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ORIGINAL ARTICLE

Isolation, Characterisation and Antibiotic Susceptibility of Staphylococcal Isolates with Special Reference to Methicillin-Resistant *Staphylococcus aureus* From the Anterior Nares of Healthcare Workers in a Tertiary Healthcare Centre

Mukul Chaurasia,[1] Neha Agrawal,[1] Ankita Chourasia,[2] Monica Bhatnagar,[2] Geeta Parihar,[1] Vijaylatha Rastogi,[1] Amit Tak[3]

(1) Department of Microbiology, JLN Medical College, Ajmer, Rajasthan, India.
(2) Department of Microbiology, Maharshi Dayanand Saraswati University, Ajmer, Rajasthan, India.
(3) ICMR - National Centre for Disease Informatics and Research, Bengaluru, Karnataka, India.

Abstract

Background: *Staphylococcus aureus* (*S. aureus*) and its resistant form methicillin-resistant *S. aureus* (MRSA) is one of the most common nosocomial pathogens causing a wide range of infections in humans. The anterior nares are the main ecological niche for *S. aureus*. Nasal carriage of *S. aureus* acts as an important reservoir of infection among the colonised healthcare workers and they transmit the infection to the community. The aim of the present study was to estimate the nasal colonisation of *S. aureus* (with special reference to MRSA) in healthcare workers (doctors and nursing staff) and its antibiotic susceptibility pattern.
Methods: A descriptive study was planned in the Department of Microbiology, JLN Medical College, Ajmer (Rajasthan, India) after due approval from the institutional ethics committee. A total of 170 healthcare workers of either sex aged between 18 to 60 years were screened for *S. aureus*. Identification was done using standard microbiological techniques, by studying their morphology, colony and biochemical characteristics. MRSA was detected by cefoxitin disc diffusion test, oxacillin disc diffusion test, minimum inhibitory concentration (MIC) of oxacillin by E-test and oxacillin screen agar test. The observations were described in proportions and Chi-squared test was used to find independence. Statistical significance was considered at 5 %.
Results: Among 170 samples, 159 (93.53 %) samples (50 doctors and 109 nursing staff) had staphylococci colonisation. Among these 159 isolates, 34 (21.38 %) were *S. aureus*. Further, 8 (5.03 %) *S. aureus* isolates were resistant to both cefoxitin and oxacillin and had oxacillin MIC values ≥ 4 µg/mL and were considered MRSA. All the MRSA were detected in the nursing staff (males: 5.50 %, females: 1.83 %). All *S. aureus* and MRSA isolates were found sensitive to linezolid, vancomycin and mupirocin (minimum inhibitory concentration ≤ 4 µg/mL).
Conclusion: Screening and treatment of healthcare workers colonised with MRSA should be an important component of hospital infection control policy. These measures will prevent spread of infection to patients and the community and thereby reduce the morbidity, mortality and healthcare costs associated with nosocomial infections.

Key words: Antibiotic susceptibility pattern; Healthcare workers; Methicillin-resistant *Staphylococcus aureus*; Minimum inhibitory concentration; Nosocomial infections.

Correspondence:
AMIT TAK
E: dramittak@gmail.com

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Introduction

Staphylococci are ubiquitous colonisers of skin and mucosa and highly successful opportunistic pathogens. *S. aureus* is one of the most harmful species of staphylococci encountered.[1] It is one of the most pathogenic bacterial species in humans causing a wide variety of infections ranging from mild skin and soft tissue infections (furuncles, carbuncles etc) to severe life-threatening infections like chronic bone infections, necrotising pneumonia, bacteraemia, septicaemia, acute endocarditis, myocarditis, pericarditis, osteomyelitis, encephalitis, meningitis, chorioamnionitis, mastitis, toxic-shock syndrome, scalded skin syndrome[2-5] and intravenous infections or at other sites where tubes enter the body (indwelling medical devices).[6] It is distinct from coagulase-negative staphylococci (CoNS) eg, *S. epidermidis*, and is more virulent despite phylogenetic similarities between them.[7, 8]

The key characteristics of *S. aureus* are colony pigmentation, production of free coagulase, clumping factor, protein-A, heat-stable nuclease, lipase and acid production from mannitol.[3] The species *aureus*, refers to those colonies that often have a golden colour when grown on solid media, while CoNS form pale, translucent, white colonies.

Staphylococcal infections occur frequently in hospitalised patients and have severe consequences, despite antibiotic therapy.[9] *S. aureus* are generally susceptible to β-lactam antibiotics, but extensive use of this class of drugs has led to increasing emergence of resistant strains.[10] The most notable example is the emergence of methicillin-resistant *Staphylococcus aureus* (MRSA), which was reported just one year after the introduction of methicillin.[11] Also known as "a superbug", MRSA has become a major problem in most medical institutions because it is creating life-threatening situations.[11] MRSA is a major healthcare-associated (HA-MRSA) as well as a community-associated (CA-MRSA) infection.[6]

Healthcare workers (HCWs) constitute an important reservoir of *S. aureus*. Nasal carriage of *S. aureus* acts as an important reservoir of infection among those colonised, who may then transmit the infection to co-workers and others in the community.[12] Approximately 20 % of individuals are persistent carriers, about 60 % are intermittent carriers and 20 % almost never carry *S. aureus*.[13] Several studies have reported that the rate of the nasal carriage of *S. aureus* among the HCWs ranges from 16.8 % to 56.1 %.[14-17] Studies conducted in different hospital settings worldwide including India, have reported the prevalence of MRSA in HCWs in the range of 5.8 % to 17.8 %.[18-22, 9, 12] The growing problem in India is that MRSA prevalence has increased from 12 % to 80.83 %.[23] The healthcare workers who are found to be colonised with *S. aureus* are advised to apply mupirocin ointment in their anterior nares and they should be retested for the nasal carriage of *S. aureus* after 3 months of treatment.[9]

The aim of the present study was to estimate the nasal carriage and antimicrobial susceptibility pattern of *S. aureus* and MRSA isolates among the HCWs in a tertiary healthcare centre. The prevalence of *S. aureus* carriers and its resistance to methicillin will help the institution develop a better MRSA infection control policy.

Methods

This descriptive study was carried out in the Department of Microbiology, Jawahar Lal Nehru (JLN) Medical College and Hospital, Ajmer, Rajasthan, India from November 2016 to December 2017. The study was approved by the Ethics Committee of JLN Medical College, Ajmer and written informed consent was obtained from all the participants.

A total of 170 HCWs aged 18 to 60 years, actively involved in healthcare provision in different departments of JLN Medical College were enrolled for the study. Each participant was interviewed using a questionnaire on general socio-demographic information, personal details and clinical symptoms. Exclusion criteria included healthcare workers not actively involved in patient care or those suffering from underlying chronic disease or respiratory tract infections, with a history of recent hospitalisation, intake of broad-spectrum antibiotics, fever or those who did not consent.

Sample collection

Nasal swabs from the anterior nares of both nostrils were collected using sterile cotton swabs with transport tubes. A swab pre-moistened with sterile saline was inserted approximately 1-2 cm

into the anterior nares and slowly rotated against the nasal mucosa five times.[24] Both nostrils were sampled using the same swab. After collection, the swabs were re-inserted in the transport tubes, labeled properly and transported to the laboratory within 30 minutes of collection for further processing.

Sample processing

All the specimens were inoculated on 5 % sheep blood agar, nutrient agar and MacConkey agar (HiMedia Laboratories Pvt Ltd Mumbai, Maharashtra, India) and incubated at 37 °C for 24 hours. After incubation, identification of genus *Staphylococcus* was done using standard microbiological techniques, by studying their morphology, colony characteristics and biochemical properties. Staphylococci were identified as Gram positive, catalase positive, furazolidone susceptible and bacitracin-resistant. *S. aureus* colonies were further identified as slide and tube coagulase positive, polymyxin B-resistant and mannitol fermenting giving yellow pigmentation on mannitol salt agar.

Antimicrobial susceptibility testing (AST)

Antibiotic susceptibility was studied by modified Kirby–Bauer disc diffusion method[25] on Mueller Hinton Agar plates (120 mm diameter) using commercially available antibiotic discs (HiMedia Laboratories Pvt. Ltd. Mumbai, Maharashtra, India): penicillin G (10 units), cephalexin (30 µg), cefoxitin (30 µg), oxacillin (1µg), gentamicin (10 µg), netilmicin (30 µg), ciprofloxacin (5 µg), ofloxacin (5 µg), levofloxacin (5 µg), erythromycin (15 µg), clindamycin (10 µg), tetracycline (30 µg), cotrimoxazole (25 µg), quinupristin dalfopristin (15 µg), vancomycin (30 µg), linezolid (30 µg), cephalothin (30 µg), amoxicillin/clavulanic acid (co-amoxiclav, 30 µg) and ampicillin (10 µg). Zone diameter interpretation for determining sensitive, intermediate or resistant isolates was done as per CLSI 2016 guidelines.[26]

Detection of methicillin-resistant *Staphylococcus aureus (MRSA)*

All confirmed *S. aureus* isolates were tested for detection of methicillin resistance by four different methods. Kirby–Bauer disc diffusion method using oxacillin 1 µg and cefoxitin 30 µg discs (HiMedia Laboratories, Mumbai, Maharashtra, India),[27] minimum inhibitory concentration (MIC) testing of oxacillin by E-test and growth on Oxacillin Resistance Screening Agar (ORSA) plates[28] as per CLSI 2016 guidelines.[26] Zone of inhibition of size ≤ 10 mm was taken as resistant, 11-12 mm as intermediate and ≥ 13 mm as sensitive for oxacillin. Zone of inhibition of size ≤ 21 mm was taken as resistant, and ≥ 22 mm as sensitive for cefoxitin. On oxacillin E-test, an MIC of ≤ 2 µg/mL was considered susceptible and ≥ 4 µg/mL as resistant. Any growth on oxacillin screen agar was considered as methicillin (oxacillin) resistant.

Detection of mupirocin-resistant *Staphylococcus aureus*

The MIC of mupirocin for isolation of *S. aureus* (Mupirocin resistance) was determined by Epsilometer test (E-test) using HiMedia, mupirocin strip (range 0.064-1024 µg/mL) and interpreted as per CLSI 2016 guidelines.[26] Isolates with mupirocin MICs ≥ 512 µg/mL were considered as high-level resistant (MuH), those with MICs 8-256 µg/mL were considered as low-level resistant (MuL), and with ≤ 4 µg/mL were considered as mupirocin sensitive.

Statistical analysis

The descriptive statistics for quantitative data was expressed as mean and standard deviation and qualitative data was expressed as proportions. Chi-squared test was used to find independence of attributes at 5 % level of significance (α = 0.5). The JASP 0.11.1.0 statistical package was used for statistical analysis.

Results

In the present study, nasal swabs were randomly collected from a total of 170 HCWs from various clinical departments and screened for the study of *Staphylococcus* colonisation. Out of a total of 170 samples, 159 (93.53 %) had staphylococci colonisation. Of these 159 HCWs, with age group ranging between 18 to 60 years, 99 (62.26 %) were males and 60 (37.74 %) were females. The colonisation rate was 31.45 %, 34.59 %, 18.24 % and 15.72 % in the age groups '18-30', '31-40', '41-50' and '51-60' years, respectively (Figure 1).

From these 159 subjects, 50 were doctors and 109 were nursing staff. Of the 50 doctors, 37 (74 %) were males and 13 (26 %) were females. Among the 109 nursing staff, 62 (56.88 %) were males

and 47 (43.12 %) were females. The maximum carriage rate in doctors was observed in the age group 31-40 years ie, 60 %, where 50 % were males and 10 % were females. In the nursing staff group, maximum carriage was seen in 18-30 years age group where 20.18 % were males and 18.35 % were females accounting for a total of 38.53 % carriage rate in their group (Figure 1).

In the present study *Staphylococcus* colonisation was detected in 159 (93.53 %) healthcare workers which comprised 34 (21.38 %) *S. aureus* and 125 (78.61 %) CoNS isolates. Dual colonisation with *S. aureus* and CoNS was observed in 10 samples. The carriage rate of *S. aureus* was significantly higher in nursing staff (26.60 %) as compared to doctors (10 %) ($\chi^2 = 5.62$, p = 0.018). Professors/associate professors/assistant professors and resident doctors were found to have *S. aureus* nasal carriage rate 16.67 % and 7.89 %, respectively (Figure 1).

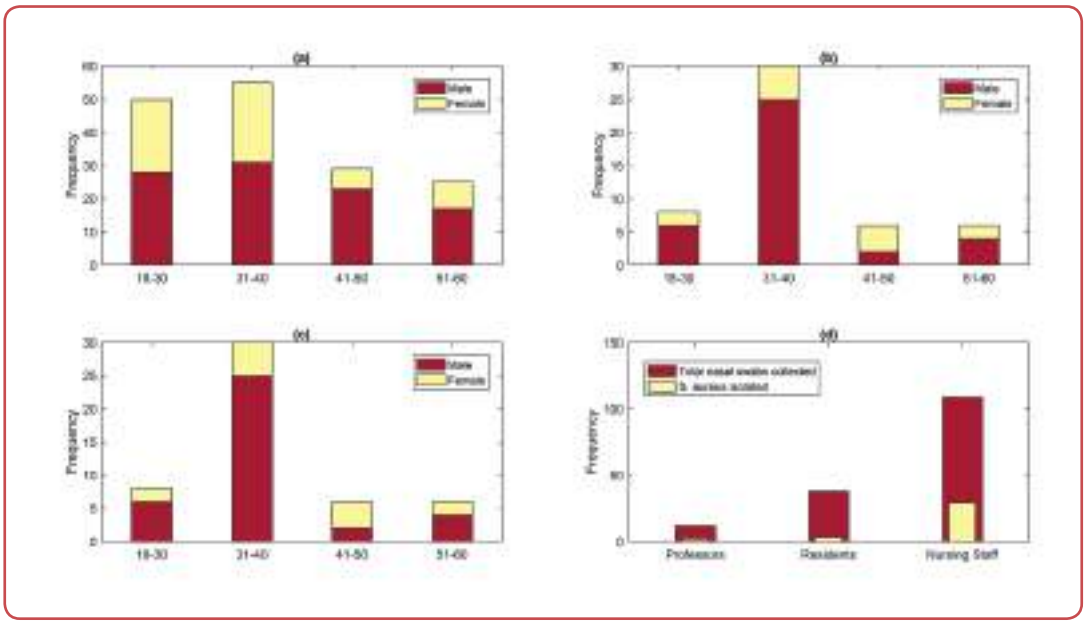

Figure 1: Stacked bar plots showing demographic profile (a) Age wise gender distribution of all healthcare workers (n = 159), (b) age-wise gender distribution of doctors (n = 50), (c) age-wise gender distribution of nursing staff (n = 109), (d) Total number of nasal swabs collected and S. aureus isolated in healthcare workers (n = 159)

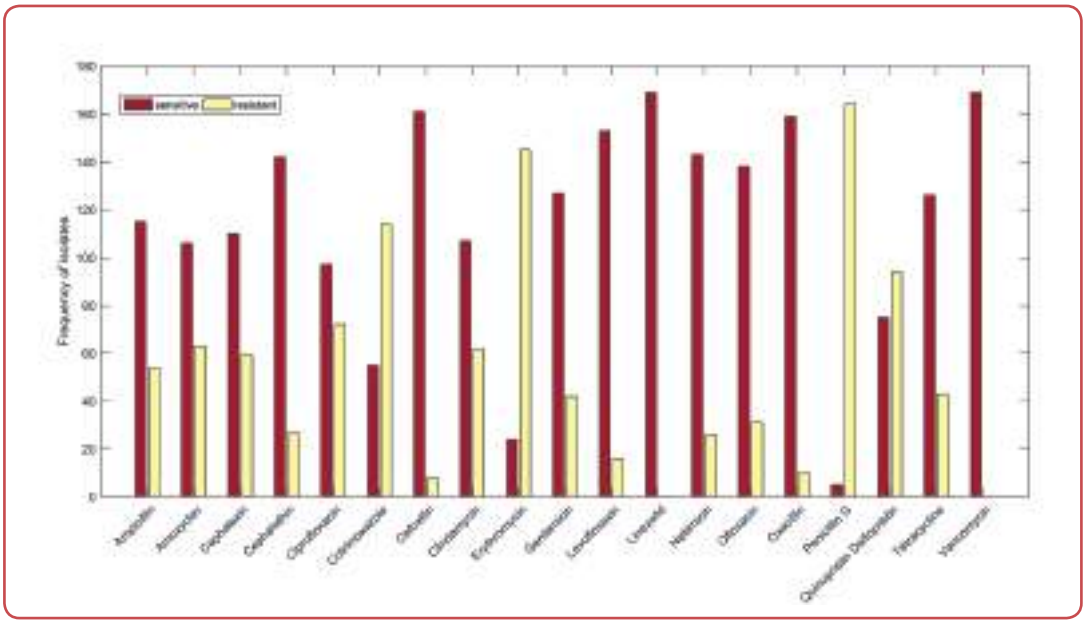

Figure 2: Bar plots showing antimicrobial sensitivity pattern of Staphylococcus isolates by modified Kirby-Bauer disc diffusion method (n = 169)

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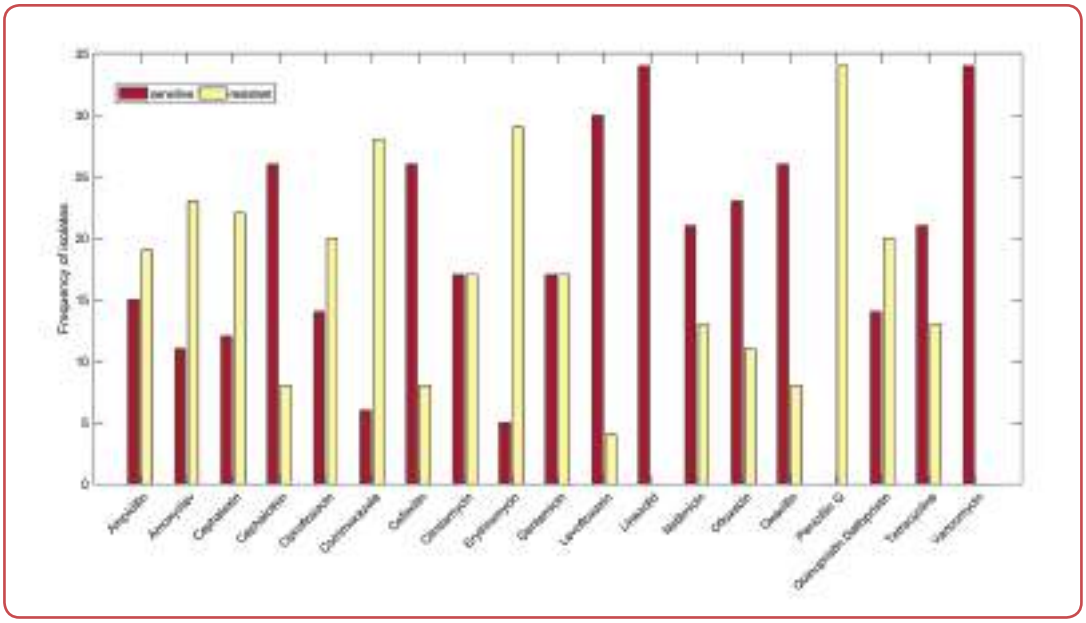

Figure 3: Bar plots showing antimicrobial sensitivity pattern of S. aureus isolates by modified Kirby-Bauer disc diffusion method (n = 34)

Antimicrobial sensitivity pattern of *Staphylococcus* isolates by disc diffusion method is shown in Figure 2. Ten subjects had concomitant colonisation of *S. aureus* and CoNS. Therefore, antimicrobial susceptibility testing was done for 169 *Staphylococcus* isolates.

Among the antibiotics tested, all the staphylococcal isolates were susceptible only to linezolid and vancomycin (100 %). Maximum resistance was shown to penicillin G (97.49 %). Resistance to cefoxitin and oxacillin was 4.73 % and 5.92 %, respectively.

All the *S. aureus* isolates were found to be susceptible to linezolid and vancomycin (100 %). All *S. aureus* isolates showed complete resistance to penicillin G (100 %). Extremely low susceptibility was shown for erythromycin (17.71 %) and cotrimoxazole (17.65 %). Resistance to cefoxitin and oxacillin was 23.53 % (Figure 3).

Detection of MRSA was done by four different phenotypic methods. Among the 34 *S. aureus* isolates studied, 8 isolates (23.53 %) were found to be MRSA. While in oxacillin screen agar testing, 6 (17.65 %) isolates were found to be MRSA. No isolate showed intermediate resistance. Thus, out of 34 *S. aureus* isolates, 8 (23.53 %) were MRSA and 26 (76.47 %) were methicillin sensitive *S. aureus* (MSSA) (Table 1). One MRSA isolate showed resistance to vancomycin disc on AST. However due to limited resources, further testing of this isolate by MIC testing of vancomycin using agar dilution method (recommended by CLSI) to determine it as vancomycin-resistant, intermediate or sensitive could not be carried out.

Table 1: Comparison of different phenotypic methods for detection of methicillin resistance in S. aureus isolates (n = 34)

S. aureus	Tests used for detection of MRSA			
	Oxacillin (1 µg) disc diffusion	Cefoxitin (30 µg) disc diffusion	Oxacillin screen agar	Oxacillin MIC by E-test
Methicillin-resistant *S. aureus* (MRSA)	8 (23.53)	8 (23.53)	6 (17.65)	8 (23.53)
Methicillin-sensitive *S. aureus* (MSSA)	26 (76.47)	26 (76.47)	28 (82.35)	26 (76.47)

Note: percentage is shown in parenthesis; S. aureus: Staphylococcus aureus

In the present study, the 8 *S. aureus* isolates that were resistant to both cefoxitin and oxacillin had oxacillin MIC values ≥ 4 µg/mL (Table 2). There were no isolates found resistant to cefoxitin and intermediate resistance to oxacillin at the same

Table 2: Comparison of cefoxitin and oxacillin disc diffusion tests with minimum inhibitory concentration (MIC) of oxacillin by E-test method (n = 34)

Results of oxacillin and cefoxitin disc diffusion	MIC of oxacillin (µg/mL)	No. of isolates (%)
Resistant to both cefoxitin and oxacillin	≥ 4	8 (23.53)
Resistant to cefoxitin and intermediate resistant to oxacilln	≥ 4	0 (0.0)
Resistance to cefoxitin and sensitive to oxacillin	≥ 4	0 (0.0)
Sensitive to cefoxitin and oxacillin	< 4	26 (76.47)

Note: percentage is shown in parenthesis

Table 3: Nasal carriage of S. aureus and MRSA among various healthcare workers (n = 34)

Healthcare workers	No. of nasal swabs collected	No. of *S .aureus* isolated (%)	No. of MRSA isolated (%)
Professors, Associate Professors, Assistant professors	12	02 (16.67)	0 (0.0)
Resident doctors	38	03 (7.89)	0 (0.0)
Nursing staff	109	29 (26.60)	08 (7.34)
Total	**159**	**34 (21.38)**	**08 (5.03)**

Note: percentage is shown in parenthesis; S. aureus: Staphylococcus aureus; MRSA: methicillin-resistant Staphylococcus aureus

time. No isolate was found resistant to cefoxitin while being sensitive to oxacillin. 26 *S. aureus* isolates that were sensitive to both oxacillin and cefoxitin by disc diffusion method had MICs ≤ 2 µg/mL, indicating their susceptibility to oxacillin. Thus, out of 34 *S. aureus* isolates, 8 (23.53 %) were MRSA and the remaining 26 (76.47 %) were oxacillin and methicillin susceptible (MSSA).

Out of 159 isolates, 21.38 % subjects had *S. aureus* colonisation out of which 5.03 % had MRSA colonisation (Table 3). All of these MRSA carriers were detected in the nursing staff. The carriage

Table 4: Sex wise distribution of nasal carriage of S. aureus and MRSA among various healthcare workers (n = 34)

Healthcare workers	No. of nasal swabs collected	No of *S. aureus* isolated (%)		No. of MRSA isolated (%)	
		Male	Female	Male	Female
Doctors	50	4 (8)	1 (2)	0	0
Nursing staff	109	17 (15.60)	12 (11.01)	6 (5.50)	2 (1.83)
Total	**159**	**21 (13.21)**	**13 (8.18)**	**6 (3.77)**	**2 (1.26)**
		34		**8**	

Note: percentage is shown in parenthesis; S. aureus: Staphylococcus aureus; MRSA: methicillin-resistant Staphylococcus aureus

Table 5: Mupirocin minimum inhibitory concentration (MIC) of nasal isolates of S. aureus (n = 34)

S. No.	Mupirocin MIC (µg/ml)	No. of *S. aureus* isolates	No. of MRSA isolates
1	≤ 0.125	13	01
2	0.25	11	03
3	0.5	06	02
4	1	03	01
5	2	01	01
6	4	00	00
7	8	00	00
Total		**34**	**08**

Note: S. aureus: Staphylococcus aureus; MRSA: methicillin-resistant Staphylococcus aureus; MIC: minimum inhibitory concentration

rate of *S. aureus* too was significantly higher in nursing staff ie, 26.60 % with MRSA carriage rate of 7.34 %. Professors/associate professors/assistant professors and resident doctors were found to have *S. aureus* nasal carriage rate 16.67 % and 7.89 % respectively with no MRSA carriage. The MRSA carriage rate was 5.5 % and 1.83 % in male and female nursing staff, respectively (Table 4).

For *S. aureus*, mupirocin MIC ≤ 4 µg/mL is considered as susceptible. MIC 8-256 µg/mL is considered as intermediate resistant and MIC ≥ 512 µg/mL is considered as resistant. All *S. aureus* isolates had MIC ≤ 4 µg/mL for mupirocin, indicating mupirocin susceptibility of all the isolates (Table 5). Further, as many as 13 *S. aureus* and 1 MRSA isolate had MIC ≤ 0.125 µg/mL and 11 *S. aureus* and 3 MRSA isolates had MIC ≤ 0.25 µg/mL.

Discussion

S. aureus is a common component of the skin flora, and 30 % to 50 % of healthy adults are colonised with it at any given time. The primary site of colonisation of *S. aureus* in humans are the anterior nares.[2, 29] Hospital workers have higher rates of MRSA nasal colonisation than the general population.[30] In the present study 21.38 % subjects had *S. aureus* colonisation. Among HCWs around the globe, the nasal carriage rates of *S. aureus* have been reported at 14 % in Nigeria, 27.5 % in Turkey, 31.1 % in Iran, 33.4 % in France and 39.3 % in Spain.[31] The growing problem in India is that MRSA prevalence has increased from 12 % to 80.83 %.[23]

Out of a total of 159 subjects (50 doctors and 109 nursing staff) *S. aureus* and CoNS appeared in 34 and 125 samples respectively. Dual colonisation of *S. aureus* and CoNS was observed in 10 samples. However, no dual isolation was observed in a study conducted by Vinodhkumaradithyaa et al.[22] The prevalence of the *S. aureus* nasal carriage was higher among the male HCWs (13.21 %) than the female HCWs (8.18 %). Similar observation was reported by Rongpharpi et al.[9] In the present study, the carriage rate of *S. aureus* was significantly higher in the nursing staff 26.60 %. Professors/associate professors/assistant professors and resident doctors were found to have *S. aureus* nasal carriage rate 16.67 % and 7.89 % respectively. Out of 34 *S. aureus,* 5.03 %

had MRSA colonisation. All of these MRSA carriers belonged to the nursing staff with MRSA carriage rate of 7.34 %. Similar studies from Barabanki, Uttar Pradesh reported 81 % *Staphylococcus*, 48 % *S. aureus* and 14 % MRSA colonization.[12] However, Bhatiani et al reported 39 % and 15 % carriage rates of *S. aureus* and MRSA in Rama Medical College, Hospital and Research Center, Kanpur which is similar to the findings presented here.[32] Shobha et al found none of the healthcare workers colonised with *S. aureus*[33] while a study from south India showed 9.3 % *S. aureus* colonization.[34]

In the present study, on MRSA detection using oxacillin disc diffusion, cefoxitin disc diffusion and MIC of oxacillin by E-test, 8 (23.53 %) isolates were found to be MRSA. In this study, 8 *S. aureus* isolates that were resistant to both cefoxitin and oxacillin had oxacillin MIC value ≥ 4 µg/mL and 26 isolates that were sensitive to both oxacillin and cefoxitin had MIC values ≤ 2 µg /mL. Oxacillin screen agar could detect only 6 (17.65 %) isolates instead of 8 detected by the other three methods. Hence it is recommended that all four methods should be used for detection of oxacillin resistance. Pramodhini et al found oxacillin disc diffusion method to be less sensitive for the detection of MRSA.[35] Mohanasoundaram and Lalitha obtained 100 % concordance in disc diffusion method and oxacillin MIC using agar dilution methods.[36]

In the present study, 97.04 % staphylococcal isolates and 100 % *S. aureus* and MRSA were found to be resistant to penicillin G. Similar findings were observed by Bala et al and Bhatiani et al where penicillin was found to be 100 % resistant to all strains of *S. aureus*,[37, 32] but Rongpharpi et al reported 90 %,[9] Duran et al reported 92.8 %,[38] Kandle et al reported 98.9 % penicillin resistance.[39] All the MRSA isolates were resistant to penicillin as reported by Agarwal et al.[12] In the present study, ampicillin and co-amoxiclav showed a resistance of 31.95 % and 37.28 % for staphylococci and 55.88 % and 67.65 % for *S. aureus.* Out of 8 MRSA isolates, 6 (75 %) and 8 (100 %) isolates were found to be resistant to ampicillin and co-amoxiclav respectively. Bhatiani et al has reported a 100 % resistance to ampicillin,[32] while 88.57 % and 82.00 % resistance to ampicillin by *S. aureus* isolates was observed by Rongpharpi et al and Jindal et al in studies conducted among HCWs respectively.[9, 40] Study conducted at a tertiary care hospital in Iran reported 89.4 % resistance among MRSA.[41]

A total of 34.91 % *Staphylococcus* isolates, 64.71 % *S. aureus* isolates and all MRSA isolates were resistant to cephalexin in the present study while 73.7 % MRSA isolates were found to be resistant to cephalexin in a similar study.[42] In the present study, 24.85 % and 15.38 % staphylococcal, 50 % and 38.24 % *S. aureus*, 87.50 % and 75.00 % MRSA isolates were resistant to gentamicin and netilmicin respectively. In studies by Hauschild et al and Schmitz et al, 24.4 % and 23 % resistance was shown in *S. aureus* isolates to the above aminoglycosides.[43, 44] In this study, of 34 *S. aureus* isolates, 38.24 % were resistant to at least one of the two aminoglycosides tested. Hauschild et al reported that 38.1 % *S. aureus* were resistant to one of the aminoglycosides tested.[43]

In the present study, 42.60 % *Staphylococcus* isolates and 58.82 % *S. aureus* isolates were resistant to ciprofloxacin. Lower incidence of resistance (10.4 %) was reported by Tahnkiwale et al,[45] 41 % by Duran et al[38] and 90 % by a Mexican study on 211 isolates.[46] In Europe resistance by region showed a 5.6 % resistance in the northern, 6.2 % in the central and 23.6 % in the southern region.[47] Resistance to ofloxacin was shown by 18.34 % *Staphylococcus* isolates and 32.35 % *S. aureus* isolates in this study. Levofloxacin resistance stood at 9.47 % and 11.76 % for *Staphylococcus* and *S. aureus* isolates, respectively. However, 87.50 %, 75.00 % and 12.50 % MRSA isolates showed resistance to ciprofloxacin, ofloxacin and levofloxacin, respectively. In contrast, Agarwal et al reported 50 % MRSA isolates resistant to ciprofloxacin and 21.4 % for levofloxacin.[12]

Erythromycin-resistant *Staphylococcus* often exerts cross resistance to other macrolides, lincosamide and streptogramin type B (MLS_B).[48] In the present study erythromycin resistance was seen in 85.80 % and 85.29 % *Staphylococcus* and *S. aureus* isolates respectively. However, a lower resistance to erythromycin ranging between 66.66 % and 67.9 % has been observed by Bhatiani et al,[32] Bala et al[37] and Kausalya et al.[49] Clindamycin resistance was shown in 36.69 % and 50 % *Staphylococcus* and *S. aureus* isolates, respectively, while in a study by Verma et al,[23] erythromycin and clindamycin resistance was found to be 52.8 % and 48.28 %, respectively in *S. aureus* isolates. In this study, 25.44 % *Staphylococcus* and 38.24 % *S. au-*

CONTENT

reus isolates respectively were tetracycline-resistant. A higher resistance was reported by Shittu and Lin and Duran et al who reported 55.9 % and 35.6 % resistance for *S. aureus* isolates, respectively.[38, 50] In the present study, 75.00 % (6/8) MRSA isolates were found to be resistant to tetracycline which is much higher as reported by Agarwal et al.[12]

During the 17-year period of the studies by Cuevas et al there was low resistance of *S. aureus* to cotrimoxazole in all the studies (0.5 to 2.1 %).[51] In this study, 82.35 % *S. aureus* isolates were resistant to cotrimoxazole while other studies conducted in India have reported a resistance of 63.84 %,[23] 73.3 %,[32] 46.1 %,[22] 31.43 %[9] and 57.1 %.[40] Present study showed that 87.50 % (7/8) MRSA isolates were resistant to cotrimoxazole which correlates with the study by Mohanasoundaram and Lalitha showing 82 % cotrimoxazole resistance among the MRSA isolates.[36] A somewhat higher resistance was reported by Pulimood et al (97.1 %)[52] while low resistance in MRSA isolates was reported by Agarwal et al (57 %).[12]

In the present study, a total of 55.62 % (94/169) *Staphylococcus* isolates and 58.82 % *S. aureus* isolates showed resistance to quinupristin dalfopristin. All the MRSA isolates (8/8, 100 %) were found to be resistant to quinupristin dalfopristin, while in a study conducted by Kaur and Chate, only 5.56 % MRSA isolates were reported as resistant to quinupristin dalfopristin.[53]

In this study, 100 % *Staphylococcus* and *S. aureus* isolates showed sensitivity to vancomycin. One MRSA isolate showed resistance to vancomycin disc on AST. However, due to limited resources, further testing of this isolate by MIC testing of vancomycin using agar dilution method (recommended by CLSI) to determine it as vancomycin-resistant (VRSA), intermediate (VISA) or sensitive (VSSA) could not be carried out. In a similar study, conducted at Kasturba Medical College, Hospital, Mangalore, no vancomycin resistance was observed in MRSA isolates.[54] Complete sensitivity to vancomycin of *S. aureus* isolates was reported by Anupurba et al and Datta et al.[55, 56] In 2003, Assadullah et al reported staphylococcal isolates with intermediate susceptibility to vancomycin in India.[57] Tiwari and Sen reported two strains of VRSA in the northern parts of India.[58] Sharma and Vishwanath studied 156 MRSA isolates which were susceptible to vancomycin by disc diffusion method but, the MIC of 18 isolates was ≥ 4 µg/mL (VISA).[59]

This study showed 100 % susceptibility to linezolid and vancomycin. Vancomycin and linezolid were found to be the most sensitive drugs against *S. aureus* in studies by Agarwal et al and Bhatiani et al.[12, 32] Golan et al reported a significant trend in increased MRSA linezolid resistance from 2002 onwards.[60] Linezolid, a member of the new oxazolidone class of antibiotics is highly active *in vitro* against MRSA and has excellent oral bioavailability and constitutes the drug of choice against MRSA infection, besides vancomycin. The present study supported this.

Resistance to mupirocin is being reported from across the globe with a prevalence of 0.5 % in Nigeria to 14.6 % in India.[50, 61] Rapid resistance to mupirocin has been reported among some strains of *S. aureus* isolated from various hospitals. In the present study of 34 *S. aureus* isolates, sensitivity to mupirocin was 88 % with isolates having MIC < 0.5 µg/mL. Mohajeri et al reported 100 % sensitivity to mupirocin in the nasal carriage isolates of the patients.[62] Though mupirocin resistance was not seen in the *S. aureus* isolates in the study by Mohajeri et al, the MIC of 9.2 % of the isolates was as high as 4 µg/mL which was very close to a low level resistance (8 µg/mL).[62] In the study by Saderi et al, 6 strains had MIC > 4 µg/mL.[63] Abimanyu et al observed all MRSA isolates showed a high level mupirocin resistance and inducible clindamycin resistance.[64]

Agarwal et al reported that 4 (2 %) isolates were found to be mupirocin-resistant of which three isolates were high levels resistant.[12] In the presence of mupirocin-resistant strains, treatment with mupirocin may be ineffective, especially with high-level resistance strains. Although low-level mupirocin-resistant strains can be controlled by normal dosage schedule of mupirocin, a few studies suggest that treatment failure may occur. This emphasizes the importance of identification of both high and low-level resistant strains.[65-67]

Simple preventive measures like hand washing, using a sterile mask, gown and avoiding touching one's nose during work, should be reinforced in all healthcare settings. This study reiterates the need for periodic surveillance, early and accurate detection and treatment of MRSA carriers. This

should be accompanied with appropriate hospital infection control measures, to prevent the nasal carriage of MRSA in hospital healthcare workers.

Conclusion

In the present study, very high carriage rate was detected in the anterior nares which are also the commonest site for *Staphylococcus* colonisation. The results obtained from the antibiogram of Staphylococci, *S. aureus* and MRSA isolates from colonised HCWs showed the increase in rates of resistance against various antibiotics. The present study confirms for the first time the presence of MRSA in HCWs working in this hospital and demonstrates the prevalence of the antibiotic resistance amongst them. Vancomycin resistance in *Staphylococcus* species is beginning to emerge as a clinical threat, yet the attention it has received is scant and serves to underscore the seriousness of the problem.

A better understanding of these issues will be a key to help in the prevention and treatment of these infections in the future and in containing the spread of these from HCWs to patients and vice versa. All the HCWs should be periodically educated and trained in the maintenance of hygiene and infection control and the effects of the use or rather, the misuse of antibiotics.

The limitations of the study

The study enrolled HCWs from a single tertiary healthcare centre, however, to generalise the results multi-centric studies are required.

Contribution of Authors

MC was involved in planning, concept design and hypothesis generation, NA did data collection, AC did data assembly, literature review and manuscript writing, MB helped in statistical analysis, GP and VR helped in data interpretation and literature review, AT helped in manuscript writing and data visualisation. All the authors collaborated and finally approved the manuscript.

Ethics Statement

The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of the JLN Medical College, Ajmer (No 42954-85, dated 28-10-2016).

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None.

Conflict of interest

None.

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ORIGINAL ARTICLE

High-Sensitive Troponin-T as a Predictive Outcome Factor in COVID-19 Hospitalised Patients: Analysis After One-Year Follow-Up

Darko Stojanović,[1] Živko Ćetojević,[1] Boris Dujaković,[2] Mirko Stanetić,[1, 3] Tamara Kovačević-Preradović,[1, 3] Bojan Stanetić[1, 2]

(1) Medical Faculty, University of Banja Luka, Bosnia and Herzegovina.
(2) Department of Cardiology, University Clinical Centre of the Republic of Srpska, Banja Luka, Bosnia and Herzegovina.
(3) Department of Pulmonology, University Clinical Centre of the Republic of Srpska, Banja Luka, Bosnia and Herzegovina.

Abstract

Introduction: Since December 2019, the humanity is constantly under affection of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Despite global dissemination, neither the treatment or the specific predictive factors have been found or strictly defined yet.
Aim: Aim of this study was to assess the long-term (1 year) predictive value of high-sensitive Troponin T (hsTnT) in COVID-19 affected, hospitalised patients.
Methods: Between 5 March 2020 and 31 March 2020, 87 consecutive patients hospitalised at University Clinical Centre of the Republic of Srpska due to SARS-CoV2-caused pneumonia, in whom hsTnT was measured, were included. The Kaplan-Meier analysis was used to assess differences in all-cause mortality between the groups. Independent predictors of all-cause mortality were identified through univariate- and multivariate Cox regression analysis.
Results: Compared with patients who had normal hsTnT levels, patients with raised hsTnT were significantly older (70.7 ± 13.23 vs 49 ± 15.29; p < 0.001). Glucose values were significantly increased in patients with raised hsTnT (9.29 ± 5.14 vs 6.76 ± 2.46 [4.1-5.9] mmol/L; p = 0.005), as well as serum creatinine (179.07 ± 225.58 vs 87.53 ± 18.16 µmol/L; p = 0.01), hsTnT (187.43 ± 387.29 vs 7.58 ± 3.40 pg/mL; p = 0.003), D-dimer (5.94 ± 13.78 vs 1.04 ± 1.26 [0-0.50] mg/L; p = 0.024), C-reactive protein (125.92 ± 116.82 vs 69.97 ± 73.09) [< 5.0] mg/L; p = 0.009) and calcium (1.32 ± 0.46 vs 1.03 ± 0.173 [2.20-2.65] mmol/L; p = 0.001). Kaplan-Meier analysis revealed that the number of all-cause deaths at 1 year was 19 of whom 18 were presented with elevated hsTnT (log-rank p < 0.001). When univariate Cox regression was applied, multiple predictors of all-cause mortality have been identified ie age, haemoglobin, haematocrit, urea, CK-MB as well as hsTnT. In a multiple regression model, hsTnT remained an independent predictor of poor outcome.
Conclusion: Results from this study showed that the value of hsTnT during hospitalisation is possibly associated with long-term poor outcome of COVID-19 patients. Therefore, hsTnT may appear as a surrogate factor to differentiate between patients at high risk who need more intensive follow-ups.

Key words: COVID-19; High-sensitive Troponin-T; All-cause mortality.

Correspondence:
BOJAN STANETIĆ
E: bojan.stanetic@med.unibl.org

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Introduction

Since December 2019, the humanity is constantly under affection of Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes illness known as Coronavirus disease 2019 (COVID-19). As the first cases were recorded in Wuhan, Hubei Province, China no one assumed it

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would have impact in global proportions. The new virus has caused a lot of repercussions in all segments and spheres of human lives, such as health issues, economic consequences and changes in global politics. All over the globe, the new virus has spread onto (over) 223 countries (areas, territories) and infected 120,164,106 people to this day. Due to absence of specific treatment, a total of 2,660,422 people died because of infection, and these numbers continue to rise every day.[1] In the Republic of Srpska, first case of SARS-CoV-2 was registered on 5 March 2020. Since then, there were 47,993 confirmed cases, from which 2,420 people died, and 37,342 have successfully recovered.[2]

Until now, many impacts of SARS-CoV-2 to the human body were found.[1] Infection can be asymptomatic, it may have mild, moderate or severe symptoms, or it could have symptoms of critical illness (acute respiratory distress syndrome, respiratory failure, shock or multiorgan system dysfunction). Respiratory symptoms are the main manifestation of COVID-19 infection, but the evidence also indicates affection of many other systems: cardiovascular, gastrointestinal, nervous, as well as renal, endocrine and haematological manifestations.[1] The heart symptomatology varies, starting with increased serum levels of heart-specific enzymes, over various kinds of arrhythmias all the way to myocardial injury. There were also reported isolated cases of COVID-19-induced myocarditis, probably caused by direct myocardial injury of SARS-CoV-2. The fact that angiotensin-converting enzyme 2, the receptor SARS-CoV-2 uses, is significantly increased in cardiac tissue, support this thesis.[3] One study found that 16.7 % COVID-19 infected patients suffered from arrhythmias, while 7.2 % had severe myocardial injury.[4]

Despite global dissemination, neither the treatment or the specific predictive factors (especially in a long-term) have been found or strictly defined yet. Therefore, in order to establish correlation between specific parameters and organ systems (cardiovascular primarily) it was decided to gather a group of COVID-19 affected patients and did a follow-up study during the time of 365 days (1 year).

Methods

Study population

Between March 5, 2020 and March 31, 2020, consecutive patients hospitalised at University Clinical Centre of the Republic of Srpska due to SARS-CoV2-caused pneumonia, in whom high-sensitive Troponin-T (hsTnT) was measured, were included (Figure 1). The study group comprised of patients with polymerase-chain-reaction (PCR)-confirmed infection only. The study was approved by the local ethics committee. Since this was an observational (ie, non-interventional) study, informed consent had been waived.

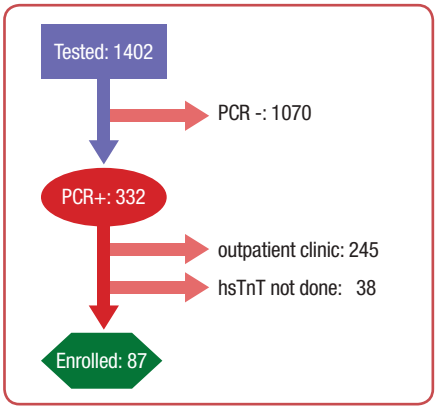

Figure 1:
Study flow chart

Data collection

Data acquisition consisted of separate entries for classical factors of affected person history, cardiovascular threat factors, laboratory exams and data on long-term 1-year follow-up. Laboratory values, which included hsTnT have been collected at admission as well as during hospitalisation. Peak values for hsTnT were used for calculation. Electrocardiogram (ECG) was not routinely recorded due to logistic reasons. Follow-ups have been retrieved from electronic and written records, as well as by phone contacts.

Patients were divided into two groups ie troponin-negative (hsTnT < 14) and troponin - positive (hsTnT ≥ 14). During the one-year follow-up of the initial presentation, data on all-cause death were acquired.

Statistical analysis

Statistical analysis was performed using SPSS (Version 25; MAC). Descriptive statistics were given as mean plus standard deviation or median plus interquartile range, based on the distribution, which was tested by Kolmogorov-Smirnov test.

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Categorical variables were presented as a number with a percentage and tested by Chi-squared test. Differences between means were assessed by the Student's t-test when normally distributed ie, Mann Whitney U-test when normal distribution could not be found. Kaplan-Meier analysis was used to assess differences in all-cause mortality between the groups. Independent predictors of mortality were identified through univariate- and multivariate Cox regression analysis.

Results

Patient characteristics

A total of 87 patients were included in this study, 44 of them (50.5 %) with raised hsTnT, and 43 (49.4 %) had normal hsTnT values. The median age of patients was 63 years (range, 22 – 88 years). Compared with patients who had normal hsTnT levels, patients with raised hsTnT were significantly older (70.7 (± 13.23) vs 49 (± 15.29); p < 0.001). Among these patients, fever (67 patients or 77 %) was the most common symptom. Cough, shortness of breath, fatigue and muscle pain were present in 44 patients (50.6 %), 39 patients (44.8 %), 51 patients (58.6 %) and 33 patients (37.9 %), respectively (Table 1). Headache (8 patients [9.2 %]), nausea (8 patients [9.2 %]) and diarrhoea (16 patients [18.4 %]) were rare. The most common coexisting conditions were diabetes (22 patients [25.3 %]) and hypertension (46 patients [52.9 %]). Six patients (6.9 %) had central nervous system disease, 2 (2.3 %) had liver disease, 9 patients (10.3 %) had renal disease and 8 patients (9.2 %) had problems with respiratory system. Twenty-seven patients (31 %) had heart issues, 10 of them ischaemic (11.5 %) and 11 of them (12.6 %) had some form of cardiomyopathy (Table 1).

Table 1: Baseline characteristics of hospitalised patients positive for COVID-19 related to hsTnT values

Variable	hsTNT ≤ 14.0	hsTNT > 14.0	p-value	Total
Gender				
Male	34	29	0.170	63
Female	9	15		24
Age				
0-50	19	4	0.000	23
> 50	24	40		64
Diabetes mellitus				
No	37	28	0.016	65
Yes	6	16		22
Dyslipidaemia				
No	41	39	0.250	80
Yes	2	5		7
Arterial hypertension				
No	31	10	0.000	41
Yes	12	34		46
CNS disease				
No	40	41	0.977	81
Yes	3	3		6
Liver disease				
No	43	42	0.157	85
Yes	0	2		2
Kidney disease				
No	41	37		78
Acute renal failure	0	1	0.158	1
Chronic renal failure	0	4		4
Other	2	2		4
Lung disease				
No	41	38	0.250	79
Obstructive lung disease	1	5		6
Other	1	1		2
Heart disease				
No	41	19		60
Ischemic heart disease	2	8		10
Valvular heart disease	0	3	0.000	3
Cardiomyopathy	0	11		11
Other	0	3		3
Carcinoma(s)				
No	43	37	0.006	80
Yes	0	7		7
Fever				
No	3	9	0.034	12
Yes	39	28		67
Cough				
No	12	16	0.334	28
Yes	24	20		44
Shortness of breath				
No	24	16	0.092	40
Yes	16	23		39
Fatigue				
No	15	13	0.580	28
Yes	24	27		51
Muscle pain				
No	20	21	0.451	41
Yes	19	14		33
Headache				
No	30	28	0.567	58
Yes	5	3		8
Nausea				
No	35	32	0.905	67
Yes	4	4		8
Diarrhoea				
No	35	28	0.197	63
Yes	6	10		16
Pneumonia				
No	3	5	0.329	8
Unilateral	7	3		10
Bilateral	32	35		67

hsTnT: high-sensitive Troponin-T, ref values: < 14.0 pg/mL; red colour: statistical significance;

CONTENT

Table 2: Laboratory findings of hospitalised patients positive for COVID-19 related to hsTnT values

Variable	hsTNT ≤ 14.0	hsTNT > 14.0	p-value
Gender	58.33 ± 16.80	65.91 ± 19.08	0.074
Age	49 ± 15.29	70.7 ± 13.23	0.000
Erythrocytes $\times 10^{12}$ (per L)	4.76 ± 0.65	4.12 ± 1.21	< 0.001
Haemoglobin (g/L)	141.58 ± 13.42	120.48 ± 2.89	< 0.001
Haematocrit (L/L)	0.43 ± 0.05	0.375 ± 0.1	< 0.001
Platelets $\times 10^9$ (per L)	201.16 ± 70.73	245.09 ± 110.45	0.030
Leukocytes $\times 10^9$ (per L)	6.41 ± 2.62	9.44 ± 5.46	0.002
Neutrophils (%)	70.0 ± 17.0	77.0 ± 22.25	0.076
Lymphocytes (%)	18.86 ± 9.66	13.73 ± 9.25	0.013
Monocytes (%)	10.33 ± 15.19	7.02 ± 4.45	0.170
Eosinophils (%)	0.55 ± 0.98	0.88 ± 1.67	0.270
Basophils (%)	0.14 ± 0.355	0.16 ± 0.370	0.860
Glucose (mmol/L)	6.76 ± 2.46	9.29 ± 5.14	0.005
BUN (mmol/L)	5.06 ± 1.69	2.01 ± 8.56	< 0.001
Creatinine (µmol/L)	88.0 ± 22.0	115.0 ± 85.0	< 0.001
AST (U/L)	45.91 ± 27.39	55.73 ± 55.18	0.303
ALT (U/L)	51.16 ± 45.15	54.08 ± 63.53	0.810
GGT (U/L)	76.73 ± 101.32	115.58 ± 100.73	0.274
LDH (U/L)	307.77 ± 134.64	368.20 ± 154.99	0.060
CK (U/L)	353.40 ± 488.97	394.63 ± 602.18	0.728
CK-MB (U/L)	22.28 ± 15.42	29.13 ± 18.68	0.101
HsTnT (pg/mL)	7.58 ± 3.40	187.43 ± 387.29	0.003
Feritin (µg/L)	1413.92 ± 1797.97	2253.37 ± 4293.38	0.380
D-dimer (µg/L)	1.04 ± 1.26	5.94 ± 13.78	0.024
CRP (mg/L)	69.97 ± 73.09	125.92 ± 116.82	0.009
PCT (ng/mL)	0.308 ± 0.73	6.53 ± 28.37	0.333
Sodium (mEq/L)	139.30 ± 2.82	139.40 ± 5.46	0.914
Potassium (mmol/L)	4.49 ± 0.59	4.65 ± 0.75	0.281
Calcium (mmol/L)	1.03 ± 0.173	1.32 ± 0.46	0.001
Chloride (mmol/L)	107.56 ± 6.73	107.69 ± 8.64	0.937
Lactate (mmol/L)	3.38 ± 1.07	3.69 ± 1.20	0.253

**BUN, blood urea nitrogen; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyltransferase; LDH, lactate dehydrogenase; CK, creatine kinase; CK-MB, creatine kinase – myocardial band; hsTnT, high sensitive troponin T; CRP, C-reactive protein; PCT, procalcitonin; red colour: statistical significance;*

Laboratory findings

Compared with patients who had normal hsTnT levels (Table 2), patients with raised hsTnT had lower red blood cell count (average, 4.11 ± 0.77) vs 4.83 ± 0.49 [4.00-6.3 x 10^{12}] cells/L; p < 0.001), haemoglobin count (120.48 (± 2.89) vs 141.58 (± 13.42) [120-180] g/L; p < 0.001), haematocrit count (0.37 ± 0.065) vs 0.42 ± 0.039) [0.40-0.52] L/L; p < 0.001), higher platelet count (245.09 ± 110.45 vs 201.16 ± 70.73) [140-440 x 10^9] cells/L; p = 0.03) and higher white blood cell count (9.44 ± 5.46 vs 6.41 ± 2.62 [4.0-10.0 x 10^9] cells/L; p = 0.002), but lower lymphocyte count (13.73 ± 9.25 vs 18.86 ± 9.66) [20.0-45.0] %; p = 0.013); other leucocyte fractions did not differ significantly (neutrophils, monocytes, eosinophils, basophils). Glucose values were significantly increased in patients with raised hsTnT (9.29 ± 5.14 vs 6.76 ± 2.46 [4.1-5.9] mmol/L; p = 0.005), as well as creatinine (179.07 ± 225.58 vs 87.53 ± 18.16) [59-104] µmol/L; p = 0.01), hsTnT (187.43 ± 387.29 vs 7.58 (± 3.40) [pg/mL]; p = 0.003), D-dimer values (5.94 ± 13.78) vs 1.04 ± 1.26 [0-0.50] mg/L; p = 0.024), C-reactive protein levels (125.92 ± 116.82 vs 69.97 ± 73.09 [<5.0] mg/L; p = 0.009) and calcium levels (1.32 ± 0.46 vs 1.03 ± 0.173) [2.20-2.65] mmol/L; p = 0.001). The liver enzymes (aspartate-aminotransferase, alanine-aminotransferase, gamma-glutamyl transferase) as well as other heart-specific enzymes (lactate-dehydrogenase, creatine-kinase, creatine-kinase myocardial band, lactate), ferritin, electrolytes (sodium, potassium, calcium and chloride) – showed no significant difference between the target groups.

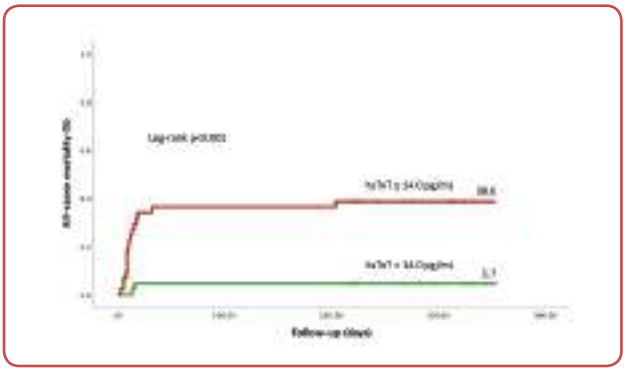

Figure 2: Kaplan-Meier analysis. The cumulative incidence rates of all-cause death between patients with and without elevated high-sensitive Troponin T at 1-year follow-up

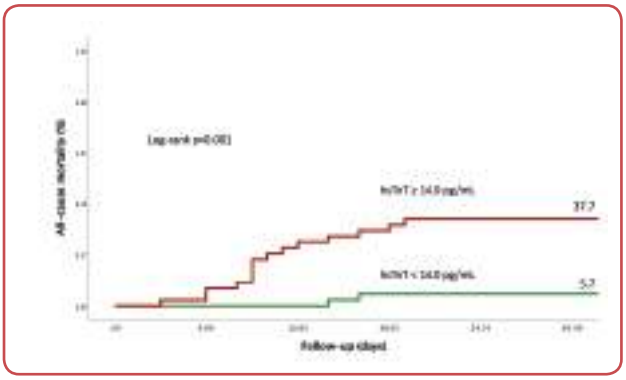

Figure 3: Kaplan-Meier analysis. The cumulative incidence rates of all-cause death between patients with and without elevated high-sensitive Troponin T at 30-days follow-up

All-cause mortality and predictors analysis

The number of all-cause deaths at 1 year was 19 of whom 18 were presented with elevated hsTnT (log-rank p < 0.001; Figure 2). The vast majority of deaths occurred in the first 30 days (Figure 3). When univariate logistic regression was applied (Table 3), multiple predictors of all-cause mortality have been identified, ie age (p = 0.003), haemoglobin (p = 0.007), haematocrit (p = 0.017), urea (p = 0.030), CK-MB (p = 0.001) as well as hsTnT (p = 0.002). In a multiple regression model, hsTnT was identified as an independent predictor

CONTENT

Table 3: Univariate and multivariate Cox regression analysis of mortality predictors of hospitalised patients positive for COVID-19

Variable	Univariate regression			Multivariate regression		
	HR	CI 95 %	p	HR	CI 95 %	p
Gender	1.223	0.465-3.217	0.684			
Age	1.059	1.020-1.099	0.003	1.047	0.958-1.143	0.314
Erythrocytes	0.453	0.255-0.804	0.007	1.842	0.105-32.212	0.676
Haemoglobin	0.976	0.956-0.995	0.015	1.014	0.861-1.195	0.868
Haematocrit	0.000	0.000-0.210	0.017	0.000	0.000-1.499E+15	0.417
Platelets	0.998	0.993-1.003	0.476			
Leukocytes	1.000	0.906-1.103	0.996			
Neutrophils	1.002	0.979-1.026	0.857			
Lymphocytes	0.969	0.922-1.019	0.222			
Monocytes	0.926	0.831-1.031	0.162			
Eosinophils	0.814	0.516-1.284	0.375			
Basophils	0.038	0.000-13.445	0.274			
Glucose	1.052	0.966-1.146	0.242			
BUN	1.049	1.005-1.095	0.030	0.880	0.731-1.060	0.178
Creatinine	1.001	0.999-1.003	0.514			
AST	0.991	0.975-1.007	0.266			
ALT	0.994	0.982-1.006	0.348			
LDH	1.001	0.999-1.004	0.339			
GGT	1.003	0.999-1.008	0.155			
CK	1.000	0.999-1.001	0.722			
CK-MB	1.035	1.015-1.056	0.001	1.055	1.024-1.087	0.000
hsTnT	10.189	2.351-44.147	0.002	1.621	1.202-1.822	0.001
Feritine	1.000	1.000-1.000	0.100			
D-dimer	1.001	0.960-1.043	0.975			
CRP	0.999	0.994-1.004	0.690			
PCT	0.991	0.948-1.037	0.708			
Sodium	0.988	0.886-1.103	0.833			
Potassium	1.186	0.613-2.293	0.613			
Calcium	1.650	0.601-4.532	0.331			
Chloride	1.011	0.950-1.075	0.739			
Lactate	1.300	0.909-1.860	0.150			
Diabetes mellitus	1.363	0.518-3.586	0.531			
Arterial hypertension	3.864	1.282-11-650	0.016	0.682	0.069-6.767	0.744
Dyslipidaemia	0.618	0.082-4.627	0.639			
Heart disease	1.703	1.283-2.260	0.000	1.127	0.671-1.895	0.651
CNS disease	3.657	1.061-12.599	0.040	992.742	21.287-46298.101	0.000
Kidney disease	1.075	0.757-1.527	0.687			
Pulmonary disease	1.490	0.816-2.722	0.194			
Liver disease	2.753	0.366-20.698	0.325			
Cancer	3.784	1.251-11.452	0.018	3.300	0.469-23.193	0.230
Fever	0.225	0.087-0.583	0.002	0.400	0.083-1.919	0.252
Cough	0.864	0.300-2.491	0.787			
Shortness of breath	2.253	0.845-6.003	0.104			
Fatigue	1.702	0.613-4.726	0.308			
Muscle pain	0.708	0.257-1.949	0.504			
Headache	2.221	0.610-8.087	0.226			
Nausea	0.565	0.074-4.294	0.581			
Diarrhoea	0.555	0.126-2.444	0.437			
Pneumonia	1.407	0.596-3.319	0.436			

BUN, blood urea nitrogen; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl-transferase; LDH, lactate dehydrogenase; CK, creatine kinase; CK-MB, creatine kinase – myocardial band; hsTnT, high sensitive troponin T; CRP, C-reactive protein; PCT, procalcitonin; CNS, central nervous system; red colour: statistical significance; HR, hazard ratio; CI, confidence interval;

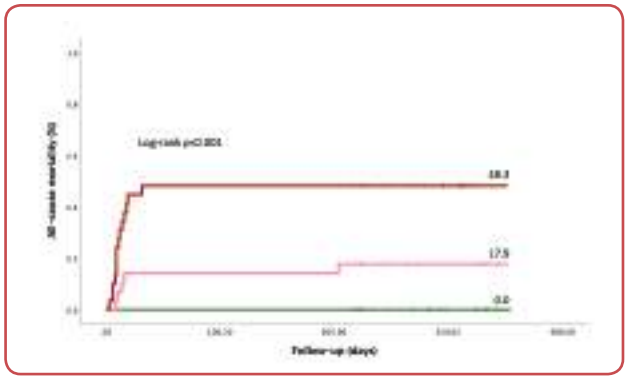

Figure 4: Kaplan-Meier analysis. The cumulative incidence rates of all-cause death stratified by high-sensitive Troponin T terciles at 1-year follow-up

of poor outcome. In addition, the value of hsTnT correlated well with incidence of mortality (Kaplan-Meier analysis by hsTnT terciles – Figure 4).

Discussion

To our best knowledge, this study represented first report with follow-up of 1 year. It was shown that correlation exists between elevated hsTnT value and mortality in patients with COVID-19. After 1 year of hospitalisation, the value of hsTnT higher or equal than 14.0 pg/mL was associated with high risk of mortality in the studied population.

Cardiac troponin is one of the most important biomarkers in indication of cardiac injury. The troponin complex regulates the contraction of striated muscles and consists of three subunits – troponin C, troponin T and troponin I. In this region, troponin T is mostly used in diagnosis of cardiac injury. After myocardial injury, troponin starts to rise in peripheral blood within three to four hours and stays increased for ten to fourteen days. The result of modification of the fourth generation of cardiac assay is the new highly sensitive troponin, which is capable of detecting more subtle elevations indicative of cardiac injury. The normal range for high-sensitive troponin T in peripheral blood is up to 14 pg/mL.[5] The role of troponin in COVID-19 in uncertain. In the Mayo clinic analysis of 367 patients, elevations of hsTnT were in 95 % due to isolated myocardial injury. Most increases are due to either chronic illness such as heart failure or renal disease, followed by acute aetiologies such as critical illness or sepsis. Myocarditis was rare; it was suspected as a potential cause of myocardial injury in only 3 patients and the diagnosis was not proven with cardiac magnetic resonance or biopsy.[6] But in the Mayo clinic study elevated hsTnT were not predictor of mortality, just of events. Another studies from China, however, showed significant impact of troponin on mortality. Multivariate Cox regression analysis on 1,717 patients from the Tongji Hospital and the Hubei Xinhua hospitals identified troponin I as an independent predictor of 30-day mortality.[7] In-hospital mortality was 10-fold higher in hospitalised patients with COVID-19 in Wuhan with myocardial injury (high-sensitive cardiac troponin above the 99th-percentile upper reference limit) which may reflect illness severity due to myocardial oxygen supply–demand imbalance.[8] In this study of 87 patients who were hospitalised a year ago in University Clinical Centre of Republic of Srpska, the value of hsTnT was monitored and it was tried to establish the correlation between its value and outcome of hospitalised patients. It was found that 44 out of 87 (50.5 %) hospitalised patients had hsTnT value higher than 14.0. The highest measured hsTnT was 2126 pg/mL and the lowest was 3 pg/mL. The number of death cases was 19, while 18 of them were presented with high value of hsTnT. This finding showed that the value of hsTnT is possibly associated with outcome of COVID-19 positive patients. The factor that contributes to high mortality and cardiac damage was age: out of 44 patients with elevated hsTnT, 40 of them were older than 50 years (Table 1). It is a strong predictor in univariate regression model ($p = 0.003$) (Table 3). Among 46 patients with chronic arterial hypertension, 34 of them (73.9 %) had hsTnT values over 14.0 pg/mL. Among 19 death outcomes, 15 of them (78.9 %) were presented with arterial hypertension and all of them had elevated hsTnT (the lowest 14.5 and the highest 2126 pg/mL). Also, the study found that among 22 patients with diabetes, 6 had death outcome (27.2 %), and all 6 were presented with hsTnT levels over 14.0 (range between 18.6 – 2126 pg/mL).

Based on many previous studies, angiotensin-converting enzyme 2 (ACE_2) is a human cell receptor with a strong binding affinity to the Spike protein of the SARS-CoV-2, and ACE_2 is also highly expressed in heart, so it can be assumed that COVID-19–induced cardiac injury might be mediated by ACE_2.[9] Autopsy report from other

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study showed interstitial mononuclear inflammatory infiltrates in heart tissue. Inflammatory biomarkers were significantly increased in patients with cardiovascular diseases, indicating that inflammatory cell necrosis promoted inflammatory response and led to cytokine storm damage to the myocardium, which can be severe and even lead to fulminant myocarditis.[4] Moreover, the systemic inflammatory response may trigger rupture or erosion of coronary plaques in patients with underlying coronary artery disease.[10, 11] The overall mortality rate in this study was 21.8 % (19 out of 87 patients). Similar findings were found in a meta-analysis of 12,262 patients from 13 studies where mortality rate was 23 % (20-26 %). Elevated troponins were found in 31 % (23-38 %) with clear association with increased mortality (odds ratio 4.75, CI 4.07-5.53; $p < 0.01$) suggesting that chance of mortality is 45 % in patients with elevated troponin and 14 % in patients with non-elevated troponin.[12] Interestingly enough, the correlation was not influenced by age ($p = 0.218$), male gender ($p = 0.707$), hypertension ($p = 0.182$), diabetes ($p = 0.906$) or coronary artery disease ($p = 0.864$).

Recently, in a meta-analysis of 51 studies,[13] elevated troponin values were associated with a higher risk of subsequent death during hospitalisation (RR 2.68, 95 % CI 2.08–3.46). The same analysis showed that after adjustment for confounders, troponin was an independent predictor of mortality in multivariable analysis.[13] These results revealed similar predictive performance in a long-term follow-up (HR 1.621, 95 % CI 1.202-1.822). Even more interesting is the fact that the meta-analysis found a post-test probability of death about 42 % for patients with raised troponins on admission (in this analysis 18 out of 44 patients (40.9 %) with hsTnT ≥ 14 died during follow-up). Another cohort study which included 1,053 patients with COVID-19 revealed that troponin I higher than 0.34 ng/mL was strong independent predictor of 1-month mortality (OR 4.38; $p < 0.001$). Patients with a mortality score using hypoxia at admission, age, and troponin I elevation, age (HA2T2) ≥ 3 had a 1-month mortality of 43.7 % whereas the remaining population had mortality of 5.9 % (AUC 0.834).

Limitations

This study has some limitations. Because of the observational and retrospective nature of the study, data collection may be incomplete. Some data were missing at the beginning of research, ie hypoxia measurements were not done in all patients. Furthermore, due to logistic reasons additional diagnostic tests were not routinely recorded to rule out STEMI or NSTEMI, ie ECG and echocardiogram. Since this was not a randomised trial, there was no possibility to exclude the potential selection bias. The sample size was small, however, follow-up at one year was collected.

Conclusion

1. Elevated values of highly sensitive troponins represent state of myocardial injury, which was not rare and strong predictor in COVID-19 patients.
2. There are multiple conditions, such as worsening of chronic ischaemia or acute inflammation, which can lead in troponin increase.
3. High-sensitive Troponin T may appear as a surrogate factor to differentiate between patients at high risk who need more intensive follow-up.

Acknowledgements

None.

Conflict of interest

None.

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Bansal et al. Scr Med 2021 Jun;52(2):104-8. DOI:10.5937/scriptamed52-32096
ORIGINAL ARTICLE

Probabilistic Model to Predict the Outcome in Acute Suicidal Chemical Poisoning Cases From Age and Gender of Patient and Type of Chemical Poison Consumed

Alka Bansal,[1] Smita Jain,[2] Ashish Agrawal,[3] Monica Jain,[1] Shivankan Kakkar,[1] Sneha Arora[1]

(1) Department of Pharmacology, SMS Medical College, Jaipur-302004, Rajasthan, India.
(2) Department of Mathematics and Statistics, JECRC University, JECRC university, Jaipur-302018, Rajasthan, India.
(3) Ashish's Clinic, A-207 Triveni Nagar, Gopalpura Bypass, Jaipur-302018, Rajasthan, India.

Correspondence:
SHIVANKAN KAKKAR
E: drshivankankakkar@gmail.com
T: +919828276839

Abstract

Background: Acute chemical poisoning is a significant global health problem. Chemical poisons include agrochemical, household and industrial poison subtypes. The present study used a probabilistic model based on age, gender and type of poison consumed by the patient to predict the outcome in acute suicidal poisoning cases.
Material and methods: A prospective observational study was conducted at emergency department of SMS Hospital, Jaipur, India, from January 2019 to February 2020. Patients over 15 years of age with poisoning severity score 2 or above were included in the study. Probabilistic model was used to predict the outcome measured in terms of cure, death and left against medical advice (LAMA) using Minitab 14.
Results: Poisoning cases were 0.32 % of all emergency presentations. Out of them, 857 (59.6 %) had consumed chemical poison. Their mean age was 32 years and men to women ratio was 1.22. Agrochemical subtype was most common followed by household and industrial poisoning. Analysis by Probabilistic model showed that person between 30-60 years is more likely to be cured and chances of death and LAMA are highest in age group 60-75. Gender-wise, men have higher possibility for recovery. Besides, a person has highest chances of recovery in case of household poisons; death is most common in industrial poisons and LAMA in agrochemical poisons.
Conclusion: The study concluded that in poisoning, patients' basic information like age, gender, type of poison consumed can be used to identify high death probability and LAMA risk patients. It will assist in designing and monitoring the most effective strategies for them.

Key words: Cure; Death; LAMA; Outcome; Poisoning; Probabilistic model.

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Introduction

Poison is any substance that by means of its exposure due to ingestion, inhalation or contact can injure, kill, or impair normal physiological function in humans and produce general or local damage in the body.[1, 2] Acute poisoning either with single or multiple exposures within 24 hours is a significant global health problem responsible for large number of emergency presentations.[3] According to WHO in 2012, it ranked 45th in total death worldwide, while in India, it accounted for the fourth common cause of mortality.[1, 2]

Poisons can be divided into four major categories: chemical poisons, drugs and medications

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overdose, poisons of biologic origin and radiation. Chemical poisons include agricultural, household and industrial poisons subtypes. Organophosphates, carbamates, paraquat, rodenticides, chlorinated hydrocarbons, pyrethroid and fertilisers are various types of agrochemicals. Household poisons are products used in kitchens, bathrooms and day to day life like detergents, carbon tetrachloride, naphthalene, boric acid, oxalic acid, bleaching agents, chlorates, hydrogen peroxide etc. Industrial poisons include organic and inorganic acidic and alkali corrosives, hydrocarbons, oxidising agents, alcohols, glycols and metallic poisons.[4, 5]

Whether taken intentionally, forcibly or accidentally, chemical poisoning is commonly found in all age groups and in both genders. Also it is on rising trend due to ever increasing use of chemicals with industrial and agricultural field revolution in recent years combined with lifestyle and behaviour change, easy availability, unrestricted sale and low cost.[6] Though many studies regarding clinic-epidemiologic pattern of poisoning are available, only few of them explore the possible relationship between patient's basic characteristics, product used for poisoning and probable outcome.[7, 8, 9] Hence, this study was exclusively planned with following objective.

Material and methods

A prospective observational study was conducted at emergency department of SMS hospital, a premier tertiary care institute of North India from January 2019 to February 2020, after the approval of the study protocol by the institutional ethics committee via letter No 2684MC/EC/2016 dated 30/9/16 and in continuation of STROBE guidelines.

All patients above 15 years of age presenting as acute suicidal poisoning cases of chemical subtype (ie agrochemical, household and industrial) and belonging to grades 2 or above for The poisoning severity score (PSS) at the time of presentation were included in the study after patient's own or their guardian's consent (if they were incapable of giving so due to their illness) was obtained.

The PSS was developed by the European Association of Poisons Centres and Clinical Toxicologists (EAPCCT), the International Programme on Chemical Safety (IPCS), and the Commission of the European Union between 1990 and 1994 to provide a simple and reliable scoring system to describe poisonings and define their severity. It classifies poison cases into four grades (also known as scores) 0, 1, 2, 3, 4. Score 0 means no symptoms/signs related to poisoning; 1 means mild, transient, and spontaneously resolving symptoms/signs; score 2 stands for moderately pronounced/prolonged symptoms/signs; score 3 identifies severe life threatening symptoms/signs and score 4 means fatal cases attributed to poison. It is to clarify here that PSS is clinically based and do not depend on dose and nature of poison consumed.[10, 11]

Drug overdoses and poisons of biologic origin as in stings, bites and food poisoning were excluded from the study as in drug overdose, patients usually have associated chronic psychiatric and biologic, radiation origin poisons are mainly accidental. Children (< 15 years) were also excluded as this hospital cares to adults only.

The data pertaining to patient's age, gender and poison consumed was collected. Data was analysed using software Minitab 14, Pennsylvania, USA. Descriptive data was expressed in terms of numbers and percentages. Chi-squared test was applied to find association between age, gender, type of poison consumed and final outcome of patient measured in terms of lethal outcome, recovery or left against medical advice (LAMA). Probabilistic model was used to predict the outcome based on rest three parameters.

Results

Total 1,427 cases were found to be related to poisoning among the 441,204 patients who visited emergency department during 14 months of study period (0.32 %). Out of them 857 cases of acute suicidal poisoning by chemicals were identified making 58.8 % of total poisoning cases. Remaining 570 patients were either accidental or homicidal in case of chemical or caused by drug overdose / poison of biologic origin/ radiation. Men outnumbered women in ratio 1.22.

Mean age in the both sexes was 32 years. As shown in Table 1, amongst chemicals, agrochemicals were most commonly used in 59.6 % of over-

Table 1: Summary of acute chemical poisoning cases

	Poison Type - N (%)				
	Agrochemical	Household	Industrial	Total	p-value
Gender					
Female	224 (43.84)	97 (47.55)	65 (45.77)	386 (45.04)	p = 0.654
Male	287 (56.16)	107 (52.45)	77 (54.23)	471 (54.96)	
Outcome					
Cure	388 (75.93)	159 (77.94)	100 (70.42)	647 (75.49)	p = 0.247
Death	81 (15.85)	31 (15.20)	33 (23.24)	145 (16.92)	
LAMA	42 (8.22)	14 (6.86)	9 (6.34)	65 (7.58)	
Age Groups*					
15-30	305 (59.69)	119 (58.33)	82 (57.75)	506 (59.04)	p = 0.869
30-45	144 (28.18)	57 (27.94)	46 (32.39)	247 (28.82)	
45-60	29 (5.68)	11 (5.39)	7 (4.93)	47 (5.48)	
60-75	33 (6.46)	17 (8.33)	7 (4.93)	57 (6.65)	
Total	511 (59.62)	204 (23.80)	142 (16.56)	857 (100.00)	

**For making the age group interval, both upper and lower limit for the first and last class were considered and excluded the lower limit for the middle two classes; LAMA: left against medical advice.*

all cases and also in all age groups followed by household and industrial poisons. Fifty-nine percentage of patients were between 15-30 years of age succeeded by 30-45 years of age group. By and large mortality was seen in 16.9 % of total cases with considerable 7.6 % patients who left the hospital against medical advice (LAMA).

Statistical analysis

To find the association between all the factors: age group, gender, outcome, poison type; Chi-squared test was applied. As shown in Table 1 and 2, p-value for all the factors was greater than 0.05 (level of significance in this study), hence it was concluded that none of them affected each other. Then the data was evaluated using conditional probabilistic model. For it first the events were defined as given below:

A = Outcome; A1: Cured, A2: Death, A3: LAMA
B = Poison type; B1: Agrochemical, B2: Household, B3: Industrial
C = Gender; C1: Men, C2: Women
D = Age group; D1: 15-30, D2: 30-45, D3: 45-60, D4: 60-75

Then the formula was applied using software. Results obtained have been summarised in Table 3.

It can be interpreted from values shown in Table 3, that according to probabilistic model, type of poison consumed affected the outcome of patient. A person randomly selected from the population has highest chances of cure if he/she has consumed household poison, highest chances of death if he/she has taken industrial poison and the highest chances of LAMA if he/she has consumed agrochemical poison.

Table 2: Age-wise distribution of outcome

	Outcome - N (%)				
	Cured	Death	LAMA	Total	p-value
Age (years)					
15-30	382 (75.49)	84 (16.60)	40 (7.91)	40 (7.91)	p = 0.446
30-45	191 (77.33)	37 (14.98)	19 (7.69)	19 (7.69)	
45-60	36 (76.60)	10 (21.28)	1 (2.13)	1 (2.13)	
60-75	38 (66.67)	14 (24.56)	5 (8.77)	5 (8.77)	
Total	647 (73.94)	145 (16.57)	65 (7.43)	857 (100.00)	

Table 3: Conditional probabilities values to depict effect of age, gender and type of poison consumed on final outcome of patients

Conditional probabilities			
	Outcome (A)		
Poison Type (B)	Cure (A1)	Death (A2)	LAMA (A3)
Agrochemical (B1)	0.76	0.16	0.08
Household (B2)	0.78	0.15	0.07
Industrial (B3)	0.71	0.23	0.06
Gender (C)			
Men (C1)	0.76	0.16	0.08
Women (C2)	0.74	0.18	0.08
Age Group (D)			
15-30 (D1)	0.75	0.17	0.07
30-45 (D2)	0.77	0.15	0.08
45-60 (D3)	0.77	0.21	0.02
60-75 (D4)	0.66	0.25	0.09

Same model revealed that men have higher possibility for recovery and women are more prone to lethal outcome in acute chemical suicidal poisoning cases. Furthermore, it was also found that a randomly selected person has highest chances of cure if he/she is between 30-60 years of age and highest chances of death and LAMA if he/she is from the age group 60-75.

Discussion

This study found 0.32 % consultations in emergency were related to poisoning. Out of them 58.8 % were exclusively pertinent to acute exposure of chemicals. A total of 59 % of patients included in the study were between 15-30 years of age and the overall men to women ratio was 1.22. Many congruent studies have also reported 0.3 to 0.6 % of emergency presentations due to poisons.[7, 12, 13]

As in this study, most studies found maximum incidence of poisoning in age group less than 40 years and with men predominance, though, Ethiopia reports mixed results related to gender pre-

ponderance in different studies.[1,12-14] The suggested explanation to high incidence of poisoning in men is that they are comparatively more exposed to stress and strain due to myriad reasons like financial difficulties, loss of job, discord at home and workplace etc, leading to mental vulnerability and impulsiveness of youth population. 59.6 % cases in the present study had agrochemical poisoning. Similar findings were reported by other researchers.[1, 12-14] However, as per analysis of calls to National Poisons Information Centre, New Delhi, 44.1 % of poison related calls were due to household agents followed by drugs (18.8 %), agricultural pesticides (12.8 %), industrial chemicals (8.9 %).[2]

As the outcome was concerned, 16.9 % of patients in the present study succumbed to poisoning, 7.6 % were LAMA cases and rest 75.5 % were cured with maximum cure rate in household poisons (77.9 %). These results were in partial agreement with study by Sharminder et al from India who also mentioned overall mortality of 9.7 %, LAMA in 3.1 % and least case fatality in household poisons.[12] However, Getie et al from Ethiopia found 93.3 % poison cases were cured, as against only 63 % of cure incidence in another study from the same country.[13, 14]

Analysis by probabilistic model in this study revealed advanced age to be an independent predictor of mortality (Table 3) as well as LAMA. These findings were in concordance with those by Gudduz et al, who also found higher survival in younger patients.[15] Accumulation of the toxins in the body owing to the increased amount of fat tissue and their slower metabolism by liver with aging might be responsible for it. Moreover, the propensity to respiratory failure, an important cause of mortality, also increases with age. However, no study to compare the high incidence of LAMA in elderly population could be found.

Contradictory to the findings presented in this paper where men had comparatively more cure rate and women showed high probability of death, Reddy et al and Lee et al reported men as an independent variable contributing to increased mortality rate (odds ratio up to 2.5).[16, 17] However, Munera Khan et al found no difference in gender-based outcome of poisoning.[18]

Consumption of industrial poison in the present study was connected to highest mortality rate followed by agrochemical and household poisons in that order. Bhadade et al had similar findings that chemical poisoning with methanol, kerosene, diesel, phenyl (industrial poisons) were associated with high mortality in 34.31 % cases followed by pesticides (agrochemical) in 25 % patients.[1] However, in study by Shan et al the mortality rate between patient groups ingesting different types of chemicals did not differ.[19] One study reported surfactant group (household) with an unexpectedly high mortality rate of 75 %, but surfactant cases in total were the least common, accounting for only 5 % of cases.[20]

Conclusion

The study concluded that elderly population and those who consume industrial poison are at higher probability to succumb to poison in contrast to patients between 30-45 years of age and consumers of household poisons who have maximum chances of recovery. Identification of high death and LAMA risk patients on the basis of basic information soon after presentation will eventually be helpful in designing and monitoring the most effective strategies for them.

Strengths of study

It is a novel study of its own kind to show that simple variables like age, gender and knowledge about type of poison consumed can be used as an outcome predictor in acute chemical poisoning cases.

Limitations of study

Other factors like socioeconomic status, marital status, reason of poisoning, seasonal changes were not considered for the sake of simplicity, saving time and due to lack of reliable information.

CONTENT

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None.

Conflict of interest

None.

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ORIGINAL ARTICLE

Effects of Esmolol Infusion on Cardiovascular Parameters and Quality of General Anaesthesia in Younger and Older Patients

Dragana Lončar-Stojiljković,[1, 2, 3]

(1) Faculty of Medicine, University of Banja Luka, Banja Luka, the Republic of Srpska, Bosnia and Herzegovina.
(2) Dedinje Cardiovascular Institute, Belgrade, Serbia.
(3) Military Medical Academy, Belgrade, Serbia.

Abstract

Background: Esmolol is a cardioselective β-adrenergic antagonist that is used during general anaesthesia to blunt the sympathetic reflex tachycardia and hypertension. The aim of the study was to investigate whether the potential beneficial and adverse effects of esmolol differ depending on the patient age.
Methods: A total of 50 ASA I/II patients scheduled for elective upper abdominal surgery were divided in two groups: younger (patients aged up to 35 years) and older (patients older than 65). After premedication with diazepam, they were infused with esmolol during the first 5 min at a rate of 0.3 mg/kg/min and 0.1 mg/kg/min thereafter. Anaesthesia was induced with thiopental sodium 3-5 mg/kg intravenously (iv) and fentanyl 1.5 μg/kg iv. Tracheal intubation was facilitated with suxamethonium 1-2 mg/kg iv. Long-term neuromuscular blockade was induced with pancuronium bromide 0.07 mg/kg iv bolus and maintained with incremental iv boluses of 0.01 mg/kg. Inhalational anaesthesia was maintained with a mixture of oxygen and nitrous oxide (O_2/N_2O) 2 : 1.
Results: The systolic blood pressure remained constant during the intubation phase in the group of older patients, at the same time being around 89 % of the pre-induction values, while in younger patients it rose up to 100 %. During the same phase of anaesthesia, the diastolic blood pressure in older patients remained at about 91 %, while in younger patients it rose up to 107 % of the pre-induction values. The consumption of drugs and the speed and quality of the recovery from anaesthesia did not differ between the two groups of patients.
Conclusion: Infusion of esmolol contributes to the concept of general balanced anaesthesia in elective patients scheduled for upper abdominal surgery equally in younger and older patients.

Key words: Esmolol; General balanced anaesthesia; Fentanyl; Thiopental sodium; Atropine.

Correspondence:
DRAGANA LONČAR-STOJILJKOVIĆ
E: dragana.loncar-stojiljkovic@med.unibl.org

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Introduction

Esmolol – methyl 3-{4-[2-hydroxy-3-(isopropylamino)propoxy]phenyl}propionate hydrochloride - is a selective, hydrosoluble β_1-adrenergic receptor blocker described first in 1982 by Zereslinski et al.[1] It has a unique pharmacokinetics, since it is rapidly hydrolysed, which results in elimination half-time between 9 and 10 min.[2, 3] It was demonstrated that the esterase responsible for such a rapid hydrolysis of esmolol is located in the cytosol of human erythrocytes.[4] These properties of esmolol make it easily titratable, which enables a perfect control of the degree of β-blockade, in con-

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trast to the undesirable effects of the intravenous (iv) preparations of propranolol and metoprolol.[5]

Esmolol has a 34-fold higher affinity for β_1- in comparison with β_2-adrenoceptors.[6] For this reason, it was first approved for treatment of supraventricular tachyarrhythmias.[7] Later adopted indications include: hypertension and tachycardia following tracheal intubation and other critical phases of anaesthesia and operation in non-cardiac[8,9,10] and cardiac surgery,[11,12] but also in non-surgical indications, such as electroconvulsive therapy,[13] thyrotoxic storm[14-17] and treatment of septic patients.[18-20]

It is now well-stablished that esmolol alleviates a reflex hypertension and tachycardia that occur due to extremely painful stimuli that accompany some critical phases of anaesthesia and operation, contributing thus to the concept of general balanced anaesthesia.[21,22] At the same time, blood pressure in humans increases in a linear manner with age,[23,24] as well as the prevalence of bradycardia.[25] Given the mechanism of action of esmolol, the question arises whether the efficacy and safety of esmolol infusions during the perioperative period can bring different beneficial and adverse effects in older patients, in comparison with the younger ones. This was the aim of the present study.

Methods

A total of 50 patients scheduled for upper abdominal elective surgery at the Military Medical Academy was enrolled in this clinical trial. It was approved by the local Ethics Committee. They had to be ≥ 18 of age and without any significant cardiovascular and metabolic morbidities (acute coronary disease, past myocardial infarction, transient ischaemic attack or stroke, diabetes) and of ASA I or II grade. Patients older than 35 but younger than 65 were excluded. In this way, two groups were formed – the younger (N = 26) and the older patient group (N = 24).

All patients received prior to induction of anaesthesia an iv infusion of esmolol in glucose solution 5 %, during the first 5 min at a rate of 0.3 mg/kg/min and 0.1 mg/kg/min thereafter. This maintenance infusion rate was selected based on the notion that hypotension rarely occurred when the esmolol infusion rate was below 0.15 mg/kg/min.[26]

All patients received the same type of anaesthesia. Premedication consisted of diazepam 10 mg intramuscularly (im), administered 30-45 min before anaesthesia. Anaesthesia was induced with thiopental sodium 3-5 mg/kg iv and fentanyl 1.5 μg/kg iv. Tracheal intubation was facilitated with suxamethonium 1-2 mg/kg iv. Long-term neuromuscular blockade was induced by pancuronium bromide 0.07 mg/kg iv bolus, while the desirable level of neuromuscular relaxation was maintained by its incremental iv boluses of 0.01 mg/kg. Inhalational anaesthesia was maintained with a mixture of oxygen and nitrous oxide (O_2/N_2O) = 2 : 1.

Episodes of increase of systolic and diastolic blood pressure and heart rate by more than 20 % of its pre-induction values were treated with iv boluses of fentanyl 1.5 μg/kg alone or in combination with droperidol 1:50 (Thalamonal®). In case of unfavourable response, isoflurane 0.5 % was added as an inhalation. Upon completion of the operation, atropine 0.5 mg iv was injected and the residual neuromuscular block was antagonised with neostigmine 1.5 mg iv. The same dose of atropine was used as an iv bolus during the operation in case of bradycardia, which was defined as a decrease of heart rate below 60/min or by more than 20 % of the pre-induction values.[25]

Registration of all major cardiovascular parameters – systolic and diastolic blood pressure and heart rate was performed in critical phases of anaesthesia and intubation: (1) before the induction (baseline), (2) induction to anaesthesia, (3) tracheal intubation, (4) first skin incision, (5) surgical manipulation with organs, (6) suture of the surgical wound and (7) tracheal extubation. Besides, total consumption of fentanyl, droperidol, atropine, pancuronium and isoflurane was registered. Quality of the post-anaesthesia recovery was based on the registration of times needed to regain ability to comply with simple commands (eg, eye opening), to open eyes spontaneously and until regaining full orientation. Adverse effects were registered and scored: 1 – mild, 2 – moderate and 3 – strong. Overall assessment of the quality of anaesthesia was performed by the experienced anaesthesiologist, by using the scale: 1 – poor, 2 – good and 3 – excellent.

Statistical analysis was performed by using para-

metric or non/parametric tests, depending on the nature of the parameters observed and the normality of their distribution. IBM SPSS 18.0 software was used for these analyses.

Results

Based on Table 1, it is obvious that other the age difference – the younger patient group was on average 2.36 times younger than the older group – there were no demographic criteria that made these two groups different from one another. However, as a direct consequence of this age difference, the pre-induction values of all three cardiovascular parameters differed significantly. Systolic and diastolic blood pressure were by 25 % and 19 % higher, while the heart rate was by 14 % lower in elderly patients.

Table 1: Demographic data and preinduction values of the cardiovascular parameters in younger and elderly patients scheduled to receive esmolol during the operation under the general balanced anaesthesia

Parameter (unit)	Younger patients (x ± SE)	Older patients (x ± SE)	Statistical significance
Age (years)	29.23 ± 78.46	68.85 ± 1.24	$p < 0.01$
Body weight (kg)	78.46 ± 3.80	74.69 ± 2.70	ns
Gender: male/female	12 vs 1	9 vs 4	ns
Systolic blood pressure	135.54 ± 6.52	168.85 ± 7.30	$p < 0.01$
Diastolic blood pressure	84.08 ± 13.02	100.39 ± 3.32	$p < 0.05$
Heart rate`	107.31 ± 11.55	92.15 ± 4.47	$p < 0.01$

**Younger patients: patients 18-35 years of age, N = 26; Older patients: patients older than 65, N = 24; ns - not significant;*

Figures 1, 2 and 3 show changes in basic cardiovascular parameters in all the critical phases of anaesthesia and surgery. It is obvious that esmolol in both groups assured good control of cardiovascular parameters, but also that there were some differences between the groups.

The systolic blood pressure remained constant during the intubation phase in the group of older patients, at the same time being around 89 % of the pre-induction values, while in younger patients it rose up to 100 % (Figure 1). During the same phase of anaesthesia, the diastolic blood pressure in older patients remained at about 91 %, while in younger patients it rose up to 107 % of the pre-induction values. The same phenomenon occurred also during the phases of the first skin incision and surgical manipulations with organs (Figure 2). It is interesting though that among the two age groups there were no statistically signif-

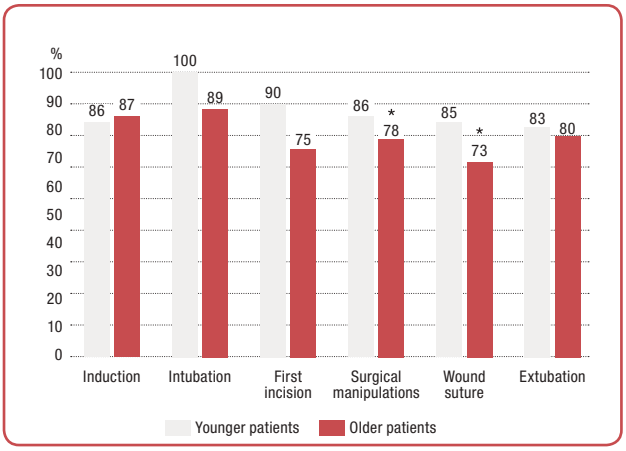

Figure 1: Systolic blood pressure in various phases of anaesthesia and operation in patients infused with esmolol

*%: Values are expressed as percentages of pre-induction values; Younger patients: patients 18-35 years of age, N = 26; Older patients: patients older than 65, N = 24; *: statistical significance;*

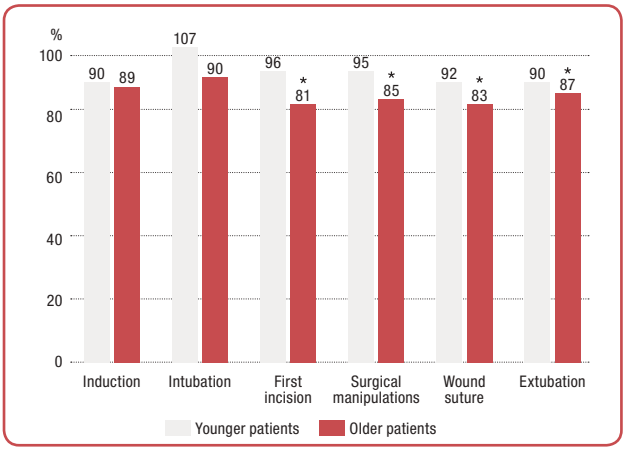

Figure 2: Diastolic blood pressure in various phases of anaesthesia and operation in patients infused with esmolol

*%: Values are expressed as percentages of pre-induction values; Younger patients: patients 18-35 years of age, N = 26; Older patients: patients older than 65, N = 24; *: statistical significance;*

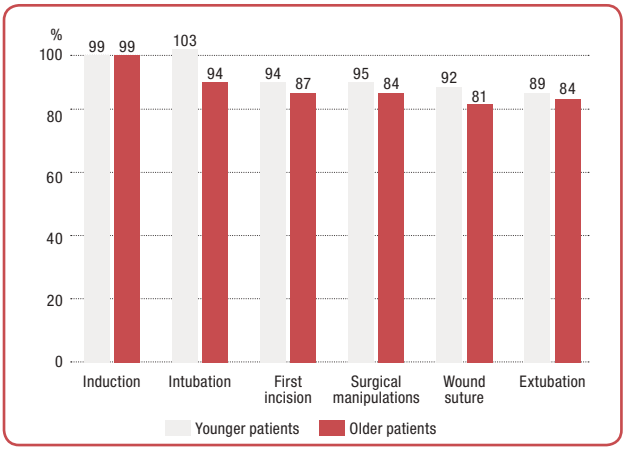

Figure 3: Heart rate in various phases of anaesthesia and operation in patients infused with esmolol

*%: Values are expressed as percentages of pre-induction values; Younger patients: patients 18-35 years of age, N = 26; Older patients: patients older than 65, N = 24; *: statistical significance;*

icant differences between the values of heart rate during the critical phases of anaesthesia and operation (Figure 3).

At the same time, the consumption of anaesthetic drugs fentanyl, droperidol, atropine and isoflurane was similar in both groups (Table 2).

Table 2: Consumption of medicines in younger and elderly patients scheduled to receive esmolol during the operation under the general balanced anaesthesia

Drug (unit)	Younger patients (x ± SE)	Older patients (x ± SE)	Statistical significance
Fentanyl (mg)	0.38 ± 0.88	0.30 ± 0.66	ns
Droperidol (mg)	6.11 ± 1.48	5.15 ± 1.93	ns
Atropine (mg)	1.02 ± 0.09	0.91 ± 0.07	ns
Pancuronium (mg)	5.98 ± 0.44	5.22 ± 0.34	ns
Isoflurane (% of patients)	15.39	12.50	ns

Younger patients: patients 18-35 years of age, N = 26; Older patients: patients older than 65, N = 24; ns - not significant;

Table 3: Effect of esmolol on speed and quality of postoperative recovery in younger and elderly patients scheduled to receive esmolol during the operation under the general balanced anaesthesia

Parameter (unit)	Younger patients (x ± SE)	Older patients (x ± SE)	Statistical significance
Duration of anaesthesia (min)	108.54 ± 8.97	120.54 ± 11.34	ns
Opening of eyes on command (min)	2.94 ± 0.44	3.02 ± 0.12	ns
Spontaneous opening of eyes (min)	5.23 ± 0.49	6.20 ± 0.55	ns
Full orientation (min)	8.44 ± 0.95	9.00 ± 1.20	ns
Extubation possible (% of patients)	96.15	87.50	ns
Evaluation of quality of anaesthesia*	2.70 ± 0.13	2.22 ± 0.11	ns

Younger patients: patients 18-35 years of age, N = 26; Older patients: patients older than 65, N = 24; ns - not significant; Scale: 1 - poor, 2 - good, 3 - excellent;

Table 4: Frequency of adverse effects in younger and older patients scheduled to receive esmolol during the operation under the general balanced anaesthesia

Adverse effect	Younger patients Number (%)	Older patients Number (%)	Statistical significance
Ventricular extrasystoles	2 (7.69)	2 (8.33)	ns
Tachycardia	1 (3.85)	0	ns
Bradycardia	0	2 (8.33)	ns
Hypotension	1 (3.85)	2 (8.33)	ns
Skin rash	1 (3.85)	0	ns
Total	5 (19.23)	6 (25)	ns

Younger patients: patients 18-35 years of age, N = 26; Older patients: patients older than 65, N = 24; ns: not significant;

Moreover, as seen in Table 3, no significant difference between the younger and older group of patients could be found regarding the speed of their recovery, possibilities for early extubation or in the subjective anaesthesiological assessment of the quality of anaesthesia in general.

Frequency of adverse effects in younger and older patients is shown in Table 4. Although there was a tendency toward more frequent occurrence of bradycardia and hypotension in the group of older patient, neither of these differences were significant.

Discussion

This clinical study has shown that there were no major differences in the change of cardiovascular parameters, consumption of drugs nor in the quality of the recovery from the balanced endotracheal anaesthesia in elective upper abdominal surgery patients infused with esmolol between the groups of younger and older patients. Since the baseline values of cardiovascular parameters were different – higher blood pressure and lower heart rate in older than in younger patients – these changes were expressed as percentages of the pre-induction values and not in absolute units.

Stability of cardiovascular function is one of the main goals in the concept of general balanced anaesthesia.[27, 28] This is why esmolol was viewed as an additional component within thus concept – a so-called 'soft' β-blocker for administration as an iv bolus or iv infusion with a rapid kinetics that allows for its titration to the needs of a specific patient and the particular phase of anaesthesia and operation.[29-31] The contribution of esmolol to the concept consists in the decrease in the doses of or the need for administration of intravenous anaesthetics,[32] inhalation anaesthetics,[32, 33] opioid analgesics[34] and even neuromuscular relaxants.[35]

There are no available publications on the differences in potential beneficial and adverse effects of esmolol regarding the age of the patients. What could be expected is that esmolol induces a sharper decrease of the already higher values of systolic and diastolic blood pressure and especially in the heart rate in the group of older patients, as compared to the younger ones. Besides, it was to be expected that esmolol is less efficient in coping with the increases in blood pressure in the older group, but neither of these happened, nor this group required higher doses of fentanyl or atropine to treat the episodes of bradycardia.

There are only two studies that focused on the desired and unwanted effects of esmolol in older patients, but neither of them included for comparison a group of younger patients. In a randomised

clinical trial in 27 patients scheduled for short eye surgery, aged 76 ± 7, half of the patients received a bolus of esmolol 2 or 3 mg/kg, while the rest of them were treated with local anaesthetic administration.[36] The goal of this study was to compare the cardiovascular effects of these two procedures. Although the type of general anaesthesia was similar to the one used in the present study, the authors administered esmolol in the form of the iv bolus, in contrast with the iv infusion, used in this study. Out of five patients treated with higher dose of esmolol – 3 mg/kg – four experienced a significant hypotension and one of these four had a vascular collapse. The regime of esmolol utilised in the present study was obviously less drastic – first, during 5 min, an infusion at a rate of during the first 5 min at a rate of 0.3 mg/kg/min and 0.1 mg/kg/min thereafter and was followed by a much more favourable cardiovascular response.

In another open clinical trial in 22 elderly patients scheduled for cataract surgery, half was treated with esmolol and the other half with labetalol.[37] The esmolol group was aged 78 ± 9 and all of them had extremely high perioperative blood pressure values – systolic pressure > 200 mmHg diastolic pressure > 100 mmHg, as defined by the inclusion criteria. The dosage regimen in this study was much more similar to the one in the present study – esmolol was administered in a small iv bolus of 0.5 mg/kg, followed by the iv infusion at a rate of 0.15-0.30 mg/kg/min. Although the therapeutic goal was assured – systolic blood pressure reduction by 20 % (from 217 ± 12.3 mmHg to 175 ± 4.0 mmHg), the level of heart rate reduction was moderate (from 81 ± 9 to 61 ± 8/min). Two out of 11 patients treated with esmolol developed a serious bradycardia (heart rate < 50/min) and for them esmolol was discontinued. In the present study, bradycardia was defined as heart rate < 60/min and it was registered in two out of 24 patients. This difference could be explained by a significantly higher age of the patients in the study published by Singh and co-workers.[37]

Conclusion

Infusion of esmolol contributes to the concept of general balanced anaesthesia in elective patients scheduled for upper abdominal surgery equally in younger (aged 18-35) and older patients (aged 65 or more). In both groups, esmolol assured perioperative cardiovascular stability, good recovery after anaesthesia and favourable profile of adverse effects.

Acknowledgements

None.

Conflict of interest

None.

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Samardžić et al. Scr Med 2021 Jun;52(2):115-8. DOI:10.5937/scriptamed52-30096
ORIGINAL ARTICLE

Comparison of Short Tandem Repeat Loci D2S1338 and D18S51 in the Populations of the Republic of Srpska and the Autonomous Province of Vojvodina

Branka Samardžić,[1] Željko Karan,[1, 2] Zoran Obradović,[1] Dijana Došen[1]

(1) Institute of Forensic Medicine of Republic of Srpska, Banja Luka, the Republic of Srpska, Bosnia and Herzegovina.
(2) Faculty of Medicine, University of Banja Luka, Banja Luka, the Republic of Srpska, Bosnia and Herzegovina.

Abstract

Introduction: Numerous human migrations between Bosnia and Herzegovina and Serbia's northern province of Vojvodina have led to the increased mixing of these population's genetic pool, leading to a reduction in genetic differences. The question is whether there are now genetic differences between the mentioned populations.
Methods: Short Tandem Repeat (STR) loci D2S1338 and D18S51 were used in this paper to compare the diversity of alleles and genotypes between the populations of Vojvodina and one of the two Bosnia's entities, the Republic of Srpska (RS). Three hundred ninety unrelated persons, 140 persons from the RS and 260 from Vojvodina, were analysed. The PowerPlex ESX16 System commercial kit was used for profiling persons from the territory of the RS and the AmpF/STR Identifiler commercial kit for persons from Vojvodina. The Mann-Whitney U-test was used for statistical analysis.
Results: Data analysis concluded a significant deviation in the allele frequency of the D18S51 locus where p = 0.021. There was no statistically significant deviation in the D2S1338 locus allele frequency between these two populations. It was also found that there is a statistically significant deviation in the genotype frequencies of these two populations for the analysed genetic markers.
Conclusion: This study confirms the existence of a significant deviation of allelic frequency for the D18S51 gene locus and a significant deviation of both gene markers frequency of genotypes.

Key words: Human genome; Alleles; Microsatellite repeats; Population genetics.

Correspondence:
BRANKA SAMARDŽIĆ
E: branka.zoric88@gmail.com

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Introduction

The DNA molecule consists of coding and non-coding regions. The non-coding parts are characterised by exceptional polymorphism. There are three main types of non-coding region polymorphisms:

- Minisatellites (Variable Number of Tandem repeats - VNTRs),
- Short Tandem Repeats (STRs),
- Single Nucleotide Polymorphisms (SNPs).[1]

STRs consist of short repetitive parts 2-10 base pairs long. These repetitions are widespread in the human genome and represent a rich source of highly polymorphic markers. Alleles of these loci differ in the number of repeats of the repetitive sequence located in the amplification region.[2] STRs have such properties as abundant, codominant, highly polymorphic, and nearly selectively neutral which makes them very useful in applications such as the construction of genetic maps, gene location, genetic linkage analysis, identifi-

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CONTENT

cation of individuals, paternity testing, as well as disease diagnosis. STR analysis has also been employed in population genetics. They can be applied to reconstruct the history of migration and evolution of the species, as well as to assess biological diversity at various levels of biological organization.[3]

Numerous human migrations between Bosnia and Herzegovina and Serbia's northern province of Vojvodina have led to the increased mixing of these population's genetic pool, leading to a reduction in genetic differences. The question is whether there are now genetic differences between the mentioned populations.

This paper's aim was to determine and compare the frequencies of alleles and genotypes of STR loci D2S1338 and D18S51 in population of the Republic of Srpska (RS) and Vojvodina. Proposed hypothesis is that there are no genetic differences between two mentioned populations.

Methods

The diversity of alleles and genotypes of the two previously mentioned gene markers was analysed on a sample of 390 unrelated individuals. One hundred forty people were analysed from the territory of the RS, and 250 from the territory of Vojvodina.

Samples from the RS were collected in the Institute of Forensic Medicine of Republic of Srpska during of disputed paternity testing and analysis biological traces of human origin at the crime scene. DNA amplification was performed according to the PowerPlex ESX 16 Systems protocol in PCR ABI-Veriti, manufactured by Applied Biosystems, USA. Separation and detection of amplified fragments was performed by capillary electrophoresis on an instrument, ABI PRISM 310 Genetic Analyzer, manufactured by Applied Biosystems, USA. The analysis of the obtained fragments was performed using the software package GeneMapper ID v3.2.1. manufactured by Applied Biosystems, USA.

The Institute of Forensic Medicine in Novi Sad provided genotypes of 250 unrelated persons from Vojvodina. A commercial set AmpF/STR Identifiler, manufactured by Applied Biosystems, USA, was used for DNA amplification.

Statistical data processing was performed using the Mann-Whitney U-test.

Results

The results of the analysis of the diversity of 2 microsatellite loci D2S1338 and D18S51 in the human populations of the RS and Vojvodina are presented here. A total of 390 people were analysed.

The D2S1338 gene marker has 12 identified alleles in the analysed population of the RS and 14 alleles in the population of Vojvodina, allele 17 being the most common in both populations (Table 1). The D18S51 gene marker has 12 identified alleles in the analysed population of the RS and 16 alleles in the population of Vojvodina. Allele 14 has the highest frequency in the RS's population and allele 15 in the population of Vojvodina (Table 2).

Table 1: The allelic frequencies of D2S1338 locus %

Alleles	Republic of Srpska	Vojvodina
13	0.000	0.200
15	0.357	0.400
16	3.571	5.000
17	18.214	21.600
18	10.357	10.800
19	11.786	9.200
20	16.786	13.200
21	3.929	3.000
22	2.500	2.800
23	15.000	10.800
24	7.500	10.200
25	8.214	11.200
26	1.786	1.400
27	0.000	0.200

According to the Mann-Whitney U-test, the significance level is $p < 0.05$. Data analysis concludes that there is no statistically significant deviation in the frequency of the alleles in the D2S1338 locus between these two populations. However, there is a statistically significant deviation in the allele frequency of the D18S51 locus where $p = 0.021$. The allelic frequencies of these two loci in the populations of the RS and Vojvodina are shown in Table 3.

In the population of the RS, the presence of 49 different genotypes for both gene markers was determined. On the D2S1338 gene marker, the same frequency of 4 different genotypes was determined, while on the D18S51 gene marker, one genotype is singled out as the most common. In the sample from the Vojvodina territory, the presence of 57

Table 2: The allelic frequencies of D18S51 locus %

Alleles	Republic of Srpska	Vojvodina
9	0.000	0.200
10	1.786	1.000
11	1.786	1.800
12	10.357	13.000
13	10.714	13.600
14	20.357	16.000
15	15.000	16.400
16	13.571	15.000
17	11.786	9.200
18	5.000	8.000
19	5.357	1.600
20	2.143	2.200
21	2.143	0.800
22	0.000	0.800
24	0.000	0.200
25	0.000	0.200

Table 3: Differences of allelic frequencies in loci according to Mann-Whitney U-test

Loci	U	Z	p-value	Z-adjusted	p-value
D2S1338	68362.50	-0.542	0.588	-0.547	0.584
D18S51	63068.50	2.296	0.022	2.317	0.021

By variable Var1 Marked tests are significant at p < 0.05 (red colour)

Table 4: Number of genotypes by loci

Loci	Republic of Srpska		Vojvodina	
	A	B	A	B
D2S1338	49	20,23-8	57	17,18-14
		17,23-8		
		17,19-8		
		17,18-8		
D18S51	49	14,15-11	60	13,15-13
				15,16-13

A. Number of genotypes in the population
B. The most common genotype-the number of repeats

Table 5: Summary of the difference of genotypic frequencies according to Mann-Whitney U-test

Loci	U	Z	p-value	Z-adjusted	p-value
D2S1338	14359.00	-2.941	0.003	-2.942	0.003
D18S51	14914.50	-2.421	0.015	-2.422	0.015

By variable Var1 Marked tests are significant at p < 0.05 (red colour)

different genotypes for the D2S1338 gene marker and 60 genotypes for the D18S51 gene marker was determined. At the D2S1338 gene locus, one genotype is singled out as the most common, and at D18S51, we see the same frequency of 2 genotypes. A comparative overview of genotype's number in the analysed loci, in each population, as well as the most common combinations of alleles are shown in Table 4. Table 5 shows the difference in genotype frequencies of these two populations for the analysed gene markers from which it can be seen that there is a significant deviation.

Discussion

Polymorphism of the D2S1338 and D18S51 gene markers was confirmed in both analysed populations (the RS and Vojvodina).

There are no earlier studies concerning similar research in RS, but there are several researches which include Bosnia and Herzegovina. In 2006, a survey was conducted for the territory of Bosnia and Herzegovina, which analysed 110 people. It was reported the presence of 11 alleles for D2S1338 marker with allele 17 being the most common allele.[4] Later study covering 1000 persons from the same territory identified presence of 15 alleles in this STR marker, where allele 14 has the highest frequency.[5] Locus D18S51 has shown bigger diversity in previous studies in Bosnia and Herzegovina. They found 15 allelic forms, with allele 16 being the one with the highest frequency.[6]

The sample of 100 unrelated, autochthonous healthy adult Serbians from Novi Sad (Vojvodina Province, Serbia and Montenegro) was profiled via AmpFLSTR Identifiler loci in 2004. Scientists have reported 12 alleles for STR marker D2S1338 (allele 17 had the highest frequency) and 14 for D18S51 marker (equally represented alleles 13 and 16).[7]

Similar results to ours, concerning the number of allelic forms have been reported earlier from the countries in the region.[8, 9]

Comparison of allele diversity and genotypes of these gene markers was made using the Mann-Whitney U-test. A significant deviation in allele frequency was observed for the D18S51 gene marker, which was expected given that this

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locus showed high allelic diversity in both populations. Both analysed genetic markers showed a statistically significant deviation in the frequency of genotypes in the human populations of the RS and Vojvodina. It follows that there are specific genetic differences between the inhabitants of the RS and Vojvodina.

Since two gene markers with a high degree of genetic variability were analysed, caution is necessary in concluding the differences between these two populations. Many possible allelic forms characterise D2S1338 and D18S51 and a significant deviation could be expected.

To conclude the actual difference or similarity in the diversity of autosomal gene markers between these two populations, it is necessary to increase both populations' samples and ensure the correct ethnic and gender representation. In other words, more representative samples are needed. It is also necessary to profile all samples with the same DNA amplification commercial kit to obtain as many genetic markers as possible for comparison.

Conclusion

In a sample of 390 unrelated persons from the territories of the Republic of Srpska and Vojvodina, allelic diversity was determined on both genetic markers. The autosomal marker D2S1338 has less allelic diversity than the range, while the allelic diversity is in the range for the marker D18S51. Based on this study, it can be concluded that a significant deviation of allelic frequency for the D18S51 gene locus and a significant deviation of the frequency of genotypes of both gene markers exist.

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Conflict of interest

None.

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CONTENT

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ORIGINAL ARTICLE

Influence of EmbryoGlue® Transfer Medium on Implantation of Human Embryos

Dragana Pavlović Vasić,[1] Sanja Sibinčić,[1] Irena Milaković,[1] Sanja Lukač,[1] Saša Vujnić,[1] Milica Gvero,[1] Danijela Madžar,[1] Aleksandra Govedarović,[1] Dragan Ivanović[1]

(1) Health institution Medico S, a member of the Pronatal group, Banja Luka, the Republic of Srpska, Bosnia and Herzegovina.

Abstract

Introduction: Thanks to ever-growing advances in medical science, couples who are in the in vitro fertilisation (IVF) now have more options than ever to encase their chances at a successful pregnancy. One of the options is the use of EmbryoGlue (EG), that creates a bridge between the embryo and the uterus and provides protection to the embryo itself during the transfer process.
Aim of this study was to determine whether EG medium is of greater importance for embryo implantation than conventional medium in assisted reproductive technology and compare the rate of embryo implantation with EG and conventional medium in relation to the quality of the embryo, the age of the patients and tobacco smoking.
Methods: The retrospective study included 50 patients who used EG medium in embryo transfer (ET) and 50 patients in the control group using conventional medium. All patients underwent ET after stimulation of the cycle according to a short protocol. ETs were done on Day 2, 3, or 5 in the blastocyst stage. Age and smoking status were recorded.
Results: Out of a total of 100 patients, 42 patients had successful implantation and positive β-hCG 15 days after ET. In a control group 38 % had positive β-hCG and in the group of patients who used EG 46 %. A higher rate of embryo implantation success was observed on the second day of transfer in the group of patients using EG. In the EG group a significant increase in the embryo implantation rate was observed in patients older than 35. In tobacco smokers the implantation rate was higher if they used EG during ET.
Conclusion: EG medium had a positive effect on the second day of ET, patients above the age of 35 and patients who were tobacco smokers.

Key words: EmbryoGlue; Embryo; Hyaluronan; Implantation.

Correspondence:
DRAGANA PAVLOVIĆ VASIĆ
E: drdragana1972@gmail.com

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Introduction

Embryo transfer (ET) is a multi-stage process and one of the most sensitive steps in the *in vitro* fertilisation (IVF) procedure. From aspiration to transfer, it is necessary to support the oocytes and later, the development of the embryo by providing optimal cultivation conditions. The combination (ingredients) of the transfer medium is considered to be important for the interaction of the endometrium and the embryo during implantation.[1] After transfer, the embryo must rely on its own ability to implant and use of hyaluronan-enriched media can improve implantation success.

IVF programs continuously seek to increase the rate of implantation and pregnancies.[2] Advances in stimulation regimen and therapy, cultivation

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media, and technology contribute to this.[3] By designing cultivation media, which mimic changes in the female reproductive tract, it is possible to optimise development and create a healthy environment for the embryo.[4]

EmbryoGlue (Vitrolife)® (EG) is a medium modelled exclusively for ET with a proven effect of increasing implantation. EG has a basic composition of blastocyst culture medium and contains high concentrations of hyaluronan (HA) and recombinant albumin. It can be used to transfer all stages of embryonic development, including blastocysts after assisted hatching, biopsy, and cryopreservation. HA is a glycosaminoglycan naturally present *in vivo* in follicles, oviducts, uterine fluids and may be involved in the implantation process,[5, 6] by binding to the CD44 glycoprotein receptor of the embryo allowing HA to enter the cell.[7, 8] HA is ubiquitous in the body, however, its action at different locations of the reproductive tract critically depends on its size, which is controlled by the balance of synthesis using one of the three isoforms.[9]

Due to its similar consistency, HA increases the viscosity of the transfer medium and may enhance the ability of the embryo to interact with uterine fluid. Thanks to these properties, it improves the apposition and binding of embryos, which are key steps in the implantation process.[10, 11]

Aim of this study was to determine whether EG medium is of greater importance for embryo implantation than conventional medium in assisted reproductive technology (ART) and compare the rate of embryo implantation with EG and conventional medium in relation to the quality of the embryo, the age of the patients and tobacco smoking.

Material and methods

The retrospective study included 50 patients who used EG medium in ET and 50 patients in the control group using conventional medium. The period from June to November 2019 was analysed through available data from the history of ART Health Institution Medico S, a member of the Pronatal group. All patients underwent ET after stimulation of the cycle according to a short protocol. ETs were done on Day 2, 3, or 5 in the blastocyst stage. Age and smoking status were recorded.

Statistical processing was performed using the IBM SPSS software, version 15. All obtained data were processed by standard procedures of comparative statistics, Student's t-test and Chi-squared test, with statistical significance set as $p < 0.05$.

Results

a) Implantation success

Out of a total of 100 patients, 42 patients had successful implantation and positive beta human chorionic gonadotropin (β-hCG) 15 days after ET. In a control group (50 patients), 19 had positive β-hCG and 31 negative β-hCG, representing 38 % of embryo implantation success rate. In the group of patients who used EG, 23 patients had positive β-hCG and 27 negative β-hCG, which makes a 46 % success rate. There is an obvious difference in implantation success rate between these two groups of 8 % in favour of EG medium, however, difference was not statistically significant (Chi-squared test, $p = 0.418$) (Figure 1).

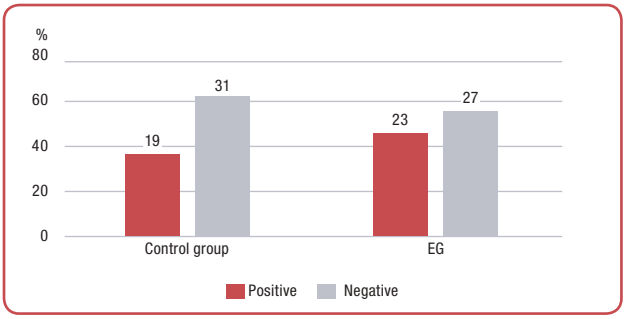

Figure 1: Embryo implantation success rate
**EG: EmbryoGlue %; percentage of total number of patients;*

b) Quality of the embryo

In the control group, the success rate of embryo implantation (positive β-hCG) on the Day 2 was 28.6 % (4 of 14 patients), on the Day 3, 37 % (10 out of 27 patients) and on the Day 5, 55.6 % (5 out of 9 patients) (Figure 2).

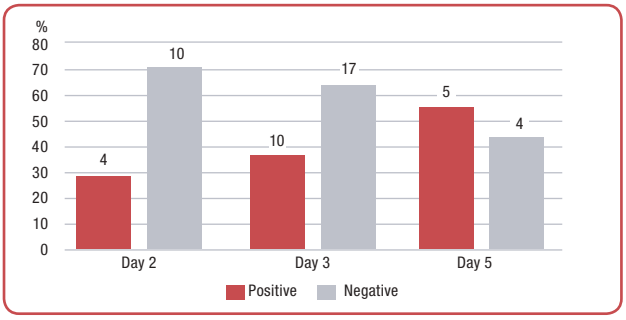

Figure 2: Implantation success in the control group
%: percentage of total number of patients;

CONTENT

In the group of patients who used EG medium, the success rate of embryo implantation (positive β-hCG) on the Day 2 was 56 % (9 out of 16 patients), on the Day 3, 45.8 % (11 out of 24 patients) and on the Day 5, 30 % (3 out of 10 patients) (Figure 3).

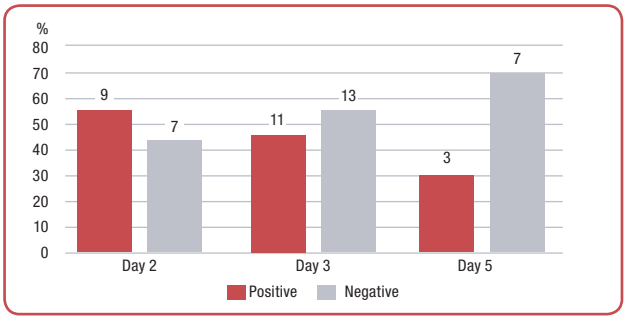

Figure 3: Success of implantation with EmbryoGlue medium
%: percentage of total number of patients;

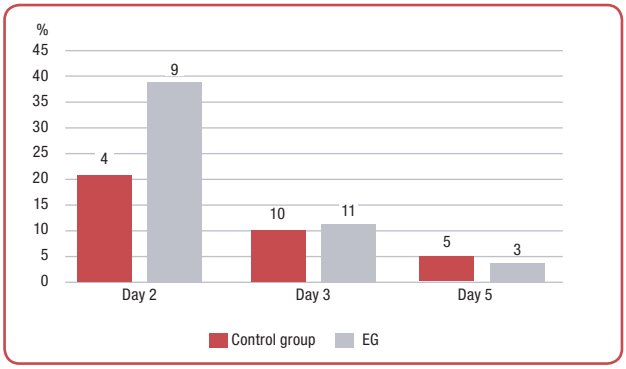

Figure 4: Comparison of implantation success depending on embryo quality between control and EmbryoGlue (EG) group
%: percentage of total number of patients

Comparative analysis using Student t-test between these two groups did not show a statistical difference p = 0.834. However, a higher rate of embryo implantation success was observed on the second day of transfer in the group of patients using EG (Figure 4).

c) Influence of patient age

Patients were classified according to age into five groups (20-25, 26-30, 31-35, 36-40 and 41-45 years). At the age of 20-25, there were no patients with positive β-hCG hormone in the control and EG groups. In the age of 26-30, positive β-hCG was in 37.5 % patients (3 of 8 patients), while in the group where EG was used as a transfer medium, 20 % (1 of 5 patients). At the age of 31-35 years, there were 50 % positive β-hCG in the group with EG medium (8 of 16 patients), while in the control group it was 43.5 % (10 of 23 patients). Of the number of patients aged 36-40 years, 48 % had positive β-hCG who used EG medium (12 of 25 patients), while in the control group it was 37.5 % (6 of 16 patients). At the age of 41-45, there were 66.7 % of patients with positive β-hCG in the group with EG medium (2 of 3 patients), and there were no implantation in the control group (Figure 5).

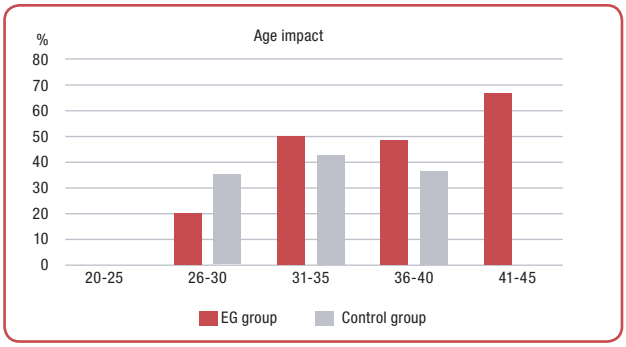

Figure 5: Comparison of implantation success between EmbryoGlue (EG) and control group in relation to age
%: percentage of total number of patients;

Student-t test did not reveal a significant difference in age between these two groups (p = 0.182). By analysing the success rate of the implantation rate within the age groups, in the EG group a significant increase in the embryo implantation rate was observed in patients older than 35 years.

d) Impact of tobacco smoking

Impact of tobacco smoking on success in the implantation of embryos was analysed. In the group with EG medium, there were 32 % of smokers and 68 % of non-smokers, and in the control group there were 38 % smokers and 62 % non-smokers.

Table 1: Impact of tobacco smoking on implantation success

Tobacco smokers	Control group				EmbryoGlue group			
	Total		Positive β-hCG		Total		Positive β-hCG	
	N	%	N	%	N	%	N	%
Yes	19	38	4	21.1	16	32	7	43.7
No	31	62	15	48.3	34	68	16	47.1

β-hCG: β-human chorionic gonadotropin

Embryo implantation (positive β-hCG) in the group of patients who used EG medium in smokers was 43.7 %, while in non-smokers it was 47.1 % (Table 1). In the control group of female patients, 21.1 % of smokers had positive β-hCG, and in the non-smoking group, β-hCG was positive in 48.3 %. Due to the small number of samples in the observed parameters, difference is not statistically significant, but it is noticed that in smokers the implantation rate was higher if they used EG during ET.

Discussion

As already mentioned, EG contains HA and nutrients that are necessary to support embryos from transfer to the moment of implantation as well as for the process of biochemical interaction during that period. The positive effect of EG medium was proven by its introduction on the market in 2003. After that, several studies published its positive effect. In 2010, an independent Cochrane Collaboration published a meta-analysis of controlled randomised studies "ET medium adhesion components".[1] Using EG for ET, the rate of clinical pregnancies increased significantly from 41 % to 50 % compared to conventional media with low or even no HA content (meta-analysis of 13 publications covering Cochrane articles in more than 3,200 patients). Analyses did not show an increase in abortions or other unforeseen circumstances (outcomes). Cochrane authors identified a clear positive effect of high concentrations of HA in transfer media. Their report published in 2020 shows an increased pregnancy rate after using high concentrations of HA in ET media. Results from this study confirm the previous conclusions of the Cochrane report. In this study, a positive effect of EG medium was proven, since the implantation rate is higher after its use, although there is no statistically significant difference in the observed parameters.

Numerous clinics in Japan that use EG transfer medium presented different results at scientific conferences between 2010 and 2013. Meta-analyses of Japanese studies involving nearly 10,000 cycles show increased implantation and pregnancy rates from fresh and cryo transfers, after the use of EG medium.[12, 13] Their results confirm the previous conclusions of the Cochrane report. HA-enriched media may increase the implantation rate[14] and clinical pregnancies in patients with previous failed implants and in the third or more attempts after cryo ET.[10, 15] They also report that clinical pregnancies and implantation rates were higher in embryos after cryo ET, indicating that the viscosity of the EG medium physically protects embryos after assisted hatching.[13]

Zhang et al[16] and Wu et al[17] investigated whether EG has an impact on pregnancy outcome during *in vitro* fertilisation compared to G2 medium. In conclusion, they cite EG as a transfer medium that can improve the rate of implantation and pregnancy in the IVF program. Based on their results, there is no statistically significant difference in the age of patients, duration of infertility, number of failed cycles, endometrial thickness, number and between embryo quality and the average number of embryos in the observed groups. The results of this study show that the level of implantation is more successful in women above 35 years of age who used EG, while in women younger than 35 the rate of implantation in both groups is approximately the same. Urman et al confirm the beneficial effect of HA-enriched medium, which is evident in women over 35 years of age, in women with poorer embryo quality and with previous unsuccessful implantation.[6]

Svobodová et al[18] observed a statistically significant beneficial effect of EG medium on embryo implantation in patients aged 30-38 years. This research confirms the positive effect of EG medium on Day 2 of transfer, which shows that embryos in the early phase of transfer have a higher chance of more successful implantation with this medium, while for transfers 3 and 5 days the results are similar between the control and EG groups. Urman et al found that HA-enriched media increased the rate of clinical pregnancies and implantation on days 3 and 5 of ET.[6]

Several authors state that HA-enriched media have not shown significant results in their study[2, 19] and concluded that future research should show whether EG is the highest quality option for all patients or only for those patients in whom the quality of the embryo is poor, for patients in whom implantation has failed and older patients.[20] Nishihara et al[21] state that pregnancies, implantation, and abortion rates where media with different concentrations of HA was used during ET gives similar results.

Toxic components of cigarette smoke affect the gametes, ovarian, tubal, endometrial and myometrial components. Uterine receptivity is also impaired.[22]

When comparison was made on the effect of EG on embryo implantation in smoking and non-smoking patients, conclusion was made that support the justified use of EG in women who smoke, because the rate of embryo implantation in women smokers who used EG as a medium is significantly higher than those who used a conventional medium. In non-smoking women there was no large difference in embryo implantation between these two media. The sample was not large enough to have a statistically significant difference.

CONTENT

Conclusion

According to the results obtained, conclusion could be made that there is a difference in the success rate of implantation with EG medium. A higher rate of embryo implantation on the second day of transfer and in older patients (> 35 years) with EG medium was observed, although the statistical analysis did not reveal a significant difference in these categories.

In smokers, the rate of implantation with EG medium was higher compared to the conventional medium, while in non-smokers there was no difference.

EG medium had a positive effect on the second day of ET, patients above the age of 35 and patients who were tobacco smokers.

Acknowledgements

None.

Conflict of interest

None.

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CONTENT

Laštro et al. Scr Med 2021 Jun;52(2):124-31. DOI:10.5937/scriptamed52-32379
ORIGINAL ARTICLE

Parents' Knowledge and Attitudes When Choosing Their Children's School Bag: an Introductory Study

Dijana Laštro,[1] Mirsad Muftić,[2] Nenad Ponorac,[3] Dubravko Bokonjić[4]

Abstract

Background/Aim: A school bag is a device whose purpose is to store things that are necessary for a child in performing daily productive activities. The aim of the research was to examine how much parents are informed about the school bag and which specifications are crucial when choosing a school bag for their children.
Material and methods: The prospective study included 150 parents of students aged 11 to 12 from Banja Luka. For the purposes of the research, a questionnaire with closed and open questions was used.
Results: More than 97 % of the surveyed parents belonged to the age range of 30 to 49 years. The largest number of surveyed parents have had secondary education 44 %, but the number of university-educated parents was also very high 39.3 %. Ninety-six percent of students used a backpack. School bags in 97.4 % of cases were purchased in stores. Only in 10 % of cases, school bags fully meet the basic criteria. In the remaining 90 % of cases, there was an absence of at least one or more desired characteristics. Using the Chi-squared test ($\chi^2 = 245.45$; $p < 0.001$), a significant difference between the desired and the achieved result was statistically proven. 77.3 % of parents did not receive information about the school bag. Salespeople (11.3 %), other parents and friends (9.3 %) were the main source of information before purchasing a school bag. In the future, 54 % of parents would like to get information about the school bag through lectures and practical workshops for children and parents.
Conclusion: Greater information enables adequate selection and reduces the possibility of marketing tricks and influences on consumers when choosing the desired product such as a school bag. Parents have an important role to play in ensuring unhindered growth and development during the educational process and in raising children's awareness and supervision of the proper use of the school bag.

Key words: Schoolbag; Parents; Knowledge; Awareness.

(1) Institute for Physical Medicine and Rehabilitation "Dr Miroslav Zotović", Banja Luka, the Republic of Srpska, Bosnia and Herzegovina.
(2) Faculty of Health Studies, University of Sarajevo, the Federation of Bosnia and Herzegovina, Bosnia and Herzegovina.
(3) Department of Physiology, Faculty of Medicine, University of Banja Luka, Banja Luka, the Republic of Srpska, Bosnia and Herzegovina.
(4) Medical Faculty of the Military Medical Academy, University of Defence, Belgrade, Serbia.

Correspondence:
DIJANA LAŠTRO
E: dijana.lastro@med.unibl.org

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Introduction

Carrying a school bag is a common way of carrying teaching aids and accessories that are necessary for a child to perform daily productive activities. Numerous studies have reported side effects associated with school bag use, prevalence, and risk factors such as back pain in younger students.[1-3] According to a University of Boston study, about 85 % of students report discomfort and pain associated with backpack use on their own. Being overweight can lead to health problems in the short and long term. Improper use of backpacks can lead to muscle imbalance that could turn into

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chronic neck and back pain.[4] A systematic review by Balamurugan (2017) reports that a school bag, that is approximately 10 – 15 % of body weight can cause overload of the cervical spine and upper limbs, when burdened by weight in the thoracic spine.[5] Weight has a significant effect on the centre of gravity of the body taking into account appropriate carrying techniques. Different bag carrying techniques are a significant factor influencing the posture and gait of students between of ages 11 - 13 years.

Studies have reported that there is no difference in lateral spinal deviation between two-strap backpacks and students without a bag, however, the belt on the school bag reduces the strain on the back, neck and shoulders.[6-8] Carrying a school bag has significant biomechanical, physiological and adverse effects on the wearer, especially with loads greater than 10 % of the student's body weight. Such effects may include changes in posture (eg, changes in spinal posture, lumbosacral angles, and thoracic kyphosis), gait, increased physical discomfort, muscle activity, and increased respiratory rate.[9] Nevertheless, concerns have been raised about the possible harmful consequences of using a school bag, especially related to overweight, back position and suboptimal design aspects such as uncomfortable shoulder straps and the absence of shoulder straps.[10-13]

American Association for Occupational Therapy, The American Academy of Orthopaedic Surgeons and the International Association of Paediatric Chiropractors suggests that the load should not exceed 10 %, the American Association of Physical Therapy suggests 15 %, and the American Association of Chiropractors 5-10 % of body weight.[14] A comprehensive understanding of the recommended measures is necessary to achieve the desired effect. Authors Dianat and Karimi (2014) concluded in the study that parental awareness should play an important role in changing children's school bag carrying habits, and especially in reducing the weight of the burden they carry. This highlights the need to incorporate guidelines and recommendations into practice to ensure that the health and safety of schoolchildren is not compromised when using a school bag.[15] Although the American Association of Occupational Therapists (AOTA, 2014) has issued guidelines for the proper use of a school bag, in our country there is a lack of such prevention programs.[16]

The goal of this research was to examine how much parents are informed about the school bag and what specifications are crucial when choosing a school bag for their children.

Methods

The research was a prospective study. Using the cluster sampling method, the study included 1507 parents of students aged 11 to 12, of seven primary city schools from Banja Luka. After meeting the criteria for inclusion and testing students with a physical activity test (The Physical Activity Questionnaire for Older Children, PAQ-C),[17, 18] respondents with their parents entered the study successively and were divided into three groups: insufficiently physically active, moderately, and highly physically active children. Sampling was performed until the number of 150 respondents of both sexes and their parents was filled.

Criteria for inclusion: children of older primary school age 11-12, both sexes, children who have signed written consent of a parent or legal representative, children who have agreed and signed written consent to participate in the research and children who have the ability to self-ambulance.

Exclusion criteria: children with intellectual disabilities, epilepsy, cerebral palsy, hemiparesis, diabetes mellitus, heart and circulatory diseases, respiratory diseases, children with a history of fractures or injuries of the lower extremities in the past year, children who used a mobility aid, fatigue, pain and inability to adapt to the work of the zebris strip.

The research was approved by the Ministry of Education and Culture of the Republic of Srpska (Approval Certificate No 07.041/059-3292/18 dated 17 December 2018 and No 07.041/059-2436/19 dated 25 September 2019 and the Ethics Committee of the Medical Faculty of the University of Banja Luka (Approval Certificate No 18/14.27/19 dated 1 July 2019 and No 18/4.27-1/20 dated 5 February 2020). All respondents and parents gave their voluntary consent, which in addition to the oral explanation was also contained in the informed consent for parents and students and written information for parents and students. The research was conducted during the school year 2019/2020 and 2020/2021.

CONTENT

For the purposes of the research, a survey questionnaire for parents based on previous research was used and adapted to the needs of the research itself.[19] The survey questionnaire is designed to assess parents' awareness of the factors associated with carrying a school bag as well as which specifications are crucial when choosing a school bag. The questionnaire consisted of 37 questions that were mostly with one or more choice answers closed (n = 34) and open type (n = 3). From the survey questionnaire, items related to information on demographic data and family structure, school bag design, type, weight, importance of school bag characteristics, place of school bag procurement, availability and request for use of information and parental education were selected.

The parents were interviewed at the school and at the Institute for Physical Medicine and Rehabilitation "Dr Miroslav Zotović" in Banja Luka. The research was conducted by a research team consisting of the main researcher, occupational therapist-physiotherapist and trainee occupational therapist.

Complete statistical analysis of data was done with the statistical software package, SPSS Statistics 18. Most of the variables were presented in a text and table as frequency (%) of certain categories. Statistical significance between groups (categorical data) was tested by Chi-squared test. In case of continuous data, variables were presented as mean value ± standard deviation (SD). All analyses were estimated at minimum $p < 0.05$ level of statistical significance.

Results

The results of the descriptive analysis of the basic characteristics of the school bag that children had, and the compliance of the desired and realised characteristics of the bag are shown in the table (Table 3 and 4), as well as demographic and socio-economic characteristics of the sample, school bag procurement (Table 1 and 2), and graphically (Figure 1, 2, 3) the importance of school bag characteristics, sources of information that parents have used and would like to use in the future about the school bag. The values of the results of the descriptive statistics are shown in absolute numbers and percentages (%).

Table 1: Basic demographic characteristics of the surveyed parents

Demographic characteristics	Total number of respondents (N = 150)	
	N	%
Gender		
Male	62	41.3
Female	88	58.7
Age		
< 30	1	0.7
30-39	68	45.3
40-49	78	52.0
50-59	3	2.0
Education		
Elementary school	1	0.7
High school	66	44.0
High school	9	6.0
Faculty	59	39.3
Master of Science	10	6.7
Doctor of Science	5	3.3
Marital status		
Married	132	88.0
Divorced	13	8.7
Unmarried	2	1.3
Divorced	1	0.7
Widow / Widower	2	1.3

Parents of both sexes participated in the survey, and mothers were numerically dominant (88; 58.7 %). More than 97 % of the surveyed parents belonged to the age range of 30 to 49 years. The largest number of surveyed parents had secondary education (44 %), but the number of university-educated parents was also very high (39.3 %). In 88 % of cases, these were, at least formally, standard marriages.

Table 2: Basic socio-economic characteristics of the surveyed families

Socio-economic characteristics	Total number of respondents (N = 150)	
	N	%
Property status of the family		
Satisfactory	120	80.0
Somewhat satisfying	29	19.3
Unsatisfactory	1	0.7
The environment where they live		
City	118	78.7
Suburban	32	21.3
Rural	0	0.0
Number of children in the family		
One	23	15.3
Two	87	58.0
Three or more	40	26.7
Family type		
Extended (2 generations)	16	10.7
Family with 1 parent	19	12.7
A family with both parents	115	76.7

Of the total number of respondents, 120 (80 %) thought that household income is satisfactory. Almost the same percentage of families lived in urban areas, and the rest (21.3 %) were located in

suburban settlements. The largest percentage of families had two children (58 %), three or more children also had an enviable percentage of respondents (26.7 %). In most cases (76.7 %) these were families with both parents.

Table 3: Basic characteristics related to the procurement of a school bag

School bag	Total number of respondents (N = 150)	
	N	%
Participant in election[1]		
Mother	93	62.0
Father	31	20.7
A child	90	60.0
The others	2	1.3
Method of procurement[2]		
Shop	146	97.4
Via the Internet	2	1.3
From family members (used)	2	1.3
From a friend (used)	0	0,0
Other	0	0,0

(1) possible multiple choice, percentages of individual answers exceed 100 %
(2) there is a possibility of only one answer, the total percentage is 100 %

A high percentage of mother and child were involved (62 % and 60 %, respectively) in the purchase of a school bag, and the fathers were involved in the purchase of only one in five children (31; 20.7 %).

When it comes to participants in the selection of school bags, in 99 cases (66.0 %) only one of the above persons participated in the selection, in 37 (24.7 %) 2 of the above persons participated in the procurement, in 13 (8.7 %) cases of 3 persons and only in 1 case (0.7 %) all 4 listed persons (data are not shown in the table).

Table 4: Type and basic characteristics of the school bag that children have

School bag	Total number of respondents (N = 150)	
	N	%
Type[1]		
Backpack	144	96.0
Wheeled bag	5	3.3
One shoulder bag	1	0.7
Sports bag	0	0.0
Characteristics[2]		
Reinforcement on the shoulders	133	88.7
Belt over chest	45	30.0
Waist belt	31	20.7
Partitions	113	75.3
Adjustable shoulder straps	116	77.3
Wheels	5	3.3
Cat's eyes	22	14.7
Rear reinforcement	94	62.7
Mobile phone pocket	58	38.7
Side pockets for storing things	116	77.3
Other	3	2.0

(1) there is a possibility of only one answer, the total percentage is 100 %
(2) multiple choice possible, percentages of individual answers exceed 100 %

School bags were predominantly procured in stores (97.4 %) and only sporadically via the Internet or by downloading used bags from other family members (2.6 %).

The dominant type of school bag purchased was the backpack model (96 %), and only sporadically the wheeled bag (3.3 %) (Table 4).

Among the characteristics that the purchased school bags had, according to their high representation, stand out reinforcement on the shoulders (88.7 %), adjustable shoulders (77.3 %) and side pockets for storing things (77.3 %).

Table 5 shows the desirable characteristics that a school bag must meet (left) and the real situation achieved by purchasing school bags (right). In a very small number of cases (15 school bags; 10 %), the basic criteria was fully met.

Table 5: Conformity of desired and realised characteristics of school bags

School bag	The presence of all 6 desired characteristics	
Minimum of desired characteristics	**No**	**Yes**
Reinforcement on the shoulders	135 (90 %)	15 (10 %)
Belt over chest	$\chi^2 = 245.45$ $p < 0.001$	
Waist belt		
Adjustable shoulder straps		
Rear reinforcement		
Side pockets for storing things		

Using the Chi-squared test ($\chi^2 = 245.45$; $p < 0.001$), a significant difference between the desired and the achieved result was completely statistically proven. In the remaining number of cases (90 %), there is an absence of at least one or more desired characteristics.

A detailed analysis of the data from the Table 4 revealed that in most cases, the purchased school bags lack 2 characteristics (chest belt and waist belt are present in only 30 % and 20.7 %, respectively) of purchased school bags.

Ranking was done according to the protocol: 1-Very important; 2-Important; 3-Not very important; 4-It doesn't matter at all; 5-I don't know. The graph presents mean values and standard deviations.

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Based on the stated ranking, it is clear that the answers with the lowest average grades indicated the fact of the importance of that characteristic of the school bag. In contrast, characteristics that were of very little importance to parents (or did not know enough about it), during the selection of the school bag, had the highest average grades (mostly above 3, dashed line) (Figure 1).

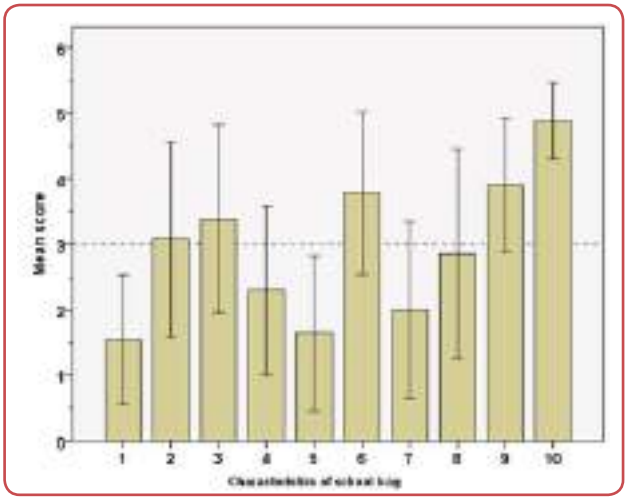

Figure 1: Parents' opinions on the importance of the characteristics that a school bag should have

1 - Shoulder reinforcement; 2 - Chest belt; 3- Waist belt; 4 - Partitions; 5 - Adjustable shoulder straps; 6 - Wheels; 7 - Reinforcement on the back of the bag; 8 - Cat's eyes; 9 - Mobile phone pocket; 10 - Other; Bars represent standard deviation of the mean;

In general, (Figure 1) most parents did not know the answer (94 %; n = 141). Parents considered that the most important characteristics that a school bag should have are reinforcement on the shoulder straps 64.7 % (n = 97), adjustable shoulders 65.3 % (n = 98) and reinforcement on the back of the bag 49.3 % n = 74). Most of the other characteristics (average grades close to 3 or higher) were rated by the parents as slightly important or unimportant. A relatively small number of parents 28 % (n = 42) considered it very important that the school bag has features as a measure of safety and security when moving.

The largest number of parents (36.7 %) estimated that a filled school bag must be lighter than 10 % of body weight, in a slightly lower percentage (35.3 %) the answer was related to the assessment that 5 % is the limit weight of a filled school bag.

Most parents did not receive information about the school bag, 77.3 % (n = 116). Vendors 11.3 % (n = 17), other parents and friends 9.3 % (n = 14) were the main source of information before purchasing a school bag.

Table 6: Parents' assessment of the importance of school bag weight

School bag	Total number of respondents (N = 150)	
	N	%
Weight[1]		
Less than 5 % of the child's weight	53	35.3
12 % of the child's weight	3	2.0
Less than 10 % of the child's weight	55	36.7
Less than 20 % of the child's weight	4	2.7
Less than 15 % of the child's weight	9	6.0
There is no standard bag weight	16	10.7
Other	10	6.7

(1) there is a possibility of only one answer, the total percentage is 100 %

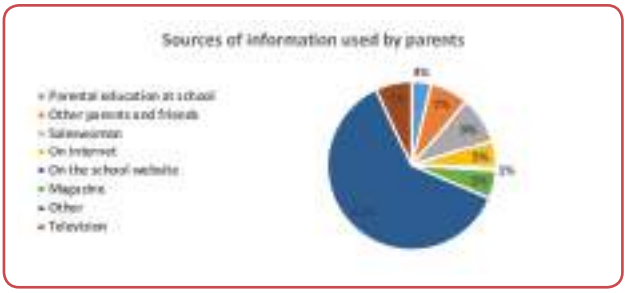

Figure 2: Sources of information used by parents

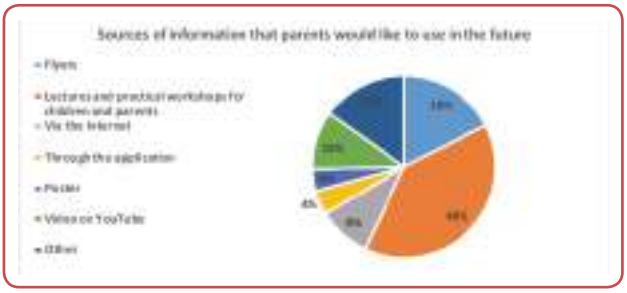

Figure 3: Source of information that parents would like to use in the future

A large number of 54 % (n = 81) of parents in the future would like to receive information about the school bag through lectures and practical workshops for children and parents, 24 % (n = 36) written materials and video platforms 14 % (n = 21).

Discussion

A large number of previous researches on the topic of carrying a school bag for school-age children have been focused on the identification of risk factors that are related to weight, back curvature, the presence of pain, exhaustion and the appearance of fatigue using statistical indicators.[20-25] However, a small number of authors considered that the role of parents in solving this problem is crucial[26-28] and that it can significantly contribute to ensuring unhindered growth and development during the child's educational process.

CONTENT

The aim of the study was to examine how much parents are informed about the school bag and which specifications are crucial when choosing a school bag for their children. 150 parents participated in the research, 54 % female (n = 81) and 46 % male (n = 69). The largest number of parents belonged to the age structure between 40-49 years - 52 % (n = 78). Within the professional structure of female respondents, the highest percentage was with undergraduate education 40.7 % (n = 61) and male with secondary education 54 % (n = 81). The authors of Das and Goswami (2020) in the conclusion of their study reported that the educational qualification of parents acts as a major factor in raising awareness in children and that there are some differences in their views regarding the weight of a school bag. Lower educated parents ignored this problem and did not have enough knowledge about the harmfulness of a school bag, which is in line with the findings of this study.[27] The analysis of the family structure showed that the largest percentage of children lived with both parents - 76.7 % (n = 132), most often the families of respondents with two children were 57.3 % (n = 85), three or more children also had an enviable percentage of respondents (26.7 %) whose income was satisfactory 120 (80 %), of the total number of respondents, statistically, the survey most often covered the first child at birth 63.3 % (n = 95). This statement is important, because the attitude and knowledge that parents possess will certainly be applied to their younger children.

In 62 % of cases, the mother and in 60 % of cases the child participated in the selection of the school bag. In 97.4 % of cases, the school bag was purchased in stores. The knowledge gained in this study is important, because greater information of parents enables adequate choice of school bag and reduces the possibility of marketing tricks and influence on consumers when choosing the desired product such as a school bag. A small percentage of children in this sample had a school bag on wheels (3.3 % of students), which indicates that parents do not consider the most suitable bag for a child to be a good choice. In accordance with the findings of previous studies, the backpack is the most common bag used by children, 96 %,[28, 29] whose characteristics in 88.7 % had a reinforcement on the straps, 75.3 % adjustable straps, 62.7 % and side pockets for disposal of items by 77.3 %. Their children's school bag had a number of satisfactory characteristics and the results were in line with the results of other studies.[30, 31]

Parents' knowledge of the importance of the specifications that a school bag should have was satisfactory. In most cases, 64.7 % (n = 97) of parents consider it very important that the straps on the bag have reinforcement, 65.3 % (n = 98) adjustable and 49.3 % (n = 74) reinforcement from the back foreign bags. Only 30.7 % (n = 46) of parents considered it important that a school bag should have a belt over the chest and 20.7 % (n = 31) a belt over the waist. The results of this study show that parents' knowledge is unsatisfactory and that the belt over the chest and waist helps distribute the load, which is in line with previous findings of other studies and the importance of health promotion and application and safety of school bags to reduce the incidence of pain in students.[31] Significantly small number of children, 14 % (n = 22) had a cat's eye on a school bag and also a small number of parents, 28 % (n = 42) considered it important that the school bag had features as a measure of safety and security when moving. This statement is important and indicates that parents' awareness of safety and security measures when moving is low. The largest number of parents, 36 % (n = 55) believed that the weight of the school bag should be less than 10 % of body weight and 35.3 % (n = 53) that the weight of the school bag should be less than 5 % of body weight, which indicates that parents were satisfactorily informed about the recommended weight of the school bag, which was stated in a study conducted among 616 parents in Saudi Arabia, which reported that only 37.6 % of fathers and 28.9 % of mothers knew the ideal weight of the school bag.[34]

Most parents did not receive information about the school bag, 77.3 % (n = 116). Salespeople 11.3 % (n = 17), other parents and friends 9.3 % (n = 14) were the main source of information before purchasing a school bag. Interestingly, 54 % (n = 81) of parents have chosen hands-on workshops to adopt new information about the school bag. A small number of parents, 24 % (n = 36) considered written materials and video platforms (14 % (n = 21)) a good way to gain knowledge and information about the school bag as a teaching tool. The results of this study contradict the results of a study that reported that parents considered written materials as the best source of knowledge acquisition.[19]

So far, no studies on information and attitudes of parents about the school bag have been pub-

lished in the Republic of Srpska and the Federation of Bosnia and Herzegovina. The parents' survey was conducted within the project for the development of a doctoral thesis, to obtain more detailed information on wearing habits and characteristics of school bags that may affect further physical development of children.

Conclusion

Most parents, 77.3 % (n = 116) did not receive information about the school bag. Parents demonstrated satisfactory knowledge of the appropriate type of school bag and the allowable weight that the school bag should have. Parents' knowledge was satisfactory about the specifications that a school bag should have, but in 90 % of cases there was the absence of at least one or more desired characteristics of their children's school bag. In most cases (70 % and 79.3 %), school bags lack 2 characteristics (chest belt and waist belt) that help distribute the load during use. Although, we live in the age of information technology, a large number, 54 % (n = 81) of parents in the future would like to get information about the school bag through lectures and practical workshops for children and parents. A small number of parents, 24 % (n = 36) considered written materials and video platforms, 14 % (n = 21) as a good way to gain knowledge and information about the school bag as a teaching tool. Preventive programs enable children and parents to adopt new values, skills for the most correct and uninterrupted functioning of the body in performing daily life activities, applications and safety of students during the use of school bags. Greater parental awareness allows for adequate school bag selection and reduces the possibility of marketing gimmicks and influencing consumers when choosing a desired product, such as a school bag. Parents have an important role to play in ensuring the unhindered growth and development during the child's educational process and in strengthening children's awareness and supervision about the proper use of the school bag.

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None.

Conflict of interest

None.

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ORIGINAL ARTICLE

Factors Influencing Efficacy of Complete Decongestive Treatment in Patients with Breast Cancer-Linked Arm Lymphoedema

Dragana Bojinović-Rodić,[1] Samra Pjanić,[1] Tamara Popović,[1, 2] Tatjana Nožica-Radulović[1, 2]

(1) Institute for Physical Medicine and Rehabilitation "Dr Miroslav Zotović", Banja Luka, the Republic of Srpska, Bosnia and Herzegovina.
(2) Faculty of Medicine, University of Banja Luka, Banja Luka, the Republic of Srpska, Bosnia and Herzegovina.

Abstract

Background/Aim: The most recommended form of lymphoedema therapy is complete decongestive treatment (CDT). Efficacy of CDT in patients with arm lymph-oedema related to malignant breast tumour has reported in many studies, but the predictive factors of outcome of this therapy have not been yet sufficiently investi-gated. The purpose of this research was to identify predictive factors of efficacy of CDT in patients with breast cancer-linked arm lymphoedema throughout the inten-sive phase of therapy.
Methods: The prospective study included 51 patients with breast cancer-linked arm lymphoedema who were subjected to a 3-week program of CDT. Patients' clini-cal and demographic features, breast cancer treatment characteristics, lymph-oede-ma and CDT characteristics were collected and assessed for their prognostic value. The influence of certain predictors on the degree of lymphoedema reduction was evaluated by multivariate linear regression analysis.
Results: Mean age was 58.1 ± 8.0 (95 % CI: 55.8 - 60.3), median of BMI was 28.4 kg/m^2 (95 % CI: 27.2 - 29.6). The average duration of lymphoedema was 36.5 ± 43.9 months (95 % CI: 24.1 - 48.8). The mean size of lymphoedema before CDT was 6.99 ± 5.36 %, and the mean degree of lymphoedema reduction was 63.7 ± 28.6 %. The mean compliance to bandages was 217.5 ± 97.8 hours (95 % CI: 190.0 - 245.0) and 7 (13.7 %) patients had a history of erysipelas of the ipsilateral arm. When observ-ing each individual predictor, statistically most significant contribution showed the size of lymphoedema before the therapy ($p < 0.001$), then history of erysipelas ($p < 0.01$), and patients' age ($p < 0.05$).
Conclusion: Size of lymphoedema before treatment is the most crucial prognostic factor of the efficacy of CDT in the patients with breast cancer-linked arm lymph-oedema. The present study also identified history of erysipelas and patients age as independent predictors of the CDT efficacy.

Key words: Breast cancer; Lymphoedema; Physical therapy modalities; Compression bandages; Drainage; Treatment outcome.

Correspondence:
DRAGANA BOJINOVIĆ-RODIĆ
E: dbojinovic@yahoo.com
M: +387 65 422 999

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Introduction

Breast cancer-linked arm lymphoedema is ab-normal accumulation of interstitial fluid due to mechanical failure of the lymphatic system of the upper limb, usually because of the breast cancer surgery, radiotherapy, infection, or trauma. In the available literature, the average incidence of breast cancer-linked arm lymphoedema caused by axillary dissection is greater than 20 %, and

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after sentinel lymph node biopsy is less than 10 %.[1-4] The overall goal of lymphoedema treat-ment is to reduce swelling, mobilise congestive interstitial fluid, reduce connective and fat tissue proliferation, control symptoms, and minimise the consequences.[5]

The most effective and most common form of lymphoedema therapy is complete decongestive treatment (CDT), which represents the gold standard in the conservative treatment.[6-9] Efficacy of CDT in patients with arm lymphoedema related to malignant breast tumour has reported in many studies,[10-14] but the predictive factors of outcome of this therapy have not been yet sufficiently in-vestigated. The purpose of this research was to identify independent predictive factors of effica-cy of CDT in patients with breast cancer-linked arm lymphoedema throughout the intensive phase of therapy.

Methods

Study design

This prospective study was carried out at the In-stitute for Physical Medicine and Rehabilitation "Dr Miroslav Zotović" in Banja Luka, and included patients with arm lymphoedema who underwent malignant breast tumour surgical procedure. The research was permitted by the Ethics Committee of the facility.

Participants

The inclusion criteria for the study were: unilateral axillary dissection, clinically verified lymphoede-ma (difference in circumference between affected and healthy arm was larger than 2 cm at minimum 1 measurement level), more than 3 months from the breast cancer surgery and radiotherapy and patient-signed informed consent form, with prior knowledge of the trial purpose.

The elimination criteria were: metastatic breast disease, clinically verified acute erysipelas; un-treated and poorly controlled hypertension, heart failure, deep venous thrombosis and anticoagulant therapy, shoulder and upper limb damage caused by neurological, orthopaedic or rheumatic diseas-es diagnosed prior to breast cancer surgery, diag-nosed and medically treated psychiatric disorders, liver cirrhosis and nephrotic syndrome. For each patients following data were collected and assessed for their prognostic value: clinical and de-mographic features (age, body mass index-BMI, co-morbidity), breast cancer treatment characteristics (time from surgery, type of breast surgery, number of lymph nodes removed and involved, therapy before and/or after surgery), lymphoedema char-acteristics (duration of lymphoedema, time until lymphoedema onset, size of lymphoedema, report-ing pain and other symptoms in the arm, history of erysipelas) and CDT characteristics (compliance to bandages).

Intervention

Patients were taken to a 3-week program of CDT, once a day, 5 days a week. The CDT protocol con-sisted of manual lymphatic drainage (MLD), short-stretch multilayer compression bandages (Rosidal® K Lymphset, Lohmann & Rauscher, Vienna, Austria) and exercises provided by therapists. Exercises were performed with compression bandages as an essential part of the decongestive phase of lymph-oedema therapy. The exercises consisted of: exer-cises of diaphragmatic breathing, remedial exercis-es, flexibility (stretching) exercises and resistance (weight-lifting) exercises of the affected arm.

Lymphoedema size

Lymphoedema was assessed by the arm circum-ference. It was measured at 7 symmetrical levels (metacarpophalangeal joints, radial styloid process, at 10, 20, 30 and 40 cm from the radial styloid pro-cess and over olecranon) of the affected and contra-lateral arm. The lymphoedema size was expressed as the ratio of the total circumference of the affect-ed and unaffected arm, and calculated according to the following formula: [(total circumference of the affected arm - total circumference of the unaffected arm) / - total circumference of the unaffected arm] x 100, where 0 % indicates the same total circum-ferences of two arms. The degree of lymphoedema reduction was calculated by the following formu-la: (total circumference of the affected arm before treatment - total circumference of the affected arm after treatment/ total circumference of the affected arm before treatment - total circumference of the unaffected arm) x 100.[14-16]

Bandage compliance

All patients received instructions for wearing the bandages as long as possible ie, until the next treat-ment day. The bandage-carrying compliance was evaluated through the so-called "bandage log" in which all patients registered the exact time of ap-plication and removal of bandages daily, based on which the total number of hours under the ban-dage was calculated.

Statistics

Descriptive statistics methods were used to describe all data in the study. Numerical data associated with the percentage of lymphoedema reduction were identified by Pearson correlation. Categorical data were analysed by the independent samples t-test. Factors with $p < 0.05$ in the stated analyses were selected as final predictors for multivariate linear regression analysis. All analyses were carried out using SPSS Version 21.0 for Windows. The result was significant if the p-value was less than 0.05.

Results

The prospective study included 51 female patients with secondary arm lymphoedema after breast cancer treatment. Mean age was 58.1 ± 8.0 (95 % CI: 55.8 - 60.3), median of BMI was 28.4 kg/m^2 (95 % CI: 27.2 - 29.6). The largest number of patients (47.1 %) were in the category of overweight, 29.4 % were obese patients, 21.5 % were in normal range and only 1 patient (2.0 %) was in the category of underweight. The average duration of lymphoedema was 36.5 ± 43.9 months (95 % CI: 24.1 - 48.8). The mean degree of lymphoedema reduction was 63.7 ± 28.6 %. The mean compliance to bandages was 217.5 ± 97.8 hours (95 % CI: 190.0 - 245.0) and 7 (13.7 %) patients had a history of erysipelas of the ipsilateral arm. Table 1 shows characteristics of the patients.

Table 1: Patients' characteristics

Characteristics	N (%)	Mean ± SD	Range (min-max)
Patients' age (years)		58.1±8.0	41.0-77.0
Time from surgery (months)		53.4±50.0	3.0-185.0
Type of breast surgery			
Rradical mastectomy	30 (58.8)		
Breast conserving surgery	21 (41.2)		
Number of lymph nodes removed		14.2±6.6	3.0-42.0
Number of lymph nodes involved		2.8±6.1	0.0-32.0
Therapy			
Chemotherapy	37 (72.5)		
Radiotherapy	38 (74.5)		
Hormonal	37 (72.5)		
Duration of lymphoedema (months)		36.5±43.9	0.5-170.0
Time until lymphoedema onset (months)		17.0±22.9	0.0-124.0
Size of lymphoedema before CDT (%)		6.99±5.36	1.99-25.0
Degree of lymphoedema reduction (%)		63.7±28.6	13.8-100
Reporting pain and other symptoms in the arm			
Yes	44 (86.3)		
No	7 (13.7)		
Hystory of erysipelas			
Yes	7(13.7)		
No	44 (86.3)		
Body mass index - BMI (kg/m^2)		28.4±4.3	17.9-37.9
Underweight (< 18.5)	1 (2.0)		
Normal range (18.50-24.99)	11 (21.5)		
Underweight (25.00-29.99)	24 (47.1)		
Obese (> 30)	15 (29.4)		
Comorbidity (medication for)			
Hypertension	23 (45.1)		
Heart disease	9 (17.6)		
Thyroid problems	12 (23.5)		
Diabetes	4 (7.8)		
Venous insufficiency in the lower limbs	5 (9.8)		
Osteoporosis	8 (15.7)		
Others	5 (9.8)		

The size of lymphoedema before therapy was statistically significantly negatively correlated with degree of lymphoedema reduction ($p < 0.001$). Also, there was statistically significant negative correlation between patients' age and percentage of lymphoedema reduction ($p < 0.05$). The degree of lymphoedema reduction in patients with history of erysipelas, was statistically significantly lower than in those who did not have erysipelas ($p < 0.01$) (Table 2).

Table 2: Factors accociated with efficacy of CDT

Variable	Pearson correlation		Independent samples T-test	
	R	p	T	p
Patients' age (years)	-0.280	**0.047**		
Time from surgery (months)	-0.129	0.367		
Type of breast surgery				
Number of lymph nodes removed	-0.186	0.191	-0.608	0.546
Number of lymph nodes involved	-0.016	0.914		
Chemotherapy				
Radiotherapy			-0.508	0.614
Hormonal therapy			0.109	0.914
Duration of lymphoedema (months)	-0.082	0.569	0.142	0.888
Time until lymphoedema onset (months)	-0.124	0.386		
Size of lymphoedema before CDT	-0.710	**0.000**		
Reporting pain and other symptoms in the arm			-0.406	0.687
Hystory of erysipelas	-0.129	0.367	-3.808	**0.002**
Body mass index - BMI (kg/m^2)				
Comorbidity (yes/no)			0.516	0.608
Number of comorbidities	0.076	0.598		
Compliance to bandages (hours)	0.008	0.956		

Table 3: Predictors of complete decongestive treatment (CDT) efficacy after multivariate analysis

Factors	β coefficient of linear regression	t-value	p
Size of lymphoedema before the CDT	-0.553	-6.207	0.000
Hystory of erysipelas	0.273	3.078	0.004
Patients' age (years)	-0.200	-2.354	0.023

Influence of certain predictors on the degree of lymphoedema reduction was evaluated by multi-variate linear regression analysis. The results showed that the model explained 65.4 % of the total variance, ($F = 24.579$, $p = 0.000$). When ob-serving each individual predictor, statistically most significant contribution showed the size of lymphoedema before the therapy ($p < 0.001$), then history of erysipelas ($p < 0.01$), and patients' age ($p < 0.05$). Table 3 shows predictors of CDT efficacy after multivariate analysis with the per-centage of lymphoedema reduction as dependent variable.

Discussion

In this study, the influence of independent predictors on the success of decongestive therapy of reast cancer-related arm lymphoedema was investigated. Younger age and lower size of lymphoedema before CDT were identified as predictors associated with better response to treatment. The history of erysipelas was associated with a poor outcome of CDT.

The most important predictor of the degree of reduction of lymphoedema was the size of lymphoedema before the therapy: the lower the size of lymphoedema before the treatment was, the greater the degree of reduction achieved. Efficacy of CDT is better, if the therapy starts as earlier as possible, when lymphoedema is less pronounced. Similar results were obtained by other authors.[14, 15, 17]

The younger age was also a predictor of better therapy response. Lia SF et al also reported that younger age would predict CDT efficacy, believing that older patients have poor compliance with bandages.14 That could not be concluded in the present study. In the study of predictors of lower limb lymphoedema, Vignes et al reported that older patients had better treatment outcomes. The average age of patients in this study (45.8; range 32-60.4) was considerably less than in mentioned study (58.1; range 41-77).[18]

The most common lymphoedema complication is erysipelas. It is an infection that involves the superficial layer of the skin with primarily affects-the lymphatic vessels (lymphangitis). Upper limb erysipelas occurs in up to 24 % of women aftersurgical treatment for breast cancer follow-inglymphatic system damage. Lymphoedema is considerable risk factor for reappearance of erysipelas.[19-21]

The only study that identified previous erysipelas as predictor of the efficacy of CDT was a study of primary lower extremity lymphoedema. But, in that study patients with previous episode(s) of erysipelas obtained higher lymphoedema volume reduction.18 History of earlier erysipelas has proved to be an individually negative significant predictor in the present research. Considering total of patients who had erysipelas in this study was 7 (13.7 %), this clinical feature requires further research.

Erysipelas may have negative impact on CDT efficacy. Every episode od erysipelas affects the lymphatic vessels, aggravating of pre-existing lymphatic impairment and worsening lymphoedema.

A surprising finding in these results is that bandage-carrying compliance was not associated with better treatment response. Bandage compliance is generally considered a factor influencing-CDT outcomes. According to the findings of some authors, bandage-carrying compliance is a dominant predictive factor of CDT effectiveness, not only after the intensive phase of treatment, but also during the maintenance phase.[15, 22]

Forner-Cordero et al concluded that good bandage
compliance improved the percent reduction of lymphoedema by 25 % compared with fair or bad bandage compliance in breast cancer-linked lymphoedema.[15]

The present study is, to authors' knowledge, the only study that evaluated bandage-carrying compliance using a "bandage log" and compared the total number of hours of wearing a bandage with the degree of lymphoedema reduction. The average number of hours of bandage wearing in this study was 217.5 ± 97.8 hours (range 81-471). If the criteria from the Forner-Cordero' study were used, 72.5 % of patients in this study would have bad bandage-carrying compliance, 25.5 % fair compliance, and only 1 patient (2 %) good bandage-carrying compliance. Since most patients

had poor bandage-carrying compliance, this factor requires further examination in a larger number of patients.

BMI and duration of lymphoedema also were not associated with better response to CDT. The results of other studies are contradictory. Vignes et al demonstrated that duration of lymphoedema and BMI were correlated with a greater absolute reduction, but not a relative decrease in lymphoedema volume.[23] Forner-Cordero at al found that the duration of lymphoedema does not affect the outcome of the therapy and that patients may benefit from treatment long time after symptoms appear.[15]

The authors consider that the most important factor in keeping lymphoedema under control is the
regular administration of CDT to reduce swelling and that the largest lymphoedemas are not the oldest.

It is well known that overweight or obesity, expressed as a BMI greater than 25.0 and 30.0 respectively, is important risk factor for secondary lymphoedema.24-26 The effect of BMI on CDT outcome has been described in some studies,[18, 22, 23] but in this one, such a result was not obtained. The
reason could be that most of the patients in this study were in the pre-obese or obese category, and only 21.5 % were in the normal range.

The breast cancer treatment characteristics (time from surgery, type of breast surgery, number of lymph nodes removed and involved, treatment before and/or after surgery) did not affect CDT efficacy in present study.

Study strengths and limitations

The present study was conducted at an institution specialised in the treatment of patients with lymphoedema and only included patients with arm lymphoedema linked to breast cancer. The study was prospective and monocentric. All patients received homogenous CDT protocol and MLD was carried out by two trained physiotherapists and under the supervision of the researcher. The main restriction of this study is little sample size. Forthcoming research with greater number of patients is necessary.

Conclusion

Size of lymphoedema before treatment is the most crucial prognostic factor of the efficacy of CDT in the patients with breast cancer-linked arm lymphoedema. The present study also identified history of erysipelas and patients age as independent predictors of the CDT efficacy.

Although this study did not show statistical significance for the bandage-carrying compliance, BMI and duration of lymphoedema, these factors should be paid attention in the further prospective studies with a larger number of patients.

Acknowledgements

None.

Conflict of interest

None.

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ORIGINAL ARTICLE

Correlation of Body Mass Index and Orthostatic Hypotension in Patients with Hypertension on ACE Inhibitor Monotherapy

Danijela Tasić,[1, 2] Zorana Kovačević,[1] Miroslav Mitrović,[3] Zlatko Maksimović,[4] Dragana Lončar-Stojiljković,[1, 2] Nebojša Tasić[1]

(1) Dedinje Cardiovascular Institute, Belgrade, Serbia.
(2) Faculty of Medicine, University of Banja Luka, Banja Luka, the Republic of Srpska, Bosnia and Herzegovina.
(3) HISPA, Hypertension, Infarction, Stroke Prevention Association, Belgrade, Serbia.
(4) Hospital "Sveti Vračevi", Bijeljina, the Republic of Srpska, Bosnia and Herzegovina.

Correspondence:
NEBOJŠA TASIĆ
T: +381 11 3601669
E: nebtasa@yahoo.com

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Abstract

Background/Aim: Orthostatic hypotension (OH) is considered to be a drop in the systolic and diastolic blood pressure (> 20 mmHg; > 10 mmHg) 3 minutes from postural changes. The objective of this study was to analyse the correlation of body mass index (BMI) and OH during the treatment with trandolapril, as a single-drug treatment of hypertension.
Methods: The study involved 255 patients (average age 54.3 ± 11.7; 54.1 % men) with poorly regulated hypertension, who were given trandolapril as a single-drug treatment. The patients were divided into two groups regarding stage of hypertension: first-degree arterial hypertension (140-149 mmHg for systolic and 90-109 mmHg for diastolic blood pressure) and second-degree arterial hypertension (> 150 for systolic and > 110 mmHg for diastolic blood pressure). Incidence of OH occurrence was then analysed regarding hypertension stage and BMI during 6 months of follow-up, on 4 control examinations.
Results: During 24-week period after trandolapril introduction into the treatment of hypertension, a statistically significant difference in systolic, diastolic and mean blood pressure values was observed. No statistically significant difference was observed in incidence of OH between the first and second as well as between third and fourth examination during the study. Regarding the incidence of OH in normal body weight and obese patients, there was also no statistically significant difference.
Conclusion: As shown in this study, trandolapril, along with some other ACE inhibitors, has shown good balance in hypertension control and OH occurrence.

Key words: Orthostatic hypotension; Trandolapril; Body mass index.

Introduction

Angiotensin converting enzyme (ACE) inhibitors are commonly used antihypertensive drugs, not just for hypertension, but also for various cardiovascular diseases.[1] The mechanism of action disables angiotensin I to angiotensin II conversion, all by blocking the ACE, which consequently decreases angiotensin II activity. Moreover, ACE inhibitors lower catecholamine activity and interfere in vascular remodelling,[2] which enables its widespread use.

Trandolapril is a non-sulfhydryl-containing type of ACE inhibitor. Affinity of binding trandolaprilat to the ACE is very high. Compared to other ACE inhibitors, required dose for blocking 50 % of ACE is similar to ramiprilat.[3] Blood pressure (BP) values

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change depending on the body position. Usually a discreet drop of systolic and a discreet increase of diastolic blood pressure (DBP) occur when rising into standing position from a horizontal one. Orthostatic hypotension (OH) occurs when these changes are greater: for systolic blood pressure (SBP) a drop more than 20 mmHg and for DBP drop more than 10 mmHg within 3 minutes from the postural changes.[4] It can occur no matter of age or sex. The OH prevalence increases with age, estimated at 5-30 % in population over 65 years of age.[5] It can present with a range of unpleasant symptoms such as dizziness, nausea, headache, weakness, palpitations, etc. Therefore, it is recommended to gradually introduce the hypertension treatment and to measure blood pressure in lying or sitting / standing position once in a while, especially in patients older than 50, in order to prevent OH and its symptoms.[6] By its aetiology, OH can be neurogenic or non-neurogenic, while in terms of symptoms onset it can be initial, classic or delayed. Some antihypertensive drugs are associated with OH as their side effect.[7] Patients suffering from essential hypertension can have various and individual response to different antihypertensive drugs. Therefore, it is necessary to examine and determine patients' response to ACE inhibitors, in order to estimate risk and occurrence of OH.[21] The selection of adequate antihypertensive drug for different patients must be done thoroughly, taking into consideration incidence of OH in certain groups of patients.

The objective of this study was to analyse the correlation of body mass index (BMI) and OH during the treatment with trandolapril, generic representative of the ACE inhibitors, as a single-drug therapy.

Methods

This prospective study involved 255 patients with poorly regulated arterial hypertension. To these patients, trandolapril was introduced as a monotherapy. Follow-up period was 24 weeks.

Poorly regulated arterial hypertension was defined as SBP greater than 140 mmHg and for DBP greater than 90 mmHg measured two times in separate examinations. Patients were distributed in two examination groups, considering values of BP. The first group included patients with first-degree arterial hypertension (SBP between 140-149 mmHg, DBP between 90-109 mmHg) and the second group considered patients with second-degree arterial hypertension (SBP ≥ 150 mmHg and DBP ≥ 110 mmHg). Excluding criteria were as follows: patients younger than 18, pregnancy (positive β-hCG test), nursing mothers, patients with OH, patients with renal failure stage II or greater, patients with microalbuminuria > 300 mg / 24 h, patients with electrolytic disbalance, cardiac arrhythmias, anaemia (haemoglobin levels < 100 g/L), liver enzymes ≥ 1.5 times greater than reference values.

At the beginning of the study, every patient underwent physical examination, body weight and height measurement, BMI was calculated, 12-lead ECG was performed with heart rate calculation. BP was measured at every examination in sitting and standing position. Measurements were performed using mercury sphygmomanometer with cuff placed to cover two thirds of upper arm surface and minimum 80 % of upper arm circumference. BP was measured consecutively three times at each arm, with one-minute breaks between the measurements and mean values were calculated afterwards. Control visits were performed at 6th, 12th and 24th week after inclusion to the study. At each visit, doses of trandolapril were corrected in order to achieve adequate BP control (≤ 120/80 mmHg). Prior the inclusion, every patient had signed informed consent for participation. The research has been granted by the Ethics Committee of the Dedinje Cardiovascular Institute in Belgrade.

Statistical analysis was performed using descriptive statistical methods and analysed in ANOVA repeated measurements. IBM SPSS 18.0 software was used.

Results

The study included 255 patients, 138 men (54.1 %) and 117 women (45.9 %), average age 54.3 ± 11.67 years (Figure 1). The average BMI value was 27.73 ± 4.7, ranging from 17.3 to 36.6. According to BMI values, 2 (0.8 %) patients were underweight (BMI < 18.5), 35.3 % had normal weight (BMI between 18.5 and 25) while 163 (63.9 %) patients were overweight (BMI > 25). In the first study group, 127 (49.8 %) patients had first-degree hypertension, while 128 patients (50.2 %) had the second-degree hypertension.

Table 1: Blood pressure values of hypertensive patients on monotherapy with trandolapril

Haemodynamic parameters (X±SD)	Measurement period				Significance #
	Prior to therapy	After 4-6 weeks	After 12-14 weeks	After 24-26 weeks	
Systolic pressure	152.60 ±11.03	141.33 ±15.41	132.93 ±10.48	127.11 ±6.05	p=0.000*
Diastolic pressure	90.60 ±8.43	85.03 ±7.52	81.35 ±6.36	78.87 ±5.20	p=0.000*
Mean arterial pressure	110.80 ±10.02	103.78 ±8.58	98.91 ±7.15	95.67 ±5.48	p=0.000*

**statistically significant difference; #Fridman's test*

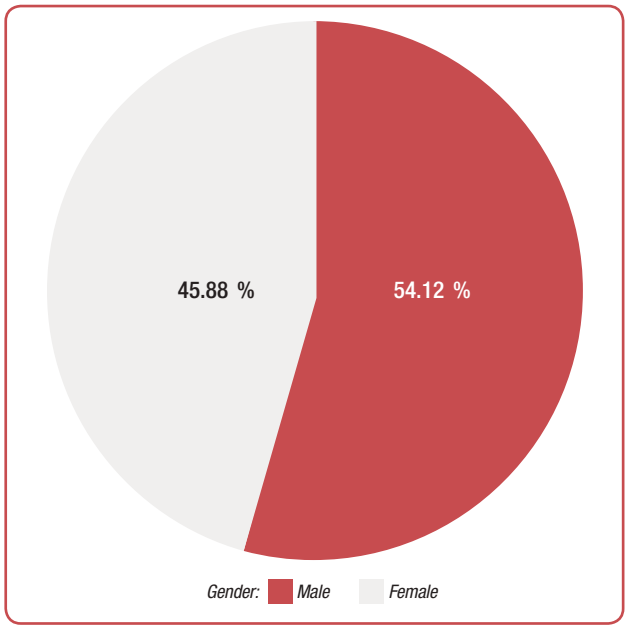

Figure 1: Distribution of subjects by gender of hypertensive patients on monotherapy with trandolapril

Table 2: Orthostatic hypotension and body mass index (BMI) of hypertensive patients on monotherapy with trandolapril

Measurement period	Orthostatic hypotension	BMI		Significance
		< 25	≥ 25	
Prior to treatment	Yes	8 (8.7 %)	17 (10.4 %)	$^{a}p = 0.863$
	No	84 (91.3 %)	146 (89.6 %)	
After 4-6 weeks	Yes	8 (8.7 %)	24 (14.7 %)	$^{a}p = 0.198$
	No	84 (91.3 %)	139 (85.3 %)	
After 12-14 weeks	Yes	9 (9.8 %)	18 (11.1 %)	$^{a}p = 0.621$
	No	83 (90.2 %)	145 (88.9 %)	
After 24-26 weeks	Yes	7 (7.6 %)	9 (5.5 %)	$^{a}p = 0.453$
	No	85 (92.4 %)	154 (94.5 %)	

**statistically significant difference; $^{a}\chi^2$-test; Mann Whitney test;*

During 24-week period after trandolapril introduction into the treatment of hypertension, a statistically significant difference in SBP, DBP and mean BP values was observed (Table 1, Figure 2). From all patients included in the study, 25 (9.8 %) were diagnosed with OH, while 230 (90.1 %) were without OH at first appointment. After initiation of the treatment, on the first visit, 10 patients (3.9 %) still had OH and 22 (8.6 %) new patients had

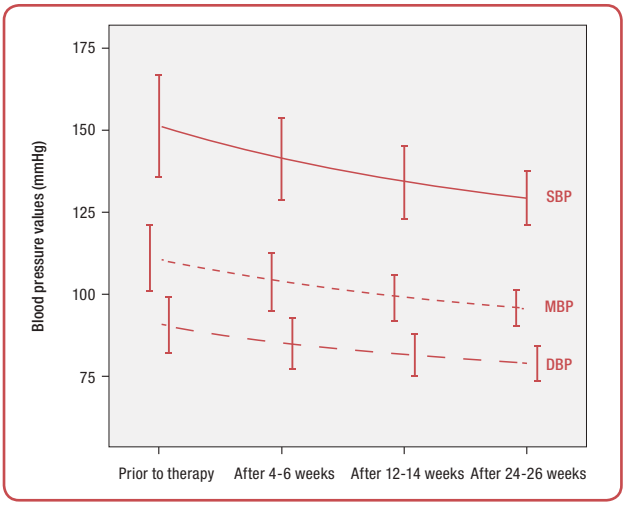

Figure 2: Systolic, diastolic and mean blood pressure of hypertensive patients on monotherapy with trandolapril
**SBP: systolic blood pressure, MBP: mean blood pressure, DBP: diastolic blood pressure;*

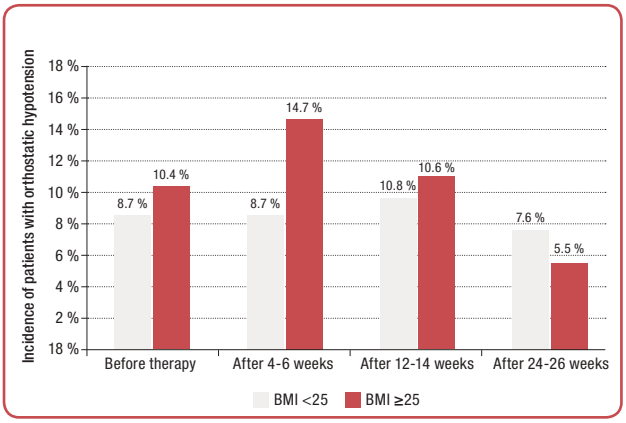

Figure 3: Incidence of orthostatic hypotension in normal weight and overweight patients (on trandolapril therapy)

Table 3: Incidence of adverse effects

Incidence of adverse effects n (%)	Time (weeks)		
	After 4-6	After 12-14	After 24-26
No adverse effects	250 (98.03 %)	250 (98.03 %)	249 (97.64 %)
Mild fatigue	2 (0.8 %)	0 (0 %)	0 (0 %)
Mild occasional a	1 (0.39 %)	0 (0 %)	0 (0 %)
Rare episodes of dry cough	1 (0.39 %)	1 (0.39 %)	1 (0.39 %)
Mild nausea	1 (0.39 %)	0 (0 %)	0 (0 %)
Mild vertigo	0 (0 %)	2 (0.8 %)	1 (0.39 %)
Occasional headache	0 (0 %)	2 (0.8 %)	0 (0 %)
Occasional moderate vertigo	0 (0%)	0 (0 %)	4 (1.56 %)

**Fridman's test;*

OH. There was no significant difference in OH incidence in this period.

Later, at the second visit, there were 7 patients (2.7 %) with prior OH and 20 patients with newly diagnosed OH.

Finally, statistically significant decrease in incidence of OH was recorded after 24-26 weeks of

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treatment ($p < 0.05$), within only 6.2 % of subjects with OH.

Regarding the incidence of OH in normal body weight and overweight patients, there was no statistically significant difference (Table 2). However, after 4-6 weeks of follow-up, in the overweight group there was slightly increased incidence of OH, which declined later in the study (Figure 3). Finally, about 1.5 % of the patients experienced one of the side effects of trandolapril (Table 3). Moreover, there were no cases of death in the study.

Discussion

This study has shown a great safety as well as efficiency of trandolapril for treating poorly regulated hypertension. Trandolapril successfully and significantly decreased SBP, DBP and mean BP in all patients during 24 weeks of follow up, with continuous antihypertensive effect throughout this period. Moreover, this research presented a low incidence of side effects of trandolapril, especially of dry cough (0.39 %). Furthermore, there was a significant lowering of OH occurrence between the third and fourth visit. After 24-26 weeks of the treatment and follow up, only 6.4 % of patients had OH. Overall, there was no statistical difference in the incidence of OH regarding BMI. Nevertheless, right after introducing trandolapril to the treatment, there was a slightly higher incidence of OH in overweight patients, but by the end of the study the incidence of OH in overweight and normal weight patients was less than 8 %.

Several studies have shown a difference in hypotensive effect of various ACE inhibitors as a result of their pharmacokinetics.

OH is more frequently found in subjects with diabetes mellitus, followed by a higher incidence of falls, fractures and early death.[8] Beside diabetes, OH is also found in the obese patients, where there is a common occurrence of autonomic dysfunction.[9] Among the reasons for OH occurrence in obese patients is cardiovascular autonomic neuropathy, which can result, in addition to OH, in exercise intolerance, silent myocardial ischaemia.[11] In the obese, a common finding is reduction of sensitivity of the cardiac sinus node, which affects sympathetic and parasympathetic activity.[12, 13]

Some studies also suggest that weight loss can improve autonomic function.[10] On the other hand, a rapid weight reduction can, in some cases of obese diabetics, result in severe OH - an autonomic dysfunction masked by long-standing hypertension and obesity.[14-16]

Occasional occurrence of OH is usually associated with the time of day and drug intake. More commonly OH happens in the morning, requiring several BP measurements to reach an accurate diagnosis. "Table-tilt" test and the "beat-to-beat" BP monitoring are new diagnostic approaches, which enable better quantification of OH.[17]

The mechanism of drug-induced OH can be explained by drugs interfering with reflexes that limit vasoconstriction, the heart rate and minute volume.[18] Numerous studies have demonstrated association of OH with cardiovascular and cerebrovascular morbidity (heart attack, stroke, heart failure).[19] Moreover, causes and mechanisms of OH hypertensive patients and diabetics have not been completely explained.[20] The Malmö Study examined the connection between different cardiovascular risk factors and OH incidence.[21] The ARIC Study showed that OH could bring a significant risk for ischaemic stroke.[22]

Also, analysis of the British Women's Heart and Health Study has shown a significant prevalence of OH in hypertensive patients. According to their findings, ACE inhibitors ere highly correlated with the prevalence of OH in women.[23]

Canney et al examined the impact of different antihypertensive drugs used as a monotherapy on the occurrence of OH in elderly patients with hypertension. It was shown that only beta-blockers had association with long-term and persistent OH.[24] On the contrary, Montastruc et al have shown a lower incidence of OH when ACE inhibitors were used compared to other drugs.[25]

When making a differential diagnosis, initial OH that can occur in the elderly and in the young should be considered. These persons suffer from a short-term and temporary drop in BP when getting to the upright position from the previous lying down position.[26] Finally, it is important to stress out that trandolapril causes a low percentage of OH compared to other ACE inhibitors, both in short-term and long-term use. The absence of OH when using trandolapril was also shown in several other studies.[27, 28]

Conclusion

OH is a common cause of treatment rejection by patients, often leading to treatment modification or even secession in hypertensive patients, which can lead to poor hypertension and cardiovascular risk control. Therefore, it is important to timely recognise, diagnose and adequately treat OH, using both pharmacological and non-pharmacological treatment. As shown in this study, trandolapril, along with some other ACE inhibitors, has shown good balance in hypertension control and OH occurrence. Therefore, they can be used in long-term treatment to prevent OH and improve patient compliance.

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None.

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REVIEW ARTICLE

Visceral Adiposity Syndrome and Cardiometabolism

Heno F Lopes,[1] Brent M Egan[2]

(1) Heart Institute (InCor) Medical School, Universidade de Sao Paulo, Sao Paulo, Brazil.
(2) University of South Carolina School of Medicine – Greenville, Greenville, SC, USA.

Correspondence:
HENO F LOPES
E: hipheno@gmail.com

Abstract

The distribution of fat in the human body is related to hemodynamic and metabolic homeostasis. Brown fat is inversely related to body mass index and is associated with a lower probability of developing diabetes. Beige adipose tissue shares some functional characteristics with brown adipose tissue. White adipose tissue constitutes the majority of the fatty tissue and is mainly distributed in the subcutaneous and abdominal cavity. Intra-abdominal white fat has gained prominence in recent years for its association with cardiovascular risk factors and higher cardiovascular mortality. This review article discusses the human adaptation in the environment, a sympathovagal and hypothalamic-pituitary-adrenal imbalance as a possible cause of increased visceral adiposity and its consequences on cardiometabolism.

Key words: Visceral adiposity; Sympathetic nervous system; Hypothalamic-pituitary-adrenal axis; Cardiometabolism.

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Introduction

The adaptation of human beings to the environment over thousands of years was gradual. Possibly the fight for survival contributed to the development of adaptive responses that were incorporated into the genes of the human organism and underwent changes over time. A response to environmental stress, well known today, was described by Cannon and De La Paz in 1911[1] as a fight-or-flight reflex. The stress coming from the environment activates the cerebral cortex and is directed to centres (areas) in the midbrain that regulate our homeostasis. According to Cannon's description,[1] the fight-or-flight reaction is characterised by neuroadrenergic activation and catecholamine release. The catecholamines released in the nerve endings and blood circulation will have an effect on different parts of the body, preparing for fight or flight. Among the changes that occur in the body due to the reflex, the following stand out: increased blood pressure, vasodilation of blood vessels in the skeletal musculature, heart and brain, vasoconstriction in the blood vessels to the intestine and kidneys, muscle relaxation smooth bowel and bladder, bronchodilation, increased heart rate and heart contraction force, mydriasis, increased gluconeogenesis, increased liver glycolysis, decreased fluid secretion in most glands.

Is there a relationship of the fight-or-flight (chronic) reaction with the clustering of cardiovascular risk factors (metabolic syndrome), with a high prevalence in the adult population in a large number of countries in the world?[2] This clustering of cardiovascular risk factors has been much discussed in the last 30 years. However, the main pathophysiological mechanism for this condition remains unclear. Insulin resistance has been identified as the main pathophysiological mechanism for the association of visceral obesity with other risk factors (dyslipidaemia, hyperglycaemia, hypertension). According to observations

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presented in this paper, there is strong evidence that sympathovagal imbalance and activation of the hypothalamic-pituitary-adrenal (HPA) axis may be responsible for the visceral adiposity syndrome (VAS).[3] In this review the different types of fat in the body, visceral adiposity and the implication of visceral adiposity in cardiometabolism will be addressed.

Adipose tissue

Fat tissue is the body's largest energy reserve. The main component of adipose tissue (triglycerides) is formed from the two largest caloric sources in nature: carbohydrates and fat. Today, it is known that adipose tissue performs different functions in the body. Fat cells are classified into brown, beige and white fat. These cells are distributed in different places in the body so that each group of fat cells performs its function independently, but integrated.

Brown adipose tissue (BAT) has the function of providing heat (thermogenesis) to the body and becomes more evident in positron emission tomography/computerised tomography (PET/CT) images when the individual is exposed to cold. Leitnera et al, using the PET/CT technique, described the anatomical distribution and functional capacity of BAT in non-obese and obese individuals after exposure to cold.[4] BAT is predominant in women and has an inverse relationship with the body mass index.[5] In morbid obese patients BAT is functionally less active and it is expressed again after weight loss in this population.[6] Due to its inverse relationship with the body mass index, ability to expend energy in form of heat, and its association with a lower risk of type 2 diabetes, BAT has been seen as a therapeutic target.[7]

The existence of adipose tissue other than brown and white, biochemically capable of producing heat, was found by Petrović et al in 2010.[8] Beige adipose tissue, adipocytes infiltrating white adipose tissue (WAT), share some functional characteristics with brown adipocytes. However, the thermogenic capacity of beige adipocytes is only 10 % of brown adipocytes.[9] Better knowledge of the origin of beige adipose tissue from physiological and pharmacological stimuli can result in a greater probability of using these adipocytes as a therapeutic target. According to Shao et al[10, 11] the use of transcription factors to recruit WAT such as thermogenic fat (brown/beige) is a promising therapeutic opportunity for the future.

WAT constitutes most of the fat tissue in the human body and is located mainly in the subcutaneous and abdominal cavity. The increase in abdominal circumference does not necessarily represent a large amount of visceral adipose tissue (VAT), as expansion of subcutaneous abdominal fat may predominate in some individuals with weight gain.[12] The visceral adipose tissue (intra-abdominal) has several characteristics that differentiate it from subcutaneous adipose tissue (SAT): larger adipocytes, venous drainage to the portal system, greater sensitivity of adrenergic receptors (β_3 and α_2) to catecholamine stimulation, higher level of beta adrenergic receptors 3.[13] In addition to the structural, functional and autonomic modulation differences, VAT is different from SAT in relation to gene expression.[14, 15] The main focus of this review is to discuss a possible mechanism for concentration of active visceral white adipose tissue in the abdominal cavity, the autonomic modulation and the cardiometabolic reflex resulting from this active adipose tissue.

Visceral adiposity syndrome - possible pathophysiological mechanism

The grouping of cardiovascular risk factors that includes visceral fat (central obesity), dyslipidaemia, high blood glucose and high blood pressure gained greater prominence based on the observations of Reaven in 1988.[16] However, in 1761 JB Morgagni had already drawn attention for the association between visceral obesity, hypertension, hyperuricaemia, atherosclerosis and the obstructive sleep apnoea syndrome.[17] Since Morgagni's observations until today, different authors have drawn attention to the association of obesity and other risk factors with cardiovascular disease.[18, 19] Based on the observations of Reaven,[16] different definitions for this grouping of risk factors emerged and are still in force today.[20-23]

However, in recent years, much more has been learned about the components of the grouping of cardiovascular risk factors described by Reaven[16] and their possible pathophysiological mechanisms. Uric acid as described by Morgagni et al[17, 24] and

other substances seem to be part of this risk factor grouping. Adiponectin, for example, emerged as an important component and biomarker for this grouping of risk factors.[25-27] For these reasons presented here and others, this grouping of risk factors is seen as a more complex syndrome, whose main pathophysiological mechanism is activation of the sympathetic nervous system (SNS).

There is a tendency in the literature to associate increased sympathetic tone with obesity and comorbidities.[28] Experts in the assessment of sympathetic activity have associated overweight, obesity and metabolic alterations as possible causes of increased sympathetic activity.[29] However, some evidence points to the increased activity of the SNS and of the HPA axis as a cause of visceral obesity and comorbidities.[30, 31] This increase in sympathetic activity and greater activation of the HPA axis would occur from environmental factors (salt, stress), especially in those who have a genetic predisposition to it. Children of hypertensive parents, for example, would be a population predisposed to develop visceral obesity and metabolic alterations. In this sense, higher values for body mass index (BMI), blood pressure, insulin, insulin-glucose ratio, norepinephrine and lower HDL-cholesterol values in normotensive young children of malignant hypertensive parents have been observed.[32] In the review by Feber et al[33] they draw attention to the importance of the sympathetic switch hyperactivity in the pathophysiogenesis of hypertension and its relationship with central obesity in children.

SNS, hypertension, and insulin resistance - the grouping of risk factors resulting from increased activity of visceral fat, VAS, has as likely and main initial mechanism and in its maintenance the hyperactivation of the SNS. Thorp and Schlaich[34] discussed the importance of the SNS in regulating metabolism and propose sympathetic hyperactivity as a possible pathophysiological mechanism of metabolic abnormalities. They also point to the chronic increase in sympathetic activity as a potential cause of "metabolic syndrome" through increased blood pressure, insulin resistance, increased triglycerides and obesity. In addition, the skeletal muscle of the obese hypertensive patient extracts less glucose when stimulated compared to the normotensive one, possibly due to changes in microcirculation resulting from hypertension.[35] On the other hand, the use of prazosin, an alpha 1 adrenergic receptor blocker, resulted in an improvement in insulin resistance in obese hypertensive patients.[36] In the study by Grassi et al,[37] sympathetic activity, assessed by microneurography, was greater in patients with clustering of risk factors and even greater in those in which hypertension was part of the risk factors. These observations point to an association of sympathetic activity with high blood pressure, insulin resistance and metabolic changes. In a study involving individuals with clustering of risk factors for cardiovascular disease (metabolic syndrome according to ATP III)[22] we showed that those with 3 risk factors or more, including hypertension, had a higher low frequency (LF) component in the spectral analysis, worse metabolic, inflammatory, prothrombotic profile, and lower level of adiponectin compared to those without the high blood pressure component.[38] In this study, the BMI and fat percentage were identical in both groups. The activity of the SNS is increased at baseline and after different types of stress in children of hypertensive patients even before the development of arterial hypertension.[39, 40] Thus, the activity of the SNS is increased before the onset of hypertension, in the initial phase and becomes more evident as the severity of hypertension increases.[41]

What is the relationship of sympathetic activity with hypertension and visceral adiposity? The increase in sympathetic activity results in the activation of other systems such as the renin-angiotensin aldosterone system (RAAS). It is known that catecholamines and angiotensin II play an important role in vascular and cardiac remodelling and are also related to glucose metabolism. Vascular remodelling begins in the endothelium, compromising vasodilation, and is influenced by the SNS.[42] Sympathetic activation compromises the endothelium and also results in cardiac and vessel smooth muscle hypertrophy.[40, 41] In addition, central noradrenergic abnormality may be responsible for the increase in peripheral vascular tone observed in arterial hypertension.[45] The SNS activates the RAAS via the β1 receptor. Activation of the RAAS contributes to greater muscle contraction and growth of the vessel involving complex mechanisms.[46] The increase in smooth muscle tone of peripheral vessels, under the influence of the sympathetic nervous system and the RAAS, will result in greater vascular resistance, increased afterload and greater cardiac work. A consequence of this hemodynamic imbalance will

be greater metabolic demand and consequently the hypertensive patient will have a higher metabolic rate at baseline and after physical activity.[47, 48] The greater sympathetic activity resulting from environmental factors (stress, salt, ...) results in what the Russians called it in the 1950s "cardiovascular neurosis" to explain the pathophysiology of high blood pressure.[49] However, this "cardiovascular neurosis" does not just result in increased blood pressure. In the present interpretation, based on current literature, the imbalance of the autonomic nervous system (increase in sympathetic activity) results in greater demand metabolic, consequently higher energy expenditure. The greatest energy expenditure will be supplied by fatty acids from visceral fat. This increased demand for visceral fat results in the differentiation of adipocytes in terms of size and function.[50] This intra-abdominal (visceral) fat deposition we call VAS resulted from a poor adaptation to the environment over many years.

Energy expenditure and visceral fat - the body's main sources of energy are carbohydrates and fat. Carbohydrates account for approximately 2 to 8 % and fat replenishes for 92 to 98 % of stored energy.[51] It is known that basal metabolism accounts for 60-70 % of the total energy expended. Since the energy reserve in the form of carbohydrates is low, the body with a high metabolic rate, as in the case of arterial hypertension,[52, 53] will use fatty acids as an energy source with greater intensity than normotensive individuals. The easiest fat to use is from VAT. In a stressful situation, the body increases sympathetic activity and this increase in sympathetic activity will result in lipolysis to provide fatty acids as an energy source.[54] The chronically activated SNS in hypertensive patients[41] will contribute to the increase in fatty acids and increased fatty acids in the circulation purportedly contribute to insulin resistance.[52, 53] Therefore, insulin resistance in VAS may be a consequence changes in microcirculation resulting from hypertension,[35] and a "competition" of glucose and fatty acid metabolism as described by Randle[57] for more than 50 years as the "fatty-acid syndrome". In summary, VAS, called metabolic syndrome, may be the result of a higher turnover of fatty acids as a result of a higher metabolic demand (increased sympathetic activity).

Visceral adiposity syndrome and cardiometabolism

Analysing something complex in a simplistic way, we can say that the growth of cardiometabolic conditions (obesity, metabolic syndrome, and diabetes) in recent years[58] may be a consequence of an autonomic imbalance (predominance of the sympathetic component) that increases the metabolic demand resulting in greater mobilisation of fatty acids from visceral fat, leading to insulin resistance and all the known consequences. Sympathetic activity, assessed by microneurography, has a better association between visceral fat (intra-abdominal) than total fat and subcutaneous fat.[59] Sympathetic activity, assessed using electrocardiographic parameters, is also associated with visceral fat.[60] In addition to activation of the SNS to the VAT the activation of the HPA axis may be related to the pathophysiogenesis of the VAS,[61] as observed by Prof Bjorntorp.[62] Everything indicates that hyperactivity of the SNS and hyperactivity of the HPA axis are supporting factors in the pathophysiology of VAS.

The body's energy homeostasis and the function of some organs are modulated by the autonomic nervous system (sympathetic component and parasympathetic). Sympathetic innervation of WAT is well known.[63] Parasympathetic innervation of VAT was reported based on an experimental study by Kreier et al.[64] Currently, the role of the parasympathetic nervous system in innervation of VAT has become increasingly evident.[65] From this information, it can be speculated about the sympathovagal imbalance in the VAT as a possible origin of several alterations such as: lipolysis/lipogenesis, increase in fatty acids, insulin resistance, production of a large number of adipocytokines with different functions in organs and cells. This results in changes in cardiometabolism and increased cardiovascular risk. This sympathovagal imbalance may have its origin in the central nervous system and may contribute to the above-mentioned alterations and to the production of more than 50 adipocytokines (biologically active molecules).[66] Thus, it seems that WAT keeps up hyperactive and exerts a pleiotropic action[66] to supply an increased metabolic demand, a consequence of sympathetic hyperactivity[47] and possible activation of the hypothalamic-pituitary-adrenal axis.

In summary, a primary change in the central nervous system results in increased sympathetic ac-

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tivity, which in turn increases blood pressure and metabolic demand, resulting in a higher concentration of intra-abdominal adipocytes. This higher concentration of intra-abdominal adipocytes supplies the increased metabolic demand at the expense of fatty acids, but results in insulin resistance. Insulin resistance initiates a metabolic imbalance characterised by increased blood glucose, triglycerides, reduced HDL-cholesterol, increased uric acid, lower adiponectin, production of cytokines and hormones with specific action in different organs and cells of the body. Experimental and clinical studies are needed to elucidate the real pathophysiology of the visceral adiposity syndrome and its cardiometabolic consequences.

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CURRENT TOPIC

Review of Therapeutic Options for Spinal Muscular Atrophy

Arun Singh,[1] Monica Jain,[1] Rupa Kapadia,[1] Dhirendra Kumar Mahawar,[1] Shivankan Kakkar,[1] Jaya Dadhich,[1] Ritesh Kumar Chandel[1]

(1) Department of Pharmacology, SMS Medical College and Hospital, Jaipur, India.

Correspondence:
ARUN SIGH
E: aruns5130@gmail.com

Abstract

Spinal Muscular Atrophy (SMA) is uncommon genetic (autosomal recessive) disease that deteriorates neuromuscular function of the affected person's body by causing lower motor neuron damage, progress in muscle atrophy and in advanced cases leads to paralysis of muscles. Mainly skeletal and respiratory muscles are involved. SMA is present due to lack of SMA proteins, which are encoded by survival motor neuron-1 (SMN-1) genes. In mutation of SMN-1 genes, deficiency of SMN proteins occurs. SMA affects all age groups, but mainly and most severely children younger than 6 months of age. At present, risdiplam is a treatment option and the drug has been approved by the US Food Drug and Administration on 7 August 2020. The availability of the drug has led to increased financial, ethical and medical problems. SMA affected populations are regularly challenged to these issues.

Key words: Spinal muscular atrophy; SMN-1; Rare disease frequency; Therapeutic approaches; Risdiplam; Nusinersen; Onasemnogene abeparvovec.

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Introduction

Spinal muscular atrophy (SMA) is cluster of hereditary disorders with both copies of the gene mutated. This kind of disorders are usually passed on by 2 carriers. Carrier's health is generally not affected, except in rare cases. SMA is depicted by degeneration of alpha motor neurons within spinal cord. Muscles atrophy gradually progress, which is manifested as muscle weakness that progress into paralysis.[1] SMA disease was first defined in 1980 by a German scientist Johann Hoffman and an Australian scientist Guido Werdning. Both scientists had observed many cases of children developing muscle weakness during the first few months of life. They also have seen that this illness appeared in next generations, but only in some members.[1, 2] SMA occurs due to bi-allelic point mutations of the SMN-1 gene, leading to degeneration of α-motor neurons in spinal cord. The presentation of this disease varies and depends upon disease starts and severity form. Crawling, sit up, walk or mobility of head is affected in children with SMA. In most severe cases of SMA, it can diminish the respiratory muscles and muscles used for swallowing.

Epidemiology

SMA is the second most typical rare autosomal recessive disease in Indian populations after incidence of cystic fibrosis. The global incidence of SMA is frequently cited as being approximated one in 10,000-11,000 live births.[3] The carrier fre-

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quency of SMA is as high as one in forty to one in sixty.[4, 5] Why SMA disease is considered as a rare disease? In order for something to be considered a rare disease it is necessary to consider 3 parameters:

1. The entire range of individuals having the disease
2. Its prevalence in population
3. Non-available treatment for this disorder.

Organisation for Rare Diseases in India (ORDI) recommends a disease to be well-defined as rare if the disease affects one in 5,000 population or less. But according to the suggestion of World Health Organisation, rare disease should be one with frequency less than 6.5–10 per 10,000 people.

Table 1 : Population based frequency of rare disease (according to CIOMS)

Rare disease frequency	In total population
Very common	Equivalent one or more than one out of ten (≥ 10 %)
Common, frequent	Equivalent one or more than one out of hundred and less than one out of ten (≥ 1 % and < 10 %)
Uncommon, infrequent	Equivalent one or more than one out of thousand and less than one out of hundred (≥ 0.1 % and < 1 %)
Rare	Equivalent one or more than one out of ten thousand less than one out of thousand (≥ 0.01 % and < 0.1 %)
Very rare	Less than one out of ten thousand (< 0.01 %)

SMA is considered as rare disease because it affects one in 5,000-8,000 of population in India. The medications used for treating SMA are called orphan drugs. An orphan drug is a pharmaceutical agent developed to treat medical conditions which, because they are so rare, would not be profitable to produce without government assistance.

Causes of SMA

The consequence of mutation in the SMN-1 gene, located on chromosome 5, is a lack of a motor neuron protein. The total number of copies of the SMN-2 gene alters the severity of SMA disease. There are two SMN-1 genes in population. In 94 % of all SMA cases, mutation involves a deletion of end of exon 7. The definite place of mutation is chromosome number five's q-arm, in the 5q13.2 region.

Normally, SMN-1 genes give outcome as full-length size and fully functional SMN protein. In spite of this, whenever the mutations occur in SMN-1 gene, it results SMA's chromosome number five form. Insufficient levels of SMN protein are formed in this SMN-1 gene. The other, SMN-2 gene that is located on chromosome five, also produces SMN proteins. The SMN-2 genes mostly produce proteins that carry commands which are non-functional. Apart from this non-functional protein, a small proportion of protein made from SMN-2 gene, approximately 10 to 15 % is functional.

The continuous reduction in the SMN protein produces lower motor neuron degeneration as result and progress into muscle atrophy and finally muscle paralysis. In SMA, mainly respiratory and skeletal muscles are involved. Patients mainly have difficulties in breathing and they are unable to sit and walk independently.

Classification of SMA

SMA is categorised into five subtypes (including 0 type). This categorisation depends upon age at onset of disease and maximum motor neuron activity execute (Table 2).[6]

Table 2 : Classification criteria for SMA on the basis of its clinical features

Types	Also known as	Onset	Achieved development milestones
0	-	Before birth	Cannot survive
I	Werdnig-Hoffmann disease; infantile	0-6 months	Never achieve sitting
II	Dubowitz disease; intermediate type	In between 7 to 18 months	Sitting, never achieve standing
III	Kugelberg-Welander disease; mild; adult	In between 18 months to 35 years	Stand and walk throughout adulthood
IV	Adult	> 35 years	Walk unaided

SMA-0 type is a rarest form of SMA. Symptoms of SMA-0 become apparent as reduced movements of foetus during intrauterine life. The affected newborn characteristically has only 1 copy of SMN-2 gene and generally the they survive only a few weeks even with intensive respiratory support.

SMA-I type (also known as Werdnig-Hoffmann disease) is a very serious disease that typically appears around six months of age. A baby may be born with respiratory symptoms that can be

threatening within a year if the child is not treated. These patients also have decreased muscle tone, severe and progressive muscle weakness, abnormal swallowing and speech, weakened suckling capacity and respiratory failure. A gynaecologist observes the SMA-I type during intrauterine life. During the last months of gestation less movement of the foetus are found.[7] If not found in last months of pregnancy, it will become evident within the first few months of baby's life after birth. With these types of SMA disease babies have multiple risks of aspiration and they are unable to thrive. SMA-I is characterised in rapid motor neuron loss. It results in death or permanent ventilator support in > 90 % of patients. Patients suffering from SMA-I usually have 2 or 3 copies of the SMN-2 gene.[8]

SMA-II or Dubowitz disease or intermediate type features usually onset between 6 and 18 months of life. Patients accomplish the strength to take a seat without support and in few of them, acquire ability to stand, however do not acquire the ability to walk independently. SMA-II patients are characterised by problems of sitting without support or they fail to stand till one year. They develop pseudohypertrophy of the calf muscle and musculoskeletal deformity. Respiratory failure can also happen, and patient can live only with ventilatory support. SMA-II affected children mostly have 3 copies of the SMN-2 gene.[9] They have predominantly proximal muscle weakness. Proximal muscles of lower extremities are mainly involved. Facial muscles or ocular muscles remain unaffected.[8]

SMA-III, mild-SMA, also known as Kugelberg-Welander disease is characterised by different levels of muscle weakness. SMA-III patients have 3-4 copies of the SMN-2 gene. SMA-III (juvenile onset) incidence is 30 % of overall SMA cases in population. Affected individuals walk without support. In these patients, weakness of proximal muscles would lead to frequent falls and issue with climbing stairs. Over time, a loss of the ability to stand and walk occurs.

SMA-IV is present in later life and incidence is less than 5 % of overall SMA patients.[9] In this type SMA, patients have 4-8 copies of the SMN-2 gene.[8] Age of onset is uncertain but it usually occurs later, at thirty years of age. SMA-IV is an insignificant form of SMA and consequently patient's life expectancy remains normal. They achieve motor milestones and maintain mobility throughout life.

Developmental milestone and diseases severity progression is generally analysed by using authenticated purposeful scales – The Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP-INTEND) / Hammersmith Infant Neurological Examination (HINE) in babies; and either the Motor Function Measure (MFM) or one of some variants of the Hammersmith Functional Motor Scale (HFMS) in elderly cases are used.

Diagnosis of SMA

Early assessment of SMA is likely during pregnancy by detection of foetal movements. In such patients, there are reduced and absent foetal movements in the intrauterine life. Respiratory distress and poor feeding are life-threatening. It takes only a few weeks for death. SMA disease is definitively confirmed by genetic testing methods. The disease progression (muscle atrophy) is detected by a muscle biopsy.

Therapeutic options for SMA

The therapeutic options for SMA vary from case to case. They are based upon the disease type and severity. Most severe SMA type (SMA types 0 / I), has extreme muscle atrophy and weakness necessitating rapid therapeutics. In less severe type (type-IV) intervention may not be necessary in childhood, until forthcoming life decades.

The SMA disease's pathophysiology is incompletely understood till now. Nevertheless, various growths in understanding to the molecular basis theory of this kind of rare disease has been done and different therapeutics are evolving over the past years.[14] On December 2016, the first approved drug for SMA disease was nusinersen (trade name - Spinraza) by the US Food and Drug Administration (FDA). It is administrated in SMA patients by intrathecal route. Later on, the first oral drug for SMA was discovered. The name of this drug was risdiplam (trade name - Evrysdi) which was approved by FDA for the treatment of SMA disease in adults and children aged two months and older on 7 August 2020.

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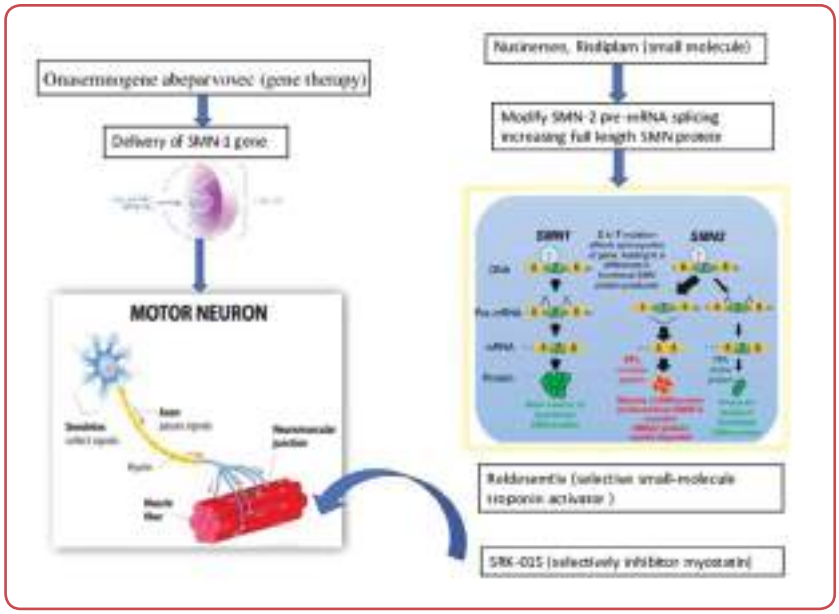

Figure 1: Core existing therapeutic options and their mechanism of action
SMN1 = Survival Motor Neuron 1; SMN2= Survival Motor Neuron 2; SMN = Survival Motor Neuron

I. SMN gene insertion therapy

(a) Splicing modification of SMN-2 gene

(i) Nusinersen is a disease modifying therapy established by Biogen (USA). It was first drug that was approved by the FDA for treatment in adults and children, as well as infants with SMA.[15] The European Union approved nusinersen in June 2017 to treat SMA. Nowadays, nusinersen is available in 29 European countries for regular reimbursement. This drug is now approved in many other countries, including Australia, Canada, Japan, Israel and Turkey. It is administered into the central nervous system by intrathecal route. This drug has orphan status under orphan drug designation program in the United States and the European Union.[16] This drug is structured as antisense oligonucleotide (ASO) in treatment of SMA that is occurred due to mutations in longer arm (q-).[17]

Nusinersen drug is an ASO. It corrects splicing of the SMN-2 gene.[16] It increases exon-7 insertion in SMN-2 gene mRNA transcripts, which finally produces the full-length SMN protein. This was shown in animal experiment of *in vitro* assays and transgenic animal models of SMA's disease studies.

The plasma concentration of nusinersen is relatively low, compared to the lowest cerebrospinal fluid concentration. The maximum concentration of the drug in plasma after administration is after 1.7 - 6 h.[16] The drug is distributed from blood to cerebrospinal fluid and peripheral tissues (skeletal muscle, liver, kidney). It is metabolised through exonuclease enzyme (3'- and 5'-) mediated hydrolysis process and it does not act as a substrate for inhibitor, or inducer of cytochrome P-450 enzymes. Elimination half-life is 135-177 days in cerebrospinal fluid and 63-87 days in plasma. The drug primary elimination route is likely by urinary excretion. After 24 h, only 0.5 % of the administered drug amount was recovered in urine. Dose (in adults and children) is 12 mg in 5 mL, single dose, via intrathecal route administration. Initial 4 loading doses are required; first three doses at 14 days interval and the fourth dose is given 30 days after the third dose. The maintenance dose is one dose every 4 months.

Nusinersen approval was grounded on the ENDEAR clinical trial. Subsequently hopeful outcomes for nusinersen in phase 1 and 2 of clinical trials with SMA type-II and -III in children has led to phase 3 (randomised, double-blind, sham procedure controlled studies were started).[19, 20] ENDEAR (ClinicalTrials.gov identifier: NCT02193074, in year 2014 to 2016) evaluated safety and clinical efficiacy of nusinersen drug in 121 infant, with infantile onset SMA type earli-

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er than 7 month of age. CHERISH trial (ClinicalTrials.gov identifier: NCT02292537, year 2014 to 2017) included 126 children with late onset/adulthood type SMA. At baseline, the median age was 4 year (range 2-9 year) in treated patient's group and 3 year (2-7 year) in control group. NURTURE phase 2 (ClinicalTrials.gov identifier: NCT02386553, initiated in 2017) open-label, single arm, multinational study is ongoing.

(ii) Risdiplam is indicated for SMA-I, II and III type in adults and children aged 2 months or older.[21] It is a first drug which is given orally. The drug was established associated with PTC Therapeutics and SMA Foundation and by Genentech company, a member of the Roche Group (USA). This drug is available as oral solution with maximal dose of 5 mg administered daily.

It is a mRNA splicing modifier for SMN-2 gene designed to treat SMA disease.[21, 22] Basically, it increases the inclusion of exon-7 throughout splicing process, which finally increase the quantity of functional SMN protein formed by SMN-2. The peak plasma time following the drug oral administration is 1-4 hours, followed by once-daily administration with a morning meal (or when breastfeeding), risdiplam reaches steady-state after 7-14 days. It is bounded to serum albumin protein, deprived of any binding to α-1 acid glycoprotein, with 11 % free fraction. The apparent volume of distribution at steady state is 6.3 L/kg. Risdiplam is primary metabolised by ketone monooxygenase 1 and 3 (FMO-1 and FMO-3) and additionally by cytochromes: 1A1, 2J2, 3A4, and 3A7. Parent drug is the main element found in plasma, accounting for 83 % of drug-related material in circulation. Elimination half-life is proximately 50 h, clearance: 2.1 L/h (for 14.9 kg patient). If the dose of 18 mg of risdiplam is administered by oral route, around 53 % of the dose is excreted by the faeces and 28 % by urine. In child at the age of 2 months and above dose is 5 mg orally once a day for one year (Table 3). Most common adverse effects are fever, diarrhoea and rashes, sometimes oral and aphthous ulcers, joint pain and infection of urinary tract.

Table 3 : Dose of risdiplam according to patient's age

Age	Dose
Age ≥ 2 months to < 2 years	0.2 mg/kg orally / once a day
Age ≥ 2 years and weight < 20 kg	0.25 mg/kg orally / once a day
Age ≥ 2 years and weight ≥ 20 kg	5 mg/kg orally / once a day

The approval of this drug by FDA was grounded on the outcomes from 2 clinical research studies: FIREFISH trial in infantile-onset SMA cases and SUNFISH trial in later-onset SMA cases. FIREFISH was an open-label type study, 2 parts pivotal clinical trial in infants aged from 2-7 months in SMA-I type.[23] Results showed 41 % (7/17) of these infants attained skill to sit without any support for at least five seconds and 90 % (19/21) infants did not required permanent ventilation at 12 months of age. Later, after minimum duration of treatment of 23 months and reaching an age of 28 months or elder, 81 % (17/21) of all children were alive without permanent ventilation. The SUNFISH study was a 2 part, double-blind, placebo controlled pivotal research trial in two-year-old children to 25 year old young adults of SMA-II and SMA-III.[24] A clinically meaningful and statistically important development in motor function of muscle among children and adults was observed as measurement of a change from baseline in the MFM-32 total score. Improved upper extremities motor function as compared from baseline, as measured by the Revised Upper Limb Module (RULM), a secondary independent muscle motor function end point of the study, also showed statistically significant improvement.

Apart from FIREFISH and SUNFISH study, risdiplam was assessed in the wide-range of SMA cases, including in JEWELFISH study, an open-label exploratory clinical trial in SMA type I, II or III aged 6 months to 60 years who were earlier treated with SMA medications, gene replacement therapy / olesoxime. Recruitment was completed by enrolment of 174 patients. RAINBOWFISH study is an open-label, single-arm, multicentre research that investigate the efficacy, safety, pharmacodynamics and pharmacokinetics of risdiplam in babies (approximately 25 patients), from 0 to 6 weeks (at first dose) in patients whom SMA was diagnosed by genetically testing, while they were still without symptoms. The study is currently recruiting.

(b) Gene replacement therapy

Onasemnogene abeparvovec (earlier called as AVXS-101) is used as a SMN-1 gene replacement therapy medication. It is given in SMA-I type cases, to children aged two years or younger.[25] The FDA approved this drug on 24 May 2019 for SMA. It is developed by the Swiss drug maker Novartis, under the trade name Zolgensma.

It is recombinant adeno-associated virus (AAV9) based on gene replacement therapy medication that is created to carry a gene copy encoding the SMN protein. It is available as suspension form for intravenous infusion. It is provided as a kit that comprises 2-9 vials, with mixture of two vial fill volumes (both 5.5 mL / 8.3 mL). Entirely vials contain nominal concentration of 2 x 10^{13} vector genomes in 1 mL. Separately vial covers an extractible volume of more than 5.5 mL / 8.3 mL. There is no establishment of the safety and efficacy of drug for children aged 2 years and above. A single intravenous infusion is administered through a venous catheter that contains 1.1 x 10^{14}/kg vector genomes. Adverse reactions in > 10 % cases is elevated aminotransferases (> ULN) (27.3 %) and in 1-10 % cases vomiting (6.8 %).

Approval was supported by phase 1 of STR1VE trial, with still ongoing phase 3.[26] In this trial, 15 SMA-I cases were enrolled, and received single dose of blood vessel adeno-associated virus serotype-9 carrying spinal muscular neuron corresponding deoxyribonucleotide encrypting the lost SMN protein. Out of 12 enrolled subjects, 11 could be seated without support, 9 set rolling over, 11 could take a meal orally, might speak and two have walked without any dependency. Four cases presented with elevated serum aminotransferase enzyme levels which was diminished by prednisolone. Phase 3 STR1VE trial is ongoing, interim data analysis showed that 95 % of subjects were alive and event-free. The median age was 9.5 months, six in out of seven (86 %) subjects aged 0.5 months or elder persisting event-free. Interim results also exhibited constant enhancement of muscle unit motor milestones (head upright hold, rolling over, unaided sitting).

II. SMN non-dependent therapy

Reldesemtiv is an advance drug which is taken orally, small molecule medication that is being established via Cytokinetics, with partnership of Astellas Pharma company, indicated for enhancement of function of skeletal muscles accompanying by neuro-muscular dysfunction, atrophy of muscles or decrease strength in SMA. It rapidly activates the skeletal muscle troponin, thus also called Fast skeletal troponin activator or FSTA. It is stimulant of troponin which is anticipated with the slow rate of calcium ion release from fast skeletal muscle fibre's regulatory troponin complex .[27] Subsequently, the Phase I study suggests confirmatory safety parameters. A Phase II, double-blind, randomised and placebo-controlled trial (ClinicalTrials.gov identifier: NCT02644668, years 2015–2018) on 70 cases SMA -II to -IV type observed its functional and respiratory performance related effects. The outcomes of the study are: in the group with higher doses, a drift towards an upsurge from baseline in the six-minute walk test (6-MWT) and of the maximal expiratory pressure (MEP). In between treated and placebo groups, there are similar adverse events reported.

A monoclonal antibody named SRK-015 selectively inhibits myostatin (also known as growth differentiation factor-8), endorsing muscle spindles growth and differentiation and improving muscle force in SMA cased mice.[28] A first phase trial (ClinicalTrials.gov identifier: NCT02644777, years 2017-2018) confirmed its safety and tolerability. A second phase study (TOPAZ, ClinicalTrials.gov identifier: NCT03921528, started in 2019 and ongoing), involved 58 SMA-II and -III cases, aged 2-21 years. These have acknowledged therapy through intravenous infusion every 4 weeks for 1 year. The results of a six-month interim study were obtained by the end of year 2020.

III. Other therapy

Valproic acid (VPA) is an anti-epileptic drug that has been used conventionally to treat patients with epilepsy. But recent research suggests that treatment with valproic acid and other drugs such as sodium phenylbutyrate, hydroxycarbamide and albuterol sulphate have been demonstrated to increase SMA transcription in laboratory findings. Apart from this, clinical research have not established markable development in disease progression.[29] The SMA CARNIVAL trials parts 1 and 2 suggests that valproic acid and L-carnitine are ineffective in improvement of strength and functions at the children 6-12 months of age.[30] Only 85 % of total cases reported adverse effects. Gabapentin, olesoxime and riluzole medications are studied for their suspected neuroprotective properties, while not vital clinically profit was noted. Creatine, sodium phenylbutyrate, gabapentin, thyrotropin releasing hormone and hy-

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droxyureas medications used for SMA have conjointly well-tried ineffective.

SMN non-dependent therapeutic goals in reference to future prospective

In upcoming years autophagy inhibition could also be option available in treatment of SMA. Previously *in vitro* and *in vivo* studies regarding SMA have informed potential autophagic characteristics in SMA-cultured motor neurons, reporting about dysregulation of autophagy, which could help in decrease of progression of SMA.[31] Autophagy is a significant intracellular process through which components of cytosol are transported by double-membrane vesicles, known as autophagosomes that are used for lysosomes to cell's degrading process. This process is extremely controlled via a sequence of proteins, that are known as autophagy-related genes (ATGs). Basically, autophagic pathways regulate degradation of cytoplasmic contents like damaged mitochondria, injured cytosolic organelles, attack pathogens and aggregate prone proteins.

Over the previous years, various spinal muscle neuron freelance factors are considered in pathophysiology of SMA, on the idea of *in vitro* and *in vivo* studies and consequently they might characterise forthcoming therapeutic goals. Besides, administration of injection via intramuscular route of brain derived neurotrophic factors and C-fragment of the tetanus toxin heavy chain may decrease the activity of autophagy markers (Atg5,Becn1, Lc3 and p62) in transcript lacking of exon-7 muscles SMA mice deprived of effects on body mass and survival time.[32] Moreover, arrestment of autophagy process by invasive injection technique of 3-methyladenine (3-MA) directly into cerebrospinal fluid in cerebral ventricles (to bye-pass the bold brain barrier) has been revealed to have better autophagic characteristic, prolong lifespan and recover motor performances in SMA pups.[31] Numerous evidences suggest that apoptotic processes have been proved to have a role in SMA pathology. *In vitro* studies suggested SMN protein decline stimulates apoptosis.[33] JNK-3 (c-Jun NH2-terminal kinase) cascades, identified as a pro-apoptotic role is triggered in transcript lacking exon-7 SMA mice and in SMA affected person.[34] Additionally, c-Jun NH2-terminal kinase pharmacological inhibition improves morphological characteristic, recovers motor performances and life expectancy of SMA mice.[18]

Current scenario for treatment of SMA disease in Rajasthan

In the month of September 2020, JK Loan Hospital, part of Sawai Man Singh Hospital Medical College and Attached Group of Hospital, had a visit from a 3-year-old child suffering from SMA from Gorakhpur City, Uttar Pradesh. The patient was brought to JK Lone Hospital in Jaipur for special treatment. When he was 8 months old, his mother noticed that child showed decreased movement and weakness in lower limbs. The child was unable to stand and walk. SMA was suspected and routine blood test, serum creatinine kinase test and electromyography were performed. Serum creatine kinase level was normal and in EMG, typically prominent fibrillation potential and markedly diminished compound action potential was found. As gold standard diagnostic method, genetic test confirmed SMA. He was treated by giving the first dose of the risdiplam drug. The best part of the treatment is that this drug is taken at home. However, pediatricians of JK Loan Hospital have asked the patient to come to the hospital every month for follow up so that they would be able to monitor his health and the muscle tone. The drug cost Rs 4 crores/year and it is to be giv-

Table 4 : Therapeutic options for the SMA patients

Therapeutic option	SMA type	Mechanism of action	Route of administration	Trial's status	FDA Status
Nusinersen	I, II, III	Antisense oligonucleotide	Intrathecal	I, II, III	Approved
Risdiplam	I, II, III	Small molecule	Oral	I, II, III	Approved
Onasemnogene abeparvosec	I, II	AAV-9-vector construct	Intravenous	I, II, III	Approved
Reldesemtiv	II, III, IV	Fast activator skeletal troponin	Oral	I, II	Not approved
SRK-105	II, III	Myostatin inhibitor	Intravenous	I, II	Not approved
Olesoxime	II, III	Anti-apoptotic agent	Oral	I, II (development finished in 2018)	Not approved

en lifelong. The medicine was provided to the patient under the compassionate use programme. This medicine has been introduced to a patient for the second time in the country. This medicine can be given to children of all types of SMA free of charge due to the rarity of the disease. Most patients die prematurely due to respiratory failure. It is expected that after this treatment, the child will be able to live a normal life.

Treatmen options of SMA are profoundly varying and are given according to SMA's type and muscle atrophy progression. Summary of treatment options is presented in Table 4.

Conclusion

SMA is rare disease that can manifested at any period from intrauterine life to birth and adulthood with different severity. To diagnose this disease, doctors are facing many difficulties because there is not enough information, relevant literature and genetic analysis. Therapeutic options for now cannot recover motor neurons or muscle spindle cells which were previously lost, but they can still delay the progression of muscle atrophy, control the severity of the disease and help recover a person's permanent muscle function. Goal is to improve quality of life as well as better life expectancy. Some above given therapeutic options are now available and they help reduce disease progression, give patient better quality of life and decrease mortality. It is expected that the scope of therapeutics option will increase gradually even further, and patients will get relief from this disease completely.

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Conflict of interest

None.

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CASE REPORT

Ogilvie Syndrome in a COVID-19 Patient with Pneumonia, Absolute Tachyarrhythmia and Heart Failure: a Case Report

Zoran Matković,[1] Nataša Đekić Matković[1]

(1) General Hospital "Sveti apostol Luka", Doboj, the Republic of Srpska, Bosnia and Herzegovina.

Abstract

The COVID-19 pandemic has recently spread worldwide presenting primarily in form of pneumonia. Gastrointestinal manifestations such as nausea, vomiting, diarrhoea and abdominal pain are less common than respiratory symptoms. However, critically ill patients may develop digestive complications including acute pseudo-obstruction of colon - Ogilvie syndrome. Gastrointestinal symptoms can manifest before the onset of typical respiratory symptoms. Common mucosal immune response underly both-pulmonary and gastrointestinal manifestations (high expression of angiotensin-converting enzyme 2 receptors). This article described a 75-year old female patient who arise Ogilvie syndrome during viral bilateral pneumonia induced by COVID-19. Patient also had an absolute tachyarrhythmia and hearth failure. Diameter of caecum, ascending and transverse colon was 12 to 14 cm. The walls of this segment of large bowel were deserosed, with threatening perforation. Right colectomy was performed. Nine days after the surgery, despite all therapeutic measures taken, there was a fatal outcome due to pulmonary thromboembolisation.

Key words: Ogilvie syndrome; COVID-19; Pneumonia; Heart failure; Gastrointestinal manifestations.

Correspondence:
ZORAN MATKOVIĆ
E: 1zona@teol.net
M: +387 65 740 459

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Introduction

The COVID-19 pandemic has recently spread worldwide and became a global challenge to public health.[1, 2] Pneumonia and other respiratory diseases are most common form of COVID-19, but, last months, gastrointestinal (GI) symptoms are recognised as one of the leading extrapulmonary characteristics of the virus.[1] In regard to the GI system COVID-19 present with nausea, vomiting, diarrhoea and abdominal discomfort and pain.[3-8, 13] GI symptoms expression are less frequent than pulmonary (about 20 %)[7, 8, 11] but Chinese patients demonstrated that more than half (50.5 %) had at least one of the GI symptom.[5] Hypomotility-associated consequences were developed in half of the patients, with 46 % of them developed ileus diagnosed clinically and radiologically.[10] SARS-CoV-2 virus can be found in faeces.[11, 12, 19] Recent studies have reported that GI symptoms can be presented before respiratory manifestation.[3, 5, 8] In cases where nasal swab is negative, faecal testing can be advised in patients with digestive manifestation.[9] One study suggested that 9/76 cases of positive COVID-19 infection were identified in patient conferred with abdominal pain without respiratory symptoms at the time of COVID-19 pandemic.[13] COVID-19 patients with GI disease had higher le-

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vels of liver transaminases, prolonged prothrombin time, lower monocyte and lymphocyte counts as well as lower total proteins and albumines. Consequently, they were treated with more antimicrobial medication when compared with patients without GI manifestations.[8, 14]

Patients with mild disease severity and critical ill may developed GI complications including bowel ischaemia, bleeding, pancreatitis, Ogilvie syndrome and severe ileus.[5, 8, 13, 15] In patients with obligatory abdominal surgical treatment, mortality rate is high as 40 %.[10] We present COVID-19 positive female patient aged 75, who developed acute colonic pseudo-obstruction (Ogilvie syndrome).

Case history

A 75 years old female patient was admitted in our hospital due to fever, cough, myalgia, anorexia, fatigue and dyspnoea. First symptoms occurred seven days before admission. Peripheral oxygen saturation was 90 % and body temperature was in normal range (36.2 °C). Blood pressure was slightly elevated (150/90 mmHg). Fast Ag test was negative, while PCR test and serologic test (increase IgM antibody) were positive. Blood analysis performed are presented in Table 1. High level of CRP, D-dimer, neutrophilia and lymphopenia were detected. Transaminase levels were in normal range (AST 21, ALT 45). X-ray of chest showed shadows in lower parts of both lungs - *Infiltratio basalis bilateralis.*

Table 1: Blood-test findings during hospitalisation of the patient

Parameter	At admission	13th day	20th day
CRP (mg/L)	362.51	91.25	27.25
D-dimer (ng/mL)	> 10 000	3600	-
Leukocytes (x 10^9/L (%))	21.47	12.01	17.01
Neutrophils (x 10^9/L (%))	19.53 (91.0 %)	10.83 (90.2 %)	-
Lymphocytes (x 10^9/L (%))	0.82 (3.8 %)	0.46 (3.8 %)	-
NLR	23.81	-	-
MLR	1.31	-	-
Monocytes (x 10^9/L (%))	1.08 (5 %)	-	-
Erythrocytes (cells/mcL)	4.3	3.76	4.84
Haemoglobin (g/L)	136	120	150
Haematocrit (1/100)	0.411	0.37	0.557
Thrombocytes (× 10^9/L)	208	-	182
Potassium (mmol/L)	4.5	5.2	3.8
Blood urea nitrogen (mg/dL)	8.9	-	10.9
Creatinine (µmol/L)	124	-	85
LDH (U/L)	981	445	-

NLR: Neutrophile/lymphocyte ratio; MLR: Monocyte/lymphocyte ratio; LDH: Lactate dehydrogenase; CRP: C-reactive protein

Antibiotics (Ceftriaxone, 2 x 2 g), low molecular weight heparins (LMWH), crystalloid solutions, vitamin and oxygen therapy was given.

Abdominal discomfort appeared ten days after admission, patient started vomiting. First native abdominal X-ray showed meteorism, without aero-liquids levels, but next two X-rays revealed signs of ileus of large bowel (Figure 1 and 2). After consultative examination by a surgeon conservative treatment was attempted (Bowel rest, NG tube, electrolyte correction, neostigmine). A patient was transferred to surgical department for COVID-19 positive patients.

Later that day, in the evening, on examination, palpation indicated acute abdomen. It was suspected that patient developed mesenteric thrombosis and intestine necrosis due to absolute arrhythmia.

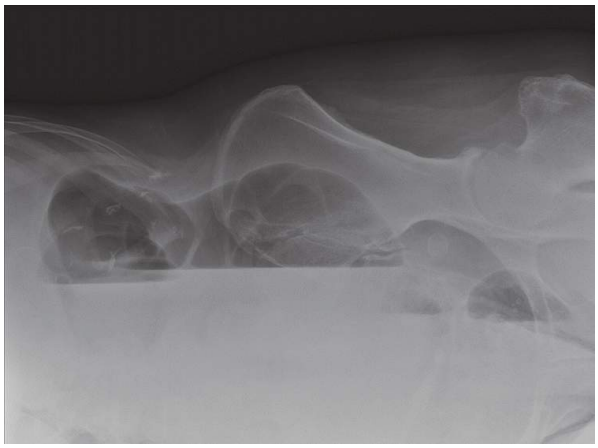

Figure 1: Native X-ray of abdomen (lateral decubitus)

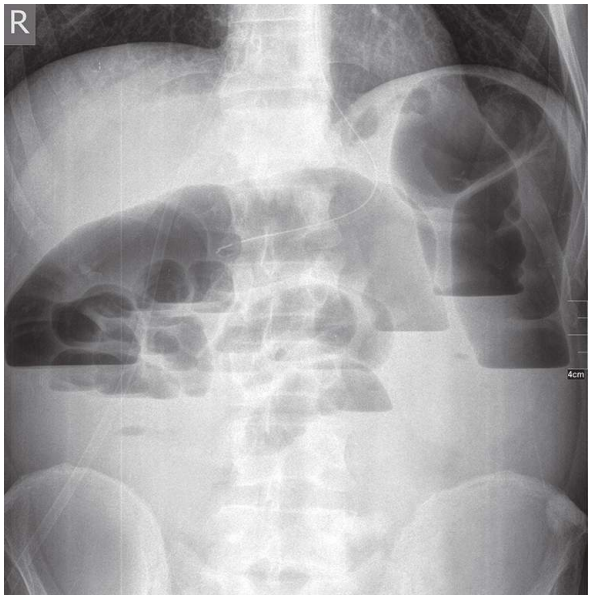

Figure 2: Native X-ray

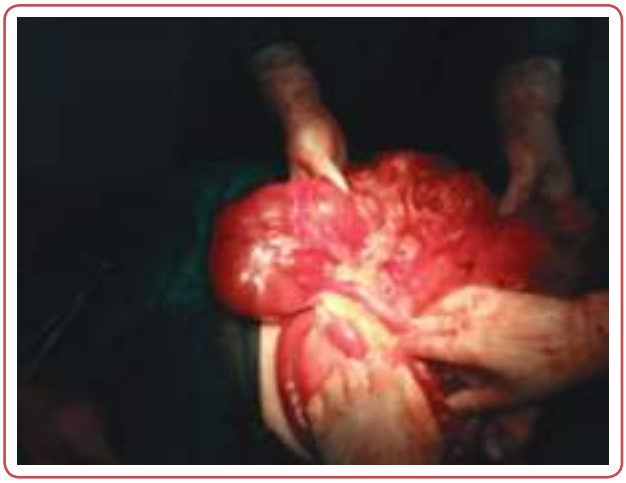

Figure 3: Intraoperative finding: megacolon (caecum, ascendens)

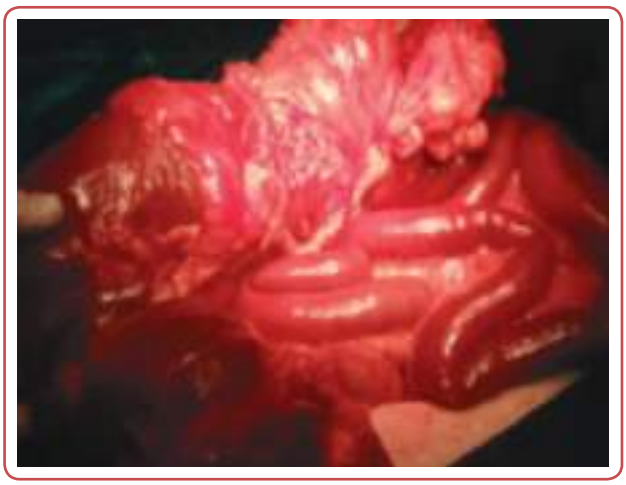

Figure 4: Intraoperative finding: megacolon (caecum, ascendens and transversum)

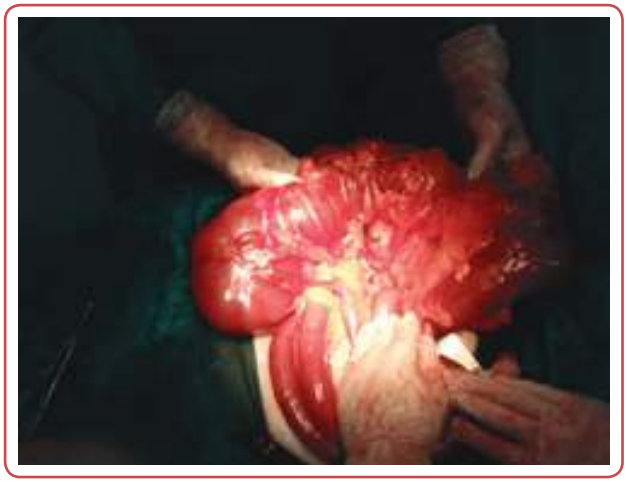

Figure 5: Intraoperative finding: dilated and normal parts of colon

Therefore, an emergency surgery was performed. Intraoperative finding: whole right colon and biggest part of transverse colon (2/3) were extremely dilated (megacolon, lumen diameter was between 12-14 cm) (Figure 3-5). Colon segment walls were deserosed, with threatening perforation. Small part of transverse colon and decedent colon were without pathological changes. No mechanical causes of the cessation of the passage was seen It was not possible to suppress content without rupturing of the bowel. It was concluded that was acute pseudo obstruction of large bowel - the Ogilvie syndrome. Extended right hemicolectomy was performed. Nine days after the surgery, despite all therapeutic measures taken, there was a fatal outcome due to pulmonary thromboembolisation.

Pathological findings - macroscopic description

The colon was with a thinned wall, about 0.1 cm. Lumen was dilated, mucosa was yellow-green, with reduced folds.

Microscopic description

The intestinal villi were broad and swollen. Mucosa was partially missing and necrotic. The fibrin exudate was seen above mucosa. Transmurally, especially in submucosa, extremely dilated blood and lymph vessels as foci of bleeding and an inflammatory infiltrate dominated by neutrophils. Fibrous-purulent exudate was also present on the serous surface of the colon.

Discussion

Laboratory and clinical data tracking are essential for increasing treatment success in COVID-19 patients. Laboratory findings are accompanied with severe form of disease. C-reactive protein, D-dimer and NLR have the most important influence on COVID-19 prognosis.[8, 15] GI epithelia with SARS CoV-2 host-cell receptors and high expression of Angiotensin converting enzyme receptor type 2 could explain abdominal organs partaking.[16] GI manifestation can be explained with SARS-CoV-2 intestinal tropism. Bowels and glandular epithelial cells of GI can be affected with SARS-CoV-2. Faecal-oral transmission is a potential way of SARS-CoV-2 transmission.[17-20] Physicians and researchers have conflicted opinions on ACE inhibitors therapy discontinuation due to increasing number and activity of ACE-2 receptors.[21]

Loss of parasympathetic spinal control of intestine motility can be one of diseases characteristics. Acute colonic pseudo-obstruction (ACPO) syndrome (Ogilvie) is characterised with colon

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distension in absence of mechanical obstruction and it is induced by deteriorated autonomic nervous system.[22, 23] In this case there is not any mechanical barrier in lumen or in wall of colon or extraluminal compression. Colonoscopic decompression is one of effective therapeutic options in patients with this syndrome. Abdominal computed tomography (CT) is the gold standard imaging modality in these cases.[24] In this case, CT was not performed due to technical issues.

Patients with COVID-19 surgical appearance can have mesenteric inflammation or congestion that can simulate appendicitis or ileus diagnosis.[25] Impact of perioral nutrition is very important in patients with anorexia as leading symptom followed by diarrhoea, nausea and vomiting and abdominal discomfort.

Probiotics are recommended from China National Health commission in treatment of patients with severe form of COVID-19 because they can reduce intestinal disbalance with bacterial displacement and consequently reduce secondary infections.[5, 8]

The caloric intake should be more than 1500-2000 calories, with 75 to 100 g of protein per day. Oral diet is desirable in patients who are capable to eat. Otherwise, enteral feeding via nasogastric or nasojejunal tube may be useful. This patient had a small oral intake due to loss of appetite. A few studies suggested that the threshold must be reduced for supplemental or full parenteral nutrition as those types of nutrition are not safe or well tolerated.[26, 27]

In one study, 58 of 141 patients had digestive hypomotility, although these GI complications could be associated with metabolic and electrolyte disturbances or pharmacologic adverse events, experienced in patients with severe form of disease. Severe acute respiratory syndrome initiated by thrombosis small vessel or viral enteropathy requires further investigation.[10, 28]

Histopathological specimen of resected bowel shows COVID-19 initiated microthrombosis, inflammatory infiltrate and necrotic fields leading to GI perforation.[1, 10, 28] In case of the presented patient similar findings were registered.

Doctors treating COVID-19 patients should be attentive of these consequences and be watchful in cases where gastrointestinal symptoms required surgical consultation. In critically ill patients there is high frequency of GI complications, due to intestinal ischaemia group of patients can required urgent surgery.

Conclusion

Early recognition and management of GI complications in COVID-19 patients, primarily conservative, are extremely important to reduce rates of mortality. As evidence for GI involvement increase, a suspicion for COVID-19 infection must be managed in all cases of patients with abdominal discomfort requiring surgical consultation. Ogilvie syndrome is one of possible GI manifestations, and one must think about this because delayed diagnosis often can lead to poor disease course and fatal outcome.

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Conflict of interest

None.

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Photos should be delivered in *jpg format, 1:1, 300 Dpi as separate files.

Papers should be prepared in accordance with the Vancouver Convention.

Scripta Medica uses the British spelling of the English orthography. American spelling should be limited to the official titles of the US institutions and the references only.

Preparation of the manuscript
Parts of the manuscript are: Title page; Abstract with Key words; Text; Acknowledgements (optional), References.

1. Title page
a) The title should be concise but informative, while subheadings should be avoided. Words in the title should be capitalised according to the Rules of the AP Title Case.
b) Full names of the authors marked with ascending Arabic numerals in superscript (eg 1, 2, 3).
c) Exact names and places of department(s) and institution(s) of affiliation where the studies were performed, city and the state for any authors, clearly marked by standard footnote signs;
d) Contact data of the corresponding author.

2. Abstract and key words.
The second page should contain the title of the article and a structured abstract (250-300 words for original articles). In short, clear sentences the authors should write the Background/Aim, major procedures – Methods (choice of subjects or laboratory animals; procedures, methods for observation and analysis), the obtained findings – Results (concrete data and their statistical significance), and the Conclusion. It should emphasize new and important aspects of the study or observations. A non-structured abstract of maximum 150 words should be used for editorials, review articles, current topics, special articles, professional articles, history of medicine articles and case reports. Letters to the editor and obituaries do not have an abstract. Below the abstract 3-5 key words should be provided that describe the topic of the article. The key words should be selected from Medical Subject Headings (MeSH). Letters to the editor and obituaries do not need to have key words.

3. Text
The text of the articles includes the following chapters: Introduction, Methods, Results and Discussion. Longer articles may need subheadings within some sections to clarify their content. Case reports should have four sections: Introduction, Case history, Discussion and Conclusion.

Introduction. After the introductory notes, the aim of the article should be stated in brief (the reasons for the study or observation), only significant data from the literature, but not extensive, detailed consideration of the subject, nor data or conclusions from the work being reported.

CONTENT

Methods. Selection of study or experimental subjects (patients or experimental animals, including controls) should be clearly described. Methods, apparatus (manufacturer's name and address in parentheses) and procedures should be identified in sufficient detail to allow other workers to reproduce the results. References should be cited for established methods, including the statistical ones. Identify precisely all drugs and chemicals used, with generic name(s), dose(s), and route(s) of administration. Statements on the study approval by the Ethics Committee for human or animal studies should be included.

Results should be presented in logical sequence in the text, tables and illustrations. Emphasise or summarise only important observations.

Discussion is aimed to emphasise new and significant aspects of the study and conclusions that result from them. Relate the observations to other relevant studies.

Conclusion should be linked with the goals of the study, but avoid unqualified statements and conclusions not completely supported by your data.

Tables
Each table should be typed single-spaced on a separate sheet, numbered in the order of their first citation in the text in the upper right corner and supplied with a brief title each. Explanatory notes are printed under a table. Each table should be mentioned in the text. If data from another source are used, acknowledge them fully. Each table should be named in order to indicate the author's name the number of the table, (eg Johnson_Table 1). The place of the table in the text should be marked as in the following example: "(Table 1 near here)".

Illustrations
Any forms of graphic enclosures are considered to be figures and should be submitted as additional databases in the System of Aseestant. Letters, numbers, and symbols should be clear and uniform, of sufficient size that when reduced for publication, each item will still be legible. Each figure should be named in order to indicate the author's name and the number of the figure (eg Johnson_Figure 1). If a figure has been published, state the original source.

Captions for illustrations are typed on a separate page, with Arabic numbers corresponding to the illustrations. If used to identify parts of the illustrations, the symbols, arrows, numbers, or letters should be identified and explained clearly in the legend. The method of staining and magnification in photomicrographs should be explained. Captions should be detailed enough to allow for understanding of the content of the figure without previous reading of the text. The place of the figure in the text should be marked as in the following example: "(Figure 1 near here)".

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Authors are encouraged to use abbreviations and acronyms in the manuscript in the following manner: abbreviations and acronyms must be defined the first time they are used in the text and thereafter must be consistently used throughout the whole manuscript, abbreviations should be used only for terms that appear more than three times in text; abbreviations should be sparingly used. Use of abbreviations in the titles should be avoided. Even if used after its definition in the abstract, the same definition and repetition of the abbreviation should be performed the first time it is used in the text. In order to assure self-explanatory nature of the tables and figures, abbreviations and acronyms should be defined in the captions and then introduced, irrespective of whether it was done earlier in the text.

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Examples of references:

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