the activity of the antioxidant system) in liver homogenates showed that the development of acute paracetamol hepatitis was accompanied by a significant decrease of catalase activity by 35.3 % ($p < 0.01$) as compared to that of intact rats. In CEP administration, this index increased by 18.2 % ($p = 0.03$) and in ACC administration, it increased by 59.1 % ($p < 0.01$) as compared with rats with paracetamol-induced hepatitis. An integral assessment of the state of the prooxidant-antioxidant system in liver homogenates showed that in paracetamol-induced hepatitis, a statistically significant decrease in the value of the API by 62.2 % was noted ($p < 0.001$). The use of CEP, as well as of ACC, resulted in a significant 2.3-fold and 1.9-fold increase ($p < 0.01$) of API, respectively, indicating a slightly more pronounced ability of CEP to restore the balance of the pro-oxidant-antioxidant system of liver tissues.

The assessment of the activity of aminotransferases in peripheral blood showed that in acute paracetamol-induced hepatitis in rats, there was a significant 2.1-fold increase ALT activity, as well as an increase of AST activity by 58.8 % as compared with indices of intact rats. A disproportionate increase in the activity of aminotransferases led to a statistically significant ($p = 0.02$) decrease of the De Ritis ratio by 26.7 % as compared with the values in intact animals. A low of De Ritis ratio can be observed during the activation of gluconeogenesis processes via a glucose-alanine shunt with participation of ALT. This process which is necessary for maintenance of an adequate blood glucose level, results in elevation of activity of transaminases. Also, low De Ritis ratio may imply a decrease of liver function.[33]

The use of ACC led to a uniform decrease in the activity of ALT and AST in the peripheral blood of rats with paracetamol-induced hepatitis by 28.0 % and 25.9 %, respectively, as compared to the parameters of animals without treatment. However, when the imbalance in the activity of aminotransferases caused by liver damage persisted,

the De Ritis ratio was 26.7 % lower (p = 0.03) as compared with the untreated animals. A significant decrease in the activity of ALT (by 44.0 %) and AST (by 29.6 %) was noted in group III when CEP was used, as compared to the respective indices of animals of the control group. In contrast to ACC, the use of CEP was associated with a significant increase of De Ritis ratio (by 27.3 %), indicating the recovery of metabolic balance in liver.[28]

Studies of liver pigment metabolism showed that in paracetamol-induced hepatitis in rats a significant (p < 0.001) 4.2-fold increase of the concentration of total bilirubin was observed as compared with intact rats. This increase was mainly due to indirect bilirubin, which increased 5.2-fold, while direct bilirubin increased by only 2.7 times compared to the values of intact rats and was. An increase in the level of total bilirubin mainly by an increase in the indirect bilirubin portion is indicative functional membrane-bound transport system disorder involved in capturing of indirect bilirubin.[28]

The use of CEP led to decrease in the concentration of indirect bilirubin in a smaller extent than the use of ACC. However, the ability to reduce the level of direct bilirubin in the peripheral blood of rats with paracetamol-induced hepatitis of the two studied agents was similar: the level of direct bilirubin significantly decreased both with the use of CEP (by 52.5 %) and with the use of ACC (by 55.3 %).

Conclusion

The development of acute paracetamol-induced hepatitis in rats was associated with activation of lipid peroxidation processes in liver tissues, as demonstrated by a statistically significant increase of TBA-AP content in liver homogenates as compared with intact animals. Also, there was a statistically significant rise in the activity of both ALT and AST as well as a statistically significant increase of total bilirubin concentration as compared with intact rats.

The cryopreserved placenta extract showed marked hepatoprotective activity in paracetamol-induced hepatitis in rats. This was demonstrated by a statistically significant increase of the antioxidant-prooxidant index, a statistically significant decrease of the ALT and AST activity, as well as a statistically significant decrease of the level of direct bilirubin in rats treated with CEP as compared with non-treated animals.

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None.

Conflict of interest

None.

References

1. Andrade RJ, Chalasani N, Bjornsson ES, Suzuki A, Kullak-Ublick GA, Watkins PB, et al. Drug-induced liver injury. Nat Rev Dis Primers 2019;5:58. doi: 10.1038/s41572-019-0105-0.
2. Garcia-Cortes M, Robles-Diaz M, Stephens C, Ortega-Alonso A, Lucena MI, Andrade RJ. Drug induced liver injury: an update. Arch Toxicol 2020;94:3381–407.
3. Alempijevic T, Zec S, Milosavljevic T. Drug-induced liver injury: Do we know everything? World J Hepatol 2017;9:491–502.
4. Reuben A, Koch DG, Lee WM, Acute Liver Failure Study Group. Drug-induced acute liver failure: results of a U.S. multicenter, prospective study. Hepatology 2010;52:2065–76.
5. Chalasani NP, Hayashi PH, Bonkovsky HL. ACG Clinical Guideline: the diagnosis and management of idiosyncratic drug-induced liver injury. Am J Gastroenterol 2014;109:950–66.
6. McAtee C. Drug-induced liver injury. Crit Care Nurs Clin North Am 2022;34:267–75.
7. Yimin M. HepaTox: The professional networking platform for promoting clinical and translational research of drug-induced liver injury in China. Chin Hepatol 2014;8:575–6.
8. Licata A. Adverse drug reactions and organ damage: the liver. Eur J Intern Med 2016;28:9–16.
9. Bjornsson HK, Bjornsson ES. Drug-induced liver injury: pathogenesis, epidemiology, clinical features, and practical management. Eur J Intern Med 2022;97:26-31.
10. Kumachev A, Wu PE. Drug-induced liver injury. CMAJ 2021 Mar 1;193(9):E310. doi: 10.1503/cmaj.202026.
11. Tajiri K, Shimizu Y. Practical guidelines for diagnosis and early management of drug-induced liver injury. World J Gastroenterol 2008;14:6774–85.
12. Jaeschke H, Akakpo JY, Umbaugh DS, Ramachandran A. Novel therapeutic approaches against acetaminophen-induced liver injury and acute liver failure. Toxicol Sci 2020;174:159–67.

CONTENT

13. Chao X, Wang H, Jaeschke H, Ding WX. Role and mechanisms of autophagy in acetaminophen-induced liver injury. Liver Int 2018;38:1363–74.
14. Hladkykh FV, Chyzh MO, Manchenko AO, Belochkina IV, Mikhailova IP. Effect of cryopreserved placenta extract on some biochemical indices of therapeutic efficiency and toxicity of diclofenac sodium in adjuvant-induced experimental arthritis. Pharm Pharmacol 2021;9:278–93.
15. Hladkykh FV. The effect of meloxicam and cryopreserved placenta extract on initial inflammatory response – an experimental study. Ceska Slov Farm 2021;70:179–85.
16. Hladkykh F, Chyzh M. Modulation of meloxicam-induced changes in gastrointestinal and motor activity of the stomach by applying placenta cryoextract. Proc Shevchenko Sci Soc Med Sci 2021;64:84–94.
17. Hladkykh FV. Experimental study of the antiulcer effect of cryopreserved placenta extract on a model of acetylsalicylic acid-induced ulcerogenesis. Curr Issues Pharm Med Sci 2021;35:89–94.
18. Hladkykh FV, Koshurba IV, Chyzh MO. Characteristics of the antiulcerogenic activity of cryopreserved placenta extract in acute and chronic lesions of the stomach. Mod Med Technol 2023;1(56):62–8.
19. Koshurba IV, Hladkykh FV, Chyzh MO. Modulation of lipoperoxidation and energy metabolism in the gastric mucosa as a mechanism of placenta cryoextract activity in the healing of stress-induced erosive-ulcerative damage. Gastroenterology 2022;56(3):149–55.
20. Koshurba IV, Hladkykh FV, Chyzh MO, Belochkina IV, Rubleva TV. Hepatotropic effects of triple antiulcer therapy and placenta cryoextract: the role of sex factors in lipoperoxidation. Fiziol Zh 2022;68:25–32.
21. Koshurba IV, Chyzh MO, Hladkykh FV, Belochkina IV. Influence of placenta cryoextract on the liver metabolic and functional state in case of D-galactosamine hepatitis. Innov Biosyst Bioeng 2022;6:64–7.
22. Chyzh MO, Koshurba IV, Marchenko MM, Hladkykh FV, Belochkina IV. Gender determinism of the effect of placenta cryoextract on the hepatotropic effects of esomeprazole, clarithromycin and metronidazole in chronic liver damage. Mod Med Technol 2023;1(56):55–61.
23. Vogel HG. Drug discovery and evaluation: pharmacological assays. Berlin, Heidelberg: Springer Berlin Heidelberg; 2008.
24. Hladkykh FV. Gastrocytoprotective properties of cryopreserved placenta extract in combined action of low temperatures and inhibition of cyclooxygenase. Acta Fac Med Naiss 2022;39:48–56.
25. Hladkykh FV. Anti-inflammatory properties of diclofenac sodium on the background of combined use with cryopreserved placenta extract in the experiment. Probl Cryobiol Cryomed 2021;31:364–7.
26. Ntamo Y, Ziqubu K, Chellan N, Nkambule BB, Nyambuya TM, Mazibuko-Mbeje SE, et al. Drug-induced liver injury: clinical evidence of N-acetyl cysteine protective effects. Oxid Med Cell Longev 2021;2021:3320325. doi: 10.1155/2021/3320325.
27. Asakawa T, Matsushita S. Coloring condition of thiobarbituric acid test for detecting lipid hydroperoxides. Lipids 1980;15:137–40.
28. Aebi H. Catalase in vitro. Methods Enzymol 1984;105:121–6.
29. Vink KL, Schuurman W, van Gansewinkel R. Use of the caffeine reagent in direct spectrophotometry of bilirubin. Clin Chem 1986;32:1389–93.
30. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol 1957;28:56–63.
31. Tokuda K. Tanimoto K. New method of measuring serum bilirubin using vanadic acid. Jap J Clin Chem 1993;22:116–22.
32. Zar JH. Biostatistical analysis. 5th edition. Prentice-Hall, Englewood; 2014.
33. Botros M, Sikaris KA. The De Ritis ratio: the test of time. Clin Biochem Rev 2013;34:117-30.

Predojević Samardžić et al. Scr Med 2023 Jun;54(2):141-7. DOI:10.5937/scriptamed54-44752
ORIGINAL ARTICLE

Spontaneous Closure of Isolated Ventricular Septal Defect in the First Year

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Abstract

Background/Aim: Ventricular septal defect (VSD) is the most common congenital heart anomaly that in many cases closes spontaneously. The spontaneous closure (SC) rate of VSD varies widely between studies. The aim of this study was to identify clinical and echocardiographic factors influencing SC of isolated VSD in the first year of life among a group of patients presented at the Paediatric Clinic.
Methods: Prospective study was performed in 60 consecutive patients with trivial, small or medium isolated VSD during the first year of life. Patients were divided into groups, according to gender and gestational age of the patient, type, number and the size of the defect and persistence of pulmonary hypertension. The size of defect was described in comparison to the diameter of the aortic annulus (VSD/Ao ratio).
Results: At the time of diagnosis, the mean VSD/Ao ratio was 0.33 mm. Muscular VSD was more common (76.7 %) than perimembranous (23.3 %). SC of VSD occurred in 60 % of all patients, in case of muscular defect in 73.9 % and in case of perimembranous in 14.3 %. There was a negative correlation between defect size and SC rate. SC probability for a given defect size was described by the formula: probability = -1.82933X+1.20145. None defect with pulmonary hypertension closed.
Conclusion: It was found that type and size of VSD and the persistence of pulmonary hypertension were significant predictors for SC, while gender and gestational age of the patient and the number of defects were not. This study can be useful in predicting the natural outcome of the VSD to make proper follow-up and management plans.

Key words: Ventricular septal defect; Spontaneous closure; Probability; The first year of life.

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Introduction

Ventricular septal defect (VSD) is an abnormal congenital communication between the two ventricles.[1] With an estimated a prevalence of 2 to 3.94 per 1000 live births, it is the most common congenital heart anomaly.[2-5] Since many cases are asymptomatic and a large number of defects close spontaneously before coming to the attention of physicians, the true prevalence is probably even higher. VSD is more common in premature infants with prevalence of 4.5 - 7 per 1000 live births.[6-9] It can occur as an isolated anomaly or with other congenital heart defects and as a component of complex congenital heart diseases. The isolated form of VSD is the most common and accounts for almost 50 % of all heart anomalies.[10] These defects are mostly classified according to their lo-

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CONTENT

cation, size and number. In terms of phenotypic features, VSD can be placed into one of three primary types. The first type, named muscular VSD, is made up of a defect that has exclusively muscular borders. In the second type, membranous VSD, the posteroinferior quadrant of the defect is made up of fibrous tissue. Since borders of this type of defect usually are partly made of muscular tissue, it is usually named perimembranous.[10] In the third type, the fibrous continuity is present between the leaflets of the aortic and pulmonary valves in the cranial margin of the defect. This type of defect is doubly committed and directly juxta-arterial because of the absence of any muscular subpulmonary infundibulum.

The most objective estimate of defect size is based on the ratio of the maximum diameter of the VSD and the diameter of aortic annulus (VSD/Ao ratio).[11] Usually, defects are considered small if their diameter is less than one-third, medium if it is between one and two-thirds and large if it is greater than two-thirds of the aortic annulus diameter. Trivial defects are small defects with a diameter less than one-quarter of the aortic annulus diameter. In some cases, there is more than one defect, mostly located in the apical part of the septum, which is called multiple VSD. The clinical manifestation of an isolated VSD is related to its size and the relationship between systemic and pulmonary vascular resistances. The symptoms typically become manifest in term infants between the fourth and eighth week, concomitant with the decrease in pulmonary vascular resistance. In premature infants the onset of symptoms is much earlier.[1] Today, echocardiography has a major role in diagnosis, monitoring and planning the appropriate treatment.

It has long been recognised that VSD in many cases closes spontaneously and there is a number of observation follow-up studies about this topic. Spontaneous closure (SC) rate of the defect depends on multiple factors, of which, the well-known are defects type, location and size. The mechanism of closure depends on the location, respectively on the type of VSD. The usual mechanism of SC of muscular VSD is thought to be muscular encroachment plus superimposed fibrosis or physical hypertrophy of the septal myocardium or by fibrous tissue around the margins leading to apposition of the edge of the defect.[12] The most considered mechanism of closure of perimembranous VSD is the adherence of tricuspid valve leaflets which create an aneurysm that closes the defect.[13] Doubly committed defects, on the other hand, usually always require surgical closure since they persistence courts the risk of development of aortic valvular prolapse.[1, 14] The SC rate of VSD varies widely between studies, from 12 % to 84 %, depending to the age of the patients, the location, size and type of VSD, diagnostic methods and the length of the follow-up period.[12, 15-17] The overall reported rate of SC of isolated VSD is between 44 % and 73 % by the end of the first year of life.[13, 14, 16, 18] After that age, the rate declines, especially after 3.5 years.[13] The size of the defect is one of the major predicting factors for SC, especially in muscular defects.[8] Defects greater than 66 % of the aortic annulus diameter mostly need surgical closure.[17]

The aim of the study was to identify clinical and echocardiographic factors influencing SC of VSD and to summarise a prediction formula including contributing factors.

Methods

Study design and subjects

Prospective study was performed in 60 consecutive patients born between June 2017 and July 2019 who presented with isolated VSD at the Paediatric Clinic of the University Clinical Centre of the Republic of Srpska. Only patients with isolated VSD or VSD in combination with an open *foramen ovale* were included in the study. Those with additional cardiac and other anomalies and with chromosomal abnormalities were excluded. Patients were divided into groups, according to gender (male or female), gestational age (mature or premature), type of VSD (muscular or perimembranous), number of defects (single or multiple), size of the defect (trivial, small or medium) and persistence of pulmonary hypertension.

The size of VSD was described in comparison to the diameter of the aortic annulus (VSD/Ao ratio) in order to negate the influence of weight and gestational age on the absolute VSD size. VSD was considered trivial if it measures less or equal to 25 % of the aortic annulus diameter, small if it measures more than 25 % but less or equal to 33 % and medium if it is more than 33 % but less or equal to 66 % of the aortic annulus diameter. This study did not include patients with large defects and patients with doubly committed and direct-

CONTENT

ly juxta-arterial VSD, since these usually always require surgical treatment, rather than follow-up observation.

Diagnostic procedure

VSD was detected with conventional two-dimensional colour Doppler echocardiography. Indications for performing echocardiography was auscultation of a heart murmur during a regular physical examination of the newborn. The ventricular septum was completely scanned and the position, number and size of the defects were determined. Defect size was measured in all planes and the largest diameter of VSD was recorded and the diameter of the aortic annulus was measured at the level of the valve (Figure 1).

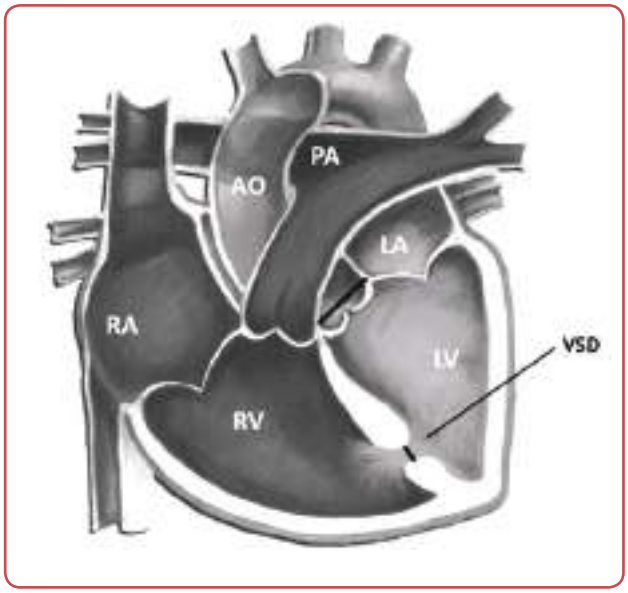

Figure 1: Picture of the heart with VSD illustrating the measurement of the VSD/Ao;

RV - right ventricle, LV - left ventricle; RA – right atrium; LA - left atrium; Ao - aorta; PA - pulmonary artery; VSD - ventricular septal defect;

In the case of multiple defects, the size of the largest one was studied, as it was considered that its natural course most faithfully reflects the course of the entire defect. Additional cardiac anomalies were excluded. All patients were followed up periodically, every three to six months depending on the size of the defect, till its closure or the end of the first year of life. This follow-up period was considered sufficient for the study since most of the defects were expected to close during the first year. The VSD was considered closed if the echocardiogram of the ventricular septum was normal, confirmed by colour Doppler mapping. Doppler echocardiography measurements were used also to estimate pulmonary artery pressure with peak tricuspid regurgitation velocity and adding right atrial pressure. Other sign of pulmonary hypertension, as right ventricle size and pressure overload, pulmonary artery acceleration time and pulmonary artery diameter, were also searched.[19]

Statistical analysis

The Chi-square test was used to compare the differences in SC of VSD between groups. A p-value of less than 0.05 was regarded as statistically significant. To estimate the SC probability for a given statistically significant variable(s), logistic regression analysis was used. The logistic regression model was generated with SC as a dependent variable (SC as 1, no closure as 0) and defect size that was described in comparison to the diameter of the aortic annulus (X) as an independent variable.

Ethics statement

The study was conducted according to the principles expressed in the Declaration of Helsinki. All parents of studied patients provided written informed consent. Ethical authorisation was obtained from the Ethics Committee of the University Clinical Centre of the Republic of Srpska (decision No 01-9-396-2/17).

Results

Out of 60 patients born with isolated VSD, 31 (51.67 %) were males and 29 (48.33 %) were females, 46 (76.67 %) were born on term and 14 (23.33 %) were born prematurely. The mean age of the patients at the time of diagnosis was 31.62 ± 58.55 days (range 1-240 days).

Muscular defect was found in 46 patients (76.67 %) and perimembranous in 14 patients (23.33 %). Among patients with muscular VSD, 26 (56.52 %) had defect in apical part, 16 (34.78 %) in middle part and 4 (8.70 %) in anterior part of muscular septum. None of patients had a defect in the posterior part of the muscular septum. At the time of diagnosis, the mean size of VSD was 2.5 ± 1.15 mm (range 1-6 mm) and the mean VSD/Ao ratio was 0.33 ± 0.14 mm (range 0.09–0.66 mm). The mean VSD/Ao ratio for muscular defects was 0.28 ± 0.08 mm and for perimembranous 0.55 ± 0.13 mm. Trivial defect was found in 22 (36.67 %), small defect in 16 (26.67 %) and medium defect in 22 (36.67 %) patients. SC of VSD till the end

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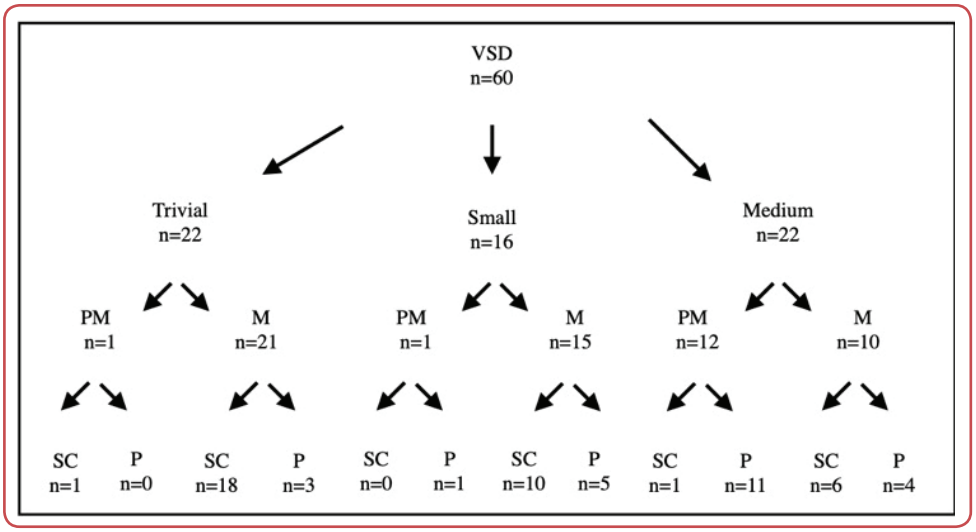

Figure 2: Flow chart showing the distribution of the studied VSDs according to size, type and natural outcome of defect

n - number; PM - perimembranous; M - muscular; SC - spontaneous closure; P - persistent; VSD - ventricular septal defect;

of the first year of life occurred in 36 (60 %) patients. The distribution of the 60 studied VSDs, according to size, type and natural outcome, is shown in Figure 2.

Table 1: Distribution of the patients according to clinical and echocardiographic indicators between the group with and the group without SC of VSD

Indicator	SC of VSD	No SC of VSD	p-value
Total, n (%)	36 (60.00 %)	24 (40.00 %)	-
Gender			
Male	21 (67.74 %)	10 (32.26 %)	0.316
Female	15 (51.72 %)	14 (48.28 %)	
Gestational age			
Mature	27 (58.70 %)	19 (31.30 %)	0.709
Premature	9 (64.29 %)	5 (35.71 %)	
Number of defect			
Single	31 (64.58 %)	17 (35.42 %)	0.263
Multiple	5 (41.67 %)	7 (58.33 %)	
Type of defect			
Perimembranous	2 (14.29 %)	12 (85.71 %)	< 0.001*
Muscular	34 (73.91 %)	12 (26.09 %)	
Size of defect			
Trivial	19 (86.36 %)	3 (13.64 %)	0.001*
Small	10 (62.50 %)	6 (37.50 %)	
Medium	7 (31.82 %)	15 (68.18 %)	
Pulmonary hypertension	0 (0.00 %)	4 (100.00 %)	-

*VSD - ventricular septal defect; SC - spontaneous closure; n - number; *p < 0.05;*

Significantly different closure rates were found for defects that differ in type ($p < 0.001$) and size ($p = 0.001$). Muscular VSD had a higher closure rate (73.91 %) compared to perimembranous VSD (26.09 %). Defects in apical and middle part had higher closure rates (76.92 % and 75 %, respectively) than defects in anterior part of the muscular septum (50 %), but this difference was not statistically significant. According to defect size, the highest closure rate was in the group with trivial VSD (86.36 %). Of 19 trivial defects that closed spontaneously, one was perimembranous and 18 were muscular defects. Three cases of trivial VSDs, all muscular, did not close. Small defects spontaneously closed in 62.50 % and medium in 31.82 % of cases. VSD closure rate was slightly higher in males (67.74 %) compared to females (51.72 %), in prematurely born patients (64.29 %) compared to those born on term (58.70 %), and in the case of a single defect (64.58 %) compared to multiple defects (41.67 %), but these differences were not statistically significant. Four patients (6.66 %) had pulmonary hypertension. In none of them, the defect was spontaneously closed till the end of the first year. Differences of results between patients with and without SC of VSD are summarised in the Table 1 and Table 2.

Table 2: Distribution of the patients with muscular VSD according to defect localisation between the group with and the group without SC of VSD

Location	SC of VSD	No SC of VSD	Total
Apical	20 (76.92 %)	6 (23.08 %)	26 (56.52 %)
Middle	12 (75.00 %)	4 (25.00 %)	16 (34.78 %)
Anterior	2 (50.00 %)	2 (50.00 %)	4 (8.70 %)
Total, n (%)	**34 (73.91 %)**	**12 (26.09 %)**	**46 (100.00 %)**

VSD - ventricular septal defect; SC - spontaneous closure; n - number; Chi-Square: 1.318; p-value: 0.517 (p > 0.05); Location: Muscular defect according to location in muscular part of ventricular septum;

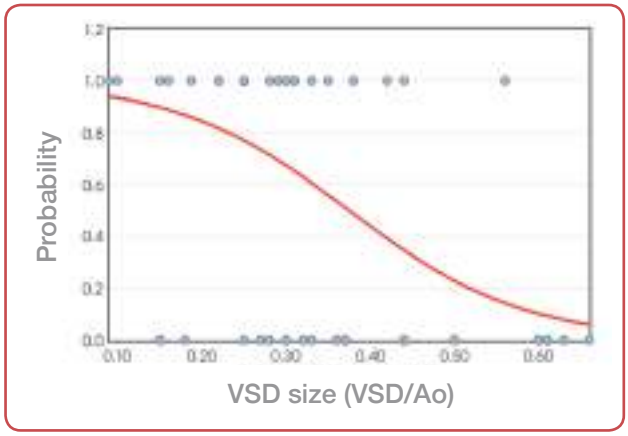

Figure 3: Logistic regression line shows the probability of SC of VSD depending on the size of the defect (VSD/Ao ratio);

1: closed VSD, 0: persistent VSD; Chi-Square: 16.765, p =0.0009; VSD - ventricular septal defect; SC - spontaneous closure; Ao - aorta;

The SC probability for a given defect size, as a statistically significant variable, was estimated using logistic regression analysis (Figure 3). The relationship between defect size (VSD/Ao ratio) and SC probability was described by the following prediction formula:

probability = - 1.82933X + 1.20145.

Discussion

The present study was conducted in order to evaluate the SC rate of isolated VSD during the first year of life. From 60 consecutively enrolled patients with isolated VSD, there was almost the same number of male and female patients, consistent with the well-established fact that there is no gender predilection in VSD.[20] Preterm born patients account for 23.33 %. Since the average incidence of preterm birth rate in Europe is 10.6 %, this result is in line with known fact that VSD is more common in premature than in term born infants.[8, 9, 21, 22] The mean age of the patient in this study at the time of diagnosis was 31.62 days, when the onset of symptoms in the case of a smaller defect, such as was studied, is usually not yet expected.[11] The explanation for this early finding is that echocardiography was performed because of the auscultation of a heart murmur as an isolated sign.

In presented study, the frequency of muscular VSD was higher than of perimembranous (76.67 % compared to 23.33 %), that differs from studies that include wide paediatric population and classically report that perimembranous defects account for approximately 75 % of total VSDs.[6, 7] This difference likely reflects the facts that the vast majority of prenatally diagnosed VSDs are muscular and that the rate of SC in the postnatal period is higher in muscular than in perimembranous defects and probably combined with the high number of muscular defects that are undiagnosed due to the common absence of clinical signs.[7, 14, 22, 23] This predominance of muscular defects in this study is consistent with the results of studies in which VSDs were screened in non-selected populations using echocardiography.[22, 23]

It was found that 60 % of all studied VSDs were closed by the end of the first year of life, which is within the range of results of many previous reports.[6, 13, 14, 18] The closure rate was significantly higher in muscular (73.91 %) compared to perimembranous VSD (14.29 %), which generally could be explained by different closing mechanisms and, in this study, with a much smaller mean size of muscular compared to perimembranous VSD. Other studies reported a similar SC rate for muscular VSD (69 % to 74 %), but higher for perimembranous VSD (22 % to 28 %) compared to presented results.[22, 23] A possible explanation for this difference in results is the relatively larger average size of the perimembranous defect in presented patients and the shorter follow-up period compared to other studies. The location of the defect within the muscular part of the septum seems that also affects the CS rate. Other studies state that it is slightly higher in defects located in the central part of the muscular septum compared to those in its apical, anterior and posterior parts.[16, 24] In this study, defects in the middle and apical parts of the septum had approximately similar SC rate (76.9 % and 75 %), which was higher than the SC rate of defects in the anterior part of the muscular septum (50 %), although this difference was not statistically significant. This difference in rates of spontaneous closure of defects in different parts of the muscular septum between this and other studies could be explained by presented small patient sample. The results of this and other studies indicating that the SC rate of VSD depends on its localisation are particularly useful for predicting the natural outcome of VSD in newly diagnosed patients.

The SC rate in this study, as in many previous studies, shows negative correlation with the size of the defect.[8, 17] The trivial VSD had, expectative, the highest closure rate (86.36 %), but in three

cases these defects have not closed till the end of the follow-up period. This is particularly unusual because they all were muscular and were not accompanied by pulmonary hypertension. This result leads us to a conclusion that other unknown factors also influence defect closure, which could be a subject of future studies. With data of the natural outcome of studied VSDs for a given defect size, the linear regression model obtained a formula for calculating the probability of SC that could be useful in predicting the natural outcome of this anomaly.

In general, multiple VSD, because of a larger shunt, has a lower SC rate than single VSD. According to one report, multiple VSD closes spontaneously in the first year only in 20 % of the cases.[16] In this study, multiple defects closed spontaneously in 41.67 % of the cases, which is not statistically significantly different from the SC rate of a single defect.

Pulmonary hypertension is a negative factor for SC of VSD according to many previous studies.[8, 12, 16] Although there were only four patients with VSD who had pulmonary hypertension in this study, it is significant to point out that in none of them defect spontaneously closed.

It seems that gender and gestational age does not affect the incidence of SC. In this study, the SC of VSD rate was slightly higher in males (67.74 %) compare to females (51.72 %), in accordance with Li et al report and opposite to Farina et al report, although these differences did not reach statistical significance in either study.[12, 25] The rate of SC in premature infants in presented study was almost similar to those born on the term, in line with other reports.[12, 17]

There were some limitations in this study. Because of small the number of patients and short the follow-up time, the results can be useful in predicting the natural outcome of the anomaly, but they do not have a diagnostic value. Only those factors that are found to be significant predictors of the outcome were examined, but other unmeasured factors could also influence defect closure and, therefore, the result. A simplified classification of VSDs was used. Determining the size of septal defects is not always easy, and therefore, results reported in relation to the size of the defect should be interpreted with caution. Finally, VSDs that were considered isolated according to the clinical findings during a follow-up time were studied, which is insufficient for detection of the possible delay in psychomotor development or growth, so some syndromic cases could have been missed.

Conclusion

It was found that type and size of VSD and the persistence of pulmonary hypertension were significant predictors for SC, while gender and gestational age of the patient and the number of defects were not. This study can be useful in predicting the natural outcome of the VSD to make proper follow-up and management plans.

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Conflict of interest

None.

References

1. Spicer DE, Hsu HH, Co-Vu J, Anderson RH, Fricker FJ. Ventricular septal defect. Orphanet J Rare Dis 2014;9:144. doi: 10.1186/s13023-014-0144-2.
2. Liu Y, Chen S, Zühlke L, Black GC, Choy MK, Li N, et al. Global birth prevalence of congenital heart defects 1970-2017: updated systematic review and meta-analysis of 260 studies. Int J Epidemiol 2019 Apr 1;48(2):455-63.
3. Hoffman J. The global burden of congenital heart disease. Cardiovasc J Afr 2013;24:141-5.
4. Van der Linde D, Konings EE, Slager MA, Witsenburg M, Helbing WA, Takkenberg JJ, et al. Birth prevalence of congenital heart disease worldwide: a systematic review and meta-analysis. J Am Coll Cardiol 2011;58:2241-7.
5. EUROCAT. Cases and prevalence (per 10,000 births) for all full member registries from 2011 to 2015. 2015. Available at: http://www.eurocat- network.eu/accessprevalencedata/prevalencetables. [Cited: 15-Nov-2021]
6. Gómez O, Martínez JM, Olivella A, Bennasar M, Crispi F, Masoller N, et al. Isolated ventricular septal defects in

CONTENT

the era of advanced fetal echocardiography: risk of chromosomal anomalies and spontaneous closure rate from diagnosis to age of 1 year. Ultrasound Obstet Gynecol 2014;(43):65-71.
7. Erol O, Sevket O, Keskin S, Yazıcıoğlu HF, Gül A. Natural history of prenatal isolated muscular ventricular septal defects. J Turk Ger Gynecol Assoc 2014;15(2):96-9.
8. Xu Y, Liu J, Wang J, Liu M, Xu H, Yang S. Factors influencing the spontaneous closure of ventricular septal defect in infants. Int J Clin Exp Pathol 2015; 8(5):5614-23.
9. Miyake T. A review of isolated muscular ventricular septal defect. World J Pediatr 2020 Apr;16(2):120-8.
10. Cho YS, Park SE, Hong SK, Jeong NY, Choi EY. The natural history of the fetal diagnosed isolated ventricular septal defect. Prenat Diagn 2017; 37(9): 889-93.
11. Eroğlu AG, Oztunç F, Saltik L, Bakari S, Dedeoğlu S, Ahunbay G. Evolution of ventricular septal defect with special reference to spontaneous closure rate, subaortic ridge and aortic valve prolapse. Pediatr Cardiol 2003;(24):31-5.
12. Li X, Ren W, Song G, Zhang X. Prediction of spontaneous closure of ventricular septal defect and guidance for clinical follow-up. Clin Cardiol 2019;42:536-41.
13. Zhang J, Ko JM, Guileyardo JM, Roberts WC. A review of spontaneous closure of ventricular septal defect. Proc (Bayl Univ Med Cent) 2015;28(4):516-20.
14. Zhao QM, Niu C, Liu F, Wu L, Ma XJ, Huang GY. Spontaneous closure rates of ventricular septal defects (6.750 consecutive neonates). Am J Cardiol 2019 Aug 15;124(4):613-7.
15. Eroğlu AG, Atik SU, Sengenc E, Cig G, Saltik IL, Oztunç F. Evaluation of ventricular septal defect with special reference to the spontaneous closure rate, subaortic ridge, and aortic valve prolapse II. Pediatr Cardiol 2017; 38(5): 915-21.
16. Cresti A, Giordano R, Koestenberger M, Spadoni I, Scalese M, Limbruno U, et al. Incidence and natural history of neonatal isolated ventricular septal defects: do we know everything? A 6-year single-center Italian experience follow-up. Congenit Heart Dis 2018;13(1):105-12.
17. Miyake T, Shinohara T, Inoue T, Marutani S, Takemura T. Spontaneous closure of muscular trabecular ventricular septal defect: comparison of defect positions. Acta Paediatr 2011;100(10):158-62.
18. Lin MH, Wang NK, Hung KL, Shen CT. Spontaneous closure of ventricular septal defects in the first year of life. J Formos Med Assoc 2001;100(8):539-42.
19. Galiè N, Humbert M, Vachiery JL, Gibbs S, Lang I, Torbicki A, et al. ESC Scientific Document Group. 2015 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension: The Joint Task Force for the Diagnosis and Treatment of Pulmonary Hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS): Endorsed by: Association for European Paediatric and Congenital Cardiology (AEPC), International Society for Heart and Lung Transplantation (ISHLT). Eur Heart J 2016 Jan 1;37(1):67-119.
20. Dakkak W, Oliver TI. Ventricular septal defect. [Updated: 8-Jun-2021]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 Jan. Available from: https://www.ncbi.nlm.nih.gov/books/NBK470330/.
21. Chawanpaiboon S, Vogel JP, Moller AB, Lumbiganon P, Petzold M, Hogan D, et al. Global, regional, and national estimates of levels of preterm birth in 2014: a systematic review and modelling analysis. Lancet Glob Health 2019;7(1):e37-e46.
22. Turner SW, Hunter S, Wyllie JP. The natural history of ventricular septal defects. Arch Dis Child 1999;81(5):413-6.
23. Meberg A, Otterstad JE, Frøland G, Lindberg H, Sørland SJ. Outcome of congenital heart defects-a population-based study. Acta Paediatr 2000;89(11):1344-51.
24. Ramaciotti C, Vetter JM, Bornemeier RA, Chin AJ. Prevalence, relation to spontaneous closure, and association of muscular ventricular septal defects with other cardiac defects. Am J Cardiol 1995;75(1):61-5.
25. Farina MA, Hook EB. Apparent sex difference in spontaneous closure of ventricular septal defect. J Pediatr 1978;93(6):1065-6.

CONTENT

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ORIGINAL ARTICLE

Association of Cardiovascular and Metabolic Diseases with Risk of Dementia in the Urban Population of North India

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Abstract

Background/Aim: Dementia has become a public health problem due to its association with biological risk factors; obesity, diabetes, hypertension and hypercholesterolaemia. Most of these risk factors, appear to be associated with dementia as well as with risk of coronary artery diseases (CADs) and stroke. This study aimed to find out the association of biological risk factors with cognitive impairment and dementia.

Methods: Cross-sectional survey in a hospital was performed. After written informed consent and approval from hospital ethic committee, all subjects (n = 2002) above 25 years of age (1016 males and 986 females) were randomly selected and recruited from urban population of Moradabad, North India. Clinical data and risk factors were recorded with the help of case record form and validated questionnaires. Assessment of cognitive decline and dementia was made by Singh's memory function rating scale and biological risk factors by physical examination, sphygmomanometer and electrocardiography. The association of biological risk factors with dementia was calculated by multivariate logistic regression analysis after adjustment of age and sex.

Results: Obesity, diabetes, hypertension, CAD and hypercholesterolemia were highly prevalent independent risk factors among patients with dementia. Multivariate logistic regression analysis showed that regardless of age and body mass index, diabetes mellitus and CAD were highly significant ($p < 0.001$) risk factors of dementia. Hypertension and family history of dementia were weakly but significantly($p < 0.05$) associated with dementia.

Conclusion: It is possible that increased frequency of obesity, diabetes, hypertension and CAD may increase the risk of dementia in an ageing population. Prevention and control of these biological risk factors may cause decline in the risk of dementia.

Key words: Western diet; Sedentary behaviour; Mastication; Cardiovascular diseases.

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Introduction

According to WHO estimates, the population of people living with dementia, could be as high as 50 million.[1,2] It seems that by 2030, it may be projected to increase to 75 million. The frequency of dementia may be estimated to be threefold more by 2050. The global count of dementia in 1990

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was 20.2 million, which became 43.8 million in 2016. This increase of 117 % contrasted with a minor increase in age-standardised prevalence of 1.7 %, from 701 cases per 100,000 population in 1990 to 712 cases per 100,000 population in 2016.[4] It seems that globally, dementia was the fifth leading cause of death, accounting for 2.4 million deaths.[4] The strength of people with dementia will increase in the Asia-Pacific region, from 23 million in 2015 to almost 71 million by 2050.

It is possible that more than half of the risk of dementia could be due to the modifiable risk factors; obesity, diabetes, smoking and due to ageing.[4] Diet and lifestyle guidelines proposed by International College of Nutrition and International College of Cardiology emphasise on desirable levels of risk factors and protective factors for prevention of dementia.[5] Apart from diet and lifestyle factors, age, obesity, diabetes and cardiovascular diseases (CVDs); are major risk factors of dementia.[1-4] Epidemiological studies indicate that diabetes mellitus,[6] hypertension,[7] obesity[8] and coronary artery disease (CAD)[9] have been demonstrated to be risk factors that predispose memory dysfunction and dementia. A previous study confirmed that late-life depression is associated with increased risk of dementia and supplied evidence that late-life depression may be an early manifestation of dementia rather than increasing risk for dementia.[10] Later research has found that a longitudinal worsening of CVD risk is associated with midlife cognitive decline and the worsening of single CVD risk factor has been shown to be associated with an increased risk of memory impairment and dementia.[11]

In previous publications based on subjects of this study, prevalence of behavioural risk factors of dementia was reported[12-14] and questionnaire for assessment of dementia.[13] This study, aimed to examine the association of CVDs and diabetes with risk of dementia in an urban population of India.

Methods

Informed consent was achieved from each subject and study was approved by the Institutional Ethic Committee, in accordance with Helsinki Declaration 1964.

This cross-sectional survey included randomly selected 20 streets from the urban area of the city of Moradabad.[15] All the subjects were randomly selected, based on voter's list, with an aim of choosing, 40-100 adults, aged 25 years and above, from each block. The 2222 subjects aged 25 years and above were invited, of which 220 (9.90 %) did not volunteer to participate and rest 2002 (1016 men and 986 women) agreed to be part of this study. Interviews were performed in detail, with the help of pretested and validated questionnaires, for assessment of behavioural risk factors and protective factors, prepared according to the guidelines of WHO and Indian Council of Medical Research. All the subjects were evaluated by a dietitian and physician administered questionnaire, a physical examination and sphygmomanometer, electrocardiogram (ECG) and blood tests.

Criteria for diagnosis of risk factors and dementia

The criteria for the diagnosis of some of the behavioural risk factors; tobacco intake, western type diet, sedentary behaviour and alcoholism as well as memory dysfunction or dementia were based on previous studies and have already been reported in earlier publications.[12-14] In brief, dementia was diagnosed based on a new questionnaire; Mild memory dysfunction (MMD) was identified if there was possible impairment of memory (score 21-40). Impairment of memory was considered if the score was 41-60. The presence of dementia was identified at score 61-80 on the basis of the instrument.[12, 13] Low cognitive activity was diagnosed based on questionnaires related with mental work. Sleep disruption was considered in presence of sleep in the night of < 6 hours duration and/or disturbed sleep with frequent awakening. Low education was considered in presence of total education of < 5 years in school.

Criteria for diagnosis of biological risk factors

The diagnosis of biological risk factors of dementia such as hypertension, CAD, obesity, diabetes was based on WHO criteria described earlier.[15] Body mass index (BMI) was calculated and obesity was defined as a BMI > 30 kg/m^2 and overweight when BMI > 25 kg/m^2 to 29.9 kg/m^2. Figures for criteria according to the Indian consensus group for overweight (> 23 kg/m^2), were also calculated. Central obesity was considered when waist – hip ratio > 0.90 in males and > 0.80 in females were observed, as suggested in previous studies.

Diabetes mellitus was diagnosed in presence of fasting blood glucose > 7.1 mmol/L (126 mg/dL) and postprandial 2 h after 75 g of oral glucose > 11.2 mmol/L (> 200 mg/dL). Glucose intolerance was diagnosed in presence of fasting glucose between 110-126 mg/dL and postprandial glucose between 180 to 200 mg/dL. The measurement of blood pressure (BP) was made in right arm after 5 minute rest, as reported earlier considering: systolic and diastolic phase V of Korotkoff, after 5 min rest as per guidelines of WHO in all subjects. The diagnosis of high BP was made if systolic BP was 140 mm Hg and above and diastolic BP 90 mm Hg and above.

CAD was identified by: (a) past or present history of myocardial infarction or angina or/and earlier identification of CAD; (b) positive Rose questionnaire and (c) specific findings in the ECG: Minnesota codes 1-1, 4-1, 5-9, 5-2 or 9-2. If all the three criteria were present, then it was diagnosed as CAD. Known CAD was also considered as presence of disease. In addition, affirmative response to Rose questionnaire and ECG with certain specific changes were also considered CAD.[11-13]

Biochemical data

Venous blood samples were collected after a fasting of about 10 hours in the morning in all subject for analysis of the blood glucose and blood cholesterol and routine blood tests.

Statistical analysis

The continuous variables were given as mean and standard deviation and prevalence rates in percent. Statistical value of association of various risk factors was found out by multivariate logistic regression analysis. The odds ratios and 95 % confidence intervals were obtained to find out level of significance using multivariate model. This was done after adjustment of age and sex using overall prevalence of cognitive deficit, as the dependent variable. Only p-values < 0.05 and two tailed t-test were considered significant.

Results

The results showed that the prevalence of dementia was significantly greater among subjects above 60 years of age in both sexes and trend was significant as reported earlier.[12, 13] The prevalence of dementia and/or memory impairment was more common among men compared to women, respectively [84 (8.26 %) vs 53 (5.27 %)] with total prevalence of 6.84 % (n = 137).[13] The prevalence of biological risk factors such as age above 60 years, diabetes mellitus, CAD were more common among men compared with women (Table 1).

Table 1: Prevalence of biological risk factors and protective factors of dementia among men and women

Risk factors and protective factors of dementia	Men (n = 1016)	Women (n = 986)	Total (n = 2002)
Depression and mood disorders	225 (22.1)	215 (21.8)	440 (22.0)
Protective factors			
Mastication of foods (> 30 mastications/bite)	231 (22.7)	197 (19.9)	428 (21.4)
Biological risk factors			
Age > 60 years	226 (22.2)*	169 (17.1)	395 (19.7)
Diabetes mellitus	81 (8.0)*	52 (5.3)	133 (6.6)
Hypertension	285 (28.0)	248 (25.1)	533 (26.6)
Hypercholesterolaemia	313 (30.8)	317 (32.1)	630 (31.4)
Overweight/obese	321 (31.6)	324 (32.8)	645 (32.2)
Coronary artery disease	132 (13.0)*	98 (9.9)	230 (11.4)

**p < 0.05, **p < 0.01 (Chi square test), Values are number (%); Hypertension: blood pressure > 140/90 mm Hg; Hypercholesterolemia: cholesterol > 5.18 mmol/L; Overweight/obese: body mass index > 25 kg/m^2;*

The frequency of biological risk factors such as age > 60 years, diabetes mellitus and obesity, were highly prevalent risk factors among patients with dementia whereas hypertension, high cholesterol, CAD and family history of dementia were not very common but frequency was significant. The frequency of risk factors among patients with dementia and those without dementia are given in Figure 1. Depression and mood disorders, diabetes mellitus, hypertension, hypercholesterolaemia, obesity, coronary artery disease and family history of dementia were significantly more common among patients with dementia compared to rest of the subjects. Tobacco and alcohol are not common in women.

Multivariate logistic regression analysis showed that regardless of age and BMI, diabetes mellitus, obesity, age > 60 years and CAD were highly significant (p < 0.001) risk factors of dementia. Hypertension and family history of dementia were weakly but significantly (p < 0.05) associated with dementia. Depression and mood disorders as well as high cholesterol were not associated with dementia (Table 2).

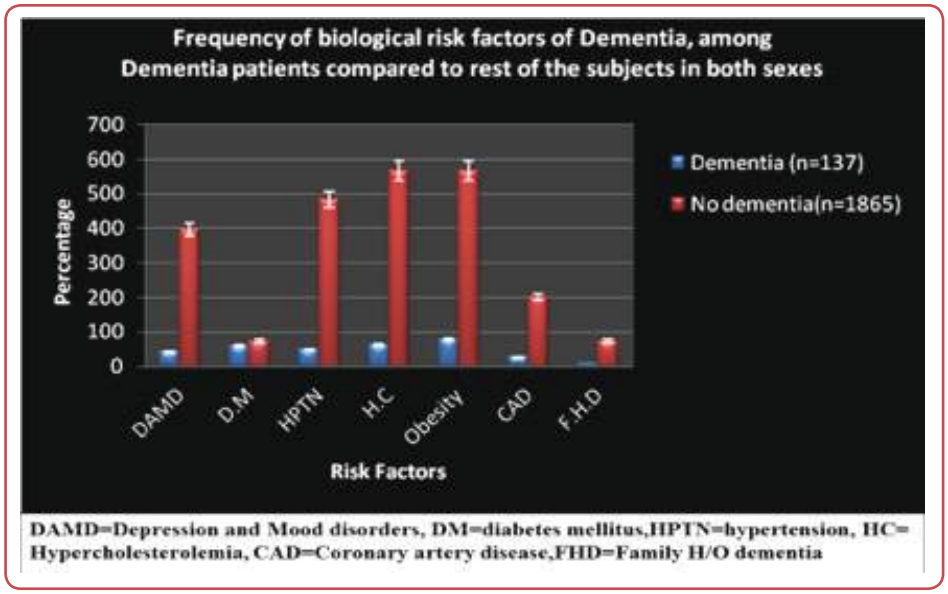

Figure 1: Frequency of biological risk factors of dementia among patients with and without dementia

Table 2: Multivariate logistic regression analysis for association of risk factors with risk of dementia after adjustment of age and body mass index, among men and women

Risk factors	Men (n = 1016)	Women (n = 986)
Depression and mood disorders	0.83 (0.71-0.97)	0.83 (0.71-0.97)
Diabetes mellitus	0.73 (0.66-0.79)**	076 (0.70- 0.84)**
Age > 60 years	0.78 (0.70-0.88) **	0.83 (0.75-0.92)**
Hypertension	0.90 (0.81- 0.99) *	0.91 (0.82-0.99)*
Overweight / obese	0.75 (0.68-0.80) **	0.73 (0.66-0.81)**
Coronary artery disease	0.88 (0.81- 0.94) **	0.90 (0.84-0.97)**
Hypercholesterolaemia	0.87 (0.71-0.1.00)	0.91 (0.73.-1.18)
Family history of dementia	0.88 (0.78-0.96) *	0.89 (0.80-0.99)*

**p < 0.05, **p < 0.001, p-value was obtained by regression analysis; Values are presented as odds ratios (95 % confidence intervals); Hypertension: blood pressure > 140/90 mm Hg; Hypercholesterolaemia: cholesterol > 5.18 mmol/L; Overweight/obese: body mass index > 25 kg/m^2;*

Discussion

This study shows that after adjustment of age and BMI, diabetes mellitus, overweight/obesity, age > 60 years and CAD were highly significant ($p < 0.001$) risk factors of dementia. Hypertension, family history of dementia were weakly but significantly ($p < 0.05$) associated with dementia. Depression and mood disorders as well as high cholesterol were not associated with dementia. The overall prevalence of dementia was 6.84 % (n = 137) and risk of dementia showed significant increase with increase in age after 55 years as reported earlier.[12, 13] The population of India is extremely diverse in terms of socio-economic, lifestyle and behaviour, cultural, linguistic, geographical and genetic factors.[16] The epidemiological transition in India from communicable diseases to non-communicable diseases as well as the unequal disease burden between different states within India is common.[17] These variations in diseases in different states, may be due to varying grades of economic development and alteration in diet and lifestyle factors.[17] Epidemiological studies conducted in India indicate that the prevalence of vascular and metabolic risk factors, as

well as white matter hyper-intensities, differ between urban and rural cohorts.[17-20] It seems that the study of the role of vascular risk factors, socioeconomic, cultural factors and genetic influences on dementia prevalence and progression in Indian populations is urgently needed.

In a recent study, involving a total of 62,372 patients, 10,417 (16.7 %) had diabetes and 43,023 (69.0 %) had obstructive CAD.[9] After a median follow-up of 5.8 years patients with both diabetes and CAD had the highest risk of dementia (aHR 1.47, 95 % CI 1.27–1.71), including Alzheimer's dementia (aHR 1.26, 95 % CI 1.01–1.56) and vascular dementia (aHR 2.60, 95 % CI 1.78–3.80), as well as ischaemic stroke (aHR 2.02, 95 % CI 1.77–2.32). Patients with either diabetes or CAD were at intermediate risk of dementia and ischaemic stroke. No significant trend was observed between the extent of CAD and risk of dementia in diabetes patients (p for trend = 0.069). It is possible that both diabetes and CAD were independent risk factors of dementia. Patients with combined diabetes and CAD had a particularly higher risk of cognitive impairment and ischaemic stroke.[9]

There are several studies from India reporting the prevalence and determinants of dementia.[21-26] In an Indian survey among 560 participants, 140 (25 %) patients were identified with impairment of cognition and the risk was higher among rural (27.6 % vs 18.5 %) and female (29.8 % vs 19.1 %) subjects compared to urban subjects, respectively.[21] The increased risk among rural subjects may be due to lower education and lower quantity of fruits and vegetables in the diet of rural population. In a cohort, comprising of 1066 subjects, 104 had dementia (98 with Alzheimer's dementia - AD) during 8.1 years of follow-up.[22] In the elderly subjects, aged ≥ 65 years, the incidence rate was 21.61 per 100,000. These rates of AD, appear to be much greater than that reported from rural north India, comparable with those reported from China and marginally lower than those reported from the Western world.[22] The prevalence of biological risk factors of dementia, in a previous study, among 595 elderly subjects > 50 years, was quite high (18.6 %, n = 111), because more than 50 % of subjects had either obesity, or hypertension or CAD or diabetes mellitus among urban subjects.[23] A community study was conducted over 5 years (2003-2008) in Kolkata, India, on 100,802 (male: n = 53,209) randomly selected subjects to assess prevalence and capture data on incident cases and deaths.[24] Standard case definitions were used. The data were used to estimate years of life lost (YLL) due to premature mortality, years of life lived with disability (YLD) and DALY, based on Global Burden of Disease 2010 approach. In a cohort study among 100,802 subjects, including 53,209 males, there were 103 (55 men) cases of dementia, with a prevalence of 1.53 % (age adjusted 1.12 %) at age ≥ 65 years.[24] In those ≥ 55 years age, average annual incidence rate of dementia was 72.57 per 100,000. In a sample of 500 subjects from hill region, mean age 69 years, no subject had dementia which may be due to enormous physical activity, natural environment and traditional diet.[25]

A rural and urban study included, 750 subjects aged 60 years and above from rural area and 1300 older people 65 years and above from urban area in South India.[26] The prevalence of dementia was higher among rural compared to urban subjects (3.5 % vs 2.7 %). It seems that, the only viable option, is to address primary as well as biological risk factors and promotion of protective factors for primary prevention of dementia.[12, 13, 18] It is possible that the development of an effective systemic health-care model for delivery of services to the families and patients with dementia keeping our sociocultural beliefs in mind may be useful. Every step should be taken to improve awareness regarding dementia and its preventive measures, to halt the epidemic, thereby contributing to the sustainable development goals.[18, 19] Low-cost, culturally appropriate and modifiable interventions need to be developed expeditiously and implemented through public health measures to reduce the growing burden of dementia.[27-30] A large cohort study, including 1,958,191 people from UK, with a median age, 55 years and a median follow-up of 9.1 years, showed that dementia occurred in 45,507 people, at a rate of 2.4 cases per 1000 person-years.[30] Compared with people of a healthy weight, underweight people (BMI < 20 kg/m^2 had a 34 % higher (95 % CI 29-38) risk of dementia. Interestingly, the incidence of dementia continued to fall for every increasing BMI category, with very obese people (BMI > 40 kg/m^2 having a 29 % lower (95 % CI 22-36) dementia risk than people of a healthy weight.

Analysis of demographic data indicate that the patterns of dementia are driven by decline in fertility in conjunction with rise in life expectancy, which together can predispose significant changes in the age structure of the population.[31] Eco-

nomic development may be associated with many societal changes alongside the rise of non-communicable disease in East Asia, which can alter the prevalence of dementia in the future once those cohorts with high risk of dementia reached their older age.[32, 33] In a cohort study, involving 6220 subjects (54.8 % females), aged 65 years and older, 463 individuals (7.4 %) had new onset of dementia ascertained in the 12 years of follow up period.[33] In the cohort born between 1926 and 1943, the hazard of developing dementia was 1.68 times greater (hazard ratio [HR] = 1.68 [95 % CI, 1.05-2.86]) for those who were relatively poor compared with those in the highest quintile of wealth, independent of education, index of multiple deprivation and health indicators. The lower risk of dementia among rich people may be due to increased consumption of fruits, vegetables and nuts and better cognitive activity as well as better spare time physical activity. Since diet and lifestyle factors can modulate all the biological risk factors of dementia, hence diet and lifestyle guidelines should be developed along with suitable drug therapy for prevention of dementia.

Conclusion

Biological risk factors such as diabetes mellitus, obesity, CAD, hypertension and stroke should be treated with optimal medical therapy along with modification of behavioural risk factors of dementia. It is possible that improvement in diet and lifestyle factors along with drug therapy of biological risk factors, may be useful in the prevention of dementia.

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Conflict of interest

None.

References

1. World Health Organization [Internet]. [Accessed Jun-2019]. Available at: http://www.emro.who.int/non-communicable-diseases/ causes/unhealthy-diets.html.
2. World Health Organization [Internet]. WHO dementia action plan. [Accessed Jan-2023]. Available at: https://www.who.int/mental_health/ neurology/dementia/action_plan_2017_2025/en/.
3. World Health Organization [Internet]. WHO risk reduction guidelines. [Accessed Jan-2023]. Available at: https://www.who.int/mental_health/mental_health/neurology/dementia/guidelines_ risk_reduction/en.
4. GBD 2016 Dementia Collaborators. Global, regional, and national burden of Alzheimer's disease and other dementias, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Neurol 2019 Jan;18(1):88-106.
5. Singh RB, Watanabe S, Li Duo, Nakamura T, Juneja LR, Takahashi T, et al. Diet and lifestyle guidelines and desirable levels of risk factors and protective factors for prevention of dementia: a scientific statement from joint symposium of JAAS and APCNS 2019;17; Biomed Sci and Tech Res BJSTR 2019;17(3):12844-64.
6. Tamura Y, Kimbara Y, Yamaoka T, Sato K, Tsuboi Y, Kodera R, et al. White matter hyperintensity in elderly patients with diabetes mellitus is associated with cognitive impairment, functional disability, and a high glycoalbumin/glycohemoglobin ratio. Front Aging Neurosci 2017 Jul 6;9:220. doi: 10.3389/fnagi.2017.00220.
7. Singh-Manoux A, Dugravot A, Shipley M, Brunner EJ, Elbaz A, Sabia S, et al. Obesity trajectories and risk of dementia: 28 years of follow-up in the Whitehall II Study. Alzheimers Dement 2018 Feb;14(2):178-86.
8. Clark LR, Koscik RL, Allison SL, Berman SE, Norton D, Carlsson CM, et al. Hypertension and obesity moderate the relationship between β-amyloid and cognitive decline in midlife. Alzheimers Dement 2019 Mar;15(3):418-28.
9. Olesen KKW, Thrane PG, C Gyldenkerne, T Thim, M Maeng. Diabetes, coronary artery disease, and risk of dementia – a cohort study from Western Denmark. Eur Heart J Nov 2020;42(S2):1507. doi:10.1093/ehjci/ehaa946.1507
10. Li G, Wang LY, Shofer JB, Thompson ML, Peskind ER, McCormick W, et al. Temporal relationship between depression and dementia: findings from a large communitybased 15-year follow-up study. Arch Gen Psychiatry 2011 Sep;68(9):970-7.
11. Bae JB, Han JW, Kwak KP, Kim BJ, Kim SG, Kim JL, et al. Is dementia more fatal than previously estimated? A population-based prospective cohort study. Aging Dis 2019 Feb 1;10(1):1-11.
12. Singh RB, Wilczynska A, Fedacko J, Horuichi R, Takahashi T, Manal MA, et al. Prevalence of behavioral risk factors and their association with dementia in the urban population of North India. MOJ Public Health 2023;12(1):46-50.
13. Singh RB, Wilkzynska A, Mojto V, Fedacko J, Fatima G, Niaz MA, et al. Development and validation of a questionnaire for assessment of memory impairment and dementia, by a new modified memory function rating scale. MOJ Public Health 2022;11(3):64-9.
14. Isaza A, Singh RB, Wilczynska A, Fedacko J, Takahashi T, Fatima G. Association of Indo-Mediterranean neuroprotective dietary (MIND) pattern with memory impairment and dementia, in an urban population of north India. Inter J Clin Nutr 2021;21:11–20.

CONTENT

15. Singh RB, Fedacko J, Pella D, Macejova Z, Ghosh S, de Amit K, et al; Five City Study Group; Gupta AK. Prevalence and risk factors for prehypertension and hypertension in five Indian cities. Acta Cardiol. 2011 Feb;66(1):29-37.
16. Ravindranath V, Sundarakumar JS. Changing demography and the challenge of dementia in India. Nat Rev Neurol 2021 Dec;17(12):747-58.
17. India State-Level Disease Burden Initiative Collaborators. Nations within a nation: variations in epidemiological transition across the states of India, 1990-2016 in the Global Burden of Disease Study. Lancet 2017 Dec 2;390(10111):2437-60.
18. Gudala K, Bansal D, Schifano F, Bhansali A. Diabetes mellitus and risk of dementia: A meta-analysis of prospective observational studies. J Diabetes Investig 2013 Nov 27;4(6):640-50.
19. Iyer GK, Paplikar A, Alladi S, Dutt A, Sharma M, Mekala S, et al; ICMR Neurocognitive Tool Box Consortium. Standardising dementia diagnosis across linguistic and educational diversity: study design of the indian council of medical research-neurocognitive tool box (ICMR-NCTB). J Int Neuropsychol Soc 2020 Feb;26(2):172-86.
20. Chandra V, Ganguli M, Pandav R, Johnston J, Belle S, DeKosky ST. Prevalence of Alzheimer's disease and other dementias in rural India: the Indo-US study. Neurology 1998 Oct;51(4):1000-8.
21. Patel RM, Singh US. Prevalence study of cognitive impairment and its associated sociodemographic variables using minimental status examination among elderly population residing in field practice areas of a medical college. Indian J Community Med 2018 Apr-Jun;43(2):113-6.
22. Mathuranath PS, George A, Ranjith N, Justus S, Kumar MS, Menon R, et al. Incidence of Alzheimer's disease in India: a 10 years follow-up study. Neurol India 2012 Nov-Dec;60(6):625-30.
23. Singh RB, Rao RS, Thakur AS, Srivastav S, Niaz MA, Shinde SN. Prevalence and risk factors of cognitive deficits and dementia in relation to socioeconomic class in an elderly population of India. J Anti-Aging Med 1999;2:141-7.
24. Banerjee TK, Dutta S, Das S, Ghosal M, Ray BK, Biswas A, et al. Epidemiology of dementia and its burden in the city of Kolkata, India. Int J Geriatr Psychiatry 2017 Jun;32(6):605-14.
25. Raina SK, Raina S, Chander V, Grover A, Singh S, Bhardwaj A. Identifying risk for dementia across populations: A study on the prevalence of dementia in tribal elderly population of Himalayan region in Northern India. Ann Indian Acad Neurol 2013 Oct;16(4):640-4.
26. Rajkumar S, Kumar S. Prevalence of dementia in the community: a ruralurban comparison from Madras, India. Aust J Ageing 1996;15:57–61.
27. Ranson JM, Rittman T, Hayat S, Brayne C, Jessen F, Blennow K, et al; European Task Force for Brain Health Services. Modifiable risk factors for dementia and dementia risk profiling. A user manual for Brain Health Servicespart 2 of 6. Alzheimers Res Ther 2021 Oct 11;13(1):169. doi: 10.1186/s13195-021-00895-4.
28. Livingston G, Huntley J, Sommerlad A, Ames D, Ballard C, Banerjee S, et al. Dementia prevention, intervention, and care: 2020 report of the Lancet Commission. Lancet 2020;396(10248):413–46.
29. Najar J, Östling S, Gudmundsson P, Sundh V, Johansson L, Kern S, et al. Cognitive and physical activity and dementia: A 44-year longitudinal population study of women. Neurology 2019 Mar 19;92(12):e1322-30.
30. Qizilbash N, Gregson J, Johnson ME, Pearce N, Douglas I, Wing K, et al. BMI and risk of dementia in two million people over two decades: a retrospective cohort study. Lancet Diabetes Endocrinol 2015 Jun;3(6):431-6.
31. GBD 2019 Dementia Forecasting Collaborators. Estimation of the global prevalence of dementia in 2019 and forecasted prevalence in 2050: an analysis for the Global Burden of Disease Study 2019. Lancet Public Health 2022 Feb;7(2):e105-e125.
32. Catindig JA, Venketasubramanian N, Ikram MK, Chen C. Epidemiology of dementia in Asia: insights on prevalence trends and novel risk factors. J Neurol Sci 22021;321(1-2):11-6.
33. Cadar D, Lassale C, Davies H, Llewellyn DJ, Batty GD, Steptoe A. Individual and area-based socioeconomic factors associated with dementia incidence in England: evidence from a 12-year follow-up in the English longitudinal study of ageing. JAMA Psychiatry 2018 Jul 1;75(7):723-32.
34. Singh RB, Wilczynska A, Shastun S, Saboo B, Maheshwari A, Singh RK, et al. Association of chrono-physiological and psychological risk factors among patients with acute coronary syndromes. World Heart J 2017;9(2):123-34.

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ORIGINAL ARTICLE

Left Gastric Artery Variants: A Cadaveric, Postmortem and Radiological Investigation

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Abstract

Background/Aim: Anatomical variations of the gastric vessels during laparoscopic surgeries of the stomach and related viscera frequently impair the surgeon's judgment, extend the duration of surgery and sometimes can lead to accidental surgical injuries, rendering it not possible to assure the safety and effectiveness of the surgical treatment. This research paper aimed to examine the variations of the left gastric artery (LGA), which could have implications for surgical and interventional procedures of the gastrointestinal tract (GIT) and related organs.

Methods: Fifty specimens, of which 22 were dissected from cadavers and 28 were acquired from post-mortems among the Indian population, regardless of age or sex were examined for variant LGA. In addition, the variation of the celiac trunk was observed in 10 patients using 3D-CT images, which were created by reconstructing multiple-slice computed tomography (CT) using 3-dimensional CT simulation software (3D-CT).

Results: The classical pattern origin of LGA from the celiac trunk was observed in 96 % specimens. In 2 % gastrophrenic trunk emerged from the abdominal aorta (AA) slightly proximal to the celiac trunk, then it branched into LGA and left and right inferior phrenic arteries. In remaining 2 %, LGA was the branch of the splenic artery. In 10 individuals radiological examination was conducted and found no abnormal pattern of celiac trunk.

Conclusion: Observing and reporting the variation in the gastric vessels by different methods has certain clinical value in upper gastrointestinal surgeries and interventions. The duration can be prolonged and the intraoperative blood loss is increased with the vascular variations. Overall, this research paper provides important information on the prevalence of anatomical variations of the LGA, which could help improve the safety and efficacy of upper gastrointestinal procedures.

Key words: Left gastric artery; Stomach; Coeliac trunk; Anatomical variations; Gastrointestinal surgery.

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Introduction

Usually, the left gastric artery (LGA) is a branch of the coeliac trunk, it travels posterior to the lesser sac within the gastro-pancreatic ligament toward the left and upward direction being enclosed here in the gastropancreatic fold. Then it runs the dorsomedial aspect of the stomach, thence along the lesser curvature, followed by it splitting it into ventral and dorsal branches

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to supply the stomach. Posterior branch in the lesser curvature anastomoses with right gastric artery.[1] The LGA not only supplies to the stomach but also supplies to the terminal part of the oesophagus. In the surgeries of the stomach and terminal oesophagus, care must be taken to spot the arteries around the stomach, even during the removal of lymph nodes.[2] Any damage or occlusion of the LGA can lead to severe complications such as gastric ulcer and bleeding. During oesophageal reconstruction usually, LGA is cut, which leads to avascular hepatic necrosis in the individuals with left hepatic artery as the branch from the LGA.[3] The LGA does not always arise from the usual site, it may arise from the aorta with a higher prevalence from a superior mesenteric artery with a lower prevalence.[4] This may lead to technical issues in the procedures of transarterial chemoembolisation and infusion therapy for liver cancer patients.[5] If the existence of a posterior gastric artery behind the stomach is a branch from the splenic artery, the significance of the exact depiction of the posterior gastric artery is vital for gastric and pancreatic surgeries.[6]

The rationale of this investigation was to study the anatomical variations in the origin and path of LGA to provide preoperative knowledge of vascular variations that would facilitate surgeons to avoid extensive dissection and vascular damage.

Methods

Institutional Ethical Committee of Sri Venkateswara Medical College, Tirupati, India was granted permission (SVMC/Institutional Ethical Committee/Acad No: 305/12/2013). All the participants, relatives of the deceased and donors of the cadaver signed a written waiver of informed consent. The study included 50 human specimens, of which 22 were obtained from cadavers in the dissecting theatre and 28, regardless of age or sex, from post-mortem examinations among Indian residents. The investigation was conducted in Anatomy Department at Sri Venkateswara Medical College, Tirupati, Kurnool Medical College, Kurnool and AIMSR, Chittoor, India. Dissection was done based on the instructions of the second volume of Cunningham's Dissection Manual. The arteries which were supplied to the stomach were traced carefully. In each instance, a schema was drawn. The source of origin, course and relations of LGA were observed. Besides, the variation of the celiac artery was evaluated in 10 individuals using 3D-CT images, which were created by reconstructing multiple-slice CT using 3-dimensional CT simulation software (3D-CT).

Results

The classical pattern origin of LGA from the celiac trunk was observed in 48 specimens. In one sample gastrophrenic trunk emerged from the abdominal aorta (AA) slightly proximal to the celiac trunk, then it branched into LGA and left and right inferior phrenic arteries (Figure 1). In another sample, LGA was the branch of the splenic artery (Figure 2).

The LGA splatted into ventral and dorsal branches immediately after a little distance from its origin to supply the corresponding surfaces of the stomach (Figure 3).

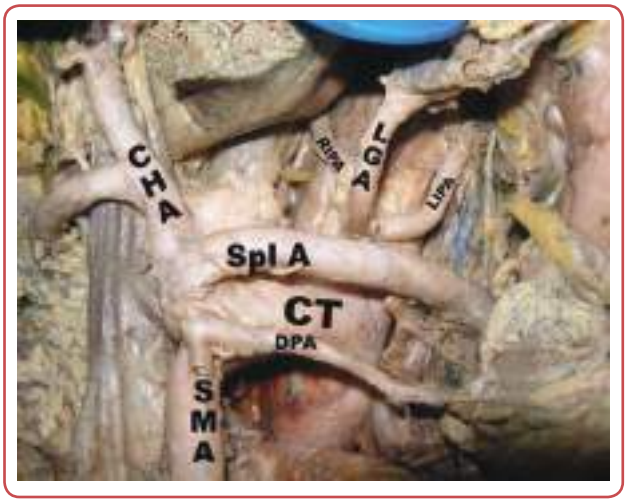

Figure 1: Hepatolienomesenteric trunk with dorsal pancreatic artery. Gastrophrenic trunk arose from the aorta

CT = Coeliac trunk; CHA = Common hepatic artery; Spl A = Splenic artery, RIPA = Right inferior phrenic artery; LGA = Left gastric artery; LIPA = Left inferior pancreatic artery; SMA = Superior mesenteric artery; DPA = Dorsal pancreatic artery;

The LGA had given branches to the oesophagus and stomach, in addition, it has given supplementary branches such as the inferior phrenic artery (IPA) and replaced left hepatic artery (LHA). The IPAs were a branch of the LGA in 2 cases. The left inferior pancreatic artery (LIPA) emerged from the LGA in another case (Figure 4). In one more specimen, the LGA was the branch of the abdom-

inal aorta and it divided into right and left IPA as the gastrophrenic trunk (Figure 1). In two instances, the LHA was substituted for the LGA (Figure 5).

Figure 2: The coeliac trunk divided into the hepatic artery and splenic artery. The left gastric artery took origin from the splenic artery

LGA = Left gastric artery; Spl A = Splenic artery; CT = Coeliac trunk; CHA = Common hepatic artery;

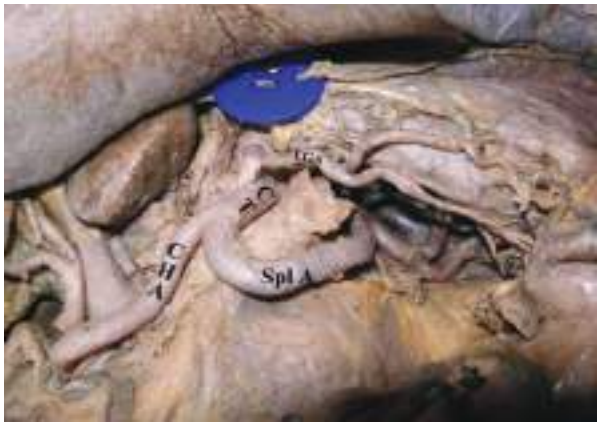

Figure 3: Left gastric artery (LGA) is divided into two branches that supply the anterior and posterior surfaces of the stomach

CT = Coeliac trunk; CHA = Common hepatic artery; Spl A = Splenic artery; LGA = Left gastric artery;

Figure 4: Left inferior phrenic artery emerged from the left gastric artery

CHA = Common hepatic artery; Spl A = Splenic artery; LGA= Left gastric artery; LIPA= Left inferior pancreatic artery;

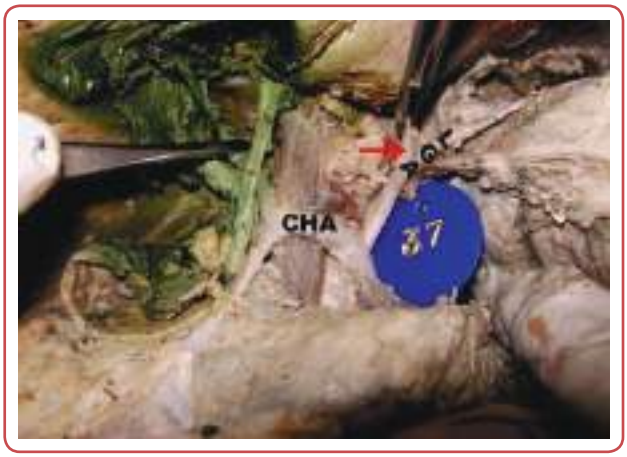

Figure 5: Replaced LHA arose from the left gastric artery. PHA continuing as RHA only

LHA = Left hepatic artery; PHA = Proper hepatic artery; RHA = Right hepatic artery; CHA = Common hepatic artery; LGA = Left gastric artery;

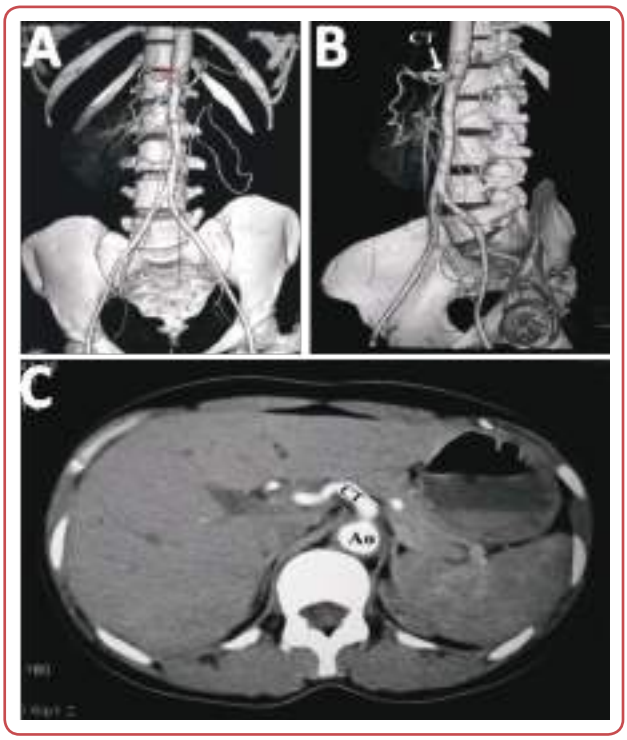

Figure 6: Representative images of reconstructed volumetric 3D rendered CT angiogram in A) AP projection B) sagittal projection showing the origin of the coeliac trunk and its branches. C) Axial section of CT angiogram showing the origin of coeliac trunk from the aorta

CT = Coeliac trunk; Ao = Aorta; AP = anterioposterior; CT = computed tomography;

To correlate cadaveric and autopsy investigation conducted CT angiogram and reconstructed volumetric 3D rendered angiogram to observe the celiac trunk anatomical variation but didn't find any variation from the classical pattern (Figure 6A, B, C).

Discussion

This study conducted a systematic investigation of cadaveric, postmortem dissections and CT angiography images to identify and report the LGA variations in comparison with normal classical patterns of the origin and course of these blood vessels. In healthy people, these anatomical differences are asymptomatic, but they will have crucial importance in individuals who have to undergo abdominal surgeries and investigation procedures.

Existing literature reported that in 94.4 % of cases, the LGA was the branch of the celiac trunk, in 2.7 % of cases it is a branch of the splenic artery (SA), in 2.1 % it arose from the aorta and in 0.3 % of cases from the common hepatic artery (CHA).[7] In this study, the LGA was the branch of celiac trunk in 96 %, the branch of SA in 2 % and the direct branch of AA and LGA as the branch of the CHA was not observed. The observations of this study are in line with earlier investigations such as LGA is the branch of AA was documented by Eaton in 4.5 %,[8] in 1.5 % by Lipshutz,[9] and in 0.5 to 1.5 % by Yildirim et al.[1] In the current investigation occurrence was 2 %, which is the same as that of Lipshutz research. The case study published in 2014 reported that the LGA emerged from the ventral aspect of AA just proximal to the hepatic trunk at the level of the first lumbar vertebra.[10] It is similar to existing literature[1] and current study. In other literature, the LGA emerged abnormally among 13 of the five hundred individuals, most commonly as a direct aortic branch.[11] The IPA and the LGA were the branches of the trunk called the gastro-phrenic trunk, which arose from the aorta.[12–14] This study also observed a similar pattern.

A recent study reported that the hepato-gastro-phrenic trunk (HGPT) is the branch of AA at the origin point of the celiac trunk. Its initial branch was the LIPA and it continued as the hepato-gastric trunk (HGT). During its course on the way to the diaphragm, the left IPA gives a branch to the left adrenal gland. HGT split into the LGA and a branch to the CHA at a length of 10.3 mm. No splenic artery (SA) emerged from the celiac trunk.[15] The CHA, SA, left IPA and LGA were all formed by the quadrification of the celiac trunk,[14] but in the current investigation, the gastrophrenic trunk was the branch of AA and it had given LGA, then it was divided into left and right IPAs. In previous research observations, the LGA had been divided into ventral and dorsal branches to supply corresponding aspects.[9, 16, 17] The findings of this study also revealed similar patterns in one individual (2 %).

The report of Piao et al revealed that in 2.9 % of cases, the IPA is a branch of LGA.[18] In a recent investigation, the LGA is given origin to the inferior RIPA and LIPA, while no celiac trunk was found.[19] In this study, the left IPA was the branch off LGA in 4 % of individuals as existing data. The hepatosplenic trunk, gastrophrenic trunk, splenic hypoplasia and auxiliary spleen were described by Yi et al,[20] whereas Shibamoto et al reported the concurrent existence of the LGA and CHA that courses retroportally called the hepatosplenomesenteric trunk and for both IPAs a common trunk in cancer stomach patient.[21] The LGA and right IPA arose from a common gastrophrenic trunk as a branch of AA.[22] This study results also demonstrated the same findings in 2 % of specimens. The previous research document that replaced LHA was the branch of LGA in 4 % of specimens[23, 24] and the LGA gave aberrant LHA.[25–28] Observations of the current study also revealed the same type of variants.

This study had some limitations since was mostly based on cadaveric and postmortem investigations, the analysis of CT images was few and further investigations are required in combination with classical and advanced methods to provide knowledge of vascular variations as they may be potential bleeding sites.

Conclusion

Considering these findings, it is assumed that the abdominal surgeons who are performing the procedures should have an idea about vascular variants and their branching pattern of LGA and also about aberrant arteries. It helps in the preoperative planning for radiological and surgical procedures to avoid possible induced damage and fatal complications and to reduce intra- and post-operative risk factors.

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None.

Conflict of interest

None.

References

1. Yildirim M, Ozan H, Kutoglu T. Left gastric artery originating directly from the aorta. Surg Radiol Anat 1998;20:303-5.
2. Yuasa Y, Okitsu H, Goto M, Kuramoto S, Tomibayashi A, Matsumoto D, et al. Three-dimensional CT for preoperative detection of the left gastric artery and left gastric vein in laparoscopy-assisted distal gastrectomy. Asian J Endosc Surg 2016;9(3):179-85.
3. Maki H, Satodate H, Satou S, Nakajima K, Nagao A, Watanabe K, et al. Clinical evaluation of the aberrant left hepatic artery arising from the left gastric artery in esophagectomy. Surg Radiol Anat 2018;40:749-56.
4. Sundgren R. Selective angiography of the left gastric artery. Acta Radiol Diagn (Stockh) 1970:Suppl 299:1+.
5. Gayretli Ö, Kocabiyik N, Kale AÇ, Yalçin B, Ozan H. Multivariations of the left gastric artery: a case report. Trak Univ Tip Fak Derg 2010;27(4):420-3.
6. Kostelic JK, Piper JB, Leef JA, Lu CT, Rosenblum JD, Hackworth C, et al. Angiographic selection criteria for living related liver transplant donors. AJR Am J Roentgenol 1996 May;166(5):1103-8.
7. Sawai K, Azuma T, Matsuda K, Izumi H, Niwa M, Kato G, Takenaka A, Tokuda H. [Angiographic analysis of vascular anatomy in gastric cancer]. Nihon Geka Gakkai Zasshi 1984 Feb;85(2):143-52. Japanese.
8. Eaton PB. The coeliac axis. Anat Rec 1917;13(6):369–74.
9. Lipshutz B. A composite study of the coeliac axis artery. Ann Surg 1917 Feb;65(2):159-69.
10. Iacob N, Sas I, Joseph SC, Pleş H, Miclăuş GD, Matusz P, et al. Anomalous pattern of origin of the left gastric, splenic, and common hepatic arteries arising independently from the abdominal aorta. Rom J Morphol Embryol 2014;55(4):1449-53.
11. Naidich JB, Naidich TP, Sprayregen S, Hyman RA, Pudlowski RM, Stein HL. The origin of the left gastric artery. Radiology 1978;126(3):623-6.
12. Hirai Y, Yamaki K, Saga T, Hirata T, Yoshida M, Soejima H, et al. An anomalous case of the hepato-spleno-mesenteric and the gastro-phrenic trunks independently arising from the abdominal aorta. Kurume Med J 2000;47(2):189-92.
13. Prasanna LC, Alva R, Sneha GK, Bhat KMR. Rare variations in the origin, branching pattern and course of the celiac trunk: report of two cases. Malaysian J Med Sci MJMS 2016;23(1):77-81.
14. Rusu MC, Jianu AM, Dincă D, Manta BA. Quadrifurcation variants of the celiac trunk. Ann Vasc Surg 2021;73:303-13.
15. Hemanth K, Garg S, Yadav TD, Sahni D. Hepato-gastro-phrenic trunk and hepato-spleno-mesenteric trunk: A rare anatomic variation. Trop Gastroenterol 2011;32(1):56-9.
16. Reeves TB. A study of the arteries supplying the stomach and duodenum and their relation to ulcer. University of Minnesota Masters thesis, 1919.
17. Vandamme JP, Bonte J. The blood supply of the stomach. Acta Anat (Basel) 1988;131(2):89-96.
18. Piao DX, Ohtsuka A, Murakami T. Typology of abdominal arteries, with special reference to inferior phrenic arteries and their esophageal branches. Acta Med Okayama 1998;52(4):189-96.
19. Szewczyk B, Karauda P, Olewnik Ł, Podgórski M, Waśniewska A, Haładaj R, et al. Types of inferior phrenic arteries: a new point of view based on a cadaveric study. Folia Morphol (Warsz) 2021;80(3):567-74.
20. Yi SQ, Li J, Terayama H, Naito M, Iimura A, Itoh M. A rare case of inferior mesenteric artery arising from the superior mesenteric artery, with a review of the review of the literature. Surg Radiol Anat 2008;30:159-65.
21. Shibamoto M, Yamada T, Ehara K, Takechi H, Kawakami H, Ito Y, et al. Simultaneous presence of the hepato-spleno-mesenteric trunk, a common trunk for both inferior phrenic arteries, the left gastric artery, and a common hepatic artery that ran behind the portal vein in a patient with gastric cancer. Clin J Gastroenterol 2022;15(3):553-9.
22. Sehgal G, Sharma PK, Kumar N, Rani A, Pankaj AK, Parihar A. Unique conglomeration of arterial variations in the upper abdomen: CT angiographic study. IOSR J Dent Med Sci 2016;15(7 Ver):35-8.
23. Kemeny MM, Hogan JM, Goldberg DA, Lieu C, Beatty JD, Kokal WA, et al. Continuous hepatic artery infusion with an implantable pump: problems with hepatic artery anomalies. Surgery 1986 Apr;99(4):501-4.
24. Waki Y, Kamiya S, Li Y, Hikage M, Tanizawa Y, Bando E, et al. Preserving a replaced left hepatic artery arising from the left gastric artery during laparoscopic distal gastrectomy for gastric cancer. World J Surg 2021;45:543-53.
25. Cirocchi R, D'Andrea V, Amato B, Renzi C, Henry BM, Tomaszewski KA, et al. Aberrant left hepatic arteries arising from left gastric arteries and their clinical importance. Surgeon 2020 Apr;18(2):100-12.
26. Okano S, Sawai K, Taniguchi H, Takahashi T. Aberrant left hepatic artery arising from the left gastric artery and liver function after radical gastrectomy for gastric cancer. World J Surg 1993;17:70-3.
27. Hiatt JR, Gabbay J, Busuttil RW. Surgical anatomy of the hepatic arteries in 1000 cases. Ann Surg 1994 Jul;220(1):50-2.
28. Ang RRG, Lee HJ, Bae JS, Zhu CC, Berlth F, Kim TH, et al. Safety of ligation of aberrant left hepatic artery originating from left gastric artery in laparoscopic gastrectomy for gastric cancer. Sci Rep 2020;10(1):5856. doi: 10.1038/s41598-020-62587-7.

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ORIGINAL ARTICLE

Association of Systemic Diseases with Chronic Pruritus

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Abstract

Background/Aim: Pruritus is an unpleasant sensation that provokes the desire to scratch. It is one of the most common reasons why patients consult a dermatologist. Aim of this study was to determine the association of chronic pruritus with skin and systemic diseases, as well as the age and sex distribution in the studied population.
Methods: The cross-sectional study included 120 patients of both sexes who, in the period from January 2017 to January 2021, received outpatient and inpatient treatment at the Skin and Venereal Diseases Clinic of the University Clinical Centre of the Republic of Srpska diagnosed with pruritus. Through the Clinical Information System insight was gained into the medical history and other documentation of the subjects from which data were taken on the age and sex of the subjects, onset, course and duration of pruritus, daily or seasonal variations in intensity, as well as the presence of associated skin and systemic diseases.
Results: Out of a total of 120 subjects, a larger number (53.3 %) of subjects with chronic pruritus were male, and 46.7 % were female, the difference was statistically significant ($p < 0.05$). The analysis of the distribution of subjects according to their age revealed that the largest number of subjects (62.6 %) was over 65 years of age, while 38.4 % of subjects were under 65 years of age. The difference in the age structure was statistically significant ($p < 0.05$). In subjects older than 65 years pruritus was most frequently (47.3 %) associated with diabetes and in subjects under 65 years of age with skin diseases and conditions. The difference was statistically significant ($p < 0.05$).
Conclusion: In people aged over 65 years, pruritus was most frequently associated with systemic diseases (diabetes mellitus) and in people aged under 65 years with dermatological diseases (*Dermatitis atopica*).

Key words: Chronic pruritus; Diabetes mellitus; Senile pruritus.

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Introduction

Pruritus or itching is defined as an unpleasant sensation that provokes the desire to scratch.[1] It is one of the most common conditions, ie symptoms causing patients to consult a dermatologist. It is a symptom not only of many skin diseases but also systemic ones. Due to its diversity both in the clinical picture and etiopathogenesis, the treatment of pruritus can be a challenge. It is equally present in all races as well as in both sexes.[2] It can be constantly present or intermittent and localised or generalised. Pruritus present for more than 6 weeks is defined as chronic one.[3] In addition to pruritus caused by skin disorders, it can also be distinguished systemic, neuropathic, psychogenic, multifactorial and idiopathic one.[1]

Skin disorders that cause pruritus mostly include dry skin, inflammatory dermatoses (primarily

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CONTENT

atopic dermatitis), then infectious dermatoses, as well as cutaneous types of lymphoma.[4, 5] Systemic pruritus occurs as a result of diseases of other organs, most frequently the liver, kidneys, endocrine and haematological disorders, tumours, infectious conditions, as well as the use of certain drugs (antihypertensives, antiarrhythmics, anticoagulants, antidiabetics, antiepileptics, cytostatics and chemotherapeutics). Neuropathic pruritus occurs as a result of damage to the nerves or the use of opioids (neurogenic pruritus), while psychogenic pruritus, which is also called somatomorphic is accompanied by numerous psychiatric conditions. Due to the large number of chronic diseases and polymedication, there is a high incidence of pruritus among the elderly population.[6,7] That is why the term senile pruritus is introduced, which means the presence of chronic pruritus of unknown cause in people aged over 65. In the elderly population, three possible reasons for the occurrence of pruritus have been described, namely dry skin, immunological and neuropathic cutaneous changes.[8] Given that the sensation and intensity of pruritus is subjective, a thorough anamnesis and dermatological examination are required for the diagnosis and successful treatment.

Clinical presentation is of great importance for the diagnosis of chronic pruritus. It can appear on clinically unchanged skin or be accompanied by various primary or secondary skin changes. Primary efflorescence can be a part of the clinical picture of the basis of the skin disease that caused pruritus, while secondary changes are the result of scratching (excoriations, crusts, lichenification, hyperpigmentation and hypopigmentation).[9] There are still many unknowns about the mechanisms of occurrence of chronic pruritus, but it is certain that it arises due to a complex interaction between keratinocytes, nerve fibres located in the dermis, prurigenic molecules and the central and peripheral nervous system.[10]

The aim of this research was to determine the association of chronic pruritus with skin and systemic diseases, as well as the age and sex distribution in the study population.

Methods

The cross-sectional study included 120 patients of both sexes who were treated at the Skin and Venereal Diseases Clinic of the University Clinical Centre of the Republic of the Srpska diagnosed with pruritus in the period from January 2017 to January 2021. Outpatients and inpatients in the specified period were included. Through the Clinical Information System insight was gained into the medical history and other documentation of the subjects from which data were taken on the age and sex of the subjects, onset, course and duration of pruritus, daily or seasonal variations in intensity, as well as the presence of associated skin and systemic diseases.

The study included subjects of both sexes aged over 18 years, in whom pruritus was present for more than six weeks. Patients in whom scabies was the cause of pruritus were not included because the duration of the disease is usually less than 6 weeks and as such it is not included within the framework of chronic pruritus. All subjects were divided into two groups. The first group consisted of patients aged up to 65 years and the second group consisted of patients aged over 65 years.

Statistical analysis of the collected data was done in the statistical software package of EZR for Windows XP (Version 2.3-0). Descriptive statistics were used to arrange and describe the data and they included the calculation of incidence. Chi-square test was used to compare the frequency of occurrence of the analysed categorical characteristics of one or more independent samples. All results were considered statistically significant if $p < 0.05$ and highly statistically significant if $p < 0.001$. In the examples where highly statistically significant results were obtained, the level of statistical significance was written.

Results

The study included a total of 120 subjects, 53.3 % of men and 46.7 % of women, who in a three-year period (from 1st January 2017 to 1st January 2021) were diagnosed with pruritus and treated at the Skin and Venereal Diseases Clinic of the University Clinical Centre of the Republic of the Srpska. The analysis of the distribution of subjects according to age revealed that 46 (38.4 %) subjects were aged under 65 years and 74 (61.6 %) were aged over 65 years. The analysis of the distribution of

subjects according to sex revealed that chronic pruritus was more frequently registered in male subjects aged over 65 years. The difference was statistically significant $p < 0.05$ (Table 1).

Table 1: Distribution of chronic pruritus by age and gender

Gender	All patients		Age				p-value
			< 65		≥ 65		
	n	%	n	%	n	%	
Male	64	53.3	18	39.1	46	62.2	0.02*
Female	56	46.7	28	60.9	28	37.8	
Total	120	100.0	46	100.0	74	100.0	

**The p-value refer to the Chi-square test.*

Dermatological diseases, atopic dermatitis and dry skin were the most common causes of pruritus in the group of subjects aged under 65 years, while systemic diseases were the most common cause in subjects aged over 65 years. Dry skin was present in 30.4 % of subjects from this group and atopic dermatitis in 21.7 % of subjects. The most common systemic diseases that caused pruritus in the population of subjects over 65 years of age were diabetes mellitus (33.8 %) and malignancies, prostate and lung cancer in men and breast cancer in women. *Mycosis fungoides* was found in two subjects from this group. Malignant diseases as a cause of pruritus were also found in the subjects from the first group (carcinoma of the breast, colon and liver) in 13.2 % of the cases. Psychogenic pruritus was present in 4 (8.7 %) of the subjects under 65 years of age and in 16 (21.6 %) of the subjects over 65 years of age, more frequently women. Chronic pruritus, of unknown cause, was recorded in 8.3 % of all subjects, mostly without skin changes (Table 2).

Table 2: The most common causes of pruritus

Variable	All patients		Age				p-value
			< 65		≥ 65		
	n	%	n	%	n	%	
Skin diseases	37	30.8	24	38.7	13	17.6	0.001*
Systemic diseases	53	44.0	12	39.2	35	47.3	
Psychogenic	20	6.7	4	8.7	16	21.6	
Idiopathic	10	8.3	0	0	10	13.5	
Total	120	100.0	46	100.0	74	100.0	

** The p-value refer to the Fisher exact test.*

Within the clinical manifestations that appeared with pruritus, primary skin changes were analysed, as well as secondary ones that occurred as a result of scratching (Table 3). In 67.9 % of the subjects of both age groups pruritus was accompanied by certain skin changes. As for the primary skin manifestations, erythema, which was described as dull and maculopapular rash, occurred most frequently. Analysis of the obtained data revealed that in all patients with malignant diseases, pruritus was accompanied by a maculopapular rash. Excoriations were present in 35 % of subjects. In all patients diagnosed with psychogenic pruritus (16.7 %) excoriations were the only clinical manifestation. There was 34.1 % of subjects without skin changes and it was determined that there was no statistical significance (Table 3).

Table 3: Correlation chronic pruritus and skin manifestations

Variable	All patients		Age				p-value
			< 65		≥ 65		
	n	%	n	%	n	%	
With skin manifestations	79	65.9	26	56.5	53	71.6	0.114*
Without skin manifestations	41	34.1	20	43.5	21	28.4	
Total	120	100.0	46	100.0	74	100.0	

** The p-value refer to the Fisher exact test.*

Seasonal variations in the intensity of pruritus were recorded in two subjects over 65 years of age, in whom pruritus lasted longer than 3 years. The subjective feeling was more pronounced in the winter months. When it comes to diurnal variations, a small number of subjects declared that they were more pronounced in the evening and night hours and this mostly relates to the women under 65 years of age, while the other subjects declared that there was no difference in the intensity of itching during the day.

Discussion

Pruritus is one of the most common conditions and/or symptoms for which patients consult a dermatologist and the reason for about 10 % of all visits to a dermatologist.[11] The problem of pruritus is not exclusively related to the field of dermatology, but it is also dealt with by doctors of various other specialties. In this paper, the incidence of pruritus among dermatological patients of different age groups and its association with systemic diseases was analysed.

The results of the studies that dealt with the sex distribution of pruritus were not harmonised. However, authors would single out the research of Dalgard and al who, based on a sample of about 18,000 subjects concluded that pruritus was the most common dermatological condition in the study population and that it occurred more often in women.[12] In presented research on a smaller

number of subjects it was found that the incidence of pruritus was higher in men.

The results of this study showed that the incidence of pruritus was significantly higher in patients older than 65 years (62.2 %) and that it was most frequently associated with various systemic diseases (44.0 %), followed by dermatological diseases and conditions (the most common of which was dry skin) and psychogenic diseases (30.8 % and 16.75 %, respectively). In the smallest number of patients (8.3 %) the cause of pruritus was not determined. The association between pruritus and systemic diseases can be explained by the more frequent presence of not only various systemic diseases in old age, but also due to the physiological process of skin aging that leads to the development of senile pruritus, as well as the more frequent use of diuretics. Diabetes mellitus was the main cause of the development of chronic pruritus in the study population and it was registered in 24.2 % of the subjects, which was in correlation with similar studies.[13, 14] In the majority of patients with diabetes skin manifestations were in the form of erythema and papular rash, accompanied by excoriations. Diabetes mellitus, in addition to affecting blood vessels, has a major impact on all skin structures. Preclinical studies on animal models and a small number of clinical studies on patients have proven that diabetes mellitus leads to disorders in the architecture of the skin. It is the cause of a damaged skin barrier, increases inflammatory infiltration, reduces the number of T lymphocytes, antimicrobial peptides, lamellar bodies, lipids of the corneal layer as well as the proliferation of keratinocytes. It also reduces skin hydration and leads to alteration of filaggrins.[15, 16] Filaggrins are structural proteins of the corneal layer that cross-link keratin filaments and are crucial for maintaining the structure and function of the corneal layer and therefore, are necessary for maintaining skin hydration.[17]

Apart from diabetes, numerous studies point out that kidney failure is one of the most common causes of systemic pruritus and that pruritus is present in 60-80 % of patients with chronic renal failure.[18-19] However, in presented study, that number was significantly lower and was found in only 1.7 % of patients older than 65 years. The result of this disproportion may be the fact that patients with chronic renal failure are under the regular supervision of nephrologists who treat pruritus in a consultation with dermatologists.

In the population under the age of 65, pruritus was more frequently registered (38.7 %) in people with some skin diseases. Atopic dermatitis represents one of the most common dermatoses associated with pruritus and in addition to it, it is also found in dry skin. Presented results are correlated with data from the literature in which atopic dermatitis is one of the most pruriginous dermatoses, in which pruritus occurs in 87-100 % of patients.[20] In presented research, it was found that pruritus associated with dry skin occurred in 10 % of women under the age of 65, while in some studies the cause is significantly higher (up to 69 %).[21] Although dry skin is the cause of pruritus in 50 % of cases in the elderly population in the world,[22] in this study the number was significantly lower and it was 17.6 %. Dry skin does not have its primary efflorescence, but in these patients excoriations and lichenification could mostly be seen as a result of scratching. In addition to genetics, the basis of dry skin is an impaired function of the skin barrier, which in the younger female population can be explained by the irrational use of various cosmetic preparations not adapted to the skin type.

Data on the incidence of psychogenic pruritus are scarce because it is difficult to draw a clear line between psychogenic and idiopathic pruritus.[23, 24] Psychogenic pruritus was confirmed in 16.7 % of the study population, of which 8.3 % was the female population over the age of 65, but there was a possibility that in some patients with idiopathic pruritus it was actually psychogenic one. Excoriations were dominant skin changes present in these patients.

In the population of subjects over 65 years of age, apart from the expressed subjective feeling of itching, which was registered in a large number of subjects (71.6 %), there were also skin manifestations, most frequently expressed in the form of erythema, maculopapular rash and excoriations. Moreover, in the group of subjects under 65 years of age cutaneous manifestations were present in a larger number of subjects (56.5 %) while 43.5 % of subjects from this group were without skin changes.

Given that pruritus is an exclusively subjective symptom, it is very difficult to assess its intensity. Diurnal and seasonal variations are described. Diurnal variations, more pronounced in the evening hours, are associated with psychogenic pruritus and this was also recorded in studied

subjects. In this research reliable data on seasonal variations of pruritus could not be provided because the disease lasted longer than a year in a small number of subjects. However, it can be said that pruritus is more pronounced in the winter months in patients with atopic dermatitis and dry skin and this is most likely the result of excessive warming, wearing clothes made of synthetic materials as well as staying in closed rooms for a long time.

Conclusion

Chronic pruritus is a problem that affects a large number of people of different ages and significantly reduces their quality of life. The results of presented study have shown that systemic diseases are the most common cause of chronic pruritus in the study population. Given that the aetiology of chronic pruritus is diverse, discovering the cause of its occurrence is a condition for successful treatment.

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Conflict of interest

None.

References

1. Weisshaar E, Szepietowski JC, Dalgard FJ, Garcovich S, Gieler U, Giménez-Arnau AM, et al. European S2k guideline on chronic pruritus. Acta Derm Venereol 2019;99(5):469-506.
2. Whang KA, Khanna R, Thomas J, Aguh C, Kwatra SG. Racial and gender differences in the presentation of pruritus. Medicines (Basel) 2019 Sep 27;6(4):98. doi: 10.3390/medicines6040098.
3. Fett N, Haynes K, Propert KJ, Margolis DJ. Predictors of malignancy development in patients with chronic pruritus. J Dermatol Sci 2016 May;82(2):123-8.
4. Pereira MP, Ständer S. Chronic pruritus: current and emerging treatment options. Drugs 2017 Jun;77(9):999-1007.
5. Moniaga CS, Tominaga M, Takamori K. Mechanisms and management of itch in dry skin. Acta Derm Venereol 2020 Jan 15;100(2):adv00024. doi: 10.2340/00015555-3344.
6. Reich A, Ständer S, Szepietowski JC. Pruritus in the elderly. Clin Dermatol 2011 Jan-Feb;29(1):15-23.
7. Welz-Kubiak K, Reszke R, Szepietowski JC. Pruritus as a sign of systemic disease. Clin Dermatol 2019 Nov-Dec;37(6):644-56.
8. Cao T, Tey HL, Yosipovitch G. Chronic pruritus in the geriatric population. Dermatol Clin 2018 Jul;36(3):199-211.
9. Sutaria N, Adawi W, Goldberg R, Roh YS, Choi J, Kwatra SG. Itch: pathogenesis and treatment. J Am Acad Dermatol 2022 Jan;86(1):17-34.
10. Mollanazar NK, Smith PK, Yosipovitch G. Mediators of chronic pruritus in atopic dermatitis: getting the itch out? Clin Rev Allergy Immunol 2016 Dec;51(3):263-92.
11. Pavlović M, Stojanović O. [Neurophysiology of pruritus]. In: Karadaglić Đ: [Dermatology]. 2nd edition. Belgrade: Birograf Comp, 2016; pp. 303-321. Serbian.
12. Dalgard F, Svensson A, Holm JØ, Sundby J. Self-reported skin morbidity in Oslo. Associations with sociodemographic factors among adults in a cross-sectional study. Br J Dermatol 2004 Aug;151(2):452-7.
13. Umičević-Šipka S, Balaban J, Bijelić R. Association between skin manifestations and glycemic control in patients with diabetes mellitus type II. Vojnosanit pregl 2021;78(3):323-30.
14. Sani H, Abubakar AB, Bakari AG. Prevalence and pattern of skin diseases in patients with diabetes mellitus at a tertiary hospital in Northern Nigeria. Niger J Clin Pract 2020 Jul;23(7):970-4.
15. Quondamatteo F. Skin and diabetes mellitus: what do we know? Cell Tissue Res 2014 Jan;355(1):1-21.
16. Zhou P, Yang C, Zhang S, Ke ZX, Chen DX, Li YQ, et al. The imbalance of MMP-2/TIMP-2 and MMP-9/TIMP-1 contributes to collagen deposition disorder in diabetic non-injured skin. Front Endocrinol (Lausanne) 2021 Oct 27;12:734485. doi: 10.3389/fendo.2021.734485.
17. Tse R, Kesha K, Morrow P, Glenn C, Stables S. Commentary on: Kenneally M, Byard RW. Increasing methamphetamine detection in cases of early childhood fatalities. J Forensic Sci doi: 10.1111/1556-4029.14321. J Forensic Sci 2020 Jul;65(4):1384. doi: 10.1111/1556-4029.14459.
18. Agarwal P, Garg V, Karagaiah P, Szepietowski JC, Grabbe S, Goldust M. Chronic kidney disease-associated pruritus. Toxins (Basel) 2021 Jul 28;13(8):527. doi: 10.3390/toxins13080527.
19. Combs SA, Teixeira JP, Germain MJ. Pruritus in Kidney Disease. Semin Nephrol 2015 Jul; 35(4): 383-91.
20. Dawn A, Papoiu AD, Chan YH, Rapp SR, Rassette N, Yosipovitch G. Itch characteristics in atopic dermatitis: results of a web-based questionnaire. Br J Dermatol 2009 Mar;160(3):642-4.
21. Mollanazar NK, Sethi M, Rodriguez RV, Nattkemper LA, Ramsey FV, Zhao H, et al. Retrospective analysis of data from an itch center: Integrating validated tools in the electronic health record. J Am Acad Dermatol 2016 Oct;75(4):842-44.
22. Fourzali KM, Yosipovitch G. Management of itch in the elderly: a review. Dermatol Ther (Heidelb) 2019 Dec;9(4):639-53.
23. Lee HG, Stull C, Yosipovitch G. Psychiatric disorders and pruritus. Clin Dermatol 2017 May-Jun;35(3):273-80.
24. Ferm I, Sterner M, Wallengren J. Somatic and psychiatric comorbidity in patients with chronic pruritus. Acta Derm Venereol 2010 Jul; 90(4):395-400.

CONTENT

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ORIGINAL ARTICLE

Prevalence of Nutraceutical Use in Younger Population of North India and the Association Between Gender and Community in Its Usage – Cross-Sectional Study

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Abstract

Background/Aim: Nutraceuticals or dietary supplements have been recognised as a fundamental part of the holistic approach to achieve complete wellness and health. Their usage is continuously increasing due to lifestyle and sport-style requirements. The present study was designed to estimate the use of nutraceuticals in the younger population and find the association between gender and community in their usage.
Methods: An observational cross-sectional study was carried out on the younger population (between 15 and 30 years of age) of North India over a period of two weeks in October 2022. The data regarding the demographic profile and nutraceutical use among participants was collected per predesigned and pre-validated questionnaire. The collected data were analysed to determine the outcomes.
Results: The study included 575 participants, with 272 males and 303 females. Out of them, 321 were from the urban background. It was found in the study that a total of 275 (47.82 %) study subjects were using nutraceuticals, with 163 (59.27 %) and 112 (40.73 %) from the urban and rural dwellings, respectively. Among the users, 122 (44.36 %) were men and 153 (55.64 %) were women. Thus, overall, 153 (50.49 %) of females and 122 (44.85 %) of males in survey were consuming dietary supplements. The study further added that 189 (68.72 %) users believed that nutraceuticals improved their health, two-thirds of participants were using nutraceuticals for lifestyle reasons and 239 (86.90 %) of consumers were using single nutraceuticals. There was no association between gender and community in nutraceuticals usage (Chi-square test, $p > 0.05$).
Conclusion: Almost half of the younger population was using nutraceuticals and there was no association between gender and community while using them.

Key words: Nutraceuticals; Dietary food; Dietary supplements; Survey; Urban/rural area; Younger population.

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Introduction

Certain foods and herbal extracts have been recognised as a fundamental part of the holistic approach to achieve complete wellness and health since ancient times, especially in the Ayurvedic system in India.[1] This recognition of the correlation between health and what we eat paved the way for the evolution of "nutraceuticals".[2] The term "nutraceutical" was coined by Dr Stephen D Felice in 1989. It is derived from the combination of the words "nutrition" and "pharmaceutical,"

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CONTENT

which mean "a nourishing food" and "a medical drug," respectively.[3]

However, they are different from pharmaceutical drugs. Pharmaceutical drugs are natural or laboratory-developed or modified substances that are primarily used to prevent or treat a disease, with their manufacturing and marketing regulated by legal authorities under relevant rules and regulations.[4] The use of pharmaceuticals is for a specific purpose and hence requires stringent clinical trials before publicising their availability.[5] Moreover, they have a potentially higher risk of side effects than nutraceuticals. On the other hand, nutraceuticals are natural substances that may be considered foods or parts of foods, usually used in addition to a traditional daily diet and suggestively provide medical and health benefits in the prevention and treatment of myriad diseases like hypertension, diabetes mellitus, osteoporosis, memory enhancers etc, with comparatively lesser regulations and adverse effects.[6] Other purposes for using nutraceuticals are to delay the aging process, increase life expectancy, or support the structure or function of the body to preserve health and general well-being.[7]

Nutraceuticals include vitamins, minerals, amino acids and proteins, herbal products, fibre, lignin, prebiotics, probiotics, antioxidants, nutritional lipids and oils, phytochemicals, metabolites, enzymes, etc.[8] Nutraceuticals available in the market can be classified into functional foods, functional beverages and dietary supplements based on product category.[9] In this manuscript the term "dietary supplement" is used interchangeably with "nutraceuticals."

In India, nutraceuticals are freely available over the counter and in grocery shops and their use is continuously increasing at a rate of 25 %.[10] The Food Safety and Standards Authority of India (FSSAI) is concerned with the regulations of licencing and registration of food businesses, manufacturing, packing and labelling, food product standards and the approval of nutraceuticals in the Indian market.[10]

Worldwide data also shows that the global nutraceuticals market, which was 498.86 billion $ in 2021, is expected to reach 1025 billion $ by 2030, with an estimated compound annual growth rate of 8.33 %.[11]

Though numerous studies evaluating the use of nutrition supplements in the geriatric population and children are available, very few studies have been done to study their use in adolescents and younger populations despite the fact nutraceuticals are increasingly becoming a part of their diet due to lifestyle or sport style requirements. As a result, a cross-sectional study was conducted to estimate the use of nutraceuticals among the younger population of North India, as well as to determine whether there was any relationship between gender and community in their usage.

Methods

This was an observational cross-sectional study conducted on a younger population of North India between 15 and 30 years of age over two weeks in October 2022. After obtaining clearance from the Ethics Committee (No IT/03/2022, dated 20 October 2012), an online survey was conducted using the Google Forms.

A self-developed questionnaire consisting of two sections: Section A concerned with the demographic details and Section B concerned with the details of the nutraceuticals usage and participants' perception of their benefits was used in the survey. The questionnaire was distributed through social media (email and WhatsApp). The study subjects were mainly contacts of friends, relatives, neighbours and colleagues of around fifty persons working as staff within the Rajasthan College Of Medical Sciences (RUHS) Hospital and the rural community within the purview of the Hospital. Only those who were between 15 and 30 years of age, literate and willing to participate were included in the study. The questionnaire was available in Hindi and English and was pre-validated by five community workers and five faculty members. As the study was confined to northern India, where the dietary habits are almost identical, cultural adaptation was not required.

A total of 575 subjects participated in the study. The data was gathered pertaining to the socio-demographic variables of participants and the usage of nutraceuticals by them. The collected data was analysed statistically and the Chi-square test was used to find the association between gender and community in using nutraceuticals.

Also, the Goodness-of-fit test was applied to test the adequacy of the model and ordinal logistic regression was performed for the categorical variable 'Do you think taking nutritional supplements is helpful for your health'. Values of 0, 1 and 2, were assigned for the responses "cannot say", "no" and "yes", respectively. Parallel regression lines were assumed and a single slope was calculated for each covariate.

Results

Overall, 575 subjects participated in the study, with the male-to-female ratio being 1:1.11 (272:303) and the urban community being 1.26 times larger than the rural population (321:254). A total of 275 (47.82 %) study subjects were using nutraceuticals, with 59.27 % and 40.73 % from urban and rural dwellings, respectively. Among the users, 44.36 % were men and 55.64 % were women. Demographic details of participants as well as statistical analysis are given in Tables 1 and 2.

Table 1: Characteristics of nutraceuticals users and non-users

Parameter	Nutraceutical users		Nutraceutical non-users		p-value
	N	%	N	%	
Gender					
Male	122	44.36	150	50.00	
Female	153	55.64	150	50.00	
Settlement type					
Urban	163	59.27	158	52.67	0.156
Rural	112	40.73	142	47.33	
Education level					
Elementary school or less	75	27.27	121	40.33	0.132
High school	110	40.00	79	26.33	
University degree	90	32.73	100	33.33	

Chi-square test;

Table 2: Logistic regression model with adjusted odds ratios (OR) and 95 % confidence interval (CI) for socio-demographic variables of nutraceuticals users

Variables	OR	95 % CI	p-value
Gender			
Male	10.83	(0.59 - 1.17)	0.286
Community settlement			
Urban	1.20	(0.85 - 1.68)	0.297
Education level			
Elementary school	0.53	(0.34 - 0.87)	
High school	1.32	(0.74 - 1.34)	0.234
University degree	1.22	(0.82 - 1.42)	

The goodness-of-fit tests, done to find the adequacy of the model, revealed the p-value to be < 0.001, thereby, indicating that the model was adequate and the relationship between the response variable and the predictors was statistically significant.

In the next step, ordinal logistic regression was performed to analyse the responses to the categorical variable "Do you think taking nutritional supplements is helpful for your health?" (Table 3).

Table 3: Logistic regression for the variable 'perception of participants of benefit of nutrition supplements' in health

Predictor	Coef	SE Coef	Z	p-value	OR	95 % CI
Const (1)	-0.71	0.12	-5.97	< 0.001		
Const (2)	0.02	0.11	0.22	0.828		
Taking nutrition						
Yes	-1.51	0.19	-7.86	< 0.001	0.22	0.15 -0.32
Yes	-21.27	12465.90	0	0.999	0	0

Coef: coefficient; SE: standard error; OR: odds ratio; CI: confidence interval; The negative coefficient and an odds ratio found to be < 1 indicates that taking more nutrients tends to be associated with better health.

As shown in Table 3, the values labelled Const (1) and Const (2) were the estimated intercepts for the logits of the cumulative probabilities of the variable helpful for health. Since the p-value was < 0.001, it indicated that for most a-levels, there was sufficient evidence to conclude that "taking nutrients" was subjectively beneficial for health. Also, the negative coefficient and an odds ratio found to be < 1 indicated that taking more nutrients tends to be associated with better health.

It was also reported in the study that two-thirds of the study subjects were using nutraceuticals for lifestyle reasons and only one-third were requiring them due to their excessive physical activity. 68.72 % of supplement users believed that nutraceuticals improved their health. 86.90 % of them were using only one food supplement and multivitamins were the most frequently used nutraceutical (62.3 %), followed by miscellaneous (16.2 %) and protein powders (11.6 %).

The Chi-square test of independence found that both the variables ie, gender and area were independent of each other and showed no association in deciding nutraceutical usage (p > 0.05).

Discussion

According to the Dietary Supplement Health and Education Act (DSHEA) of 1994, dietary supplements, or nutraceuticals are products intended to supplement the diet.[12] In recent years, diet and

lifestyle-related illnesses have become important healthcare concerns. In a similar vein, all age groups, including youngsters, have shown an increase in interest and awareness in health care that uses nutritional supplements as a non-specific biological therapy for the treatment and prevention of various diseases.[3]

The present study, which included 575 individuals between the ages of 15 and 30 years in northern India, discovered that 47.82 % of them were consuming dietary supplements. According to comparable research conducted in other regions of Asia, 32.7 % and 33.8 % of the subjects used dietary supplements.[13, 14] One similar research found that up to 80 % of people use nutritional supplements or herbal medicines.[11] Americans are most likely to consume dietary supplements, followed by Europeans who live in Germany, Italy and Russia.[9, 15]

Presented study showed that overall, a greater number of females in northern India consume nutraceuticals than males. Similarly, Shade et al[16] mentioned a significant association between gender and multivitamin use in favour of females. However, Piórecka et al[17] found that dietary supplement consumption was significantly higher among boys compared to girls (37.3 % vs 27.8 %) and among children who lived in rural areas as opposed to metropolitan areas (39.3 % vs 26.5 %). A study by Svendsen K et al[18] revealed that females are more likely to assert their knowledge of all dietary recommendations. In contrast, a study on athletes by Aguilar-Navarro et al found that male athletes consumed significantly more supplements than female athletes.[19]

Rural living differs from urban living in many ways that could affect dietary supplement consumption and related consequences, besides other demographic factors.[20] Several epidemiological studies also opine that demographic factors may influence supplement consumption.[19, 20] Old age, female gender, degree of education, affordability and employment status are a few prominent demographic characteristics linked to increasing dietary supplement usage.[21]

The current study reported that overall, 59.27 % of nutraceutical users belonged to urban dwellings. However, the findings of various research studies on nutraceutical intake in urban and rural settings vary greatly across populations.[16, 17, 22] Studies suggest that, though there is a higher calorie intake in urban areas, they are deficient in micronutrients despite the wider availability of fresh fruits and vegetables in larger cities. It could be the reason why more adolescents are using supplements in urban areas. In contrast, Shade et al[17] discovered that older people in rural areas use more oral dietary supplements. It should be noted, however, that rural areas have fewer medical facilities and a slightly higher percentage of elderly people.[23]

In presented research, the majority of consumers (68.72 %) believed that taking nutraceuticals improved their overall health. Similarly, a study mentioned that 61.6 % of patients reported feeling satisfied after using nutraceuticals, while 12.4 % of patients reported feeling unsatisfied. 25.8 % of patients had no idea that nutraceuticals existed.[9] Nevertheless, a survey found that 78 % of respondents were undecided regarding the effectiveness and safety of nutraceuticals, particularly for children and the elderly.[24]

The present study also evaluated the association between gender and community in using nutraceuticals, as in countries like India, males are usually given priority over females when it comes to nutrition and education, especially in rural settings. However, in terms of the use of nutrition supplements, the study found no significant association between gender parity and dwellings. Researchers did not find any similar studies evaluating the association between gender and community in dietary supplement usage.

Strengths and limitations of the study

The present study recruited the participants online thereby covering a larger area of northern India and the limitation was that only a small techno-savvy population could be included from the eligible group.

Conclusion

Almost half of the younger population was using nutraceuticals, with somewhat more consumption in urban areas. Also, there was no association between gender and the living dwelling while using it.

CONTENT

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Conflict of interest

None.

References

1. AlAli M, Alqubaisy M, Aljaafari MN, AlAli AO, Baqais L, Molouki A, et al. Nutraceuticals: transformation of conventional foods into health promoters/disease preventers and safety considerations. Molecules 2021 Apr 27;26(9):2540. doi: 10.3390/molecules26092540.
2. Das L, Bhaumik E, Raychaudhuri U, Chakraborty R. Role of nutraceuticals in human health. J Food Sci Technol 2012 Apr;49(2):173-83.
3. Bhowmik D, Gopinath H, Kumar BP, Duraivel S, Kumar KS. Nutraceutical-a bright scope and opportunity of Indian healthcare market. The Pharma Innovation 2013;1(11, Part A):29-41.
4. Mathur S, Hoskins C. Drug development: Lessons from nature. Biomedical reports 2017 Jun 1;6(6):612-4.
5. Poongothai S, Unnikrishnan R, Balasubramanian J, Nair MD, Mohan V. Why are clinical trials necessary in India? Perspect Clin Res 2014 Apr;5(2):55-9.
6. Nasri H, Baradaran A, Shirzad H, Rafieian-Kopaei M. New concepts in nutraceuticals as alternative for pharmaceuticals. Int J Prev Med 2014;5:1487-99.
7. Gupta C, Prakash D. Nutraceuticals for geriatrics. J Tradit Complement Med 2015 Jan 1;5(1):5-14.
8. Mali S, Rathod S, Kale N, Shinde N. Overview of Nutraceuticals. AJPRes 2022;12(1):61-70.
9. Gosavi S, Subramanian M, Reddy R, Shet BL. A study of prescription pattern of nutraceuticals, knowledge of the patients and cost in a tertiary care hospital. Clin Diagn Res 2016 Apr;10(4):FC01-4.
10. Putta S, Yarla NS, Lakkappa DB, Imandi SB, Malla RR, Chaitanya AK, et al. Probiotics: supplements, food, pharmaceutical industry. In: Grumezescu AT, Holban AM, editors. Therapeutic, probiotic, and unconventional foods. UK, London: Academic Press; 2018. pp. 15-25.
11. Nutraceuticals product market by name. [Internet]. [Cited: 28-Dec-2022]. Available at: https://www.precedenceresearch.com/nutraceuticalsmarket.
12. Chauhan B, Kumar G, Kalam N, Ansari SH. Current concepts and prospects of herbal nutraceutical: A review. J Adv Pharm Technol Res 2013 Jan;4(1):4-8.
13. Naqvi AA, Ahmad R, Elewi AA, AlAwa AH, Alasiri MJ. Dietary supplement use among undergraduate male students in health and non-health cluster colleges of a public-sector university in Dammam, Saudi Arabia. BMC Complement Altern Med 2018 Oct 1;18(1):269. doi: 10.1186/s12906-018-2332-4.
14. Anjali VG, Dhiman A, Dutt R, Ranga S. Health benefits of nutraceuticals. Pharma Innov J 2018;7(3):178-81.
15. Singh J, Sinha S. Classification, regulatory acts and applications of nutraceuticals for health. IJPBS 2012;2(1):177-87.
16. Shade MY, Witry M, Robinson K, Kupzyk K. Analysis of oral dietary supplement use in rural older adults. J Clin Nurs 2019 May; 28(9-10):1600-6.
17. Piórecka B, Koczur K, Cichocki R, Jagielski P, Kawalec P. Socio-economic factors influencing the use of dietary supplements by school children from Małopolska voivodship (southern Poland). Int J Environ Res Public Health 2022 Jun 26;19(13):7826. doi: 10.3390/ijerph19137826.
18. Svendsen K, Torheim LE, Fjelberg V, Sorprud A, Narverud I, Retterstøl K, et al. Gender differences in nutrition literacy levels among university students and employees: a descriptive study. J Nutr Sci 2021 Jul 30;10:e56. doi: 10.1017/jns.2021.47.
19. Aguilar-Navarro M, Baltazar-Martins G, Brito de Souza D, Muñoz-Guerra J, del Mar Plata M, Del Coso J. Gender differences in prevalence and patterns of dietary supplement use in elite athletes. Res Q Exerc Sport 2021 Dec;92(4):659-68.
20. Islam MA, Al-Karasneh AF, Rizvi M, Nisa ZU, Albakheet AM, Alshagawi MA, et al. Prevalence, reasons and determinants of dietary supplements use among undergraduate female students of health and non-health colleges in a Saudi public university. PLoS One 2021 Mar 3;16(3):e0247295. doi: 10.1371/journal.pone.0247295.
21. Tarn DM, Karlamangla A, Coulter ID, Paterniti DA, Knox L, Khang PS, et al. A cross-sectional study of provider and patient characteristics associated with outpatient disclosures of dietary supplement use. Patient Educ Couns 2015 Jul 1;98(7):830-6.
22. Nabdi S, Boujraf S, Benzagmout M. Evaluation of rural-urban patterns in dietary intake: A descriptive analytical study–Case series. Ann Med Surg (Lond) 2022 Nov 17;84:104972. doi: 10.1016/j.amsu.2022.104972.
23. Bolin JN, Bellamy GR, Ferdinand AO, Vuong AM, Kash BA, Schulze A, et al. Rural healthy people 2020: new decade, same challenges. J Rural Health 2015 Summer;31(3):326-33.
24. Puri V, Nagpal M, Singh I, Singh M, Dhingra GA, Huanbutta K, et al. A comprehensive review on nutraceuticals: therapy support and formulation challenges. Nutrients 2022 Nov 3;14(21):4637. doi: 10.3390/nu14214637.

CONTENT

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ORIGINAL ARTICLE

The Prevalence of *VKORC1* Alleles in the Population of the Republic of Srpska, Bosnia and Herzegovina

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Abstract

Background/Aim: Warfarin is one of the most common orally prescribed anticoagulant in patients with deep venous thrombosis, myocardial or cerebral infarctions. The main side effects of non-adequate dose of these drugs are prolonged peripheral or internal bleeding. *VKORC1* 1173C>T polymorphism (rs9934438) is of particular importance, since carriers of non-wild type allele correlates with the lower dosage of warfarin therapy. Thus, the aim of the research was to determine the distribution of 1173C>T polymorphism in population of the Republic of Srpska, Bosnia and Herzegovina (RS) and to compare results with frequencies in other populations.
Methods: A total of 124 healthy participants of both genders were enrolled in the study, from all parts of the RS. Molecular genotyping was performed by real-time PCR, using drug metabolism assays according to the manufacturer's instructions.
Results: Of the total number, 22 subjects (17.74 %) were genotyped as CC, 69 subjects (55.65 %) as CT and 33 subjects (26.61 %) as TT. The frequencies of alleles C and T were 45.18 % and 54.82 %, respectively. No statistical significance was found among allele distribution between genders (χ^2 = 0.236; p = 0.627). All observed genotype frequencies were in Hardy-Weinberg equilibrium. No statistical significance was observed among the frequency of minor T allele between presented findings and other European countries, besides Russia (p = 0.021).
Conclusion: This was the first study analysing the distribution of rs9934438 alleles in population of the RS. These findings will be helpful in better and more precise drug prescribing in patients who require anticoagulant therapy.

Key words: *VKORC1*; Allele distribution; Polymorphism; Warfarin.

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Introduction

Vitamin K epoxide reductase enzyme is encoded by homonymous *VKORC1* gene located on the chromosome 16p.[1] *VKORC1* enzymatic complex catalyses reduction of vitamin K 2,3 epoxide to the metabolically active forms of vitamin K in two steps, first to vitamin K quinone and afterwards vitamin K hydroquinone.[2] Thus, vitamin K hydroquinone acts as a cofactor for the gamma-carboxylation of glutamic acid residues of vitamin K-dependent proteins (VKDP) which include coagulation factors II, VII, IX, X, haemostatic proteins C, S and Z.[3] The essential role of this enzyme complex in the coagulation process represents the drug target of oral anticoagulants,

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CONTENT

notably warfarin.[4] Warfarin binds to the VKOR and lowers the concentration of vitamin K hydroquinone, which decreases the amount of biologically active vitamin K to produce the factors of coagulation.[5] The main issue with prescribing anticoagulant therapy are adverse drug reactions, such as prolonged gastrointestinal, intracranial, or peripheral bleeding. For prescribing anticoagulant therapy, genetic variations among *VKORC1* and CYP2C9 are of particular importance, since genotype characteristics among these genes could explain the individual differences in response to warfarin.[6]

Numerous studies have reported that there is a firm connection between *VKORC1* SNPs and sensitivity to warfarin which varies due to inter-individual and inter-ethnic differences. According to data obtained from pharmacogenetic studies, two SNPs - 1639G>A and 1173C>T, are commonly genotyped. The 1173C>T rs9934438 is located in the first intron of *VKORC1* gene.[7] It is in near linkage disequilibrium with G3673A. The 1173C>T was the first known SNP to correlate with the low-dosage warfarin therapy.[8] This indicates a lower activity of the coagulation system, which is partly related to a decreased activity of key enzyme.[9] Accordingly, individuals with CT or TT genotype require lower warfarin doses in comparison to the carriers of the CC genotype.[7, 10]

The unpredictable pharmacodynamics and pharmacokinetics represent a big challenge in choosing the right dose of a drug.[11] Nevertheless, the patient's response to the recommended dosage of warfarin is inter-individual, assigned to genetics polymorphisms within the genes responsible for the pharmacokinetics and pharmacodynamics of warfarin.[12]

The aim of the research was to investigate the frequencies and distribution of *VKORC1* 1173C>T polymorphism in population of the Republic of Srpska, Bosnia and Herzegovina, as well as to compare results with the obtained frequencies in other populations.

Methods

This study included 124 healthy participants from the Republic of Srpska, of which 74 (61.29 %) participants were men and 48 (38.71 %) were women aged between 18 and 86 (median: 59). Participants were randomly selected from all parts of the Republic of Srpska. Exclusion criteria were mental and physical illness.

The study was conducted in accordance with the Declaration of Helsinki. Ethical Committee of the Faculty of Medicine at the University of Banja Luka approved this study (No 01-19-521-2/20). Also, all participants signed a statement providing their written informed consent.

The molecular genetic analysis was performed at the Laboratory for Molecular Biology and Genetics of the University of Banja Luka, Faculty of Medicine, Centre for Biomedical Research. Total genomic DNA was isolated using PureLink® gDNA Blood Kit (Invitrogen, Carlsbad, CA, USA). The Real-Time PCR instrument 7500 by Applied Biosystems and TaqMan® Drug Metabolism Genotyping Assay (C_30204875_10) were used for determination of genotypes for *VKORC1* (1173C>T, rs9934438). The real-time polymerase chain reaction was proceeded according to the manufacture's protocol.

Statistics

The χ^2 test was used to detect if the gene distribution accorded with the Hardy-Weinberg equilibrium and to compare genotype and allele frequency between different ethnic groups. For all analyses the Social Science Statistics online calculator was used (https://www.socscistatistics.com/tests/).[32]

Results

The alleles and genotypes distribution of the investigated gene in the population of the Republic of Srpska are summarised in Table 1. Observed genotype frequencies were in Hardy-Weinberg equilibrium ($\chi^2 = 1.837$; $p = 0.175$).

For the *VKORC1* 1173C>T polymorphism, 22 subjects (17.74 %) were genotyped as CC, 69 subjects (55.65 %) as CT and 33 subjects (26.61 %) as TT. The frequencies of alleles C and T in *VKORC1* 1173C>T in a total of 124 subjects were 45.18 % (113 alleles) and 54.82 %.

Table 1: Frequencies of the VKORC1 1173C>T genotypes and alleles in a sample of the population of the Republic of Srpska

Gene	Genotype	N	%	CI (95 %)	Allele	N (%)
VKORC1 1173C>T	CC[a]	22	17.74	11.47 – 25.62	C	113 (45.18)
	CT	69	55.65	46.45 – 64.56	T	135 (54.82)
	TT	33	26.61	19.08 – 35.30		

a - referent genotype; CI - 95 % confidence interval; N – number of participants;

The allele frequencies of *VKORC1* gene were compared across several different populations as shown in Table 2.

Table 2: The prevalence of the VKORC1 allelic variants in the population of the Republic of Srpska (this study) compared to other populations

Gene	Allele	Alleles frequencies in different populations (%)[a]								
		This study	Croatian	French	Italian	Slovenians	German	Arabian	Austrian	Chinese
VKORC1 1173C>T	C	0.409	0.593	0.587	0.602	0.567	0.585	0.573	0.570	0.084
	T	0.591	0.407	0.413	0.398	0.433	0.415	0.427	0.430	0.916

a – references 1, 13-21;

The data for potential gender differences were also analysed. However, there were no statistically significant differences in *VKORC1* 1173C>T polymorphisms distribution in relation to gender in study group ($\chi^2 = 0.236$; $p = 0.627$).

Discussion

Warfarin inhibits the enzyme *VKORC1*, which leads to the reduction of vitamin K and the production of hypofunctional coagulation factors.[22] It is estimated that the influence of *VKORC1* genetic variants on warfarin dose determination is approximately 25 %.[23] A narrow therapeutic range of warfarin is known with high inter-individual sensitivity to the drug. The presence of *VKORC1* gene polymorphism affects the pharmacodynamics of warfarin. One of the most common single nucleotide polymorphisms (SNP) *VKORC1* is 1173C>T where cytosine is replaced by thymine.[24] It was estimated that due to the presence of the T allele, the dose of warfarin should be reduced by 20-28 %. Therefore, the carriers of the *VKORC1* CC genotype require a higher daily dose of warfarin, the heterozygous genotype involves a medium dose and the carriers of the *VKORC1* TT genotype the lowest dose of the drug.[25]

In authors' previous research, the prevalence of pharmacologically most important allelic variants of the *CYP2C9* in the general population of the Republic of Srpska was analysed. CYP2C9 metabolises around 15 % of all drug in modern use, involving oral anticoagulants.[26] The two most common allelic variants are *CYP2C9*2* and *CYP2C9*3*, where the **2* allele is classified as a loss-of-function variant and the **3* allele as a no-function variant.[26, 27] According to the Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for Pharmacogenetics-Guided Warfarin Dosing, individuals with *CYP2C9*2* and *CYP2C9*3* have decreased warfarin maintenance dose.[28] Thus, the prescription of warfarin therapy depends not only on *VKORC1*, but also on *CYP2C9*.

This is the first study performed in the population of the Republic of Srpska. Study showed that the *VKORC1* gene polymorphism differs between ethnicities. The frequency values for polymorphic alleles and genotypes corresponded to the frequencies for other European populations. The prevalence of *VKORC1* 1173T allele found in presented study are similar to the respective ones reported in other counties such as the French population (40.7 %; $p = 0.55$),[13] Italian (39.8 %; $p = 0.059$),[14] Slovenian (43.3 %; $p = 0.134$),[15] German (41.5 %; $p = 0.074$),[16] Arabic (42.7 %; $p = 0.13$)[17] and Austrian (43 %; $p = 0.115$).[18]

The *VKORC1* 1173T frequency was significantly different in comparison with Russian population (38.2 %; $p = 0.021$),[19] Chinese (91.6 %; $p < 0.001$)[20] and Egyptian (72.3 %; $p = 0.001$).[21]

The *VKORC1* 1173T allele is present in Caucasians

CONTENT

with a frequency of about 40 % and the rarest in African-Americans (14 %).[9, 29] Following, allele T is mostly present in the East Asian population (90 %) and this explains the use of significantly lower doses for Asians compared to Caucasians.[8, 30] In the rest of the Asian countries (South East, West and Central Asia) the prevalence of this allele oscillated between 14 and 80 %.[31]

Conclusion

In conclusion, this study showed that *VKORC1* gene was polymorphic in population of the Republic of Srpska, with a similar distribution as noticed in other European populations (about 40 %). Opposite, there was a statistically significant difference in other populations like Chinese (1 %) where the prevalence of *VKORC1* polymorphic allele was lower compared to studied population or Egyptian (72 %) where the frequency of T allele was higher than in this study. The importance of population-genetic studies, such as this one is to have a precise knowledge of the prevalence of specific genetic variants in population, thus the prescribing of particular therapy could be as precise as possible.

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None.

Conflict of interest

None.

References

1. Mandic D, Mandic S, Horvat V, Samardzìja M, Samardìja M. Vitamin K epoxide reductase complex 1 (VKORC1) gene polymorphisms in population of eastern Croatia. Coll Antropol 2013;37(4):1321–6.
2. Oldenburg J, Marinova M, Müller-Reible C, Watzka M. The Vitamin K cycle. Vitam Horm 2008;78:35–62.
3. Aquilante CL, Langaee TY, Lopez LM, Yarandi HN, Tromberg JS, Mohuczy D, et al. Influence of coagulation factor, vitamin K epoxide reductase complex subunit 1, and cytochrome P450 2C9 gene polymorphisms on warfarin dose requirements. Clin Pharmacol Ther 2006;79(4):291–302.
4. Cavallari LH, Momary KM. Pharmacogenetics in cardiovascular diseases. In: Pharmacogenomics: Challenges and Opportunities in Therapeutic Implementation. Amsterdam, Netherlands: Elsevier; 2018. p. 133–79.
5. Makar-Ausperger K, Krželj K, Benčić ML, Aumiler MR, Turk VE, Božina N. Warfarin dosing according to the genotype-guided algorithm is most beneficial in patients with atrial fibrillation: A randomized parallel group trial. Ther Drug Monit 2018;40(3):362–8.
6. Akdeniz CS, Cevik M, Canbolat IP, Yurdakul S, Cagatay P, Ciftci C, et al. The effects of and VKORC1 gene polymorphisms on warfarin maintenance dose in Turkish cardiac patients. Future Cardiol 2020;16(6):645–54.
7. Li Y, Zhu J, Ding J. VKORC1-1639G/A and 1173 C/T Genetic Polymorphisms influence individual differences in warfarin maintenance dose. Genet Test Mol Biomarkers 2015;19(9):488–93.
8. Kosaki K, Yamaghishi C, Sato R, Semejima H, Fuijita H, Tamura K, et al. 1173C>T polymorphism in VKORC1 modulates the required warfarin dose. Pediatr Cardiol 2006;27(6):685–8.
9. Owen RP, Gong L, Sagreiya H, Klein TE, Altman RB. VKORC1 pharmacogenomics summary. Pharmacogenet Genomics 2010;20:642–4.
10. Soltani Banavandi MJ, Satarzadeh N. Association between VKORC1 gene polymorphism and warfarin dose requirement and frequency of VKORC1 gene polymorphism in patients from Kerman province. Pharmacogenomics J 2020;20(4):574–8.
11. Biswas M, Bendkhale SR, Deshpande SP, Thaker SJ, Kulkarni DV, Bhatia SJ, et al. Association between genetic polymorphisms of CYP2C9 and VKORC1 and safety and efficacy of warfarin: Results of a 5 years audit. Indian Heart J 2018;70:S13–9.
12. al Ammari M, AlBalwi M, Sultana K, Alabdulkareem IB, Almuzzaini B, Almakhlafi NS, et al. The effect of the VKORC1 promoter variant on warfarin responsiveness in the Saudi WArfarin Pharmacogenetic (SWAP) cohort. Sci Rep 2020 Jul 15;10(1):11613. doi: 10.1038/s41598-020-68519-9.
13. Moreau C, Pautas E, Gouin-Thibault I, Golmard JL, Mahé I, Mulot C, et al. Predicting the warfarin maintenance dose in elderly inpatients at treatment initiation: Accuracy of dosing algorithms incorporating or not VKORC1/CYP2C9 genotypes. J Thromb Haemost 2011 Apr;9(4):711-8.
14. Lucia RD, di Perna P, Chetta M, Santacroce R, Brancaccio V, Grandone E, et al. A polymorphism in the VKORC1 gene is associated with an interindividual variability in the dose-anticoagulant effect of warfarin. Blood 2005;105(2):645-9.
15. Herman D, Peternel P, Stegnar M, Breskvar K, Dolzan V. The influence of sequence variations in factor VII, γ-glutamyl carboxylase and vitamin K epoxide reductase complex genes on warfarin dose requirement. Thromb Haemost 2006;95(5):782–7.
16. Geisen C, Watzka M, Sittinger K, Steffens M, Daugela L, Seifried E, et al. VKORC1 haplotypes and their impact on the inter-individual and inter-ethnical variability of oral anticoagulation. Thromb Haemost 2005;94(4):773–9.
17. Alzahrani AM, Ragia G, Hanieh H, Manolopoulos VG. Genotyping of CYP2C9 and VKORC1 in the arabic population of Al-Ahsa, Saudi Arabia. Biomed Res Int 2013;2013:315980. doi: 10.1155/2013/315980.
18. Cadamuro J, Dieplinger B, Felder T, Kedenko I, Mueller T, Haltmayer M, et al. Genetic determinants of acenocou-

CONTENT

marol and phenprocoumon maintenance dose requirements. Eur J Clin Pharmacol 2010;66(3):253–60.
19. Panchenko E, Kropacheva E, Dobrovolsky A, Titaeva E, Zemlyanskaya O, Trofimov D, et al. CYP2C9 and VKORC1 genotyping for the quality of long-standing warfarin treatment in Russian patients. Pharmacogenomics J 2020;20(5):687–94.
20. Miao L, Yang J, Huang C, Shen Z. Contribution of age, body weight, and CYP2C9 and VKORC1 genotype to the anticoagulant response to warfarin: Proposal for a new dosing regimen in Chinese patients. Eur J Clin Pharmacol 2007;63(12):1135–41.
21. El Din MS, Amin DG, Ragab SB, Ashour EE, Mohamed MH, Mohamed AM. Frequency of VKORC1 (C1173T) and CYP2C9 genetic polymorphisms in Egyptians and their influence on warfarin maintenance dose: Proposal for a new dosing regimen. Int J Lab Hematol 2012;34(5):517–24.
22. Au N, Rettie AE. Pharmacogenomics of 4-hydroxycoumarin anticoagulants. Drug Metab Rev 2008;40:355-75.
23. Schwarz UI, Stein CM. Genetic determinants of dose and clinical outcomes in patients receiving oral anticoagulants. Clin Pharmacol Ther 2006;80(1):7–12.
24. Nakai K, Tsuboi J, Okabayashi H, Fukuhiro Y, Oka T, Habano W, et al. Ethnic differences in the VKORC1 gene polymorphism and an association with warfarin dosage requirements in cardiovascular surgery patients. Pharmacogenomics 2007;8(7):713–9.
25. Vidovic S, Skrbic R, Stojiljkovic MP, Vidovic V, Becarevic J, Stoisavljevic-Satara S, et al. Prevalence of five pharmacologically most important CYP2C9 and CYP2C19 allelic variants in the population from the Republic of Srpska in Bosnia and Herzegovina. Arh Hig Rada Toksikol 2021:28;72(3):129–34.
26. Theken KN, Lee CR, Gong L, Caudle KE, Formea CM, Gaedigk A, et al. Clinical Pharmacogenetics Implementation Consortium Guideline (CPIC) for CYP2C9 and nonsteroidal anti-inflammatory drugs. Clin Pharmacol Ther 2020;1;108(2):191–200.
27. Johnson JA, Caudle K, Gong L, Whirl-Carrillo M, Stein CM, Scott SA, et al. Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for pharmacogenetics-guided warfarin dosing: 2017 update. Clin Pharmacol Ther 2017 Sep;102(3):397-404.
28. McClain MR, Palomaki GE, Piper M, Haddow JE. A Rapid-ACCE review of CYP2C9 and VKORC1 alleles testing to inform warfarin dosing in adults at elevated risk for thrombotic events to avoid serious bleeding. Genet Med 2008;10(2):89–98.
29. Li S, Zou Y, Wang X, Huang X, Sun Y, Wang Y, et al. Warfarin dosage response related pharmacogenetics in chinese population. PLoS One 2015 Jan 16;10(1):e0116463. doi: 10.1371/journal.pone.0116463.
30. Gaikwad T, Ghosh K, Shetty S. VKORC1 and CYP2C9 genotype distribution in Asian countries. Thromb Res 2014 Sep;134(3):537-44.
31. 31. Gaikwad T, Ghosh K, Shetty S. VKORC1 and CYP2C9 genotype distribution in Asian countries. Thromb Res 2014 Sep;134(3):537-44.
32. Social Science Statistics [Internet]. [Cited: 1-Jan-2021]. Available at: https://www.socscistatistics.com/tests/.

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REVIEW ARTICLE

Regulatory Role of Some Protein Kinases in Signal Transduction Pathways in Heart Health and Disease

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Abstract

Various protein kinases including protein kinase A (PKA), Ca^{2+}-calmodulin kinase (CaMK), phosphoinositide 3-kinase (PI3K), protein kinase C (PKC) and mitogen-activated protein kinase (MAPK: ERK1/2, p38-MAPK and JNK) are integral part of different signal transduction pathways, which are known to regulate cardiac structure, function and metabolism. In addition, these signal transducing proteins are involved in the regulation of cation transport, cellular growth, gene expression, apoptosis and fibrosis by modifying the function of different target sites of subcellular organelles in the myocardium. However, the information regarding these signal transducing molecules is scattered and mechanisms of their involvement in diverse regulatory processes are poorly understood. While PKA, CaMK, PI3K and PKC are activated by different hormones and mechanical stimuli, MAPKs are activated by growth factors and some cellular stresses such as oxidative stress, inflammation and Ca^{2+}-overload. Each type of these protein kinases is expressed in the form of two or more isozymes showing different biochemical characteristics and distinct biological functions. It has been demonstrated that all specific isoforms of these kinases produce both beneficial and detrimental effects on the heart, which are dependent upon the intensity and duration of stimulus for their activation. While PKA, PKC and CaMK are mainly involved in augmenting cardiac function as well as inducing cardiac hypertrophy and arrhythmias, PI3K is mainly involved in maintaining β-adrenoceptor function and inducing inflammation as well as arrhythmias. On the other hand, ERK1/2 mainly participate in the genesis of cardiac hypertrophy and cytoprotection whereas p38-MAPK and JNK are primarily involved in cardiac dysfunction, apoptosis and fibrosis. Since the activities of most protein kinases are increased under prolonged pathological conditions, a wide variety of their inhibitors have been shown to produce beneficial effects. However, extensive research needs to be carried out to understand the pathophysiology of different isoforms of each protein kinase as well as for the development of their isoform-specific inhibitors.

Key words: Protein kinase A; Protein kinase C; Ca^{2+}-calmodulin kinase; Phosphoinositide 3-kinase; MAP kinase; Extracellular regulated protein kinase; p38-MAP kinase; Cardiac hypertrophy; Arrhythmias; Cardiac function.

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Introduction

It is now well known that a wide variety of signal transduction pathways are activated by different extracellular (chemical and mechanical) stimuli as well as cellular stresses to modify the structure and function of cardiomyocytes in health and disease.[1-11] One of the common components

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of these signal transducing systems is a group of several protein kinases, which not only transmit the signals to their target sites but also regulate the functions of different subcellular organelles such as sarcolemma, sarcoplasmic reticulum, mitochondria, myofibrils, nucleus and extracellular matrix in the heart.[12-14] Several types of protein kinases are present in the myocardium and each of these enzymes have two or more isoforms with some overlapping structural characteristics but distinct biological functions. Some of these signal transducing proteins include protein kinase A (PKA), protein kinase C (PKC), Ca^{2+}-calmodulin dependent kinase (CaMK), phosphoinositide 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK). These protein kinases have been demonstrated to regulate cation transport, cellular growth, gene expression, cellular apoptosis and fibrosis as well as myocardial metabolism[15-17] and are thus considered to play a major role in the regulation of cardiac function.

It is pointed out that all protein kinases, upon activation by diverse agents or interventions, evoke immediate biological actions for the regulation of several subcellular protein activities. A schematic representation of different protein kinases and their activators is shown in Figure 1 whereas that for major sites and targets of the activated transducing proteins is given in Figure 2. The process of activation involves the transfer of γ-phosphate group from ATP to hydroxyl group of serine/threonine protein kinases or tyrosine residue of tyrosine protein kinases. It should be noted that most of these protein kinases exert both beneficial and detrimental effects depending upon the intensity and duration of the stimulus as well as isoform of the enzyme involved in the process of signal transduction. A few beneficial effects of some inhibitors of the activated protein kinases are shown in Figure 3. In the present article, the existing information regarding some protein kinases for their activation, actions as well as participation in various signal transduction pathways in normal and diseased hearts is updated. In view of the complex mode of involvement of various protein kinases in diverse signal transduction pathways, it is intended to briefly discuss

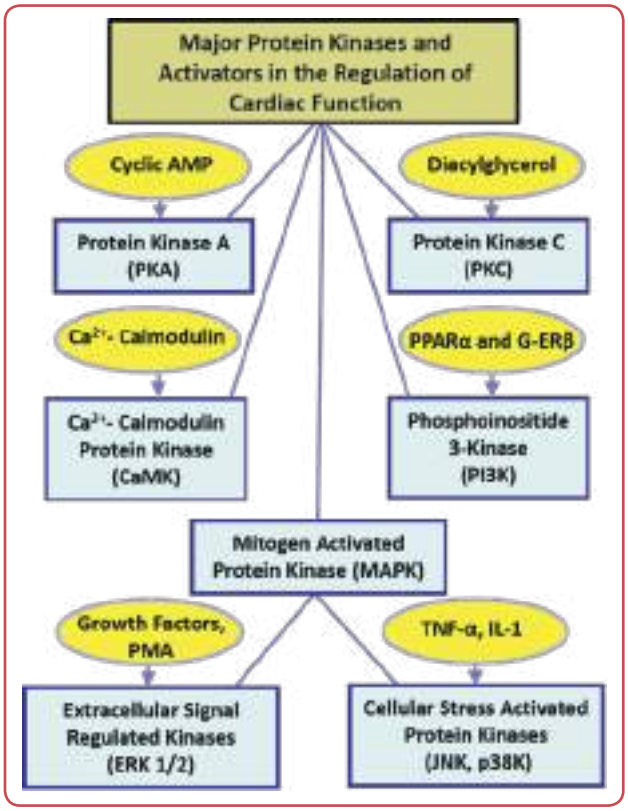

Figure 1: Activation of different protein kinases in the heart by some intracellularly produced complex factors and metabolites as well as extracellular growth factors and agents

PPAR α, peroxisome proliferator-activated receptor α; G-ERB, G-protein coupled estrogen receptor B; PMA, phorbol myristate acetate; TNF-α, tumor necrosis factor -α; IL-1, interleukin-1; JNK, c-jun N-terminal protein kinase;

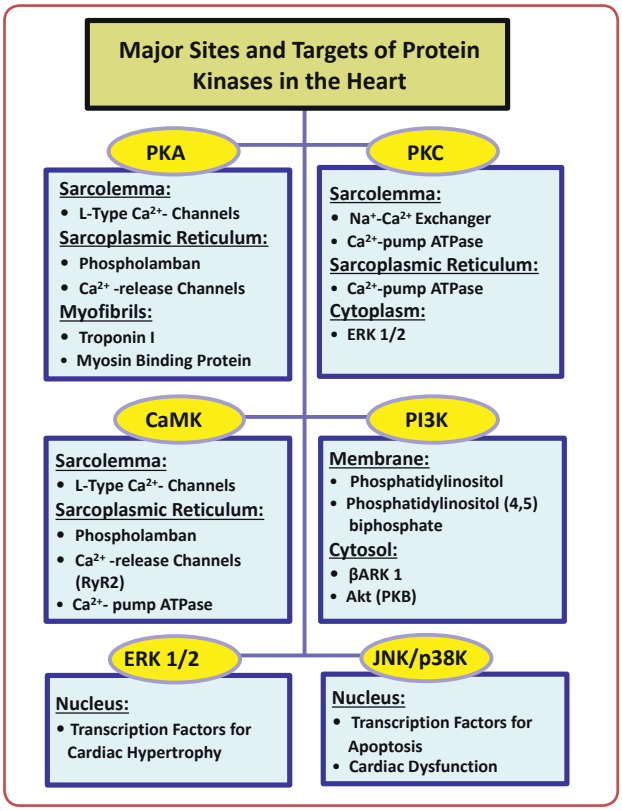

Figure 2: Major sites and targets of some activated protein kinases in the heart

PKA, protein kinase A; PKC, protein kinase C; CaMK, Ca^{2+}- calmodulin protein kinase; RyR2, ryanodine receptors; PI3K, phosphoinositide 3-kinase; ERK1/2, extracellular regulated protein kinase 1 and 2; JNK/p38K, cellular stress activated MAP kinases; βARK1, β-adrenergic receptor kinase 1; Akt, protein kinase B;

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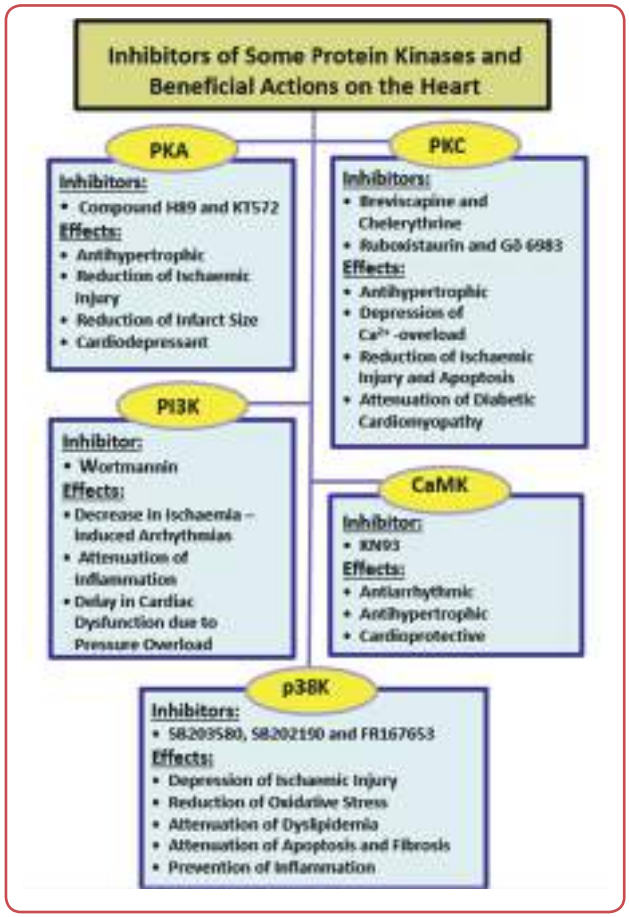

Figure 3: Beneficial effects of different inhibitors of some protein kinases in heart disease

PKA, protein kinase A; PKC, protein kinase C; CaMK, Ca^{2+}- calmodulin protein kinase; PI3K, phosphoinositide 3-kinase; p38-Kinase, cellular stress activated MAP kinase;

the role of these signalling proteins in modifying different biological processes. Since all protein kinase are activated during the development of heart disease, an attempt has also made to identify appropriate protein kinases as targets for drug development for improving cardiac performance.

Role of Protein Kinase A

PKA is a tetramer holoenzyme, which consists of two regulatory subunits and two catalytic subunits. The catalytic subunits harbour the active site, a sequence of canonical amino acid residues that bind and hydrolyse ATP and a domain that binds the regulatory subunit. The regulatory subunits have three domains – one that binds cyclic AMP, another domain which interacts with catalytic subunit and an autoinhibitory domain.[18] Upon binding of cyclic AMP to regulatory subunits, dissociation and release of active regulatory monomers occur for the transduction of signal.[19] There are four types of PKA – RIα, PKA-IIα, PKA-Iβ and PKA-IIβ, which are expressed in cardiomyocytes.[20] It is noteworthy that PKA is responsible for phosphorylation of numerous proteins involved in the regulation of myocardial contraction and relaxation. The activation of PKA has been reported to phosphorylate sarcolemma (SL) Ca^{2+} channels to increase the influx of Ca^{2+} into cardiomyocytes for the occurrence of an increase in cardiac contractibility. Several studies addressing the interaction of PKA with sarcoplasmic reticulum (SR), at phospholamban (PLB) and ryanodine receptor 2 (RyR_2, Ca^{2+}- release channel proteins), have appeared in the literature to promote Ca^{2+}-uptake and Ca^{2+}-release activities for the regulation of myofibrils for affecting the overall cardiac performance.[21-23] Furthermore, it has been reported that PLB, a SERCA regulatory protein, is phosphorylated by PKA in cardiomyocytes in response to stimulation by β-adrenoceptor (β-AR) agonists, catecholamines, which relieve the inhibitory effect of PLB on SERCA2 and thus increase the SR Ca^{2+}-pump activity.[24-28] PKA was also shown to phosphorylate and stimulate the activity of RyR2 to release Ca^{2+} from the SR Ca^{2+}-stores.[29] PKA-dependent SR phosphorylation following β-AR stimulation has also been observed to produce an increase in the leakage of SR Ca^{2+}.[30] In addition, to increasing the intracellular concentration of Ca^{2+}, phosphorylation of myofibrillar proteins by PKA has been reported to cause stimulation of cardiac filament change in orientation and affect contractility of the heart.[31] Moreover, stimulation of cardiomyocytes by β-AR is associated with phosphorylation of both myosin C protein and troponin I (cTn1) by PKA in thick myofilaments and thin myofilaments, respectively.[32] It has also been reported that cTn1 phosphorylation by PKA increases cardiac rate of relaxation and crossbridge cycle kinetics and is associated with frequency and after-load dependent enhancement of heart function.[33-35]

It should be pointed out that phosphorylated PKA in the cytoplasm also enters the nucleus to activate appropriate transcription factors to induce cardiac hypotrophy and improve cardiac function.[1, 2, 17] Since PKA induced phosphorylation of various subcellular proteins increases cardiac function, inhibition of the PKA can be seen to depress heart performance. Such an effect of PKA inhibitors was demonstrated to reduce infarct size and prevent complications following myocardial infarction.[28, 36] Because proteasome assembly is

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facilitated by PKA phosphorylation, this assembly of proteasome is also blocked upon PKA inhibition.[37] It is noteworthy that PKA inhibition has been reported to play a role in decreasing apoptosis in cardiomyocytes.[38, 39] Furthermore, PKA inhibition was shown to exert cardioprotective effect during ischaemic injury and promote growth hormone induced cardioprotection during ischaemia reperfusion as well.[28, 40] On the other hand, prolonged activation of PKA invariably results in cardiac dysfunction for the development of heart failure. These observations are consistent with the view that the activation of PKA not only induces cardiac hypertrophy and improves cardiac performance upon β-AR stimulation, but also plays a role in cardioprotection under different pathological situations such as ischaemia-reperfusion injury.

Role of Ca^{2+}-Calmodulin Dependent Protein Kinase

CaMK is a ubiquitous mediator of Ca^{2+}-linked signalling, which phosphorylates various substrates to regulate Ca^{2+}-mediated modifications in cardiac function.[41] CaMK exits in four isoforms which are encoded by highly related genes α, β, γ and δ.[42] Although, the activation of CaMK by Ca^{2+}-calmodulin is brief in nature, the oligomerisation of CaMK subunits is associated with a prolonged activation process.[43, 44] It may be noted CaMKII is one of the main effector enzymes involved in Ca^{2+} signalling in eukaryotic cells but the position of the catalytic domain, Ca^{2+} sensitivity and autophosphorylation are altered by hetero-oligomerisation processes for α, β and δ subunits of the variable domain spacers of CaMK II.[42] The involvement of CaMKII in the modification of excitation-contraction coupling by phosphorylating RyR, PLB and SERCA2, makes it a major player in modulating cardiac function and performance.[6, 45, 46] While the activation of CaMK for a short period is known to improve cardiac performance, a damaging effect on heart function has been reported to be due to excessive or prolonged activation of CaMKII in the myocardium, which is associated with the development of hypertrophic and apoptotic cardiomyopathy.[47-49] It has been demonstrated that reactive oxygen species (ROS) increase the sensitivity of CaMKII to Ca^{2+} and this ROS-dependent CaMKII activation has been shown to initiate angiotensin II induced apoptotic cascade.[50] Therefore, the positive and negative effects of CaMKII activation are dependent on the intensity and duration of stimulation. Different CaMKII inhibitors have also reported to exert both negative and positive effects on the heart. Activation of CaMKII was observed to augment oxidative stress and cause lethal ventricular arrhythmias.[51-53] CaMKII inhibition was also shown to prevent maladaptive remodelling due to excessive β-AR stimulation, Ca^{2+} handling abnormalities, as well as arrhythmias under *in vivo* conditions.[52, 54-56] An increase in RyR2-dependent Ca^{2+} leakage due to increased phosphorylation of the RyR2 has been suggested to explain the enhancement of CaMKII activity in subjects susceptible to atrial fibrillation[57] and this effect of CaMKII was also demonstrated to play a role in arrhythmias in a mouse model of heart failure.[58] Furthermore, it has been shown that induction of CaMKII by pressure overload may enhance protein synthesis and cause hypertrophy, a process that is considered to be adaptive initially but when prolonged it becomes pathological.[59]

It has been reported, that δ isoform of CaMKII plays a major role in the pathophysiological remodelling of the heart due to pressure overload.[60, 61] In fact, various CaMKII isoforms has been demonstrated to affect the heart function differently. It was shown that the levels of δ and γ isoforms of CaMKII were increased, following cardiac hypertrophy induced by aortic constriction[62, 63] and a substantial reduction in cardiac hypertrophy was observed when δ isoform was inhibited with a minimal disruption of CaMKII function. Functionally, cardiac CaMKII-δB and δC are also different as these isoforms are inversely regulated in response to IR injury and oxidative stress. While, δB inhibits myocyte apoptosis, δC triggers the opposite effect.[64, 65] Additionally, it has been reported that CaMKII δB overexpression is cardioprotective against hypoxia, oxidative stress and angiotensin-II induced apoptosis, probably as a consequence of CaMKII δC inhibition.[66, 67] It has been shown that acute overexpression of CaMKII δC alters RyR function, leading to enhanced SR Ca^{2+} leakage, which may cause ventricular arrhythmia in mice.[57, 58] This damaging effect was prevented when CaMKII inhibitors such as KN93 and autocamtide2-related inhibitory peptides were used.[69, 70] Collectively, these reports support the concept that both positive and negative effects of CaMKII signal transduction pathway depend on the involvement of specific isoform of the enzyme.

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While cardiomyocyte hypertrophy has been shown to be induced by CaMKII δ3 via the activation of apoptosis signal-regulating kinase 1 (ASK-1), phosphorylation of Ca^{2+}-induced ASK-1 was reported to be inhibited by KN93.[71, 72] Since numerous studies have revealed that CaMKII is a key player in addition to CaMKI and CaMKIV for the induction of hypertrophy in cardiomyocytes *in vivo*,[73-75] it has been suggested that CaMKII inhibition may reduce the development of hypertrophy in the heart. Although, it was shown that the left ventricular end-diastolic diameter is increased and the fractional shortening is decreased upon overexpression of CaMKIV in mice,[73, 74] the advantage of CaMK inhibition in other pathological settings is speculative. Nonetheless, it has been reported that the recovery of cardiac function after I/R is facilitated by ischaemic preconditioning and is inhibited by KN93; these findings provide evidence for the activation of CaMKII in the preconditioning process.[76, 77] The impairment in the SR function in the myocardium has also been shown to be due to modification in SR CaMK-facilitated phosphorylation, as well as due to reduction in the level of SR proteins and activity of SR CaMKII.[78, 79] Regardless of the involvement of CaMKII δC in the process of hypertrophy, it has been reported to phosphorylate calcineurin and cause inhibition of its activity. It was also shown that cardiac hypertrophy, dysfunction and arrhythmias are caused by calcineurin[80, 81] indicating that inhibition by calcineurin of active CaMKII δ is probably more efficient than inhibition of the inactive form of CaMKII δ. These observations support the view regarding the differential significance of CaMK isoforms in affecting the cardiac function.

Role of Protein Kinase C

PKC plays an important role in relaying information for a variety of extracellular signals across the membrane to regulate several Ca^{2+}-linked activities. The PKC family has many isoforms that share a conserved kinase domain with an ATP-binding site at the carboxyl terminal.[3, 82] Major PKC isoforms expressed in hearts are α and β with α being more human specific.[83] Depending upon the mode of activation, PKC is divided into 3 classes namely conventional (cPKC), novel (nPKC) and atypical (aPKC). Conventional ones respond to Ca^{2+} and diacylglycerol (DAG) to express their activities and have α, βI, βII and γ isoforms. The novel ones are activated by DAG, independent of Ca^{2+} and exhibit δ, ε, η and θ isoforms. Atypical ones respond to phosphoinositide-dependent kinase-1, independent of Ca^{2+} and show ζ and λ isoforms.[4, 84, 85] It has been reported that phorbol esters increase the activity of PKC associated with membranes to produce cardiac dysfunction.[86] It was shown that PKC isoforms α, β, ε, ζ and Ca^{2+}-independent activity are increased in cytosolic and homogenate fractions of diabetic hearts; this increase demonstrates a link between subcellular modifications and cardiac activity in these hearts.[87] Additionally, hormone induced hypertrophy in hearts was linked to PKC activation as it was found that both membrane and nuclear cytoskeletal fractions of hypertrophied heart due to pressure overload were associated with increase in levels and activities of specific PKC isoforms, PKC-β1,2 and PKC-ε.[88] Braun and coworkers[89] have also reported an increase in PKC δ, unlike PKC α or ε isoforms, in the left ventricular hypertrophy induced by volume overload whereas an increase in the activity and protein expression of both α and δ isoforms of PKC was observed in the right ventricular hypertrophy. It was shown that the left ventricular dilatation due to angiotensin II was associated with the activation of phospholipase C, causing phosphatidylinositol (PI) hydrolysis and PKCε activation. Similarly, an increase in the autophosphorylation of PKC-δ was seen to occur prior to the development of left ventricular hypertrophy as well as during the transition to heart failure. Although the expression of PKC-α was unchanged during the induction of cardiac hypertrophy, it was augmented prior to the development of left ventricular hypertrophy.[91, 92] Therefore, it was concluded that activation of specific PKC isoforms plays an important role in either the positive or the negative effect of the PKC signal transduction pathway. Nonetheless, inhibition of PKC was reported to prevent the occurrence of abnormal myocyte mechanics in diabetes and improve contractility of the heart.[83, 93] It is pointed out that an increased expression of PKC-α and PKC-β isoforms was linked to the loss of cardiac contractile function in diabetes, which eventually caused heart failure because of the regulatory effect on Ca^{2+}-cycling proteins.[83, 94-97]

It has been reported that ruboxistaurin, an inhibitor of PKC-β, attenuates diastolic dysfunction, myocyte hypertrophy, as well as collagen deposition for preserving cardiac contractility in diabetic cardiomyopathy.[98, 99] Additionally, Boyle

CONTENT

and coworkers[100] reported that inhibition of PKC with ruboxistaurin attenuated the pathological fibrosis and impairment of cardiac function in experimentally induced myocardial infarction. Deterioration in cardiac contractility and depression of metabolic activities in the ischaemic heart conditions were shown as an effect of aldosterone induced vasoconstriction through PKC-dependent pathways.[101, 102] Furthermore, Wang et al[91] reported a close association between the upregulation of PKC-α, PKC-β and PKC-ε expressions and the activity of PKC in cardiac dysfunction following MI. It was observed that inhibition of PKC by ruboxistaurin decreased ventricular dilation, improved ventricular performance and reduced fibrosis in mice following 10 weeks of pressure-overload.[98] It was also shown that PKC-α -/- mice were less vulnerable to heart failure, while PKC-β/γ -/- mice showed severe heart failure by a longstanding pressure overload.[98] Upon subjecting transgenic mice to inhibition with ruboxistaurin, an increase in cardiac contractility was noticed in the PKC-β/γ -/- model but not in the PKC-α -/- model.[98] These results provide further evidence concerning the significance of PKC isoform inhibitor specificity. It was also observed that Ro-318110, a PKC-α selective inhibitor, caused an increase in cardiac contractile function of the heart and improved the pump function in heart failure in a mouse model.[83] These studies also indicated the effectiveness of the specific inhibition of PKC-α by Ro-320432 in improving heart contractile function.

It was claimed that the compound Gö 6983 provides better cardioprotection than other PKC inhibitors when administered at the start of reperfusion, because it averts intracellular Ca^{2+} overload following ischaemic reperfusion injury and in addition to PKCξ inhibition, it also depressed other PKC isoforms.[103, 104] Inhibition of PKC-βII was shown to improve contractility following cardiac dysfunction due to the ischaemia reperfusion induced by polymorphonuclear leukocytes (PMN). This action was due to inhibition of the release of PMN superoxide and the increase in the nitric oxide release from the endothelium.[105-107] Although the extent of inhibition of PKC was found crucial, the expression level of δ-specific PKC as well as inhibition of its translocation were also shown to impact heart function.[107] In fact, an improvement in heart dysfunction without significant changes in heart structure, function or gene expression has been reported in the presence of low levels of this inhibitor.[107] On the other hand, severe consequences were observed upon overexpression of PKC-δ V1, which were lethal with depressed cardiac contractile activity, increased expression of foetal cardiac genes and formation of myocyte protein aggregates.[108]

Inhibition of PKC attenuated the effects of ischaemic preconditioning on cardiac function and lipid peroxidation indicating that cardioprotection involving the activation of PKC may be a consequence of the depression in the reactive oxygen species (ROS) induced damage.[109, 110] In fact, the activation of PKC has been reported to play an important role in exercise-induced cardioprotection against ischaemia-reperfusion injury.[111, 112] Furthermore, the activation of PKC by angiotensin II, preceding ischaemia, was observed to limit myocardial infarct size.[113] Since the overexpression of the active cardiac-specific PKCε mutant in transgenic mice has been shown to cause concentric hypertrophy with normal *in vivo* cardiac function[114, 115] the association of cardioprotection and the activation of PKC, especially the isoform ε, has been established during ischaemia-reperfusion injury.[116, 117] While cardioprotection by improving vascular endothelial nitric oxide release was achieved when PKC-ε was activated upon the administration of a peptide activator prior to ischaemia, but not during preconditioning, a PKC-ε inhibitor was observed to eliminate the cardioprotective effects of PKC-ε activator.[118] PKC-ε translocation from the cytosol to the membrane has also been shown to modify the cardioprotective effects of opioid receptor stimulation, causing decrease in cellular injury due to lethal ischaemia.[119, 120] Since cardioprotection due to ischaemic preconditioning occurs in two phases, the mechanisms for the involvement of PKC in early and late phases of cardioprotection have been shown to be of a complex nature.[121-125] While cardioprotection in early phase involves posttranslational modification of redox sensitive proteins whereas the late phase is mediated by cardioprotection of gene expression.[121] Furthermore, neither the activation and translocation of any specific PKC isoforms nor the role of associated signal transduction pathways involving mitochondrial KATP channels, formation of oxyradicals species and generation of nitric oxide in both phases of cardioprotection by ischaemic preconditioning is fully understood.[122-125] On the other hand, translocation of PKC-ε has been demonstrated to be involved in exercise preconditioning induced by both early and late phases of cardioprotection.

Different cardiac PKC isoforms were found to modulate apoptosis induced by hyperglycaemia and cardiac PKCε activation was observed to protect ventricular myocytes in rats from death signals induced by hyperglycaemia.[127, 128] In addition, cardiac specific expression of the PKC-ε translocation activator ψε-RACK (a PKC ε-agonist) has been reported to protect cardiomyocytes from apoptosis signals induced by hyperglycaemia.[127, 129] Since PKC is an essential signalling component for the fibroblast growth factor 2 (FGF2)-induced cardioprotection, various interconnections between the MAPK and PKC pathways during ischaemia-reperfusion injury are considered to play a key role in cardioprotection produced by FGF2.[130, 131] Furthermore, Das and coworkers[132] have reported that selective translocation of PKC isoforms α, δ and θ from cytosol to membrane fractions and have thus suggested their probable role in cardioprotection induced by sildenafil. Accordingly, it is evident that not only there is a great deal of specificity of PKC isoforms but the selective transduction as well as translocation of some PKC isoforms are also a special feature of cardioprotection under various conditions.

Role of Phosphoinositide 3-Kinase

PI3K is a heterodimeric molecule which consists of 2 subunits; a regulatory p85 subunit and catalytic p110, subunit harbouring two SH2 domains.[133, 134] These subunits form adaptor motifs connecting tyrosine kinases and their substrates;[135] both catalytic p110 and regulatory p85 form *in vivo* obligate heterodimers.[136] The catalytic protein forms a bilobal structure with an ATP-binding pocket between N-terminal and C-terminal sections and the catalytic amino acid residues in the phosphate-binding subsite.[137, 138] Various isoforms of PI3K are involved in the regulation of different types of biological functions in the heart. For instance, PI3Kα isoform mediates changes in the size of cells whereas PI3Kγ isoform inhibits the production of cAMP and thus reduces cardiac contractile activity.[139, 140] PI3K inhibition has also been reported to decrease cardiac arrhythmias due to ischaemia as well as inflammation following MI.[141, 142] It was shown in an animal model with chronic pressure overload overexpressing PI3K that active endogenous PI3K was replaced by inactive PI3K and this shift resulted in delay in the development of cardiac dysfunction[143] indicating the role of PI3K in heart failure. Perrino and coworkers[144] have reported that targeting of PI3K at the site of activated βAR plays a central role in the downregulation of βAR. In this regard, competitive removal of PI3K from βARK1 was found to conserve βAR signalling in heart failure, defer cardiac dysfunction progression and prolong lifespan of the genetic heart failure animal model. These investigators have also reported that adenovirus gene transfer of PI3K domain resulted in a decrease in the activity of the receptor-localised PI3K and caused normalisation of contractility in failing pigs hearts.[145] Nienaber et al[146] have shown that PI3K participates in the regulation of the level as well as sensitivity of βAR function in hearts and observed a significant role of this kinase in βAR dysfunction in pressure overload-induced heart failure. Therefore, it was suggested that inhibition of PI3K may preserve βAR signalling to avert heart failure.

A decline in cardiac function due to free radicals has been linked to PI3Kγ activation by tumour necrosis factor-α (TNF-α) for causing remodelling of the myocardium.[147] Perrino et al[148] have also reported that pathological pressure overload enhances gene expression of PI3Kγ, suggesting that its inhibition may attenuate cardiac hypertrophy. Gene knockout animal models of PI3K regulatory subunit p85 has also revealed a decrease in heart size and preservation of cardiac contractility and structure.[149, 150] It was observed that G protein-coupled oestrogen receptor was upregulated in isolated rat hearts following ischaemia-reperfusion and thus may improve cardiac function recovery and reduce the infarct size; such positive effects were eliminated when PI3K was subjected to inhibition by wortmannin.[151, 152] It should be noted that PI3K inhibitor, wortmannin, eliminated the improvement in cardiac function recovery in epoxyeicosatrienoic acid treated hearts but did not inhibit the recovery of rBNP-treated hearts.[153] Furthermore, wortmannin has been observed to attenuate adrenomedullin-induced positive effects, such as infarct size reduction, haemodynamic improvements and apoptosis inhibition and therefore it was suggested that adrenomedullin induces cardioprotective effects through the PI3K/Akt pathway.[154, 155] In a genetic mouse model of atrial fibrillation associated with heart failure, it was shown that a reduction in PI3K (p110α) activity increases the susceptibility to atrial fibrillation, whereas an increase in the activity decreases atrial fibrosis and improve

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cardiac conduction.[156] Additionally, contractile defects have been reported in cardiac myocytes lacking PI3K subunit p110α.[157] On the other hand, it was observed that an increase in the activity of PI3K p110α has positive effects on cardiac function in animal models of heart failure.[158] Absolute deficiency of PI3K-β subunit was found to result in dramatic reduction in myocardial contractile performance, while absolute deficiency of PI3K-γ resulted in myocardial infarction in mice.[159] Thus, PI3K can be seen to play an important regulatory role in inducing both beneficial and detrimental effects in heart function depending upon the involvement of its isoforms and subunits, which are activated by either by growth factors or by proinflammatory interventions.

Role of P38 Mitogen-Activated Kinases

MAPKs are intracellular signalling molecules which are activated by either growth factors or by proinflammatory cytokines[10, 11, 160] and are known to exert both beneficial and detrimental effects on the heart. Cardiac dysfunction and apoptosis have been linked to the activation of p38-MAPK when changes in Ca^{2+} status (Ca^{2+} depletion and Ca^{2+} repletion) occur.[161] While apoptosis has been shown to occur upon the activation of α-isoform of p38-MAPK, cardiac hypertrophy has been observed when p38-MAPK β-isoform was overexpressed.[162] Nonetheless, inhibition of p38-MAPK has been reported to be cardioprotective against ischaemic damage.[163-166] Otsu and coworkers[167] have demonstrated that a p38-MAPK knockout model was resistant to ischaemia/reperfusion injury. ROS has been reported to increase p38-MAPK activation and subsequently results in myocardial damage.[168] It was reported that rats treated with selective p38- MAPK inhibitors showed a decrease in angiotensin II-induced ROS production, hypertension and cardiac hypertrophy.[169] Since cardiac dysfunction due to inflammatory response has been associated with p38-MAPK activation as well as expression of inflammatory cytokines, the inhibition of p38-MAPK with specific inhibitors has been reported to avert the negative inflammatory effects on the heart function.[170-172] Li et al[173] have shown that p38-MAPK-α inhibition in a rat model of acute myocardial injury has a cardioprotective effect and substantially improved cardiac function. On the other hand, P38-MAPK-knock-out hamster model has been reported to develop early heart failure and cardiac dysfunction in comparison with the control group.[172] Although most of the regulatory actions of JNK are similar to those of p38-MAPK[10, 11] the effects of JNK and p38 inhibitors on cardiac dysfunction in hamsters are opposite to each other.[172] These observations are consistent with the view that p38-MAPK and JNK may exert both good and bad regulatory actions in the heart.

It needs to be emphasised that in addition to p38-MAPK and JNK, both ERKI and ERK2 belong to MAPK family of serine-threonine specific protein kinases.[10, 11, 16, 17] These proteins and their different isoforms and subunits share 60 to 70 % similarity and thus a great of caution be used while interpreting the effects of their inhibitors.[160, 162] Furthermore, it is pointed out that while p38-MAPK and JNK are mainly concerned with the regulation of transduction pathways for ischaemia-reperfusion injury, apoptosis and cardiac dysfunction, both ERK1 and ERK2 are known to play a major regulatory role in the processes concerned with the development of cardioprotection and cardiac hypertrophy.[10, 16, 173] Upon activation by PKC in the cytosol, both ERK1/2 enter the nucleus to phosphorylate different transcription factors for the regulation of hypertrophic response in the heart.[15, 174] Activation of ERK1/2 upon the induction of pressure overload has been reported to promote cardiomyocyte survival.[175] Multiple circulating hormones and growth factors such as angiotensin II, insulin, platelet derived growth factor and epidermal growth factor as well as tumour promoting phorbol esters have been shown to activate ERK1/2 and induce cardiac hypertrophy.[176-179] It should be also pointed out that prolonged activation of ERK1/2 has been demonstrated to result in the development of heart failure[180, 181] and thus antihypertrophic agents such as angiotensin II antagonists and angiotensin converting enzyme inhibitors can be seen to produce beneficial effects in heart failure as a consequence of reduction in the activity of ERK1/2.[12-14] It should be noted that both p38-MAPK and JNK are activated by the ischaemic insult[182, 183] whereas the effects of ischaemia-reperfusion on ERK1/2 activities are controversial.[184, 185] In fact, inhibition of ERK1/2 has been reported to enhance ischaemia-reperfusion injury as well as apoptosis.[186] Furthermore, ERK1/2 signal transduction pathway has been reported to

induce compensated cardiac hypertrophy.[187] Not only the effects of ERK1/2 activation on apoptosis are opposing to those p38K-JNK,[188] ERK1/2 signal transduction pathways have been reported to play an antiapoptotic role in the cell survival.[189] These observations support the view that ERK1/2 pathway is mainly cytoprotective.

Conclusion

From the foregoing discussion, it is evident that there are five major types of protein kinases, which participate in different signal transduction pathways for relaying information to target sites at the subcellular organelles in the heart. One group of these signal transducing proteins includes PKA, PKC, CaMK and PI3K (which are activated by various hormones and mechanical stimuli) whereas the other group (MAPK), includes two classes of proteins namely ERK1 and ERK2 (which are activated by growth factors) as well as p38-MAPK and JNK (which are activated by proinflammatory cytokines: TNF-α and IL-1). It is pointed out that in spite of the presence of numerous protein kinases, which may participate in diverse signal transduction pathways in the myocardium, some protein kinases such as PKA, PKC and CaMK are mainly involved in regulating cardiac function, cardiac hypertrophy and arrhythmias whereas PI3K is involved in inducing inflammation as well as modifying β-adrenoceptor-mediated pathway. Furthermore, ERK1/2 mainly participate in the development of cardiac hypertrophy and cytoprotection whereas p38-MAPK and JNK are involved in cardiac dysfunction as well as cellular injury. It also needs to be emphasised that all these protein kinases have been demonstrated to participate in inducing both beneficial and detrimental effects with respect to cation transport, cellular injury, apoptosis, fibrosis, cellular growth, gene expression, cardiac hypertrophy and cardiac dysfunction. However, these actions are of complex nature and are dependent upon the intensity and duration of stimulus. Accordingly, extensive work needs to be carried out in order to establish which effects is adaptive and which effect is harmful in nature. Nonetheless, it appears that these protein kinases are not only involved in the transmission of signals to subcellular organelles but are also involved in the regulation of their activities.

It is noteworthy that each type of protein kinase is expressed in the form of two to four isozymes which have been shown to produce distinctly different biological functions in the myocardium. In fact, the activation of some isoforms of a protein kinase have been reported to induce opposite actions in response to different interventions and thus both the beneficial and detrimental effects of various protein kinases upon their activation are difficult to interpret. Although a wide variety of inhibitors are available, their isoform specificity needs to be carefully determined during the development of various cardiovascular diseases. It is therefore suggested that time-course studies for different isoforms of each type of protein kinase be examined. Likewise, appropriate genetic animal models for each isoform of different protein kinases be developed to gain the information regarding their exact functional significance in different diseases. Since various protein kinases are activated during the development of heart failure, the status of each isoform of these signal transducing proteins be examined in order to understand their role in the pathophysiology of this major cardiac disorder as well as for the development of isoform specific inhibitors for the treatment of heart disease.

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Conflict of interest

None.

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References

1. Taylor SS, Buechler JA, Yonemoto W. cAMP-dependent protein kinase: Framework for a diverse family of regulatory enzymes. Annu Rev Biochem 1990;59:971-1005.
2. Dhalla NS, Wang J. Role of protein kinase C and protein kinase A in heart function in health and disease. Exp Clin Cardiol 1999;4:7-14.
3. Kikkawa U, Nishizuka Y. The role of protein kinase C in transmembrane signalling. Annu Rev Cell Biol 1986;2:149–78.
4. Mellor H, Parker PJ. The extended protein kinase C superfamily. Biochem J 1998;332:281-92.
5. Hook SS, Means AR. Ca2+/CaM-dependent kinases: From activation to function. Annu Rev Pharmacol Toxicol 2001;41:471-505.
6. Hudmon A, Schulman H. Neuronal Ca2+/calmodulin-dependent protein kinase II: the role of structure and autoregulation in cellular function. Annu Rev Biochem 2002;71:473–510.
7. Walker EH, Perisic O, Ried C, Stephens L, Williams RL. Structural insights into phosphoinositide 3-kinases catalysis and signaling. Nature 1999; 402:313-20.
8. Djordjevic S, Driscoll PC. Structural insight into substrate specificity and regulatory mechanisms of phosphoinositide 3-kinases. Trends Biochem Sci 2002;27:426–32.
9. Chan TO, Rittenhouse SE, Tsichlis PN. AKT/PKB and other D3 phosphoinositide-regulated kinases: Kinase activation by phosphoinositide-dependent phosphorylation. Annu Rev Biochem 1999;68:965-1014.
10. Zhang W, Elimban V, Nijjar MS, Gupta SK, Dhalla NS. Role of mitogen-activated protein kinase in cardiac hypertrophy and heart failure. Exp Clin Cardiol 2003;8:173-83.
11. Krishna M, Narang H. The complexity of mitogenactivated protein kinases (MAPKs) made simple. Cell Mol Life Sci 2008;65:3525–44.
12. Bhullar SK, Shah AK, Dhalla NS. Role of angiotensin II in the development of subcellular remodeling in heart failure. Explor Med 2021;2:352-71.
13. Bhullar SK, Dhalla, NS. Angiotensin II-induced signal transduction mechanisms for cardiac hypertrophy. Cells 2022;11: 3336. doi: 10.3390/cells11213336.
14. Bhullar SK, Shah AK, Dhalla, NS. Mechanisms for the development of heart failure and improvement of cardiac function by angiotensin-converting enzyme inhibitors. Scr Med 2022;53:51-76.
15. Bogoyevitch MA, Sugden PH. The role of protein kinases in adaptational growth of the heart. Int J Biochem Cell Biol 1996;28:1–12.
16. Sugden PH, Bogoyevitch, MA. Intracellular signaling through protein kinases in the heart. Cardiovasc Res 1995;30:478-92.
17. Dhalla NS, Muller AL. Protein kinase as drug development targets for heart disease therapy. Pharmaceuticals 2010;3:2111-45.
18. Turnham RE, Scott JD. Protein kinase A catalytic subunit isoform PRKACA; History, function and physiology. Gene 2016;577:101–8.
19. Heldin C-H. Dimerization of cell surface receptors in signal transduction. Cell 1995;80:213–23.
20. Di Benedetto G, Zoccarato A, Lissandron V, Terrin A, Li X, Houslay MD, et al. Protein kinase A type I as type II define distinct intercellur signaling compartments. Circ Res 2008;103:836-44.
21. Baryshnikova OK, Li MX, Sykes BD. Modulation of cardiac troponin C function by the cardiacspecific N-terminus of troponin I: influence of PKA phosphorylation and involvement in cardiomy-opathies. J Mol Biol 2008;375:735–51.
22. Shaffer JF, Kensler RW, Harris SP. The myosinbinding protein C motif binds to F-actin in a phosphorylation-sensitive manner. J Biol Chem 2009;284:12318–27.
23. Jones PP, Meng X, Xiao B, Cai S, Bolstad J, Wagenknecht T, et al. Localization of PKA phosphorylation site, Ser2030, in the three-dimensional structure of cardiac ryanodine receptor. Biochem J 2008;410:261–70.
24. Hakim K, Fischer M, Günnicker M, Poenicke K, Zerkowski HR, Brodde OE. Functional role of β2-adrenoceptors in the transplanted human heart. J Cardiovasc Pharmacol 1997;30:811–6.
25. Surdo NC, Berrera M, Koschinski A, Brescia M, Machado MR, Carr C, et al. FRET biosensor uncovers cAMP nanodomains at β-adrenergic targets that dictate precise tuning of cardiac contractility. Nat Commun 2017;8:1–14.
26. Böhm M, Reiger B, Schwinger RH, Erdmann E. cAMP concentrations, cAMP dependent protein kinase activity, and phospholamban in non-failing and failing myocardium. Cardiovasc Res 1994;28:1713–9.
27. Mattiazzi A, Hove-Madsen L, Bers DM. Protein kinase inhibitors reduce SR Ca transport in permeabilized cardiac myocytes. Am J Physiol Heart Circ Physiol 1994;267:H812–20.
28. Robinet A, Hoizey G, Millart H. PI 3-kinase, protein kinase C, and protein kinase A are involved in the trigger phase of β1-adrenergic preconditioning. Cardiovasc Res 2005;66:530–42.
29. Wehrens XH, Lehnart SE, Reiken S, Vest JA, Wronska A, Marks AR. Ryanodine receptor/calcium release channel PKA phosphorylation: a critical mediator of heart failure progression. Proc Natl Acad Sci 2006;103:511–8.
30. Ogrodnik J, Niggli E. Increased Ca2+ leak and spatiotemporal coherence of Ca2+ release in cardiomyocytes during β-adrenergic stimulation. J Physiol 2010;588:225–42.
31. Sadayappan S, Gulick J, Osinska H, Corbalán R, Foncea R, Ebensperger R, et al. A critical function for Ser-282 in cardiac Myosin binding protein-C phosphorylation and cardiac function. Circ Res 2011;109:141–50.
32. Piddo AM, Sánchez MI, Sapag-Hagar M, Corbalán R, Foncea R, Ebensperger R, et al. Cyclic AMP-dependent protein kinase and mechanical heart function in ventricular hypertrophy induced by pressure overload or secondary to myocardial infarction. J Mol Cell Cardiol 1996;28:1073–83.
33. Kentish JC, McCloskey DT, Layland J, Palmer S, Leiden JM, Martin AF, et al. Phosphorylation of troponin I by protein kinase A accelerates relaxation and crossbridge cycle kinetics in mouse ventricular muscle. Circ Res 2001;88:1059–65.
34. Zhang R, Zhao J, Mandveno A, Potter JD. Cardiac troponin I phosphorylation increases the rate of cardiac muscle relaxation. Circ Res 1995;76:1028–35.
35. Takimoto E, Soergel DG, Janssen PM, Stull LB, Kass DA, Murphy AM. Frequency-and afterload-dependent cardiac modulation in vivo by troponin I with constitutively active protein kinase A phosphorylation sites. Circ Res 2004;94:496–504.
36. Sanada S, Kitakaze M, Papst PJ, Asanuma H, Node K, Takashima S, et al. Cardioprotective effect afforded by transient exposure to phosphodiesterase III inhibitors: the role of protein kinase A and p38 mitogen-activated protein kinase. Circulation 2001;104:705–10.
37. Zhang F, Hu Y, Huang P, Toleman CA, Paterson AJ, Kudlow JE. Proteasome function is regulated by cyclic AMP-dependent protein kinase through phosphorylation of Rpt6. J Biol Chem 2007;282:22460–71.
38. Zhao ZQ, Velez DA, Wang NP, Hewan-Lowe KO, Nakamu-

CONTENT

ra M, Guyton RA, et al. Progressively developed myocardial apoptotic cell death during late phase of reperfusion. Apoptosis 2001;6:279–90.
39. Saraste A, Pulkki K, Kallajoki M, Heikkilä P, Laine P, Mattila S, et al. Cardiomyocyte apoptosis and progression of heart failure to transplantation. Eur J Clin Invest 1999;29:380-6.
40. Makaula S, Lochner A, Genade S, Sack MN, Awan MM, Opie LH. H-89, a non-specific inhibitor of protein kinase A, promotes post-ischemic cardiac contractile recovery and reduces infarct size. J Cardiovasc Pharmacol 2005;45:341–7.
41. Hudmon A, Schulman H. Structure–function of the multifunctional Ca2+/calmodulin-dependent protein kinase II. Biochem J 2002;364:593–611.
42. Tombes RM, Faison MO, Turbeville JM. Organization and evolution of multifunctional Ca2+/CaM-dependent protein kinase genes. Gene 2003;322:17–31.
43. De Koninck P, Schulman H. Sensitivity of CaM kinase II to the frequency of Ca2+ oscillations. Science 1998;279:227–30.
44. Meyer T, Hanson PI, Stryer L, Schulman H. Calmodulin trapping by calcium-calmodulin-dependent protein kinase. Science 1992;256:1199–202.
45. Soderling TR. The Ca2+–calmodulin-dependent protein kinase cascade. Trends Biochem Sci 1999;24:232–6.
46. Netticadam T, Temsah R, Osada M, Dhalla NS. Status of Ca2+/calmodulin protein kinase phosphorylation of cardiac SR proteins in ischemia-reperfusion. Am J Physiol Cell Physiol 1999;277:C384-91.
47. Koval OM, Guan X, Wu Y, Joiner ML, Gao Z, Chen B, et al. CaV1.2 β-subunit coordinates CaMKII-triggered cardiomyocyte death and afterdepolarizations. Proc Natl Acad Sci 2010;107:4996–5000.
48. Swaminathan PD, Purohit A, Soni S, Voigt N, Singh MV, Glukhov AV, et al. Oxidized CaMKII causes cardiac sinus node dysfunction in mice. J Clin Invest 2011;121:3277–88.
49. Kolodziej SJ, Hudmon A, Waxham MN, Stoops JK. Three-dimensional reconstructions of calcium/calmodulin-dependent (CaM) kinase IIα and truncated CaM kinase IIα reveal a unique organization for its structural core and functional domains. J Biol Chem 2000;275:14354–9.
50. Salas MA, Valverde CA, Sánchez G, Said M, Rodriguez JS, Portiansky EL, et al. The signalling pathway of CaMKII-mediated apoptosis and necrosis in the ischemia/reperfusion injury. J Mol Cell Cardiol 2010;48:1298–306.
51. Xie L-H, Chen F, Karagueuzian HS, Weiss JN. Oxidative stress–induced afterdepolarizations and calmodulin kinase II signaling. Circ Res 2009;104:79–86.
52. Maier LS, Bers DM. Role of Ca2+/calmodulin-dependent protein kinase (CaMK) in excitation–contraction coupling in the heart. Cardiovasc Res 2007;73:631–40.
53. Wagner S, Dybkova N, Rasenack EC, Jacobshagen C, Fabritz L, Kirchhof P, et al. Ca2+/calmodulin-dependent protein kinase II regulates cardiac Na+ channels. J Clin Invest 2006;116:3127–38.
54. Wu Y, Roden DM, Anderson ME. Calmodulin kinase inhibition prevents development of the arrhythmogenic transient inward current. Circ Res 1999;84:906–12.
55. Wu Y, Temple J, Zhang R, Dzhura I, Zhang W, Trimble R, et al. Calmodulin kinase II and arrhythmias in a mouse model of cardiac hypertrophy. Circulation 2002;106:1288–93.
56. Zhang R, Khoo MS, Wu Y, Yang Y, Grueter CE, Ni G, et al. Calmodulin kinase II inhibition protects against structural heart disease. Nat Med 2005;11:409–17.
57. Chelu MG, Sarma S, Sood S, Wang S, van Oort RJ, Skapura DG, et al. Calmodulin kinase II–mediated sarcoplasmic reticulum Ca 2+ leak promotes atrial fibrillation in mice. J Clin Invest 2009;119:1940–51.
58. van Oort RJ, McCauley MD, Dixit SS, Pereira L, Yang Y, Respress JL, et al. Ryanodine receptor phosphorylation by calcium/calmodulin-dependent protein kinase II promotes life-threatening ventricular arrhythmias in mice with heart failure. Circulation 2010;122:2669-79.
59. Zhu W, Zou Y, Shiojima I, Kudoh S, Aikawa R, Hayashi D, et al. Ca2+/calmodulin-dependent kinase II and calcineurin play critical roles in endothelin-1-induced cardiomyocyte hypertrophy. J Biol Chem 2000;275:15239–45.
60. Backs J, Backs T, Neef S, Kreusser MM, Lehmann LH, Patrick DM, et al. The δ isoform of CaM kinase II is required for pathological cardiac hypertrophy and remodeling after pressure overload. Proc Natl Acad Sci 2009;106:2342–7.
61. Ling H, Zhang T, Pereira L, Means CK, Cheng H, Gu Y, et al. Requirement for Ca 2+/calmodulin–dependent kinase II in the transition from pressure overload–induced cardiac hypertrophy to heart failure in mice. J Clin Invest 2009;119:1230–40.
62. Hagemann D, Bohlender J, Hoch B, Krause EG, Karczewski P. Expression of Ca2+/calmodulin-dependent protein kinase II δ-subunit isoforms in rats with hypertensive cardiac hypertrophy. Mol Cell Biochem 2009;220:69–76.
63. Colomer JM, Mao L, Rockman HA, Means AR. Pressure overload selectively upregulates Ca2+/calmodulin-dependent protein kinase II in vivo. Mol Endocrinol 2003;17:183–92.
64. Peng W, Zhang Y, Zheng M, Cheng H, Zhu W, Cao CM, et al. Cardioprotection by CaMKII-δB is mediated by phosphorylation of heat shock factor 1 and subsequent expression of inducible heat shock protein 70. Circ Res 2010;106:102–10.
65. Zhu W, Woo AY, Yang D, Cheng H, Crow MT, Xiao RP. Activation of CaMKIIδC is a common intermediate of diverse death stimuli-induced heart muscle cell apoptosis. J Biol Chem 2007;282:10833–9.
66. Erickson JR, He BJ, Grumbach IM, Anderson ME. CaMKII in the cardiovascular system: sensing redox states. Physiol Rev 2011: 91;889–915.
67. Mollova MY, Katus HA, Backs J. Regulation of CaMKII signaling in cardiovascular disease. Front Pharmacol 2015; 6:178. doi: 10.3389/fphar.2015.00178.
68. Maier LS, Zhang T, Chen L, DeSantiago J, Brown JH, Bers DM. Transgenic CaMKIIδC overexpression uniquely alters cardiac myocyte Ca2+ handling: reduced SR Ca2+ load and activated SR Ca2+ release. Circ Res 2003;92:904–11.
69. Kohlhaas M, Zhang T, Seidler T, Zibrova D, Dybkova N, Steen A, et al. Increased sarcoplasmic reticulum calcium leak but unaltered contractility by acute CaMKII overexpression in isolated rabbit cardiac myocytes. Circ Res 2006;98:235–44.
70. Sag CM, Wadsack DP, Khabbazzadeh S, Abesser M, Grefe C, Neumann K, et al. Calcium/calmodulin-dependent protein kinase II contributes to cardiac arrhythmogenesis in heart failure. Circ Heart Fail 2009;2:664–75.
71. Kashiwase K, Higuchi Y, Hirotani S, Yamaguchi O, Hikoso S, Takeda T, et al. CaMKII activates ASK1 and NF-κB to induce cardiomyocyte hypertrophy. Biochem Biophys Res Commun 2005:27:136–42.
72. Takeda K, Matsuzawa A, Nishitoh H, Tobiume K, Kishida S, Ninomiya-Tsuji J, et al. Involvement of ASK1 in Ca2+-induced p38 MAP kinase activation. EMBO Rep 2004;5:161–6.
73. Passier R, Zeng H, Frey N, Naya FJ, Nicol RL, McKinsey TA, et al. CaM kinase signaling induces cardiac hypertrophy and activates the MEF2 transcription factor in vivo. J Clin Invest 2000;105:1395–406.
74. Zhang T, Johnson EN, Gu Y, Morissette MR, Sah VP, Gigena MS, et al. The cardiac-specific nuclear δB isoform of Ca2+/calmodulin-dependent protein kinase II induces

hypertrophy and dilated cardiomyopathy associated with increased protein phosphatase 2A activity. J Biol Chem 2002;277:1261–7.
75. Woischwill C, Karczewski P, Bartsch H, Luther HP, Kott M, Haase H, et al. Regulation of the human atrial myosin light chain 1 promoter by Ca2+-calmodulin-dependent signaling pathways. FASEB J 2005;19:503–11.
76. Osada M, Netticadan T, Kawabata K, Tamura K, Dhalla NS. Ischemic preconditioning prevents I/R-induced alterations in SR calcium-calmodulin protein kinase II. Am J Physiol Heart Circ Physiol 2000;278:H1791–8.
77. Benter IF, Juggi JS, Khan I, Yousif MH, Canatan H, Akhtar S. Signal transduction mechanisms involved in cardiac preconditioning: role of Ras-GTPase, Ca2+/calmodulin-dependent protein kinase II and epidermal growth factor receptor. Mol Cell Biochem 2005;268:175–83.
78. Netticadan T, Temsah RM, Kawabata K, Dhalla NS. Sarcoplasmic reticulum Ca2+/calmodulin-dependent protein kinase is altered in heart failure. Circ Res 2007; 86:596–605.
79. Mishra S, Sabbah HN, Jain JC, Gupta RC. Reduced Ca2. calmodulin-dependent protein kinase activity and expression in LV myocardium of dogs with heart failure. Am J Physiol Heart Circ Physiol 2003;284:H876-83.
80. MacDonnell SM, Weisser-Thomas J, Kubo H, Hanscome M, Liu Q, et al. CaMKII negatively regulates calcineurin–NFAT signaling in cardiac myocytes. Circ Res 2009;105:316–25.
81. Kreusser MM, Lehmann LH, Keranov S, Hoting MO, Oehl U, Kohlhaas M, et al. Cardiac CaM Kinase II genes δ and γ contribute to adverse remodeling but redundantly inhibit calcineurin-induced myocardial hypertrophy. Circulation 2014;130:1262–73.
82. Newton AC. Protein kinase C: structure, function, and regulation. J Biol Chem 1995;270:28495–8.
83. Hambleton M, Hahn H, Pleger ST, Kuhn MC, Klevitsky R, Carr AN, et al. Pharmacological and gene theraphy-based inhibition of protein kinase C alpha/beta enhances contractility an attentuates heart failure. Circulation 2006;114:574-82.
84. Newton AC. Regulation of protein kinase C. Curr Opin Cell Biol 1997;9:161–7.
85. Nishizuka Y. Protein kinase C and lipid signaling for sustained cellular responses. FASEB J 1995;9:484–96.
86. Kraft AS, Anderson WB. Phorbol esters increase the amount of Ca2+, phospholipid-dependent protein kinase associated with plasma membrane. Nature 1983;301:621–3.
87. Toker A, Newton AC. Cellular signaling: pivoting around PDK-1. Cell 2000;103:185–8.
88. Gu X, Bishop SP. Increased protein kinase C and isozyme redistribution in pressure-overload cardiac hypertrophy in the rat. Circ Res 1994;75:926–31.
89. Braun MU, LaRosée P, Simonis G, Borst MM, Strasser RH. Regulation of protein kinase C isozymes in volume overload cardiac hypertrophy. Mol Cell Biochem 2004;262:135–43.
90. Paul K, Ball NA, Dorn GW, Walsh RA. Left ventricular stretch stimulates angiotensin II–mediated phosphatidylinositol hydrolysis and protein kinase C ε isoform translocation in adult guinea pig hearts. Circ Res 1997;81:643–50.
91. Wang J, Liu X, Sentex E, Takeda N, Dhalla NS. Increased expression of protein kinase C isoforms in heart failure due to myocardial infarction. Am J Physiol Heart Circ Physiol 2003;284:H2277–87.
92. Bayer AL, Heidkamp MC, Patel N, Porter M, Engman S, Samarel AM. Alterations in protein kinase C isoenzyme expression and autophosphorylation during the progression of pressure overload-induced left ventricular hypertrophy. Mol Cell Biochem 2003;242:145-52.
93. Landau D, Chayat C, Zucker N, Golomb E, Yagil C, Yagil Y, et al. Early blood pressure-independent cardiac changes in diabetic rats. J Endocrinol 2008;197:75–84.
94. Wang M, Zhang WB, Zhu JH, Fu GS, Zhou BQ. Breviscapine ameliorates cardiac dysfunction and regulates the myocardial Ca2+-cycling proteins in streptozotocin-induced diabetic rats. Acta Diabetol 2010;47:209–18.
95. Beckman JA, Goldfine AB, Gordon MB, Garrett LA, Creager MA. Inhibition of protein kinase Cβ prevents impaired endothelium-dependent vasodilation caused by hyperglycemia in humans. Circ Res 2002;90:107–11.
96. Bowling N, Walsh RA, Song G, Estridge T, Sandusky GE, Fouts RL, et al. Increased protein kinase C activity and expression of Ca2+-sensitive isoforms in the failing human heart. Circulation 1999;99:384–91.
97. Braz JC, Gregory K, Pathak A, Zhao W, Sahin B, Klevitsky R, et al. PKC-α regulates cardiac contractility and propensity toward heart failure. Nat Med 2004;10:248–54.
98. Liu Q, Chen X, Macdonnell SM, Kranias EG, Lorenz JN, Leitges M, et al. Protein kinase Cα, but not PKCβ or PKCγ, regulates contractility and heart failure susceptibility: implications for rubox-istaurin as a novel therapeutic approach. Circ Res 2009;105:194–200.
99. Connelly KA, Kelly DJ, Zhang Y, Prior DL, Advani A, Cox AJ, et al. Inhibition of protein kinase C–β by ruboxistaurin preserves cardiac function and reduces extracellular matrix production in diabetic cardiomyopathy. Circ Heart Fail 2009;2:129–37.
100. Boyle AJ, Kelly DJ, Zhang Y, Cox AJ, Gow RM, Way K, et al. Inhibition of protein kinase C reduces left ventricular fibrosis and dysfunction following myocardial infarction. J Mol Cell Cardiol 2005;39:213–21.
101. Schmidt K, Tissier R, Ghaleb B, Drogies T, Felix SB, Krieg T. Cardioprotective effects of mineralocorticoid receptor antagonists at reperfusion. Eur Heart J 2010;31:1655–62.
102. Chai W, Garrelds IM, de Vries R, Batenburg WW, van Kats JP, Danser AH. Nongenomic effects of aldosterone in the human heart: interaction with angiotensin II. Hypertension 2005;46:701–6.
103. Young LH, Balin BJ, Weis MT. Gö 6983: a fast acting protein kinase C inhibitor that attenuates myocardial ischemia/reperfusion injury. Cardiovasc Drug Rev 2005;23:255–72.
104. Peterman EE, Taormina P. Harvey M, Young LH. Gö 6983 exerts cardioprotective effects in myocardial ischemia/reperfusion. J Cardiovasc Pharmacol 2004;43:645–56.
105. Omiyi D, Brue RJ, Taormina P 2nd, Harvey M, Atkinson N, Young LH. Protein kinase C βII peptide inhibitor exerts cardioprotective effects in rat cardiac ischemia/reperfusion injury. J Pharmacol Exp Ther 2005;314:542–51.
106. Phillipson A, Peterman EE, Taormina P Jr, Harvey M, Brue RJ, Atkinson N, et al. Protein kinase C-ζ inhibition exerts cardioprotective effects in ischemia-reperfusion injury. Am J Physiol Heart Circ Physiol 2005;289:H898–907.
107. Hahn HS, Yussman MG, Toyokawa T, Marreez Y, Barrett TJ, Hilty KC, et al. Ischemic protection and myofibrillar cardiomyopathy: dosedependent effects of in vivo δPKC inhibition. Circ Res 2002;91:741–8.
108. Inagaki K, Hahn HS, Dorn GW, Mochly-Rosen D. Additive protection of the ischemic heart *ex vivo* by combined treatment with δ-protein kinase C inhibitor and ε-protein kinase C activator. Circulation 2003;108:869–75.
109. Fantinelli JC, Mosca SM.Comparative effects of ischemic pre and postconditioning on ischemia-reperfusion injury in spontaneously hypertensive rats (SHR). Mol Cell Biochem 2007;296:45–51.
110. Arbeláez LFG, Pardo AC, Fantinelli JC, Mosca SM. Cyclosporine-A mimicked the ischemic preand postconditioning-mediated cardioprotection in hypertensive rats: Role of PKCε. Exp Mol Pathol 2016;100:266–75.
111. Penna C, Alloatti G, Crisafulli A. Mechanisms involved in cardioprotection induced by physical exercise. Antioxid Redox Signal 2020; 32:1115-34.
112. Borges JP, da Silva Verdoorn K. Cardiac ischemia/reper-

fusion injury: the beneficial effects of exercise. In: Exercise for Cardiovascular Disease Prevention and Treatment. Berlin: Springer, 2017; pp 155–179.
113. Diaz RJ, Wilson GJ. Selective blockade of AT1angiotensin II receptors abolishes ischemic preconditioning in isolated rabbit hearts. J Mol Cell Cardiol 1997;29:129-39.
114. Takeishi Y, Ping P, Bolli R, Kirkpatrick DL, Hoit BD, Walsh RA. Transgenic overexpression of constitutively active protein kinase C ε causes concentric cardiac hypertrophy. Circ Res 2000;86:1218–23.
115. Newton AC, Antal CE, Steinberg SF. Protein kinase C mechanisms that contribute to cardiac remodelling. Clin Sci 2016;130:1499–510.
116. Ping P, Takano H, Zhang J, Tang XL, Qiu Y, Li RC, et al. Isoform-selective activation of protein kinase C by nitric oxide in the heart of conscious rabbits: a signaling mechanism for both nitric oxide–induced and ischemiainduced preconditioning. Circ Res 1999;84:587–604.
117. Qiu Y, Ping P, Tang XL, Manchikalapudi S, Rizvi A, Zhang J, et al. Direct evidence that protein kinase C plays an essential role in the development of late preconditioning against myocardial stunning in conscious rabbits and that epsilon is the isoform involved. J Clin Invest 1998;101:2182–98.
118. Teng JC, Kay H, Chen Q, Adams JS, Grilli C, Guglielmello G, et al. Mechanisms related to the cardioprotective effects of protein kinase C epsilon (PKC ε) peptide activator or inhibitor in rat ischemia/reperfusion injury. Naunyn Schmiedebergs Arch Pharmacol 2008;378:1–15.
119. Wang G-Y, Zhou JJ, Shan J, Wong T-M. Protein kinase C-ε is a trigger of delayed cardioprotection against myocardial ischemia of κ-opioid receptor stimulation in rat ventricular myocytes. J Pharmacol Exp Ther 2001;299:603–10.
120. Wu S, Li HY, Wong TM. Cardioprotection of preconditioning by metabolic inhibition in the rat ventricular myocyte: involvement of κ-opioid receptor. Circ Res 1999;84:1388–95.
121. Otani H. Reactive oxygen species as mediators of signal transduction in ischemic preconditioning. Antioxid Redox Signal 2004;6:449-69.
122. Uchiyama Y, Otani H, Wakeno M, Okada T, Uchiyama T, Sumida T, et al. Role of mitochondrial KATP channels and protein kinase C in ischaemic preconditioning. Clin Exp Pharmacol Physiol 2003;30:426-36.
123. Bolli R, Dawn B, Tang XL, Qiu Y, Ping P, Xuan YT, et al. The nitric oxide hypothesis of late pre-conditioning. Basic Res Cardiol 1998;93:325-38.
124. Nakano A, Liu GS, Heusch G, Downey JM, Cohen MV. Exogenous nitric oxide can trigger a pre-conditioned state through a free radical mechanism, but endogenous nitric oxide is not a trigger of classical ischemic preconditioning. J Mol Cell Cardiol 2000;32:1159-67.
125. Tappia PS, Shah AK, Ramjiawan B, Dhalla NS. Modification of ischemia/reperfusion-induced alterations in subcellular organelles by ischemic preconditioning. Int J Mol Sci 2022;23:3425. doi: 10.3390/ijms23073425.
126. Hao Z, Pan SS, Shen YJ, Ge J. Exercise preconditioninginduced early and late phase of cardioprotection is associated with protein kinase C epsilon translocation. Circ J 2014;78:1636-45.
127. Malhotra A, Kang BPS, Hashmi S, Meggs LG. PKCε inhibits the hyperglycemia-induced apoptosis signal in adult rat ventricular myocytes. Mol Cell Biochem 2005;268:169-73.
128. Shizukuda Y, Reyland ME, Buttrick PM. Protein kinase C-δ modulates apoptosis induced by hyperglycemia in adult ventricular myocytes. Am J Physiol Heart Circ Physiol 2002;282:H1625–34.
129. Malhotra A, Begley R, Kang BP, Rana I, Liu J, Yang G, et al. PKC-ε-dependent survival signals in diabetic hearts. Am J Physiol Heart Circ Physiol 2005;289:H1343–50.
130. House SL, Melhorn SJ, Newman G, Doetschman T, Schultz Jel J. The protein kinase C pathway mediates cardioprotection induced by cardiacspecific overexpression of fibroblast growth factor-2. Am J Physiol Heart Circ Physiol 2007;293:H354–65.
131. Liao S, Bodmer JR, Azhar M, Newman G, Coffin JD, Doetschman T, et al. The influence of FGF2 high molecular weight (HMW) isoforms in the development of cardiac ischemia–reperfusion injury. J Mol Cell Cardiol 2010;48:1245–54.
132. Das A, Ockaili R, Salloum F, Kukreja RC. Protein kinase C plays an essential role in sildenafil-induced cardioprotection in rabbits. Am J Physiol Heart Circ Physiol 2004;286:H1455–60.
133. Otsu M, Hiles I, Gout I, Fry MJ, Ruiz-Larrea F, Panayotou G, et al. Characterization of two 85 kd proteins that associate with receptor tyrosine kinases, middle-T/pp60c-src complexes, and PI3-kinase. Cell 1991;65:91–104.
134. Skolnik EY, Margolis B, Mohammadi M, Lowenstein E, Fischer R, Drepps A, et al. Cloning of PI3 kinase-associated p85 utilizing a novel method for expression/cloning of target proteins for receptor tyrosine kinases. Cell 1991;65:83–90.
135. Vanhaesebroeck B, Stephens L, Hawkins P. PI3K signalling: the path to discovery and understanding. Nat Rev Mol Cell Biol 2012;13:195–203.
136. Miled N, Yan Y, Hon WC, Perisic O, Zvelebil M, Inbar Y, et al. Mechanism of two classes of cancer mutations in the phosphoinositide 3-kinase catalytic subunit. Science 2007;317:239–42.
137. Huang CH, Mandelker D, Schmidt-Kittler O, Samuels Y, Velculescu VE, Kinzler KW, et al. The structure of a human p110α/p85α complex elucidates the effects of oncogenic PI3Kα mutations. Science 2007;318:1744–8.
138. Walker EH, Pacold ME, Perisic O, Stephens L, Hawkins PT, Wymann MP, et al. Structural determinants of phosphoinositide 3-kinase inhibition by wortmannin, LY294002, quercetin, myricetin, and staurosporine. Mol Cell 2000;6:909–19.
139. Patrucco E, Notte A, Barberis L, Selvetella G, Maffei A, Brancaccio M, et al. PI3Kγ modulates the cardiac response to chronic pressure overload by distinct kinase-dependent andindependent effects. Cell 2004;118:375–87.
140. Hua R, Adamczyk A, Robbins C, Ray G, Rose RA. Distinct patterns of constitutive phosphodiesterase activity in mouse sinoatrial node and atrial myocardium. PLoS One 2012:7:e 47652. doi: 10.1371/journal.pone.0047652.
141. Kolár F, Jezková J, Balková P, Breh J, Neckár J, Novák F, et al. Role of oxidative stress in PKC-δ upregulation and cardioprotection induced by chronic intermittent hypoxia. Am J Physiol Heart Circ Physiol 2007;92:H224–30.
142. Collier PN, Martinez-Botella G, Cornebise M, Cottrell KM, Doran JD, Griffith JP, et al. Structural basis for isoform selectivity in a class of benzothiazole inhibitors of phosphoinositide 3-kinase γ. J Med Chem 20015;58:517–21.
143. Durrant TN, Hers I. PI3K inhibitors in thrombosis and cardiovascular disease. Clin Transl Med 2020;9:1–21.
144. Perrino C, Naga Prasad SV, Patel M, Wolf MJ, Rockman HA. Targeted inhibition of β-adrenergic receptor kinase-1–associated phosphoinositide-3 kinase activity preserves β-adrenergic receptor signaling and prolongs survival in heart failure induced by calsequestrin overexpression. J Am Coll Cardiol 2005;45:1862–70.
145. Perrino C, Naga Prasad SV, Schroder JN, Hata JA, Milano C, Rockman HA. Restoration of β-adrenergic receptor signaling and contractile function in heart failure by disruption of the βARK1/phosphoinositide 3-kinase complex. Circulation 2005;111:2579–87.
146. Nienaber JJ, Tachibana H, Naga Prasad SV, Esposito G,

Wu D, Mao L, et al. Inhibition of receptor-localized PI3K preserves cardiac β-adrenergic receptor function and ameliorates pressure overload heart failure. J Clin Invest 2003;112:1067–79.
147. Awad AE, Kandalam V, Chakrabarti S, Wang X, Penninger JM, Davidge ST, et al. Tumor necrosis factor induces matrix metalloproteinases in cardiomyocytes and cardiofibroblasts differentially via superoxide production in a PI3Kγ-dependent manner. Am J Physiol Cell Physiol 2010;298:C679–92.
148. Perrino C, Naga Prasad SV, Mao L, Noma T, Yan Z, Kim HS, et al. Intermittent pressure overload triggers hypertrophy-independent cardiac dysfunction and vascular rarefaction. J Clin Invest 2006;116:1547–60.
149. Luo J, McMullen JR, Sobkiw CL, Zhang L, Dorfman AL, Sherwood MC, et al. Class IA phosphoinositide 3-kinase regulates heart size and physiological cardiac hypertrophy. Mol Cell Biol 2005;25:9491–502.
150. Shioi T, Kang PM, Douglas PS, Hampe J, Yballe CM, Lawitts J, Cantley LC, et al. The conserved phosphoinositide 3-kinase pathway determines heart size in mice. EMBO J 2000;19:2537–48.
151. Deschamps AM, Murphy E. Activation of a novel estrogen receptor, GPER, is cardioprotective in male and female rats. Am J Physiol Heart Circ Physiol 2009;297:H1806–13.
152. Bopassa JC, Eghbali M, Toro L, Stefani E. A novel estrogen receptor GPER inhibits mitochondria permeability transition pore opening and protects the heart against ischemia-reperfusion injury. Am J Physiol Heart Circ Physiol 2010;298:H16–H23.
153. Chaudhary KR, Batchu SN, Das D, Suresh MR, Falck JR, et al. Role of B-type natriuretic peptide in epoxyeicosatrienoic acidmediated improved postischaemic recovery of heart contractile function. Cardiovasc Res 2009;83:362–70.
154. Okumura H, Nagaya N, Itoh T, Okano I, Hino J, Mori K, et al. Adrenomedullin infusion attenuates myocardial ischemia/reperfusion injury through the phosphatidylinositol 3-kinase/Aktdependent pathway. Circulation 2004;109:242–8.
155. Yin H, Chao L, Chao J. Adrenomedullin protects against myocardial apoptosis after ischemia/reperfusion through activation of Akt-GSK signaling. Hypertension 2004;43:109–16.
156. Pretorius L, Du XJ, Woodcock EA, Kiriazis H, Lin RC, Marasco S, et al. Reduced phosphoinositide 3-kinase (p110α) activation increases the susceptibility to atrial fibrillation. Am J Pathol 2009;175:998–1009.
157. Lu Z, Jiang YP, Wang W, Xu XH, Mathias RT, Entcheva E, et al. Loss of cardiac phosphoinositide 3-kinase p110α results in contractile dysfunction Circulation 2009;120:318-25.
158. McMullen JR, Amirahmadi F, Woodcock EA, Schinke-Braun M, Bouwman RD, Hewitt KA, et al. Protective effects of exercise and phosphoinositide 3-kinase (p110α) signaling in dilated and hypertrophic cardiomyopathy. Proc Natl Acad Sci 2007;104:612–7.
159. Curcio A, Noma T, Naga Prasad SV, Wolf MJ, Lemaire A, Perrino C, et al. Competitive displacement of phosphoinositide 3-kinase from β-adrenergic receptor kinase-1 improves postinfarction adverse myocardial remodeling. Am J Physiol Heart Circ Physiol 2006; 291:H1754-60.
160. Schindler JF, Monahan JB, Smith WG. p38 pathway kinases as antiinflammatory drug targets. J Dent Res 2007;86:800–11.
161. Xu YJ, Saini HK, Zhang M, Elimban V, Dhalla NS. MAPK activation and apoptotic alterations in hearts subjected to calcium paradox are attenuated by taurine. Cardiovasc Res 72:163–74.
162. Wang Y, Huang S, Sah VP, Ross J Jr, Brown JH, Han J, et al. Cardiac muscle cell hypertrophy and apoptosis induced by distinct members of the p38 mitogen-activated protein kinase family. J Biol Chem 1998;273:2161–8.
163. See F, Thomas W, Way K, Tzanidis A, Kompa A, Lewis D, et al. p38 mitogen-activated protein kinase inhibition improves cardiac function and attenuates left ventricular remodeling following myocardial infarction in the rat. J Am Coll Cardiol 2004;44:1679-89.
164. Barancik M, Htun P, Strohm C, Kilian S, Schaper W. Inhibition of the cardiac p38-MAPK pathway by SB203580 delays ischemic cell death. J Cardiovasc Pharmacol 2000;35:474–83.
165. Nagarkatti DS, Ramadan IS. Role of p38 MAP kinase in myocardial stress. J Mol Cell Cardiol 1998;30:1651–64.
166. Martin JL, Avkiran M, Quinlan RA, Cohen P, Marber MS. Antiischemic effects of SB203580 are mediated through the inhibition of p38α mitogen-activated protein kinase: evidence from ectopic expression of an inhibition-resistant kinase. Circ Res 2001;89:750-2.
167. Otsu K, Yamashita N, Nishida K, Hirotani S, Yamaguchi O, Watanabe T, et al. Disruption of a single copy of the p38α MAP kinase gene leads to cardioprotection against ischemia–reperfusion. Biochem Biophys Res Commun 2003;302:56–60.
168. Fan L, Sawbridge D, George V, Teng L, Bailey A, Kitchen I, et al. Chronic cocaine-induced cardiac oxidative stress and mitogen-activated protein kinase activation: the role of Nox2 ocidase. J Pharmacol Exp Ther 2009;328:99-106.
169. Bao W, Behm DJ, Nerurkar SS, Ao Z, Bentley R, Mirabile RC, et al. Effects of p38 MAPK Inhibitor on angiotensin II-dependent hypertension, organ damage, and superoxide anion production. J Cardiovasc Pharmacol 2007;49:362–8.
170. Sato H, Tanaka T, Kasai K, Kita T, Tanaka N. Role of p38 mitogen-activated protein kinase on cardiac dysfunction after hemorrhagic shock in rats. Shock 2007;28:291–9.
171. Westermann D, Rutschow S, Van Linthout S, Linderer A, Bücker-Gärtner C, Sobirey M, et al. Inhibition of p38 mitogen-activated protein kinase attenuates left ventricular dysfunction by mediating proinflammatory cardiac cytokine levels in a mouse model of diabetes mellitus. Diabetologia 2006;49:2507–13.
172. Kyoi S, Otani H, Matsuhisa S, Akita Y, Tatsumi K, Enoki C, et al. Opposing effect of p38 MAP kinase and JNK inhibitors on the development of heart failure in the cardiomyopathic hamster. Cardiovasc Res 2006;69:888–98.
173. Li Z, Ma JY, Kerr I, Chakravarty S, Dugar S, Schreiner G, et al. Selective inhibition of p38α MAPK improves cardiac function and reduces myocardial apoptosis in rat model of myocardial injury. Am J Physiol Heart Circ Physiol 2006;291:H1972–7.
174. Malhotra A, Kang BP, Opawumi D, Belizaire W, Meggs LG. Molecular biology of protein kinase C signaling in cardiac myocytes. Mol Cell Biochem 2001;225:97–107.
175. Muslin AJ. MAPK signaling in cardiovascular health and disease: Molecular mechanisms and therapeutic targets. Clin Sci 2008;115:203-18.
176. Stefanovsky VY, Pellitier G, Hannan R, Gagonon Kugler T, Rothblum LI, Moss T. An immediate response of ribosomal transcription to growth factor stimulation is mediated by ERK phosphorylation of UBF. Mol Cell 2001;81:1063-73.
177. Heft MA, Harder BA, Eppenberger H. Schaub MC Signaling pathways in cardiac myocycte hypertrophy. J Mol Cell Cardiol 1997;29:2873-92.
178. Shao Q, Takeda N, Temsah R, Dhalla KS, Dhalla NS. Prevention of hemodynamic changes due to myocardial infarction by early treatment with imidapril. Cardioasc Pathol 1996;1:180-6.
179. Weinberg EO, Schoen FJ, George D, Kagaya Y, Douglas

CONTENT

PS, Litwin SE, et al. Angiotensin converting enzyme inhibitors prolong survival and modifies the transition to heart failure in rats with pressure overload hypertrophy due to ascending aortic stenosis. Circulation 1994;90:1410-22.
180. Haq S, Choukroun G, Lim H, Tymitz KM, del Monte F, Gwathmey J, et al. Differential activation of signal transduction pathways in human hearts with hypertrophy versus advanced heart failure. Circulation 2001;103:670-7.
181. Yano M, Kim S, Izumi Y Yamanaka S, Iwao H. Differential activation of cardiac c-jun amino-terminal kinase and extracellular signal-regulated kinase in angiotensin II-mediated hypertension. Circ Res 1998;83:752-60.
182. Yoshida K, Yoshiyama M, Omura T, Nakamura Y, Kim S, Takeuchi K, et al. Activation of mitogen-activated protein kinases in the nonishemic myocardium of an acute myocardial infarction in rats. Jpn Circ J 2001;65:808-14.
183. Knight R, Buxton D. Stimulation of c-jun kinase and mitogen-activated protein kinase by ishemia and reperfusion in the perfused rat heart. Biochem Biophys Res Commun 1996;218:83-8.
184. Behrends M, Schulz R, Post H, Alexandrov A, Belosjorow S, Michel MC, et al. Inconsistent relation of MAPK activation to infarct size reduction by ischemic preconditioning in pigs. Am J Physiol Heart Circ Physiol 2000;279:H1111-9.
185. Bogoyevitch MA, Gillespie-Brown J, Ketterman AJ, Fuller SJ, Ben-Levy R, Ashworth A, et al. Stimulation of the stress-activated mitogen-activated protein kinase subfamilies in perfused heart. p38/RK mitogen-activated protein kinases and c-jun N-terminal kinases are activated by ischemia/reperfusion. Circ Res 1996;79:162-73.
186. Yue TL, Wang C, Gu JL, Ma XL, Kumar S, Lee JC, et al. Inhibition of extracellular signal-regulated kinase enhances ischemia/reoxygenation-induced apoptosis in cultural cardiac myocyctes and exaggerates reperfusion injury in isolated perfused heart. Circ Res 2000;86:692-9.
187. Bueno OF, De Windt LJ, Tymitz KM, Witt SA, Kimball TR, Klevitsky R, et al. The MEKI-ERK½ signaling pathway promotes compensated cardiac hypertrophy in transgenic mice. EMBO J 2000;19:6341-50.
188. Xia, Z, Dickens M. Raingeaud J, Davis RJ, Greenberg ME, Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. Science 1995;270:1326-31.
189. Horiuchi M, Akishita M, Dzau VJ. Recent progress in angiotensin II type 2 receptor research in the cardiovascular system. Hypertension 1999;33:613-21.

CONTENT

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CURRENT TOPIC

Development of Critical Care Medicine in Post-War Republic of Srpska - Banja Luka Region

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Abstract

Critical care medicine as relatively young discipline, started developing in mid-1950s in response to epidemy of poliomyelitis. This branch of medicine evolved much faster in high-income countries (HIC) than in low resource settings (LRS) where the Republic of Srpska (Western Balkan) belongs. The experience of setting up a modern critical care program under the LRS constraints as a promising way forward to meet the increased demand for critical care worldwide is described. Main tool was systematic analysis of written documents related to the establishment of the first multidisciplinary MICU and its development to the present day. Successful development is contingent on formal education and continued mentorship from HIC, establishment of a multidisciplinary team, the support from local healthcare authorities, development of a formal subspecialty training, academic faculty development and research. Critical care medicine is a critical public health need in HIC and LRS alike.

Key words: Critical care medicine; Low-middle-income countries; Development.

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Introduction

The development of critical care medicine began in mid-1950s in response to a poliomyelitis outbreak at the time, but the modern critical care as an independent specialty began to develop in the 1970s in the United States, while in Western European countries this process was delayed by ten to twenty years.[1-5]

When it comes to developing critical care in low- to middle-income countries (LMICs), the data is very scarce. In these settings, surgical critical care has traditionally been provided by anaesthesiologists in most areas while access to medical critical care is still limited.[4, 5] Currently, the World Bank classifies Bosnia and Herzegovina (as well as the Republic of Srpska) as an upper-middle income country,[6] but it's healthcare system and problems related to treatment of critically ill patients are quite similar to LMICs and accordingly it can be defined as a low resource settings (LRS). When talking or writing about LRS, it is necessary to know that these countries are not situated somewhere in Africa, but in the heart of Europe (the Western Balkan where the Republic of Srpska is situated). The definition of LRS is used throughout this article to refer to healthcare systems in LMICs (as well as upper middle-income countries), acknowledging that LRS exist even in HICs.[7]

In post-war Republic of Srpska (as well as Bosnia and Herzegovina), no Medical Intensive Care Unit (MICU) existed until December 2008, when the first modern MICU in the country opened in

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CONTENT

Banja Luka.[8] Until the establishment of the MICU, medical critically ill patients were treated in poorly equipped classical general wards without cardiovascular and respiratory support and usually without haemodynamic monitoring (Figure 1). Invasive mechanical ventilation and other advanced life support interventions were only possible in surgical intensive care units, to which only a small number of medical critically ill patients gained access to.[5, 8]

Figure 1: Medical Intensive Care Unit 2007 at Clinic for Pulmonary Diseases, University Clinical Centre of the Republic of Srpska. Treatment of critically ill patient without haemodynamic and respiratory monitoring

A similar situation can still be found in other countries of Eastern Europe and in low- and middle-income countries worldwide.[9] In the case of Bosnia and Herzegovina, a four-year war caused the country's health system to collapse. The immediate post-war years resulted in isolation from the surrounding countries and the flow of information and exchange of medical knowledge was very poor. All these events – the war and the post-war period, devastation of medical structures and equipment and the transition of the country from one political structure to another – were a great burden on the health system.[10] Especially for new and young medical disciplines, it was practically impossible to establish effective patient care. Generally speaking, all three crucial links of medicine (clinical practice, education and research) had stagnated and regressed during the war and post-war period. The devastated health and education system did not recognise critical care as a discipline; critical care topics were not taught during undergraduate and postgraduate training and there was no significant research work in this field.[4, 5, 8, 11]

In order to aid its post-war recovery, the European Commission and other European institutions like the *Coimbra* group and the *Union Internationale Contre Le Cancer* (UICC) have created various projects to help the mobility of young researchers and doctors from Bosnia and Herzegovina.[5] The main objective was to assist the health and education system and to facilitate the exchange of knowledge and experience between clinicians and researchers in the region. The first physician to receive such a grant for the purpose of education in critical care was Dr Peđa Kovačević of the University Clinical Centre of the Republic of Srpska (UCCRS). During his training periods at the University Hospital Heidelberg between 2005 and 2007, Dr Kovačević was mentored by Prof. Dr F Joachim Meyer, a board-qualified specialist in the field of internal medicine, cardiology, respiratory medicine and critical care. He was head of the MICU at Heidelberg University Hospital. Dr Kovačević added training in invasive bronchoscopy techniques at Heidelberg's renowned *Thoraxklinik* under the supervision of Prof. Dr Heinrich Becker, then chief of the endoscopy unit. In 2006, under the guidance and referral of Prof. Meyer, Dr Kovačević applied successfully for the prestigious Travel Award for Young Fellows in Developing Countries by the American Thoracic Society (ATS). Dr Kovačević attended the ATS meeting in San Diego, where he met Bosnian-born critical care specialist Prof. Ognjen Gajić, MD from the Mayo Clinic in Rochester, USA.

Prof. Meyer and Prof. Gajić organised the first donation of non-invasive ventilators to the Pulmonary Department of the UCCRS. After his return from Heidelberg in 2007, Dr Kovačević was able to develop a modern medical critical care service there. In the same year, Dr Kovačević visited the Critical Care Department at Mayo Clinic under the supervision of Prof. Gajić, mainly for intensified training in mechanical ventilation, both invasive and non-invasive. One year later, the management of UCCRS and the Ministry of Health of the Republic of Srpska, an entity of Bosnia and Herzegovina, approved the establishment of the first modern MICU in Bosnia and Herzegovina.[5, 8] Dr Kovačević was assigned a responsible leadership role in this process.

During the following ten years, Prof. Meyer (Figure 2) and Prof. Gajić (Figure 3) provided con-

tinuous support for the development of medical intensive care, which proved to be a difficult process with multiple obstacles. Prof. Meyer visited UCCRS and worked as a visiting physician and mentor on several occasions in 2007, 2014 and 2016. He gave a series of lectures on intensive care medicine, carried out the first invasive monitoring by pulmonary catheter, the first percutaneous tracheostomy and provided education at the bedside. These activities were also supported by the Erasmus Mundus Plus project of the European Commission.

Figure 2: Prof. Dr Joachim Meyer, FCCP, University of Heidelberg

Figure 3: Prof. Ognjen Gajić, MD, MSc, FCCP, FCCM, Mayo Clinic, Rochester, MN, USA

In order to enhance the development of critical care in Bosnia and Herzegovina, the first draft of the curriculum for critical care specialisation training was proposed in 2010 by Dr Kovačević, Prof. Dr Guillaume Thiéry (University Hospital of Saint-Etienne, France), Prof. Ognjen Gajić MD, MSc (Mayo Clinic, USA) and Prof. F Joachim Meyer. In the spring of 2014, this proposal was accepted with Dr Kovačević becoming the country's first critical care training fellow. Prof. Meyer became the program's first international mentor in Bosnia and Herzegovina. In September 2016, Dr Kovačević successfully passed the board exam in front of an international jury and became the first critical care specialist in Bosnia and Herzegovina and among the first in ex-Yugoslavia region. Two years later, he was assigned as the first program coordinator for the critical care fellowship at the University of Banja Luka Medical School.

Figure 4: Very first modern Medical Intensive Care Unit at University Clinical Centre of the Republic of Srpska, December 2008

Figure 5: Modern MICU at the University Clinical Centre of the Republic of Srpska, December 2022, today this is the only ICU lever III with possibilities for veno-venous extracorporeal membrane oxygenation (vvECMO)

Together with Prof. Meyer and Prof. Gajić, Dr Kovačević started the first research projects in the field of respiratory and critical care at UCCRS. In June 2019, Dr Kovačević published a paper titled "Impact of weekly case-based tele-education on quality of care in a limited resource medical

CONTENT

intensive care unit" in the prestigious journal Critical Care.[12] With this, the development of all three crucial links in critical care medicine has begun: the treatment of the critically ill, education and research (Figure 4 and 5).

Conclusion

"It takes a village" – many highly motivated enthusiasts were necessary to create and realise the care of the sickest medical patients in post-war Republic of Srpska (and Bosnia and Herzegovina as well). Today critical care medicine is an enormous public health need of all communities, in HIC and LRS alike. Today, the MICU in Banja Luka is certified by ISO standard (9001:2015 and EN:15224) and serves as a tertiary referral centre for medical intensive care for the Republic of Srpska and provides health services for a population of 1,000,000 inhabitants. The contribution of High Income Countries (Heidelberg University, Mayo Clinic and University Clinical Centre of Ljubljana) for this project is indisputable and enormous. The great challenge of the COVID-19 pandemic should serve as a wake-up call for rapid development of critical care programs around the world. One of benefits that should come out of this pandemic is a more rapid implementation of critical care medicine programs into LRS. It is important to emphasise that trained staff is far more important than "ICU bed" or equipment. HIC in Europe are moving toward critical care medicine harmonisation with the main goal of standardising critical care practice. LMIC have to follow this pathway without delay to include modern multidisciplinary, evidence based critical care medicine into their healthcare systems.

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Conflict of interest

None.

References

1. Lassen HCA. A preliminary report on the 1952 epidemic of poliomyelitis in Copenhagen with special reference to the treatment of acute respiratory insufficiency. Lancet 1953;1:37–41.
2. Reisner-Sénélar L. The birth of intensive care medicine: Bjorn Ibsen's records. Intensive Care Med 2011;37:1084–6.
3. Tang W, Sun S. Max Harry (Hal) Weil – a leader, mentor, friend, and wonderful colleague. Resuscitation 2011;82:1481–2.
4. Vukoja M, Riviello ED, Schultz MJ. Critical care outcomes in resource-limited settings. Curr Opin Crit Care 2018;24(5):421–7.
5. Kovacevic P, Meyer FJ, Gajic O. Successful implementation of modern critical care in the low-resources country Bosnia and Herzegovina: Single-center experience. Med Klin Intensivmed Notfmed 2022;117:269-75.
6. GDP per capita (current US$) [Internet]. World Bank national accounts data, and OECD National Accounts data files. [Cited: 1-Apr-2023] Available at: https://data.worldbank.org/indicator/NY.GDP.PCAP.CD?locations=-BA.
7. Losonczy LI, Papali A, Kivlehan S, Calvello Hynes EJ, Calderon G, et al. White paper on early critical care services in low resource settings. Ann Glob Health 2021 Nov 3;87:105. doi: 10.5334/aogh.3377.
8. Thiery G, Kovacević P, Straus S, Vidovic J, Iglica A, Festic E, et al. From mechanical ventilation to intensive care medicine: a challenge for Bosnia and Herzegovina. Bosn J Basic Med Sci 2009;9(Suppl 1):69–76.
9. Rosenthal VD, Yin R, Lu Y, Rodrigues C, Myatra SN, Kharbanda M, et al. The impact of healthcare-associated infections on mortality in ICU: A prospective study in Asia, Africa, Eastern Europe, Latin America, and the Middle East. Am J Infect Control 2022 Sep 6:S0196-6553(22)00658-7. doi: 10.1016/j.ajic.2022.08.024.
10. Tokalić R, Viđak M, Kaknjo MM, Marušić A. Antifragility of healthcare systems in Croatia and Bosnia and Herzegovina: Learning from man-made and natural crises. Lancet Reg Health Eur 2021;7;9:100216. doi: 10.1016/j.lanepe.2021.100216.
11. Kovacevic P, Djajic V, Momcicevic D, Zlojutro B, Jandric M, Kovacevic T, et al. Boosting ICU capacity during the COVID-19 pandemic in the western Balkan region, the Republic of Srpska experience. J Public Health Res 2023;25;12: 22799036231151762. doi: 10.1177/22799036231151762.
12. Kovacevic P, Dragic S, Kovacevic T, Momcicevic D, Festic E, Kashyap R, et al. Impact of weekly case-based tele-education on quality of care in a limited resource medical intensive care unit. Crit Care. 2019 Jun 14;23(1):220. doi: 10.1186/s13054-019-2494-6.

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PROFESSIONAL ARTICLE

Risky Behaviour Among Adolescents

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Abstract

Background/Aim: Nowadays, adolescents are exposed to some negative challenges. Bullying is one of the horrible phenomena that can be seen in the physical and mental harassment of children and teenagers. Another challenge is addiction to computer games as well as writing on social networks. Gambling addiction, habituation and exposure to psychostimulants are also a trap for causing severe pathology. The aim of this study was to present an insight into the possible challenges of today to which adolescents are exposed and how they affect their personality and maturation.
Methods: The authors collected data and materials on the mentioned topics from their daily medical work. They also used articles in professional magazines and newspapers and television shows.
Results: Society must make efforts to eradicate such events in schools. We are witnessing various challenges and pitfalls that affect youth, society, culture and civilisation. Among the bad things, drug addiction, alcoholism, smoking, addiction to the Internet and computer games are in the first place.
Conclusion: Due to negative challenges, the youth neglect their schoolwork and duties. The school environment can be a place for violent behaviour, brachial attacks (bullying and harassment). Recognising social interactions, family relationships and understanding the underlying psychiatric conditions which may be associated with risky behaviour in adolescents could be essential for treatment planning.

Key words: Adolescent; Risky behaviour; Social networks; Today's society.

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Introduction

Adolescence is the transitional period from childhood to adulthood, in which the physical, intellectual and social maturation of the personality occurs. Since the growth, development and maturation of various functions occur at different levels, maturity and assuming the role of adults depend on many social factors. The basic problem in that period is the search for one's own identity, which is accompanied by a pronounced desire for independence from adults and confrontation with peers.[1] Prolonged schooling, economic and social dependence of youth in our society, makes the period of adolescence long and often dramatic and young people are faced with many challenges that are dangerous for young people.[2] Considering that, society should pay more attention to youth than it does. Society consists of every group of people connected with their specific human action. One of the challenges for the youth is how to gain social respectability. It is an assessment of the value of a young person's social role and it is based, first of all, on the profession that the individual performs, but also on his other social and personal characteristics. An individual's reputation also depends on how he performs certain activities, not only which

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CONTENT

activities he performs, how he relates to the prevailing social values, whether he is traditionally or innovatively oriented. When determining the reputation of a young man, the reputation enjoyed by his family as a whole is also taken into account. Incomplete families, those from the margins of society, will mostly become problematic individuals.[3] They opt for local groups that indulge in crime in their neighbourhood, city or street. These are criminals, bullies and violators of the rules of conduct. Belonging to such anti-social groups is also one of the challenges for certain young people. By doing so, they gain a negative reputation, ie "reputation", which is valued by the group of perpetrators of criminal acts.[4]

Since the beginning of time, people have placed bets on the unpredictable outcomes of events. Gambling was well known to the Babylonians, Etruscans, Romans, Greeks and Chinese. Examples of early gambling appear in ancient art and literature. Gambling becomes visible in the works of Homer, Chaucer and Shakespeare.[5] Among the challenges faced by the youth in our country is betting, ie pathological gambling. Always eager for material means - money and at the same time naive and inexperienced, they are easily persuaded to try their luck by drawing lots. Modern society, unlike the socialist one, offers many offers in this sense.[6] There have long been bars with machines for gambling games, lotteries, bingos, which are all very attractive for young people. Since they do not yet have a built-in instance of personality (superego), they easily lose control and indulge in excessive spending of money.[7] They often come to them through violent appropriation, robbery, kidnapping and blackmail from peers or theft from parents (money, jewellery, gold or expensive items) which they then resell to get cash.[8]

There are several different types of Internet addiction and they can be distinguished based on the activity that the user is most engaged in while using the Internet. There can be distinguished addiction to different social networks, addiction to information, addiction to playing video games, addiction to online betting.[9] When they are seized by the gambling passion for never before experienced winnings, they do not choose the means. Authors have had cases of parents who suspected their son, so they secretly searched his computer, smartphone and even followed him to casinos to see what he was spending so much money on. The same was the case with young patients addicted to computer games, chatting on the Internet, who could not tear themselves away from it, but spent their nights in front of the screen. The next day, they were so sleepy and tired that they were unable to follow the lessons, deconcentrated for any kind of learning and participation in the educational process in general. Those with a weaker mental constitution have succumbed to the challenges that modern society generously offers.[10]

Pathological gambling and betting is based on "feeling" and the hope that Fortune and Kairos will strike them and generously provide them with material gratification and satisfaction. According to one theory, gamblers actually like to lose, because if they happen to win something, they immediately invest it in the next attempt to challenge the gods of luck.[11] If the aetiology is searched, exploring the early phase of growing up of these patients, one often encounters a lack of love at the earliest age.[12] From the experience of not being loved, they fantasise that they are essentially bad and then, frustrated, they charge with an imagined possibility of winning. And so indefinitely, they prove to themselves unconsciously that they are still good, if luck ever rewards them. Or they come to terms with the fact that they are bad and build their psychopathic personality, later indulging more and more in criminal activities. They defensively project their bad sides onto their parents and/or society. They take revenge on them for not being loved, they pay for the necessary love denied to them in early childhood.[13]

Bullying is a phenomenon of physical confrontation between peers or young people who are weaker than themselves. Harassment usually precedes brachial triggering of aggression in the form of teasing, verbal abuse, insults or humiliation. By doing so, they strengthen their conceit about their own power. In the absence of identity, they stick to the group and together they bully the weaker ones. This is Fromm's well known escape from freedom, where they identify with the group and feel stronger.[14] Bullying myths are common disputes and fights between schoolmates. It happens that two girls fight for the love and attention of the same guy in front of a group that knows what it's about and enjoys fighting and rooting for one side. There are many cases of severe physical injuries, manifestation of sadistic perverse intentions, beatings, rape or murder.[15]

Alcoholism, drugs, tobacco smoking are also challenges that young generations face today.[16] Surveys and research show that a large percentage of young people consume these unhealthy and addictive substances. Drinking beer and smoking is almost a common habit in school age. Deadly alcohol intoxications at parties as well as murders and perversions under its influence are not rare, as reported by black chronicles. Death from overdose occurs not only in drug addicts, but also in exceptional unaccustomed users of drugs, which are available everywhere.[17]

The aim of the research was to gain an insight into the possible challenges of today, to which children and adolescents are exposed. Another goal was to determine the connections with other habits and disorders in the juvenile population and how they affect their personality and maturation.

Methods

The authors collected information and material on the described topics through their daily medical practice, articles in magazines, texts in newspapers, TV and other electronic forms of information.

Discussion

Pressured by exogenous and endogenous problems of depression or some other illness, some adolescents may resort to self-destructive behaviour. From injury by "carving" the skin, the greater part of the left forearm to attempted or committed suicide.[18] It would also be some psychopathological internal challenge stimulated by numerous possible external stimuli.[19] For example, unemployment after graduating from high school or college fills young people with pessimism, resignation to the life ahead, melancholy, depression and even depression. The maladapted, eager for life, but burdened by the impossibility of fulfilment, reach for the rather Thanatos opposite "exits" of their own hand. Incidental deaths in traffic accidents or due to drug or alcohol overdose and intoxication are also challenges faced by psychopathologically predisposed young people.[20] Newspaper reports are full of information about accidents with serious injuries and even deaths of young people. Returning from rural and urban parties, they lost control of the steering wheel and in uncontrolled conditions killing not only themselves, the passenger(s), but also innocent bystanders.[21, 22] One of the challenges for young people is the so-called going abroad. "Brain drain" is a social and state problem, because the funds invested in the long-term education of young people will never be returned by work.[23] The pauperisation of the middle and lower classes took off. The middle class has effectively disappeared. All this affects young people as one of the external causes of depression and consecutive suicidal behaviour.[24] Disappointment in the life that they are just on the threshold of, de-idealisation and disillusionment push young people to take unwanted steps.[25] Disappointed in the social system, they become apolitical.[26] In a state of plutocracy, an oligarchy of the incompetent, which decapitates young people in an attempt to survive from today to tomorrow, they often indulge in juvenile crime and delinquency.[27] Juvenile crime is on the rise, participation in peer violence as abusers, young people expose themselves to victimisation. Peer violence and physical confrontations in early and late adolescence are often associated with psychopathological behaviours.[28] The connection of these behaviours as correlates with the intolerance of frustrating situations, irritability and internalising disorders such as depression is completely clear. (Un)intentional injury sometimes requires medical attention. Peer abuse of the victim along with alcohol and drug abuse was assessed through three questions: "I often drink alcohol, what do I drink?" "I drink excessively, why do I drink?" "I used drugs, why do I take drugs" - family attitudes, experience of family relations, cohesion and control in the family.[29]

One of the few challenges of unstable personalities of young people is joining various occult sects. Dumbed down by prejudices, mentally unbalanced, they fall under the influence of a group of like-minded people. The hierarchy of the sect refers to a wise leader and blind subjects.[30] They are manipulatively managed to eg all their earnings, property they have, legally transferred to the sect. Sects seek to declare themselves religions and be registered as civil associations. Indoctrination in the sect goes so far that the followers agree to sacrifices, injuries to the skin, giving blood in ritual rites and even

persuasion to commit suicide.[31] Followers of sects participate in obscure esoteric cults and rites, isolate themselves from normal society and life, simply disappear from the family, without a trace. There are statistically verified data about this phenomenon. Since these are secret societies, investigations are difficult to access and appropriate measures to protect misguided youth are difficult to take. Only the church is bothered the most by sects because they steal their believers.[32] Depending on their upbringing, from earlier experiences of abuse they experienced in childhood, some children are very aggressive, easily lose control and take advantage of weaker classmates. What others have done to them before, they are now doing to their younger selves.[33] Learned behaviour, victimisations, are passed down from generation to generation. Of course, there is no justification for this and such phenomena should be comprehensively investigated and appropriate punitive measures should be taken.[34] The phenomenon of bullying has taken on large proportions. It must not become a common occurrence. Violence is taught from an early age. Hatred for everyone who is different, as well as one's own frustrations and stresses, are projected through violence and transferred to school premises and in front of the school, to playgrounds and other meeting places for young people.[35] Corridors, alleys and hidden corners of yards, where there is no supervision of the elderly, are especially dangerous. It is dangerous to be alone in a tram at night, because criminals are returning from bars and parties and are especially aggressive and willing to deal with unprotected young people. Beatings also occur with a fatal outcome.[36] Recidivism is common in such cases, because the abusers even receive a gratuity if they remain undetected and unpunished. Then they don't learn from the experience of the punishment served, but from the abuse on their own skin.[37] Bullying also uses surrounding objects - chairs, levers, pipes, knives, baseball bats, bricks. Shoe-shoeing is common when the victim falls to the floor, so bloody traces remain. The police sometimes do not act promptly and appropriately towards violent people, as many experiences show. It happened that criminals used sadistic torture on their victims.[38] Terrible events also happened at student parties and drunken parties. Aggression as a drive component, from the id, is abused in bullying. Aggressiveness itself is necessary for survival in a cruel world. However, aggressiveness in bullying serves to release the accumulated dissatisfaction and unconscious revenge of those who mistreated the bully in childhood.[18]

Adolescents also do not consciously control hidden and unconcealed aggressive outbursts on the streets, sports fields and in their own families, counting on avoiding punishment. They are aware of the analyses of psychologists and sociologists and use the "rights of their age" to reward their aggressive impulses.[33] Adolescence also carries impulses to acquire the ability to use a form of progressive gratification. They belong to maturity, accepting obligations and the joy of overcoming life's difficulties. Understanding the period of adolescence as a boundary between the tendencies of regressive and progressive gratification, the therapist supports progressive elements and enables the future mature person to easily reject the tendency of regressive gratification.[39]

Conclusion

Adolescence is a time of transition from a child to an adult, a time of great parting. Separation from previous authorities, emotional security of childhood and economic dependence on parents is a painful and frustrating experience. Anxiety, which accompanies this frustration, leads, along with the biological and sociological impulse towards separation, to defences against that separation. The most common defence in that period of life is regression. The adolescent becomes ambivalent. Both tendencies: both separation and regression frustrate, but also gratify. The adolescents are aware of their position in the family and in society. They know that a lot of things have been forgiven them in advance because of the "crazy years". That is why they allows themself such forms of behaviour, which they are aware are inappropriate for their age. They could easily control them, but the adolescents manage, due to their specific position, to mostly avoid the unpleasant consequences of such behaviour that gives them gratification. They are not motivated to look for other more constructive and progressive forms of behaviour.[37] Aggression in youth can be expressed in visible and invisible ways: through feelings, phantasms, desires, thoughts and movements. Pathological aggression exhausts the psychic apparatus that defends it-

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self and exhausts it with increased discharge through the body and repeated defences such as repression, projection, projective identification, splitting, denial, rationalisation, reactive formation and conversion and compulsive symptoms.

Recognising social context, family relationships and understanding the underlying psychiatric conditions which may be associated with risky behaviour in adolescents are essential for treatment planning.

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References

1. Selestrin Z. [The relationship between the prevention of mental, emotional and behavioural problems and the promotion of mental health]. Kriminologija i socijalna integracija 2021;29:248-67. Croatian.
2. Kušević Z, Melša M. [Aggression in children and adolescents]. Soc psih 2017;45:105-16. Croatian.
3. HejrGasht H, MousaviPanah SM, Rezaei S, Amini I. Social factors young people turn to the crime of theft: A case study of criminals accused of robbery confined to prisons in Tehran and Alborz provinces. Crime Prev Appr 2021;4(3):91-118.
4. Zalewski M, Stepp SD, Whalen DJ, Scott LN. A qualitative assessment of the parenting challenges and treatment needs of mothers with borderline personality disorder. J Psychother Integr 2015;25(2):71–89.
5. Pejnović Franelić I. [Affinity of first year university students towards gambling and betting] [dissertation]. Zagreb: Medicinski fakultet Sveučilišta u Zagrebu; 2013. Croatian.
6. Bilić V, Opić S. [Adolescent gambling: the role of sex, some family and school factors]. Šk vjesn 2013;62(4):455-78. Croatian.
7. Torre R, Zoričić Z, Škifić B. [The phenomenon and the legislative regulations on gambling]. Med Jadert 2010;40(1-2):27-31. Croatian.
8. Miliša Z, Tolić M. [The crisis in upbringing and expansion of contemporary addictions]. Medianali 2010;4:135-64. Croatian.
9. Mavar M, Vučenović D. [Tendency to addictive behavior and school problems in adolescents]. Klin psih 2014;7:5-21. Croatian.
10. Kryszajtys DT, Hahmann TE, Schuler A, Hamilton-Wright S, Ziegler CP, Matheson FI. Problem gambling and delinquent behaviours among adolescents: A scoping review. J Gambl Stud 2018;34(3):893–914.
11. Hopwood CJ, Kotov R, Krueger RF, Watson D, Widiger TA, Althoff RR, et al. The time has come for dimensional personality disorder diagnosis. Pers Ment Health 2018;12(1): 82–6.
12. Duval J, Ensink K, Normandin L, Fonagy P. Mentalizing mediates the association between childhood maltreatment and adolescent borderline and narcissistic personality traits. Adolesc Psychatry 2018;8(3):156–73.
13. Floros, G. Gambling disorder in adolescents: Prevalence, new developments, and treatment challenges. Adolesc Health Med Ther 2018;9:43–51.
14. Yalch M, Levendosky A. Influence of betrayal trauma on pathological narcissism. J Aggress 2020;29(9):1035–46.
15. Zhang GB, Wang GF, Han AZ, Xu N, Xie GD, Chen LR, et al. Association between different stages of precollege school bullying and murderrelated psychological behaviors among college students in Anhui Province China. Psychiatry Res 2019;282:112593. doi: 10.1016/j.psychres.2019.112593.
16. Bagley SM, Hadland SE, Schoenberger SF, Gai MJ, Topp D, Hallett E, et al. Integrating substance use care into primary care for adolescents and young adults: Lessons learned. J Subst Abuse Treat 2021;129:108376. doi: 10.1016/j.jsat.2021.108376.
17. Patrick ME, Rhew IC, Lewis MA, Abdallah DA, Larimer ME, Schulenberg JE, et al. Alcohol motivations and behaviors during months young adults experience social role transitions: Microtransitions in early adulthood. Psychol Addict Behav 2018:32(8):895–903.
18. Vergara A, Stewart G, Cosby A, Lincoln H, Auerbach RP. Nonsuicidal self-injury and suicide in depressed adolescents: Impact of peer victimization and bullying. J Affect Disord 2019;245:744-9.
19. Kernberg OF. Some implications of new developments in neurobiology for psychoanalytic object relations theory. Neuropsychoanalysis 2022;24(1):3-12.
20. Jellinek EM. Phases of alcohol addiction. Quarterly JSAD 1952;13:673-84.
21. Kyroglou G, Henn M. Political consumerism as a neoliberal response to youth political disengagement. Societies 2017;7(4):34. doi: 10.3390/soc7040034.
22. Khan J. European academic brain drain: A meta-synthesis. Eur J Educ 2021;56(2):265-78.
23. Beckman K, Lindh AU, Waern M, Stromsten L, Renberg ES, Runeson B, et al. Impulsive suicide attempts among young people–A prospective multicentre cohort study in Sweden. J Affect Disord 2021;243:421-6.
24. Shapero BG, Farabaugh A, Terechina O, DeCross S, Cheung JC, Fava M, et al. Understanding the effects of emotional reactivity on depression and suicidal thoughts and behaviors: Moderating effects of childhood adversity and resilience. J Affect Disord 2019;245:419-27.
25. Delanty G. The future of capitalism: Trends, scenarios and prospects for the future. J Class Sociol 2019:19(1);10-26.
26. Cavanagh C. Healthy adolescent development and the juvenile justice system: Challenges and solutions. Child Dev Perspect 2022;16(3);141-7.
27. Thota S, Baireddy R, Changalasetty B, Pemula R. Juvenile crime categorization with EM clustering. In intelligent systems and sustainable computing. Singapore: Springer; 2022. p. 39-48.

CONTENT

28. Lugonja L, Keleman A, Sarić M. [Review of research on the connection between socio-emotional competences and peer violence among students]. Synesis: Int J Humanit Soc 2021:2(2):19-33. Serbian.
29. Abramovitch, H. When is it time to stop? When good enough becomes bad enough. J Anal Psychol 2021;66(4):907-25.
30. Van Den Heuvel W, Stikkelbroek A, Bodden H, Van Baar L. Coping with stressful life events: Cognitive emotion regulation profiles and depressive symptoms in adolescents. Dev Psycho-pathol 2020;32(3):985-95.
31. Hardy SA, King PE. Processes of religious and spiritual influence in adolescence: Introduction to a special section. J Adolesc Res 2020;29(2):244-53.
32. Fang N. Depression reconsidered in Fairbairn's object relations theory. Psychodyn Pract 2020;26(1):20-33.
33. Lin S, Yu C, Chen J, Zhan W, Cao L, Liu L. Predicting adolescent aggressive behavior from community violence exposure, deviant peer affiliation and school engagement: A one-year longitudinal study. Child Youth Serv Rev 2020;111:104840. doi: 10.1016/j.childyouth.2020.104840.
34. van den Bedem P, Dockrell JE, van Alphen M, Kalicharan SV, Rieffe C. Victimization, bullying, and emotional competence: Longitudinal associations in (pre) adolescents with and without developmental language disorder. J Speech Lang Hear Res 2018 Aug 8;61(8):2028-44.
35. Moore CC, Hubbard JA, Bookhout MK, Mlawer F. Relations between reactive and proactive aggression and daily emotions in adolescents. J Abnorm Child Psychol 2019;47:1495-1507.
36. Jackson KM, Rogers L, Sartor E. Parental divorce and initiation of alcohol use in early adolescence. Psychol Addict Behav 2016;30(4):450–61.
37. Olver ME. Treatment of psychopathic offenders: A review of research, past, and current practice. Psych Crim Behav 2022;469-81.
38. Romer D, Duckworth AL, Sznitman S. Can adolescents learn self-control? Delay of gratification in the development of control over risk taking. Prev Sci 2010;11:319–30.
39. Greš A, Esapović Greš N. [The relation of secondary profit to progressive gratification]. Soc psihijatr 2015;43(3):151-5.

CONTENT

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CASE REPORT

Role of the Double Muscle Gastrocnemius-Soleus Flap in Soft Tissue Defect Reconstruction of the Leg in Children: Case Series

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Abstract

The reconstructive surgeon faces a problem when dealing with larger soft tissue lesions over the knee and the proximal two thirds of the tibia, two crucial sites of the lower limb. Large free flaps or pedicled local flaps are two solutions of the problem. The soleus or gastrocnemius flaps by itself are insufficient. Without using free flaps, combined gastrocnemius-soleus flaps can be employed effectively. Indicators for this kind of flap are still quite uncommon in children. In two clinical cases, authors will share their expertise and talk about the use of this kind of flap in the treatment of soft tissue abnormalities in children. Two cases were outlined: the use and outcome of the combination pedicled gastrocnemius-hemisoleus double muscle flap to repair significant defects around the knee and leg in children.

Key words: Gustilo classification; Gastrocnemius-hemisoleus; Vacuum assisted closure; Necrosectomy.

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Introduction

Local pedicled muscle flaps are frequently used to resurface soft tissue lesions that expose crucial structures at the knee and the upper two-thirds of the leg. Typically, the soleus muscle flap is utilised for the middle third of the leg and the gastrocnemius muscle flap is used for the knee and upper third of the leg.[1-3] For bigger critical wounds, a single pedicled muscle flap is insufficient. This is typically true for continuous wound abnormalities that span the middle portion of the leg and the knee. A broad free flap is one option for covering, but it has inherent hazards. The use of a combined pedicled gastrocnemius and hemisoleus flap, initially published in 2004 by Hyodo et al is a local flap treatment for this issue.[4] Based on the inter-muscular perforators between them, this flap uses either the medial or lateral head of the gastrocnemius and a hemisoleus to create a continuous double muscle flap. In a further development of this method, Pu et al[5] covered mid-tibial wounds by combining the gastrocnemius and soleus. The intra-muscle perforators were retained in the aforementioned two methods, which limited the flap's range of motion. The combination pedicled gastrocnemius and hemisoleus double muscle flap was used in presented case series to address significant leg deformities that would typically be covered with free flaps. Paediatric plastic surgery procedures are actually as conservative as they can be given the unique characteristics of children, including their greater capacity for wound healing and skin laxity. Authors' experiences are presented through two clinical instances.

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Case History 1

Consent was obtained or waived by all participants in this study. Karnataka Institute of Medical Sciences Ethics Committee issued approval No 22/2022-23. The study was approved by the institutional Ethics Committee.

As a result of a crush injury to the upper and middle third of her right leg and a compound open fracture (Gustillo class type 3B) of the tibia and proximal dislocation of the fibula, an 8-year-old girl who had been in a severe car accident presented with a soft tissue defect. Reduction and stability of fractures was achieved through external fixation. No vascular lesions were seen. The wounded skin soon developed necrosis. The anterior side of the tibia had soft tissue necrosectomy, leaving a 20-cm-diameter skin defect with the fracture site clearly visible. The vacuum assisted closure (VAC) treatment was used. It was recommended to utilise a medial gastrocnemius-soleus flap in the absence of adequate bone protection. This method was the same as that employed with adults (Figures 1A-1D). The cosmetic results following surgery were great. Notwithstanding, the child required a bone transplant and osteosynthesis since bone consolidation could not be accomplished.

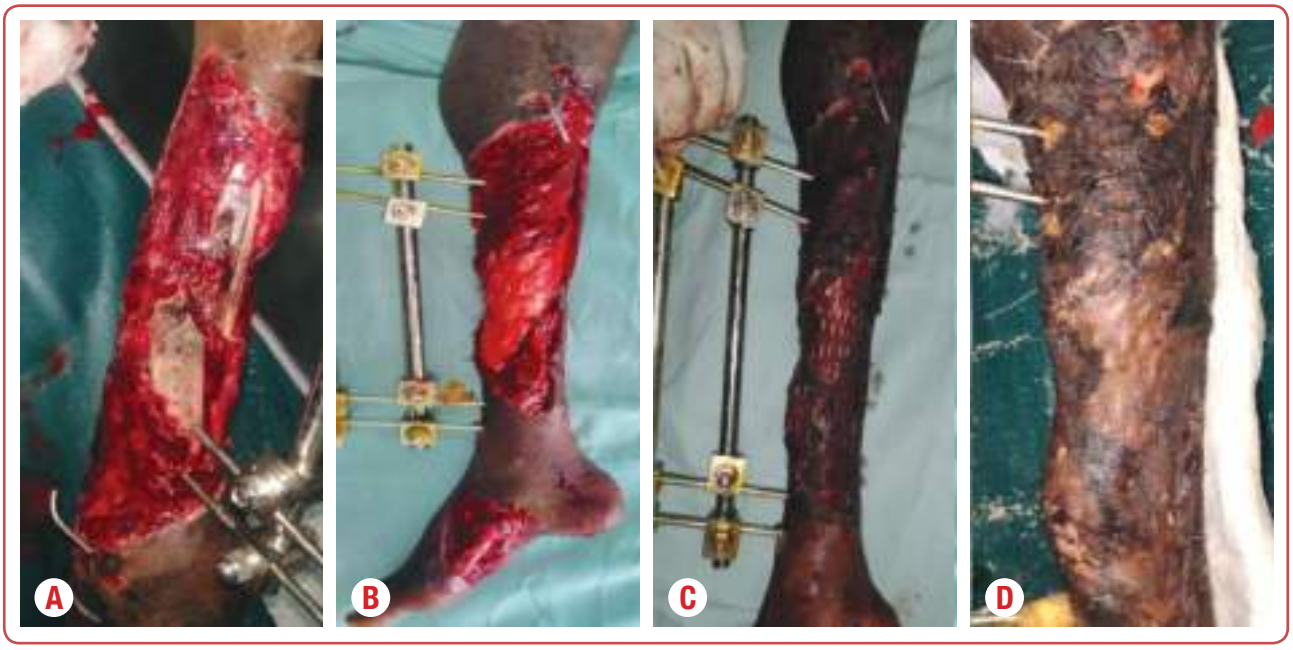

Figure 1: *The vacuum assisted closure (VAC) treatment with gastrocnemius-soleus flap in the absence of adequate bone: A) At the operation; B) One week later, C) Two weeks later; D) Three weeks post-operation.*

Case History 2

A 12-year-old child who was injured in a serious car accident was diagnosed with a soft tissue abnormality as a result of a crush damage to the middle third of his tibial diaphysis. On the anterior side of the proximal section of the leg, a soft tissue skin defect (11 cm in diameter) persisted after the implantation of osteosynthesis material. The adoption of the medial gastrocnemius soleus muscle flap was recommended since rapid wound healing was required. The patient's cosmetic results were good a year after the procedure. The patient was pleased with the cosmetic results at both the donor and recipient locations (Figures 2 and 3).

Figure 2: *A crush damage to the middle third of tibial diaphysis in a 12-year-old patient*

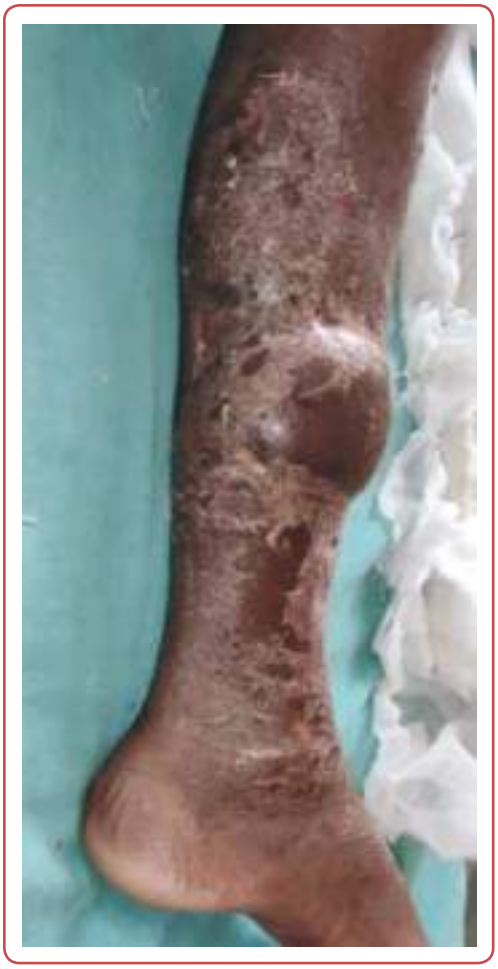

Figure 3: *One year after the adoption of the medial gastrocnemius soleus muscle flap due to a crush damage to the middle third of tibial diaphysis in a 12-year-old patient*

Surgical technique

Under the observation of a tourniquet and with the patient lying supine, the flap dissection was carried out. The soleus and gastrocnemius muscles' joint tendon was found at the length that was required for distal extension of the incision, which was then prolonged distally towards the tendon Achilles in the posterior midline. First, the area's potential harvestable muscles were located. The soleus and gastrocnemius muscles were distinguished and elevated as distinct muscle flaps (Figure 1B). Before the hemisoleus, the more superficial gastrocnemius head was elevated. When the medial gastrocnemius was harvested, the lateral gastrocnemius muscle was separated from it along the midline raphe and the medial gastrocnemius was dissected free in a distal to proximal way while retaining the pedicle arising from the sural artery. The gastrocnemius and soleus intermuscular perforators were sacrificed. It was possible to track and split the muscles' distal tendinous insertion at the tendon Achilles. The medial hemisoleus was then freed by performing a similar distal to proximal dissection beginning at the tendon Achilles insertion. The flap, which was raised based on the proximal pedicle from the posterior tibial artery, was made from the muscular component of the hemisoleus. Depending on the size of the mid-tibial wounds, the medial hemisoleus was divided longitudinally from the medial hemisoleus for wound covering. This extension was utilised on large wounds that were beyond the reach of one gastrocnemius

CONTENT

and soleus muscle. After elevating both muscles, the proximal section of the tibial incision was resurfaced with a medial gastrocnemius flap, and the distal portion was covered with a hemisoleus muscle (Figure 1C). In presented research, authors discovered that raising the gastrocnemius and hemisoleus independently might increase the reach of the double muscle flap. Unfortunately, this treatment could only be carried out in uninjured the gastrocnemius and soleus muscles.

Discussion

The combined gastrocnemius or soleus muscle flap offers various benefits, including the removal of the requirement for microsurgery, which makes the procedure easier and quicker while still delivering the same level of soft-tissue coverage and aesthetic outcome for a large tibial lesion. Also, it is more economical. Van Halen,[6] employed the gastrocnemius and soleus elevated individually with their origins and blood supply intact, allowing each to be mobilised separately, in the resurfacing of massive tibial deformities following removal of tibial sarcomas. Compared to the techniques previously reported by the other authors, these improved the flexibility and range of motion of each muscle and permitted the resurfacing of a greater region. The knee and upper part of the leg had been resurfaced by Hyodo and Pu using their bi-muscle flaps.[4, 5] When necessary, the lateral hemigastrocnemius and hemisoleus are included, expanding its usefulness to resurface abnormalities up to 30 cm^2. This includes the knee, upper and middle thirds of the leg. Wound care in children is more challenging due to rapid wound healing and probable skin retractions. The use of one-step wound closure procedures is facilitated by skin laxity. Despite everything said above, medial gastrocnemius muscle flaps can be used to repair soft tissue defects in children's legs when there is an open fracture or bone exposure. In order to reduce the dangers of infections and non-unions in the case of fractures, this option should really be used as soon as possible. The use of this flap for repair following tumour removal has been documented in adults.[7]

Conclusion

This method can promote speed and proper defect repair in surgical regions that need to recover quickly. Gustilo class type 3B open fractures were the main indications for the flap. In two cases, the medial gastrocnemius and medial hemisoleus combined flaps were utilised to cover the incision. A split-thickness skin graft was used to cover the muscle flaps principally. The average amount of time between surgery and final flap surgery for the two patients with open tibia fractures was seven days. The patients had no major donor morbidity. After being discharged from the hospital, no patient needed additional surgery for the flap. This flap is important for paediatric plastic surgery and should be detected and employed right once in certain situations, such as traumatic soft tissue defects (open fractures).

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Conflict of interest

None.

References

1. Suda AJ, Cieslik A, Grützner PA, Münzberg M, Heppert V. Flaps for closure of soft tissue defects in infected revision knee arthroplasty. Int Orthop 2014 Jul;38:1387-92.
2. Ong SW, Gan LP, Chia DS. The double muscle gastrocnemius-soleus flap in resurfacing large lower limb defects: Modifications and outcomes. J Orthop 2020 Jan 1;17:13-6.
3. Ahmad I, Akhtar S, Rashidi E, Khurram MF. Hemisoleus muscle flap in the reconstruction of exposed bones in the lower limb. J Wound Care 2013 Nov;22(11):635-42.
4. Hyodo I, Nakayama B, Takahashi M, Toriyama K, Kamei Y, Torii S. The gastrocnemius with soleus bi-muscle flap. Br J Plast Surg 2004 Jan 1;57(1):77-82.

5. Pu LL. Soft-tissue coverage of an extensive mid-tibial wound with the combined medial gastrocnemius and medial hemisoleus muscle flaps: the role of local muscle flaps revisited. J Plast Reconstr Aesthet Surg 2010 Aug;63(8):e605-10.
6. Thornton BP, Rosenblum WJ, Pu LL. Reconstruction of limited soft-tissue defect with open tibial fracture in the distal third of the leg: a cost and outcome study. Ann Plast Surg 2005 Mar 1;54(3):276-80.
7. Ver Halen JP, Soto-Miranda MA, Hammond S, Konofaos P, Neel M, Rao B. Lower extremity reconstruction after limb-sparing sarcoma resection of the proximal tibia in the pediatric population: case series, with algorithm. J Plast Surg Hand Surg 2014 Aug 1;48(4):238-43.

CONTENT

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CASE REPORT

Emerging Non-Pharmacological Refractory Intervention for Pain Relief in Fibromyalgia: A Case Report

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Abstract

Fibromyalgia (FM) is a common disorder characterised by widespread musculoskeletal pain often associated with fatigue, sleep, memory and mood disturbances. Females are more likely to suffer from FM and experience a reduced quality of life. This is a case report of a 21-year-old female patient diagnosed with FM whose pain was managed by a non-pharmacological method, weightlifting exercises. The patient suffered from chronic generalised muscular pain, muscle stiffness, fatigue, depression and anxiety. She was started on a tight progressive weight-lifting program to increase muscle mass. The program led to a resolution of symptoms after 3 months of gradual improvement, in addition to stopping taking analgesics for the pain. Notably, a relapse occurred after halting the exercise program suggesting that the weight-lifting regime was correlated to reducing symptom severity and better quality of life. Exercises involving weight-lifting could potentially provide an affordable treatment option for patients with FM.

Key words: Fibromyalgia; Pain; Pain relief; Non-pharmacological.

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Introduction

Fibromyalgia (FM) is a disorder characterised by widespread musculoskeletal pain often accompanied by fatigue, sleep, memory and mood disturbances. It is a very common cause of chronic musculoskeletal pain and its prevalence in the United States is estimated to be around 2-8 %.[1] Its incidence is five times greater in women than men.[2] FM is a challenging disease to diagnose due to the non-specific nature of symptoms which are associated with the predominant symptom of chronic pain. Despite it being non-life threatening and non-progressive, chronic pain due to FM has a significant impact on patients' quality of life.[1, 3]

The aetiology of FM is unknown but it is theorised that a disruption of neuroendocrine transmitters is at play.[2] Somatic and psychological symptoms may range from mild to severe and include sudden generalised musculoskeletal pain, muscle stiffness, especially in the morning, fatigue, gastrointestinal (GI) upset and mood and sleep disorders.[2, 3] There is no gold standard for treatment. Treatment options include symptomatic relief and pharmacological interventions. Specifically, several meta-analyses and clinical trials have concluded that pharmacological intervention (such as the three FDA-approved medications: Duloxetine, Milnacipran and Pregabalin) alleviates FM pain.[4] However, it is a complex condition that necessitates multiple treatment modalities and no single therapy outperforms the others in terms of efficacy.

Weight lifting exercises involves lifting weights

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or exercising with body weight to provide resistance to movement and result in building muscles. When considering weight lifting exercises in treating FM, results found that it significantly improved overall well-being and wellness.[5] In addition, having fatigue as a major symptom in women with FM, exercise dramatically decreased feeling of fatigue in many patients.[6] So, when comparing to no exercise, weight lifting exercises successed in reducing pain, tenderness and depression.[7]

The primary goal of this case study is to provide insights and substantiate the evidence on how patients with FM can manage their pain through strength training, which includes resistance exercises and weight lifting.

Case History

A 21-year-old female patient of Arab ethnicity, a student, presented to the Internal Medicine Outpatient Clinic at Princess Basma Teaching Hospital due to generalised muscular pain that lasted for several months' and was increasing progressively in an unpredictable pattern. Patient reported that her pain was aggravated by walking or standing for prolonged periods of time and had no clear alleviating factors. The patient reported muscle stiffness and foot pain that required more than 30 minutes to resolve spontaneously. Stiffness and pain were suspected to be caused by the patient's abnormal posture, as a result, suspicion of anterior pelvis tilt or lordosis was high. Then, anterior pelvis tilt and lordosis were ruled out by imaging studies and after exclusion of other secondary causes, pelvic muscle weakness found to be the culprit. In addition to the constant fatigue that was not relieved by rest, patient also reported anxiety and depression as major complaints which yielded her to a psychologist. The symptoms negatively impacted the patient's quality of life and social and academic performance. Patient's medications included Amitriptyline (*Tryptizol*) - tricyclic antidepressant, 10 mg twice daily and Paracetamol with Orphenadrine citrate (*Muscerol extra*) 2 pills twice daily. Patient otherwise had no previous significant medical or surgical history. Family history was unremarkable. Patient's body mass index (BMI) was 18.7. Thyroid stimulating hormone level was within normal range 2.49 uIu/mL and liver function tests were normal. Rheumatological panel was negative. After ruling out any secondary cause, FM was considered and exercise treatment was directly initiated.

Treatment protocol and results

The patient's exercise program was based on a progressive overload on muscles to induce hypertrophy. This was accomplished through gradual increases (minimum 2.5 kg - 3.5 kg) in weights lifted or increases in repetitions (reps) and sets. Reps were the actions of one complete exercise and sets were the number of reps performed in a row between rest periods. The first month's program consisted of a full-body routine of six exercises: push ups, squats, chin ups, military press, burpees and lunges, performed five days per week. Each exercise was performed in three sets of 20-25 reps with 20-25 seconds rest between each rep. For the second month, the patient increased the intensity of her routine by increasing the weights used in her exercises and completed three sets of 15-20 reps with 30-35 seconds of rest between reps. She continued to do a full body workout routine involving six exercises for five days per week. The subsequent months, the intensity was increased by either increasing weights or the number of sets and reps.

A specialised nutritionist prescribed a strict diet of 1900 calories to meet her basal metabolic rate to maintain BMI within normal range according to age and height thus maximise muscular hypertrophy. An improvement in her BMI (Table 1) and strength was notices as well as a decrease in her symptoms. The alleviation of symptoms highly motivated patient adherence for 8 months on the program. Patient noticed a reduction in muscle stiffness, leg pain, fatigue, depression and anxiety from the first week and by the end of the first month her symptoms completely resolved. Her medications were slowly tapered after the first month and by the third month she was no longer taking any medications and still reported no symptoms. Due to personal circumstances, the patient could not train for one week and soon began to experience muscle pain and stiffness. She then restarted her program and three months later reported major improvements, she regained her energy and vigour which had been absent for several years. Patient continued to be mostly adherent to her exercise program. In the rare occasion when she missed training, she noted relapses of symptoms. She became self-sufficient in her training program and learned how to keep

Table 1: Improvement in the patient's body and muscle mass, body fat, BMI and BMR after treatment

Comparison between the parameters before and after the exercise program		
Scales	Before initiating the exercise program (At the time of diagnosis)	After 6 months
Body mass	49.6 kg	56 kg
Muscle mass	63.1 %	67.5 %
Body fat	22.8 %	26.5 %
BMI	18	21
BMR	1176	1241

BMI: Body mass index, BMR: Basal metabolic rate

her muscles in a progressive overload to induce muscle growth. She reported that her quality of life dramatically improved and no limitations in her social and academic performance.

Discussion

Pain is the primary symptom of FM and it is theorised that FM is neurogenic in origin because it is associated with a central amplification of pain perception.[4] However, the exact aetiology remains unknown. A disruption of neuroendocrine transmitters, such as serotonin, growth hormone and cortisol, appears to be implicated.[2] There is no gold standard for treatment of FM but it remains challenging and both pharmacological and non-pharmacological options should be considered.[4] Three FDA-approved medications, duloxetine, milnacipran and pregabalin, have been found to be effective in reducing FM pain by several meta-analyses and clinical trials.[4] The majority of patients take medications from a variety of classes which leads to significant financial burden for the patient. Medical consultation and medication costs are estimated to cost 951 USD over a three-month period.[8] The exercise program followed by this patient presents a more affordable, yet still effective treatment option. The costs involved only include a gym membership or weightlifting equipment.

It may seem counter-intuitive to purposely stress muscle in patients who have muscle pain. However, a growing body of evidence challenges the assumption that resistance (strength) training worsens muscle pain in people with FM. In fact, the latest evidence indicates that when resistance training is tailored to individual needs, people with FM can obtain worthwhile improvements in FM severity.[9] Several studies empirically suggested that strength training can significantly improve the quality of life for people with FM.[5-7, 10]

The therapeutic protocol is based on ensuring muscular hypertrophy by weightlifting exercises tailored to patient's needs and abilities. The core of this approach is the abnormal baseline of neuroendocrine hormones which can be improved with consistent exercises.[2] A strict diet is also necessary to guarantee maximum muscular hypertrophy. The recurrence of the patient's symptoms with program interruption emphasises the link between exercise and FM pain management and shows that the program may induce remission but it does not result in a permanent cure.

Conclusion

This report shows a significant improvement in FM symptoms: complete remission of muscle pain, stiffness, fatigue, depression and anxiety. Furthermore, the report introduces a new method of treating functional limitations experienced by patients for minimal cost, namely a gym membership or weight lifting equipment at home. The weight-lifting program was extremely effective in reducing the patient's symptoms, therefore pharmacological interventions were not needed. However, a pilot study is needed to test this approach on a larger number of patients with FM to evaluate its potential for inclusion in management guidelines.

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None.

Conflict of interest

None.

CONTENT

References

1. Clauw DJ. Fibromyalgia: a clinical review. JAMA 2014 Apr 16;311(15):1547–55.
2. Jahan F, Nanji K, Qidwai W, Qasim R. Fibromyalgia syndrome: an overview of pathophysiology, diagnosis and management. Oman Med J 2012 May;27(3):192–5.
3. García-Ríos MC, Navarro-Ledesma S, Tapia-Haro RM, Toledano-Moreno S, Casas-Barragán A, Correa-Rodríguez M, et al. Effectiveness of health education in patients with fibromyalgia: a systematic review. Eur J Phys Rehabil Med 2019 Apr;55(2):301–13.
4. Thomas SA, Knight L, Balian A. Treatment of fibromyalgia pain. US Pharm 2016;41(3):51-4.
5. Busch AJ, Webber SC, Richards RS, Bidonde J, Schachter CL, Schafer LA, et al. Resistance exercise training for fibromyalgia. Cochrane Database Syst Rev 2013 Dec 20;2013(12):CD010884. doi: 10.1002/14651858.CD010884.
6. Ericsson A, Palstam A, Larsson A, Löfgren M, Bileviciute-Ljungar I, Bjersing J, et al. Resistance exercise improves physical fatigue in women with fibromyalgia: a randomized controlled trial. Arthritis Res Ther 2016 Jul 30;18:176. doi: 10.1186/s13075-016-1073-3.
7. Busch AJ, Barber KA, Overend TJ, Peloso PM, Schachter CL. Exercise for treating fibromyalgia syndrome. Cochrane Database Syst Rev 2007 Oct 17;(4):CD003786. doi: 10.1002/14651858.CD003786.pub2.
8. Lacasse A, Bourgault P, Choinière M. Fibromyalgia-related costs and loss of productivity: a substantial societal burden. BMC Musculoskelet Disord 2016 Apr 16;17:168. doi: 10.1186/s12891-016-1027-6.
9. Jones KD. Recommendations for resistance training in patients with fibromyalgia. Arthritis Res Ther 2015 Sep 17;17(1):258. doi: 10.1186/s13075-015-0782-3.
10. Hackney AC. Stress and the neuroendocrine system: the role of exercise as a stressor and modifier of stress. Expert Rev Endocrinol Metab 2006 Nov 1;1(6):783–92.

CONTENT

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IMAGES IN MEDICINE

A Quadricuspid Aortic Valve Combined with Coronary Artery Disease

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Case history

Quadricuspid aortic valve (QAV) is a rare and purely understood congenital malformation with an estimated incidence of 0.013 % - 0.043 %. Here are presented images of an incompetent QAV found during coronary artery bypass grafting (CABG).

A 68 year old male was transferred from outside hospital with a sign of left heart failure. After admission, urgent coronarography was performed and significant stenosis (≥ 75 %) was found on the proximal left anterior descending artery (LAD), ramus intermedius (RIM) and circumflex artery (RCx). Transoesophageal echocardiography revealed severe aortic regurgitation due to incompetent QAV, with hypokinetic anterior wall of the left ventricle.

After heart exposure, using cardiopulmonary bypass, cardiac arrest was induced as well as standard myocardial protection. A transversal aortothomy revealed enlarged annulus and four equal-sized aortic cusps (Type A)[1] with central gap and consequent severe malcoaptation of the QAV (Figure 1 and 2). The aortic valve was replaced with a #21 pericardial tissue prosthesis and myocardial revascularisation was successfully achieved with triple bypass using skeletonised left internal mammary artery to LAD and saphenous venous grafts to RIM and RCx. Patient had an uneventful postop course and was asymptomatic a year after surgery.

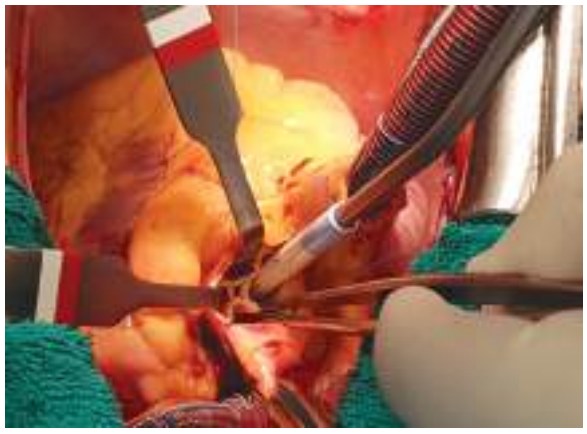

Figure 1: Intraoperative gross view of the quadricuspid aortic valve

Figure 2: Gross view of the specimen of the quadricuspid aortic valve

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CONTENT

Acknowledgements

None.

References

1. Lin Y, Yin K, Wang Y, Yang D, Luo R, Dong L, et al. Clinical characteristics and surgical outcomes of dysfunctional quadricuspid aortic valve. J Surg Res 2018 Sep;229:223-9.

Conflict of interest

None.

CONTENT

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Gillespie NC, Lewis RJ, Pearn JH, Bourke ATC, Holmes MJ, Bourke JB, et al. Ciguatera in Australia: occurrence, clinical features, pathophysiology and management. Med J Aust 1986;145:584-90.

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 programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi 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 2007 Jan 5];27(1):34-7. Available from: http://www.journals.uchicago.edu/ICHE/journal/issues/v27n1/2004069/2004069.web.pdf.

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