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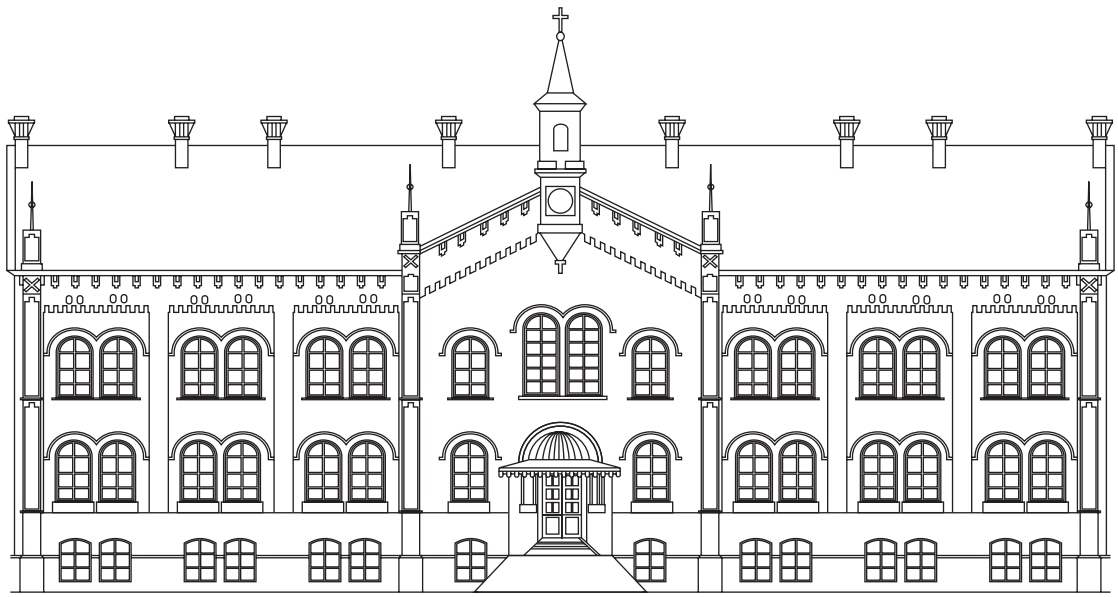

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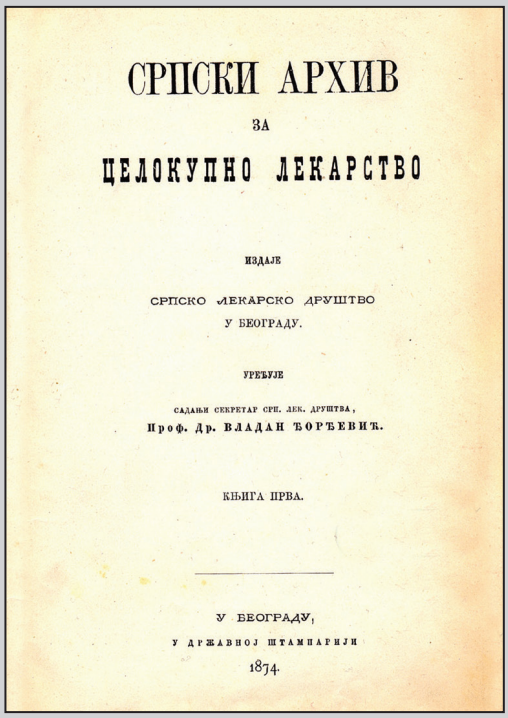

СРПСКИ АРХИВ

ЗА

ЦЕЛОКУПНО ЛЕКАРСТВО

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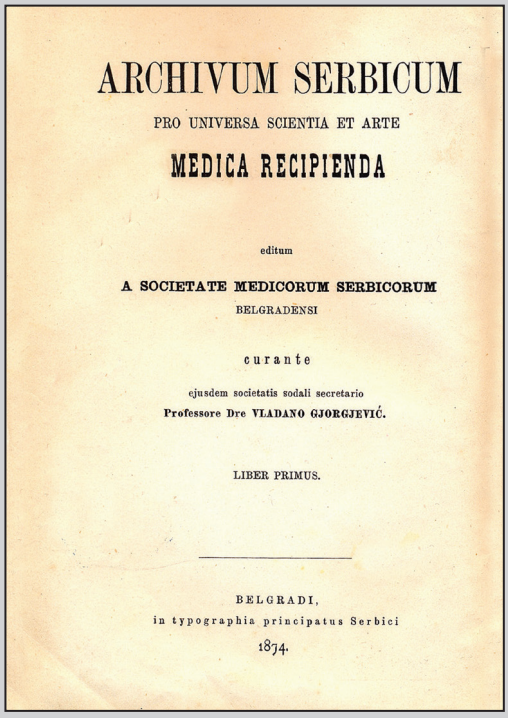
1874.

Прва страна првог броја часописа на српском језику



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BELGRADI,

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1874.

The title page of the first journal volume in Latin

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Application of dental implant robots and conventional dental implants in oral implantology – a propensity score matching study

Wenjie Ma, Xinglin Chen, Huifen Liu, Meng Yang, Xin Tong

Nanjing University, The Medical School, Nanjing Stomatological Hospital, Department of Oral Implantology, Nanjing, Jiangsu, China

SUMMARY

Introduction/Objective The aim of this study is to evaluate the application value of dental implant robot (DIR) in dental implant restoration of patients with tooth loss (TL), so as to provide reference for clinical practice.

Methods In total, 47 patients with TL who received DIR oral implantation in our hospital during the period from March 2021 to August 2023 were selected as the research subjects. By propensity score matching, according to the ratio of 1:1, the nearest neighbor matching algorithm was used to select 47 patients who received conventional oral implantation as the control group. The matching variables included age, sex, history of diabetes, history of hypertension, location of missing teeth, cause of missing teeth, and number of missing teeth. To compare the implant errors of the two groups and to test their oral function after oral implantation. In addition, we investigated the patients' pain using the visual analogue scale (VAS) and assessed their aesthetic appearance. Finally, the incidence of complications in the patients was recorded.

Results Compared to the control group, the implant error was lower in the observation group ($p < 0.05$). After implantation, there was no difference in verbal expression and occlusal ability between the two groups ($p > 0.05$), but VAS was lower in the observation group than in the control group at one week and one month after surgery ($p < 0.05$). There was no difference in the complication rate between the two groups ($p > 0.05$), but the observation group had better aesthetic appearance.

Conclusion DIR effectively enhances the accuracy of oral implantation and ameliorates the aesthetic outcome for patients.

Keywords: robotics; treatment outcome; tooth loss; plantation accuracy; treatment outcome; oral implantation

INTRODUCTION

Tooth loss (TL) constitutes one of the highly prevalent oral diseases in clinical settings, predominantly affecting middle-aged and elderly patients. It can be induced by a multiplicity of factors, including dental caries, various oral pathologies, or accidental trauma [1]. Statistically, the global incidence rate of TL ranges from approximately 23–53%, and this figure exhibits an upward trend year by year [2]. The onset of TL not only compromises the integrity of the dentition, resulting in occlusal dysfunction, alveolar bone atrophy, and decreased masticatory function, but also precipitates the development of other periodontal disorders [3]. The gold standard to treat TL is oral implantation, which involves the insertion of pure titanium implants into the alveolar bone to replace the absent teeth [4]. In recent years, with people's increasing attention to oral health and the advancement of medical technology, oral implantation technology has become more and more sophisticated and has currently become the preferred treatment option for more than 70% of TL patients [5]. The research focus of modern dental implant medicine centers on how to further curtail the surgical treatment duration, enhance patient comfort, and guarantee the success rate of the surgical procedure.

In 2016, the first dental implant robot (DIR) was granted approval for clinical medical application, presenting a brand-new solution for enhancing the accuracy and predictability of implant surgeries [6]. DIR works by using digital scanning and 3D reconstruction technology to accurately measure and analyze the patient's oral cavity, and then relies on a high-precision robotic arm to perform oral implants [7]. However, as a cutting-edge technology, the clinical application of DIR has received mixed reviews. For example, Dibart et al. [8] believe that the practical application ability of DIR is not yet sufficient to meet clinical needs, especially when dealing with complex anatomical conditions or when real-time decision adjustment is required. Li et al. [9] pointed out that the application of DIR needs more clear clinical evidence support, especially in terms of long-term success rate and cost-benefit ratio. These controversies highlight the need for further evaluation of DIR effectiveness and applicability in real clinical settings. Secondly, due to the relatively stringent requirements of DIR

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regarding hospital facilities and the operational proficiency of surgeons, it has not yet achieved comprehensive popularization throughout China. Related reports are usually reviews, lacking of exact clinical studies [10, 11].

Since 2020, our hospital has been engaged in the promotion of DIR usage within its premises. At present, a sufficient caseload has been amassed. In light of this situation, we conducted a retrospective analysis to verify the application value of DIR in oral implantation, thereby remedying the existing deficiency in DIR-related research in China. In view of the limited clinical application data of DIR, the aim of this study is to compare the differences between DIR-assisted oral implantation and traditional oral implantation in the treatment of TL through retrospective analysis, in order to provide reference and guidance for future clinical decision-making of oral implant treatment.

METHODS

Research subjects

Patients with TL who received oral implantation in Nanjing Stomatological Hospital during the period from March 2021 to August 2023 were selected as the research subjects for retrospective analysis. The Power Analysis and Sample Size software (PASS, NCSS, LLC, Kaysville, UT, USA) was used to calculate the required sample size based on the significance level $\alpha = 0.05$ (two-sided test) and statistical power $1-\beta = 0.8$. The expected effect size was set as (mean difference of apical error between the two groups was 0.6mm, standard deviation was 0.2mm). In addition, we calculated that a minimum of 47 samples per group would be required to account for a 10% risk of dropout. The treatment options for the patients were either DIR-assisted implantation (observation group) or conventional dental implants (control group).

Inclusion and exclusion criteria

Inclusion criteria were: (1) Normal mouth opening and occlusal function, with no loosening of adjacent teeth; (2) Healthy gums, good bone density, and sufficient and intact thickness of the labial wall; (3) Good overall health status, with no contraindications for oral implantation. Exclusion criteria were: (1) Inability to tolerate the implantation surgery; (2) Presence of bad occlusal habits; (3) Refusal to accept regular follow-up; (4) Existence of communication disorders or mental illnesses.

Data collection

Patients' baseline data and clinical features were collected, including but not limited to the following variables: demographic data such as sex, age, smoking history, drinking history, and place of residence; clinical features like the location, quantity, and reason of TL. All data were extracted through the electronic medical record system to ensure the accuracy and integrity of the data.

Surgical approaches

Conventional dental implants: Preoperatively, patients were informed about the surgical workflow. Relevant laboratory and imaging examinations were carried out, along with an assessment of both the intra-oral and general health status. Additionally, the environment, equipment, and preparatory items in the operating room were introduced. Following routine disinfection and draping, local infiltration anesthesia was administered to the patient using articaine (1.7 mL). During the surgical procedure, the dental implantologist performed gingival incision, flap reflection, sequential osteotomy for cavity preparation, implant placement, and wound suturing. Postoperatively, spiral computed tomography (CT) scans were re-performed to evaluate the outcomes.

DIR: (1) Preoperatively, cone-beam CT (CBCT) was performed to verify the patient's eligibility for implantation, and intra-oral scanning was conducted to obtain the dentition data (Figure 1A). The digital imaging and

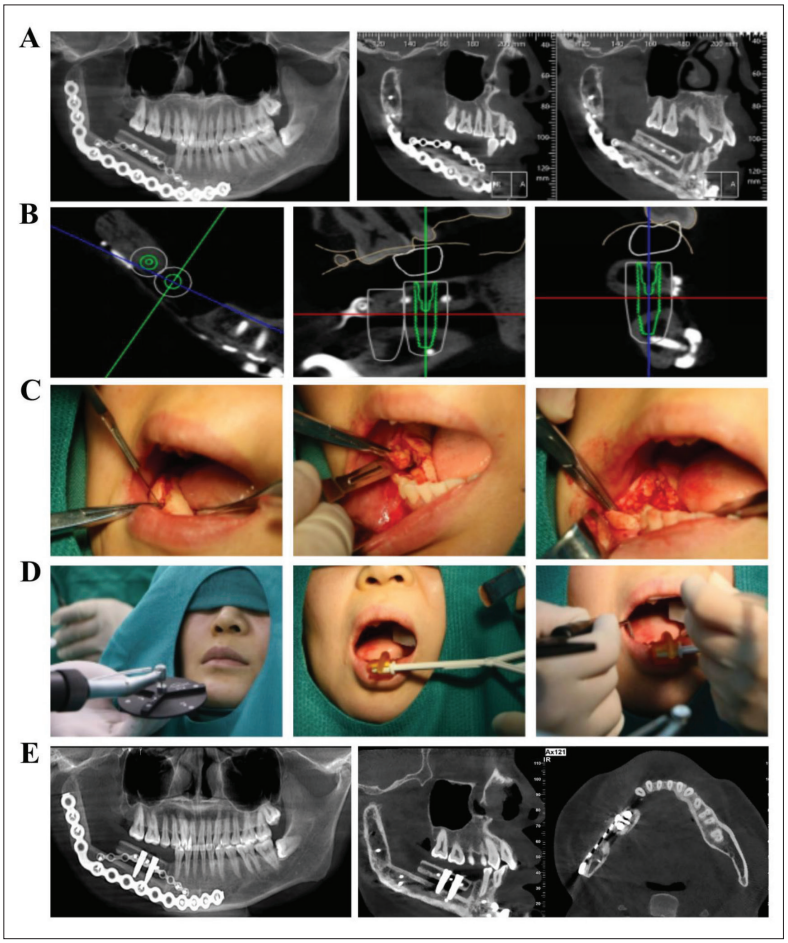

Figure 1. Schematic diagram of the surgical procedure; A – cone-beam computed tomography taken before surgery; B – design of implant position and implant path; C, D – the process of surgical operation; E – review of cone-beam computed tomography after surgery

communications in medicine data of the CBCT and the standard template library data of the intra-oral scan were then imported into the implant robot system. Through the system software, the position of the implant was designed, and the implantation path of the robot was planned (Figure 1B). Subsequently, an intra-oral positioning guide was fabricated, which was then connected to the calibration component to realize the spatial position relationship transformation between the robot and the intra-oral implantation site. Thereafter, the implantation steps were designed, and the corresponding sequential relationship between the selected tools and implantation steps was established to plan the implantation protocol. (2) After calibration, the osteotomy site was prepared. Drill bits were replaced according to the preset sequence for sequential cavity preparation. During the entire drilling process, the robotic arm was adjusted in real time to ensure that the implantation point, and the three-dimensional orientation of the implant were in accordance with the preoperative design (Figure 1C). (3) Under the instruction of the surgeon, the robotic implantation system completed the preparation of the implantation socket according to the preoperative plan. Depending on the patient's mouth-opening degree, the implant was either placed by the robot or manually (Figure 1D). (4) CBCT was repeated after surgery to confirm the results obtained (Figure 1E).

Follow-up for prognosis

All patients were subjected to a one-year prognostic follow-up investigation that was conducted regularly at two-month intervals. After one year, all implant restorations were completed, and the implant success rate was computed. The criteria for successful implantation were defined as follows: the implant remained stable with no evidence of loosening; X-ray examination revealed no radiolucent zones in the peri-implant bone tissue; and the patient reported a favorable condition without any abnormal sensations.

Outcome measures

(1) Based on the preoperative and postoperative CBCT scan results of patients, the apical point error and implant angle error between the preoperatively planned implant and the actual implant were measured. (2) The Chinese language articulation test was employed for patient assessment, with the score calculated as (the number of correctly articulated words/the total number of test words) × 100% [12]. (3) The T-scan computerized occlusal analysis system (Tekscan Inc., Norwood, MA, USA) was utilized to detect the pressure exerted by the dental implant during occlusion. Additionally, the percentage of pressure during occlusion with the contralateral homologous control tooth was recorded. (4) The visual analogue scale (VAS) [13] was adopted to investigate the pain status (scored from 0–10) at the surgical site during the preoperative stage (T0), one week after the implantation (T1), one month after the implantation (T2), and six months after the implantation (T3). A higher score on the VAS indicated a more pronounced pain level. (5) One year after the implantation, the pink esthetic score (PES) and the white esthetic score (WES) were employed to evaluate the esthetic outcome [14]. The PES includes seven parameters: mesial and distal papilla, labial gingival margin curvature and height, and root convexity, as well as soft tissue color and texture (with a total score ranging from 0 to 14 points). The WES consists of five elements: crown color, crown shape, crown contour, crown surface texture, and crown transparency (with a total score ranging from 0 to 10 points). Higher scores in PES and WES signify enhanced esthetic outcomes following restoration. (6) The incidence of complications such as postoperative gingival inflammation, infection, and periodontal discomfort in patients was recorded. (7) The self-developed satisfaction survey scale of our hospital was utilized to evaluate patient satisfaction regarding this implant treatment. This scale encompasses dimensions including the medical environment, treatment efficacy, and service attitude. The total score was 100 points, with a score above 85 indicating satisfaction, a score between 60–85 denoting basic satisfaction, and a score below 60 indicating dissatisfaction.

Statistical analysis

Statistical analysis was conducted using IBM SPSS Statistics for Windows, Version 26.0 (IBM Corp., Armonk, NY, USA). For categorical variables, the χ^2 test or Fisher's exact test was employed. The independent sample T-test or Mann–Whitney U test was used for continuous variables. Subsequently, the propensity score matching (PSM) approach was employed for 1:1 matching. The matching variables included age, sex, diabetes history, hypertension history, location of TL, cause of TL, and the quantity of missing teeth. The nearest-neighbor matching algorithm was adopted during the matching process, with a matching ratio of 1:1. After matching, the balance of baseline data in both groups was re-evaluated. A caliper value of 0.02 was set. A p-value less than 0.05 was regarded as statistically significant.

Ethics: The Ethics Committee of Nanjing Stomatological Hospital approved the study (NJSH-2023NL-064).

RESULTS

Comparison of baseline data between observation group and control group before PSM

After screening based on the inclusion and exclusion criteria, 47 patients in the observation group and 73 patients in the control group were finally determined. As shown in Table 1, the two groups were not statistically different in sex and age ($p > 0.05$). However, the observation group had more patients with a smoking history and single-tooth loss than the control group, with higher treatment costs ($p < 0.05$). In addition, the number of people in the

observation group whose place of residence was rural and whose educational level was junior high school or below was less than that in the control group, the participants were of a younger age, and the operation time was shorter ($p < 0.05$).

Evaluation of the balance of baseline variables in patients before and after PSM

We screened 47 patients in the observation group through PSM. As shown in Figure 2, the standardized mean differences of multiple variables between the two groups were relatively high before matching, and significant differences were present in the distribution of propensity scores, indicating substantial differences in these variables between the two groups. After matching, the standardized mean differences of most variables approached 0, and the distribution conformed more closely to the normal distribution.

Comparison of baseline data between observation group and control group after PSM

We found no notable differences in age, sex, and number of missing teeth between the observation group and the control group after PSM ($p > 0.05$), suggesting significantly improved comparability of baseline data between the two groups. Nevertheless, with respect to treatment costs, the observation group still exhibited higher values compared to the control group ($p < 0.05$, Table 2).

Comparison of plantation accuracy

After the follow-up, the implantation success rate of the observation group was 100% (47/47), versus 97.87% (46/47) of the control group, showing no statistical inter-group significance ($p < 0.05$). Although not reaching a statistically significant level, the 100% success rate in the observation group suggests that DIR-assisted implantation may have a clinical trend towards improved implantation success. The apical point error and implant angle error of patients in the observation group following implantation were both 0.57 ± 0.16 mm and 2.78 ± 0.34, which were lower than those in the control group ($p < 0.05$, Table 3).

Comparison of oral function

In terms of oral function, no significant differences were identified between the two groups with respect to language articulation and bite force ($p > 0.05$). However, the ratio of occlusal pressure to the contralateral homologous control tooth in the observation group was significantly higher than that in the control group ($p < 0.05$). Concerning pain assessment, no differences were observed in the VAS scores between groups at T0 and T3 ($p > 0.05$); nevertheless, lower VAS scores were determined in the observation group versus the control group at T1 and T2 ($p < 0.05$, Table 4).

Table 1. Baseline information before propensity score matching

Variables		Control group (n = 73)	Observation group (n = 47)	t (or χ^2)	p
Age		48.90 ± 11.86	52.53 ± 9.69	1.753	0.082
Sex	Male	49 (67.12)	30 (63.83)	0.138	0.710
	Female	24 (32.88)	17 (36.17)		
Smoking history	Yes	19 (26.03)	21 (44.68)	4.477	0.034
	No	54 (73.97)	26 (55.32)		
Drinking history	Yes	21 (28.77)	18 (38.3)	1.184	0.277
	No	18 (38.3)	29 (61.7)		
Education level	Junior high school and below	29 (39.73)	10 (21.28)	4.436	0.035
	High school and above	44 (60.27)	37 (78.72)		
Place of residence	Urban	36 (49.32)	34 (72.34)	6.237	0.013
	Rural	37 (50.68)	13 (27.66)		
Number of tooth loss	Single	31 (42.47)	29 (61.7)	4.232	0.040
	Double and	42 (57.53)	18 (38.3)		
Location of tooth loss	Premolars	18 (24.66)	9 (19.15)	0.498	0.481
	Molar	55 (75.34)	38 (80.85)		
Reason for tooth loss	Periodontics	34 (46.58)	26 (55.32)	0.898	0.638
	Dental caries	29 (39.73)	16 (34.04)		
	Trauma	10 (13.7)	5 (10.64)		
Operation time (min)		34.77 ± 10.92	27.51 ± 6.05	4.158	< 0.001
Treatment costs (yuan)		5008.84 ± 784.54	8438.57 ± 598.93	25.550	< 0.001

Table 2. Baseline information after propensity score matching

Variables		Control group (n = 47)	Observation group (n = 47)	t (or χ^2)	p
Age		49.04 ± 12.48	52.53 ± 9.69	1.514	0.133
Sex	Male	27 (57.45)	30 (63.83)	0.401	0.527
	Female	20 (42.55)	17 (36.17)		
Smoking history	Yes	17 (36.17)	21 (44.68)	0.707	0.401
	No	30 (63.83)	26 (55.32)		
Drinking history	Yes	14 (29.79)	18 (38.3)	0.758	0.384
	No	33 (70.21)	29 (61.7)		
Education level	Junior high school and below	17 (36.17)	10 (21.28)	2.546	0.111
	High school and above	30 (63.83)	37 (78.72)		
Place of residence	Urban	28 (59.57)	34 (72.34)	1.706	0.192
	Rural	19 (40.43)	13 (27.66)		
Number of tooth loss	Single	26 (55.32)	29 (61.7)	0.394	0.530
	Double and	21 (44.68)	18 (38.3)		
Location of tooth loss	Premolars	10 (21.28)	9 (19.15)	0.066	0.797
	Molar	37 (78.72)	38 (80.85)		
Reason for tooth loss	Periodontics	22 (46.81)	26 (55.32)	1.056	0.590
	Dental caries	17 (36.17)	16 (34.04)		
	Trauma	8 (17.02)	5 (10.64)		
Operation time (min)		30.21 ± 9.11	27.51 ± 6.05	1.694	0.094
Treatment costs (yuan)		5036.40 ± 856.23	8438.57 ± 598.93	22.320	< 0.001

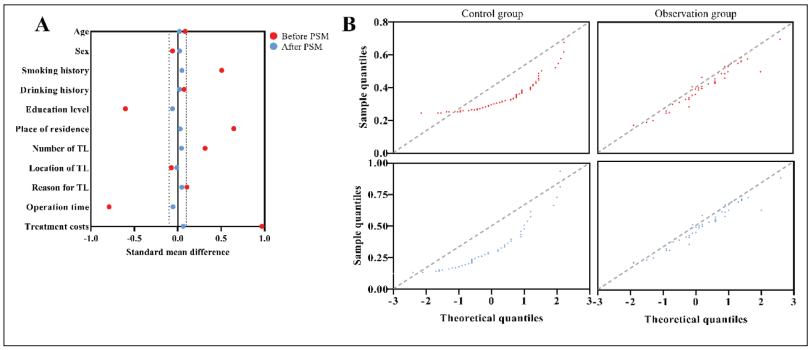

Figure 2. Effectiveness check of propensity score matching (PSM); A – standardized mean differences changes in variables before and after PSM; B – distribution of variables before and after PSM; TL – tooth loss

Table 3. Plantation accuracy of the two groups of patients

Variables	Control group (n = 47)	Observation group (n = 47)	t (or χ^2)	p
Implantation success rate	46 (97.87)	47 (100%)	1.011	0.315
Apical point error (mm)	0.66 ± 0.23	0.57 ± 0.16	2.298	0.024
Implant angle error (°)	3.24 ± 1.07	2.78 ± 0.34	2.818	0.006

Table 4. Oral function of the two groups of patients

Variables		Control group (n = 47)	Observation group (n = 47)	t	p
Oral function	Respect to language articulation (%)	89.92 ± 2.65	90.4 ± 2.19	0.963	0.338
	Bite force (N)	20.06 ± 2.84	20.96 ± 3.84	1.291	0.200
	Ratio of occlusal pressure to the contralateral homologous	0.79 ± 0.13	0.92 ± 0.17	4.303	< 0.001
Visual analogue scale	T0	5.38 ± 1.01	5.28 ± 1.06	0.498	0.619
	T1	3.49 ± 1	2.72 ± 0.8	4.108	< 0.001
	T2	2.3 ± 0.88	1.87 ± 0.99	2.198	0.031
	T3	0.72 ± 0.45	0.60 ± 0.5	1.304	0.196

Table 5. Treatment safety of the two groups of patients

Groups	Loose implants	Inflammation of the gums	Infection	Severe pain	Tearing of the wound	Incidence rate
Control group (n = 47)	1 (2.13)	2 (4.26)	1 (2.13)	2 (4.26)	1 (2.13)	14.89
Observation group (n = 47)	0 (0)	1 (2.13)	0 (0)	2 (4.26)	1 (2.13)	6.38
χ^2						1.790
p						0.181

Table 6. Aesthetic effects and treatment satisfaction of the two groups of patients

Parameters		Control group (n = 47)	Observation group (n = 47)	t	p
Aesthetic effects	Pink Esthetic Score	9.32 ± 1.07	10.60 ± 10.35	5.099	< 0.001
	White Esthetic Score	8.89 ± 1.03	9.79 ± 1.88	2.865	0.005
Satisfaction	Satisfaction	29 (61.7)	38 (80.85)	4.209	0.040
	Basic satisfaction	18 (38.3)	9 (19.15)		
	Dissatisfied	0 (0)	0 (0)	-	-

Comparison of treatment safety

Statistical analysis revealed that the incidence rate of postoperative complications in the observation group was 6.38%, with no infected cases. In contrast, the control group exhibited an incidence rate of 14.89%, with one infected patient. The comparison demonstrated no significant difference in the incidence rate of complications between the two groups ($p < 0.05$, Table 5).

Comparison of aesthetic effects and treatment satisfaction

Finally, in the comparison of post-implantation aesthetics, it was evident that both the PES and the WES were higher in the observation group than in the control group ($p < 0.05$). The results of the satisfaction survey indicated that there were no dissatisfied patients in either group. However, a greater number of satisfied patients was found in the observation group compared with the control group ($p < 0.05$, Table 6).

DISCUSSION

In this study, we reported the application effect of DIR through PSM. It was found that DIR significantly enhanced the accuracy of oral implant restoration and was more conducive to improving the occlusal function of patients. These findings provide a reliable data for future dental implant medicine.

Notably, the baseline data before PSM showed that the proportion of patients living in rural areas and the proportion of patients with education level of junior high school or below in the observation group were significantly lower than those in the control group. It is speculated that this is because the place of residence and education level may indirectly affect the implant effect (such as complication rate, pain score VAS, aesthetic satisfaction) by affecting the patient's oral hygiene habits, compliance with postoperative doctor's advice, or perception and reporting of pain. However, the primary outcome measures (implant accuracy, bite force, and speech intelligibility) in this study were mainly affected by the surgical technique and the implant itself, and were relatively unlikely to be directly affected by the above socio-demographic factors, and we ensured comparability between the two groups by PSM. However, more attention should be paid to these potential confounding factors

in the design and analysis of future studies. There was basically no difference in baseline data between the two groups after PSM, confirming that PSM can effectively control potential confounding variables and lay a more reliable foundation for the evaluation of the effect of DIR. The comparison results showed that the apical point error and implant angle error in the observation group were both lower than those in the control group, while the ratio of occlusal pressure to the contralateral homologous control tooth was higher. This is also in line with the research findings of Bahrami et al. [15], further validating the excellent application effect of DIR. As it is widely known, the traditional conventional dental implants primarily depend on dental implantologists' evaluations, which are based on preoperative CBCT results and the status of intra-oral dentition loss. In addition, the processes of cavity preparation and implant insertion during the surgical procedure rely on the surgeons' clinical expertise and tactile sense during implantation [16]. Research by Wang et al. [17] has pointed out that due to differences in the experience of dental implantologists, there may be deviations in the neck and angulation of the implant, or substantial deviations in the apical portion and depth of the implant during cavity preparation, affecting the path of insertion of the superstructure restoration. In contrast, the DIR manipulator can precisely operate by moving instruments in three-dimensional space, avoiding human errors caused by operational fatigue, suboptimal body positioning, or visual blind spots, and reducing the complexity of the operation, thus further enhancing implantation accuracy [18]. In terms of oral function recovery, there were no significant differences in speech clarity and bite force between the two groups. This is because the recovery of bite force depends mainly on the osseointegration quality of the implant, the design of the upper prosthesis, and the neuromuscular adaptation of the patient. In this study, both DIR-assisted and conventional implants followed standard osseointegration and repair procedures, which may be the main reason for the comparable bite force recovery between the two groups. The advantages of DIR in implant accuracy (such as more accurate insertion angle and position) may be more reflected in the accuracy of prosthesis insertion and long-term stability, while the effect on maximum bite force at one year follow-up post-surgery is limited. Additionally, Feng et al. [19] also mentioned that DIR guides the robotic arm through navigation to automatically complete the preparation of the implant cavity according to the preoperative plan. In the event of a slight displacement of the patient's head during the operation, the robotic arm can perform real-time updates and calibrations to ensure the precision and safety of the cavity preparation process. However, no significant difference was observed in the comparison of the incidence of complications between the two groups, which may be due to the accident caused by the small number of cases included in this study. Currently, the utilization of DIR has not achieved high prevalence, making it challenging for us to conduct a large-scale retrospective analysis. In the future, we will remain vigilant regarding this limitation.

On the other hand, the influence of TL extends beyond the pathological aspect and directly impacts the maxillofacial appearance of patients as well [20]. In traditional implant surgeries, the flap-elevation technique employed during the operation can prolong the surgical duration and cause pronounced postoperative pain. To a certain degree, this can impede the postoperative recovery of patients and lead to poor restoration outcomes. In this study, the PES and WES scores of patients in the observation group were both higher than those in the control group, suggesting that DIR provides better results to improving the aesthetics of patients. Reasons for analysis include: (1) The high-precision operation of DIR ensured that the implant was placed in the best three-dimensional position designed before the operation, and provided an ideal exit profile and support foundation for the prosthesis, which was conducive to the formation of a coordinated gingival margin curve, a full gingival papilla, and a natural crown shape. (2) The precise navigation of DIR reduces the exploration and adjustment of soft and hard tissues during the operation. In general, the socket preparation and implantation can be completed without extensive flap surgery, and the original soft and hard tissue structure and blood supply in the planting area can be preserved to the maximum extent. Minimally invasive surgery can reduce tissue edema and scar formation after operation, and is conducive to the stability and recovery of soft tissue aesthetic morphology [7]. (3) DIR can avoid implantation deviation caused by visual error or operator fatigue during free-hand operation, which may lead to poor contour of the crown or abnormal crown shape after insertion, which may affect the aesthetic effect. In a clinical application study of DIR, Wu et al. [21] also obtained the same results as this paper.

However, in this PSM study, it was observed that following the matching process, the treatment costs of the observation group remained significantly higher than those of the control group. This elevation in cost is associated with the utilization expense of the DIR and is, unfortunately, an unavoidable consequence. Although DIR has shown advantages in accuracy and aesthetic results, its high cost is an important challenge for clinical promotion. Future studies should conduct a more comprehensive cost-effectiveness analysis that considers not only the initial cost of treatment, but also the possible long-term benefits of DIR. The higher initial cost of DIR may be amortized if it significantly reduces the long-term complication rate or extends the lifespan of the prosthesis. Therefore, when evaluating the value of DIR, the cost and benefit need to be weighed from the perspective of the whole treatment cycle. Meanwhile, the following issues cannot be overlooked: (1) Currently, DIR cannot completely perform the implantation surgery independently. Instead, it necessitates surgeons to engage in preoperative planning, surgical protocol design, and comprehensive intraoperative monitoring. Moreover, it cannot timely predict and make real-time adjustments for various unexpected situations during the operation. Therefore, in order to promote DIR technology, it is necessary to strengthen the professional and systematic training of dental implantologists, and develop

more intelligent intraoperative monitoring and auxiliary decision-making systems to reduce the difficulty of operation and the absolute dependence on the experience of the doctor. (2) DIR is generally bulky and needs sufficient operating space. (3) Since DIR is a new technology, many patients find it difficult to accept it psychologically. In clinical practice, it is therefore essential to strengthen education and public awareness of DIR to improve patients' understanding and acceptance.

Limitations

The number of cases in this study was small and we need to increase the number of cases to improve the representativeness and comprehensiveness of the results. In addition, there were fewer observational indicators in this study, and we should add more objective indicators (e.g., inflammatory factors, oxidative stress indicators, etc.) to observe the full impact of DIR. Finally, the follow-up time in the current study was short, which resulted in our inability to assess the impact of DIR on the long-term prognosis of TL. Therefore, we also need to conduct a longer follow-up investigation on the subjects of this study.

CONCLUSION

DIR effectively enhances the accuracy of oral implantation, reduce the apical error by about 13.6%, and ameliorates the aesthetic outcome for patients. This represents a high clinical value. It is recommended that the use of DIR be promoted and popularized in clinical practice, thereby furnishing a more reliable treatment guarantee for dental implant medicine. The data emerging from this study are limited, and a larger prospective randomized clinical trial would be crucial to better study the application of this new technology.

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Conflicts of Interest: None declared.

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Примена роботског система и конвенционалних техника постављања денталних имплантата у оралној имплантологији – студија заснована на подударању по склоности

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САЖЕТАК

Увод/Циљ Циљ ове студије био је да се процени вредност примене роботског система за постављање зубних имплантата у оралној имплантологији код пацијената са губитком зуба, како би се обезбедиле корисне информације за клиничку праксу.

Методе Укупно 47 пацијената са губитком зуба, који су у периоду од марта 2021. до августа 2023. године у нашој болници подвргнути оралној имплантацији помоћу роботског система за постављање зубних имплантата, укључени су у студију као испитаници. Применом методе подударања по склоности, у односу 1 : 1, и коришћењем алгоритма најближег суседа изабрано је 47 пацијената који су подвргнути конвенционалној оралној имплантацији и они су чинили контролну групу. Варијабле које су се користиле за упаривање укључивале су старост, пол, анамнезу дијабетеса и хипертензије, локацију зуба који недостају, узрок и број зуба који недостају. Упоређиване су грешке у постављању имплантата између две групе, као и функционалност усне дупље након имплантације. Такође су процењивани интензитет бола пацијената коришћењем визуелне аналогне скале и естетски резултат. На крају, забележен је број компликација код пацијената.

Резултат У поређењу са контролном групом, грешка у постављању имплантата била је значајно мања у испитиваној групи ($p < 0,05$). Након имплантације није било статистички значајне разлике у вербалном изражавању и оклузивној способности између две групе ($p > 0,05$), али је вредност на визуелној аналогној скали била нижа у испитиваној групи него у контролној групи недељу и месец дана после операције ($p < 0,05$). Није било разлике у стопи компликација између две групе ($p > 0,05$), али је естетски исход био бољи у испитиваној групи.

Закључак Роботски систем за постављање зубних имплантата ефикасно повећава прецизност оралне имплантације и побољшава естетски исход код пацијената.

Кључне речи: роботика; исход лечења; губитак зуба; прецизност имплантације; орална имплантација

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Teach-back model-based health education in patients undergoing oral implant surgery

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SUMMARY

Introduction/Objective The objective of this study was to observe the application effectiveness of health education based on the teach-back model in oral implant surgery patients.

Methods A total of 480 patients who underwent oral implant surgery in our hospital from June 2023 to December 2023 were selected, and the patients were divided into the observation group (n = 241) and the control group (n = 239) based on the random-number-table method. The control group adopted the traditional preoperative oral instruction method, while the observation group adopted the preoperative teach-back combined with multimedia-education method, with the same content. A comparison was made between the two groups regarding patients' health-knowledge mastery, patient-satisfaction scores, and patient compliance.

Results The degree of health-knowledge mastery, patient satisfaction, and patient compliance in the observation group was significantly higher than in the control group, with statistically significant differences.

Conclusion Health-education based on the teach-back model can effectively improve the level of health-knowledge mastery, patient satisfaction, and patient compliance among oral-implant-surgery patients.

Keywords: teach-back; dental implantation; patient education

INTRODUCTION

Starting from April 20, 2023, the financial burden on dental implant patients has significantly decreased with the implementation of centralized-procurement prices for dental implant systems in China, lowering the barriers for patients to seek medical care, which has led to an expansion in the age and educational levels of the patients seeking treatment [1]. On the one hand, dental implants have become one of the mainstream options for tooth restoration, bringing a peak in implant surgeries for dental institutions. On the other hand, the national "Healthy China 2030" planning outline and the National Health Commission's "Healthy Oral Action Plan (2019–2025)" emphasize the need to improve health promotion and education mechanisms, enhance the effectiveness of health education services, and promote the widespread dissemination of health knowledge and the effective cultivation of health skills [2]. Therefore, it is of great significance to provide effective health education for patients undergoing implant surgery.

In recent years, various communication methods have gradually been introduced in the dental field, including tell–tell–tell, ask–tell–ask, and teach-back (TB), aiming to cultivate the personal skills of patients in managing their oral health [3]. Specifically, the tell–tell–tell method is described as "the doctor's monologue," primarily involving one-way information transmission from the doctor, lacking interaction with the patient [3]. In contrast, the ask–tell–ask method uses a three-step format of "ask–inform–ask" to assess the information needs and understanding of patients, helping to meet their emotional needs [3]. However, the TB method, as a more advantageous educational strategy, actively involves patients in the educational process by asking them to repeat or demonstrate what they have learned, significantly improving educational outcomes. In this regard, a systematic review by Yen and Leasure [4] indicated TB as an effective method for enhancing or providing health education for both children and adults. Additionally, the TB method has been recommended by various organizations, including the Joint Commission, the National Quality Forum, the Institute for Healthcare Improvement, and the Agency for Healthcare Research and Quality, as one of the effective methods for "comprehensive preventive measures" [5]. The application of TB by Seely et al. [6] in the informed-consent process for surgical patients showed that this method helped both doctors and patients focus on key discussion points, ensuring that patients truly understood the information while improving their understanding of surgical risks. Moreover, after applying TB to guide heart-failure patients in disease-related knowledge and post-discharge continuity of health education, Vellone et al. [7] found a significant increase in patients' grasp of medical information and a reduction in readmission rates. In China, the TB method was initially applied in the field of education,

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with its application in the medical field being relatively late. Since 2015, research on the application of the TB method in health education has gradually increased, covering various diseases such as diabetes, hypertension, coronary heart disease, chronic obstructive pulmonary disease, malignant tumors, and orthopedic surgeries [8]. These studies have indicated that the TB method can effectively enhance the health-knowledge retention and compliance of patients.

However, despite the remarkable success of the TB method in other medical fields, its application in dental implants remains limited. Traditional preoperative oral instruction in dental implants lacks standardization, personalization, and advancement, making it difficult to meet the comprehensive health-knowledge needs of patients, ultimately resulting in poor knowledge retention and low patient compliance [9]. In contrast, the TB method employs a two-way information-exchange model, emphasizing the learner's absorption and understanding of the educational content, thereby effectively enhancing the effectiveness of education [10]. Multimedia education uses images, animations, and videos to vividly and intuitively present the actual surgical process to patients, making it practical and engaging, with timely updates of information, which enables it to better meet the urgent information needs of patients compared to traditional oral education methods [11]. Notably, simultaneous demonstration and presentation during education – first informing patients about the main surgical process, the diagnostic and therapeutic instruments to be used, and the reasons for their use, followed by using the model and device to demonstrate the diagnostic and treatment process and working status, along with the possible sound, light, smell, and touch – can alleviate patients' psychological anxiety. In this study, health education based on the TB model was provided to 241 patients undergoing dental implant surgery at our hospital, aiming to improve the effectiveness of health education, fully meet the comprehensive health-knowledge needs of patients, and enhance their compliance and satisfaction [12, 13].

METHODS

Clinical data

This study was approved by the Ethics Committee of our hospital, with all patients voluntarily signing informed-consent forms. A total of 480 patients who underwent dental implant surgery at the Implant Center of our hospital from June to December 2023 were selected as study subjects. The patients were randomly divided into an observation group (n = 241) and a control group (n = 239) using a random-number table inclusion criteria: ① patients who met the surgical indications and received surgical treatment; ② patients without mental disorders. Exclusion criteria: ① patients unable to communicate normally; ② patients without a smartphone and unaccompanied by family members; ③ patients with combined organ dysfunction (heart, brain, kidney, etc.).

Methods

In the control group, traditional oral instruction methods were used for preoperative education: After scheduling the surgery, outpatient nurses provided verbal health education, with the content as follows: preoperative precautions (cleaning teeth, avoiding colds, abstaining from smoking and alcohol, etc.), the surgical process, key points for intraoperative cooperation, and postoperative precautions (medication guidance, dietary guidance, oral care, follow-up appointment times, etc.).

In the observation group, the TB method combined with multimedia education was used for preoperative education: After scheduling the surgery, patients were arranged in the preoperative education room at the Implant Center. For patients with mobility issues or poor comprehension, family members accompanied them, with the education provided by health-education nurses.

(1) *Multimedia education*

The educational content, developed in accordance with relevant health-education guidelines (such as "Health Guide for the Elderly in China" [14]) and tailored to oral implant surgery, was created by a team of prosthodontists, dental nurses, and geriatric specialists after reviewing clinical protocols and patient feedback. Converted into 15-minute animated videos, it covers preoperative (e.g., medication adjustments, caregiver arrangements), intraoperative (e.g., anesthesia steps, movement guidelines), and postoperative (e.g., incision care, soft-food examples) details specific to implant procedures. The videos use 3D animations, live-action demos, and large-font subtitles with pinyin/dialectal phonetics. Dialect versions (Cantonese, Shanghainese, etc.) include region-specific food examples. Features like adjustable playback speed and repeat functions aid accessibility. Before the session, nurses check language preferences, play the videos, and pause for TB, helping patients grasp the knowledge effectively.

(2) *Implementation of the teach-back health-education model*

The specific content was as follows.

① The nurses completed a one-day training program on the precautions of the TB method (including theoretical courses on the principles of the TB method, role-playing with simulated patients, and receiving expert guidance during the initial clinical application).

② Education explanation: Nurses explained the content again in simple and understandable language, demonstrating with models and instruments the surgical processes such as implant insertion, screw closure, and healing-abutment installation. Patients could touch some surgical instruments on-site and experience the noise produced by the surgical equipment; they also demonstrated oral-care practices such as the Bass brushing technique and using mouthwash with a dental model.

③ Question assessment: After the explanation, nurses used open-ended questions to ask patients to repeat or demonstrate the educational content, assessing their grasp of relevant knowledge.

④ Correction and clarification: When patients repeated or demonstrated correctly, they were positively encouraged and praised, concluding the education session. If incorrect or incomplete, corrections and clarifications were provided, supplemented by revisiting weak areas through videos and on-site demonstrations.

⑤ Assessment consolidation: Randomly selected knowledge points were questioned for re-evaluation and consolidation.

Evaluation criteria

Mastery of health knowledge: A health-education questionnaire created by our hospital for implant surgery was utilized, with a QR code generated using the SoJump software, allowing patients to scan and fill it out on-site after the preoperative education session (see Figure 1). The questionnaire covers four aspects: preoperative precautions, surgical process, intraoperative cooperation, and postoperative precautions, with a total of 10 items, and the specific content of the questionnaire can be viewed by scanning the QR code below. Each item is scored from 0–10 based on accuracy, with a total score of 100 points, and patients were categorized into three groups based on the results: fully mastered (> 90 points), partially mastered (60–90 points), and not mastered (< 60 points). Prior to formal use, the questionnaire underwent psychometric validation: content validity was assessed by the expert panel, resulting in a content validity index of 0.92, indicating good relevance and comprehensiveness; internal consistency was tested with a Cronbach's α coefficient of 0.87, demonstrating reliable internal consistency; and test–retest reliability was confirmed with an intraclass correlation coefficient of 0.85 when administered to 30 patients at a two-week interval, ensuring stable results over time. The mastery of oral-implant health-education content was calculated as follows:

Mastery = (Fully Mastered + Partially Mastered) / Total Number × 100%

Figure 1. Health education questionnaire for implant surgery

Patient satisfaction

The "Patient Satisfaction Survey for Preoperative Health Education in Implant Surgery" was developed by our hospital in accordance with the satisfaction indicators set by the National Health Commission, tailored to our hospital's actual situation, and consisted of nine items, including service attitude (four items: whether patients are respected, proactive communication, patience in answering questions, and use of polite expressions), education experience (three items: whether the methods are appropriate, content is comprehensive, and language is easy to understand), nurses' professional knowledge, psychological care, etc. Each item has four levels: very satisfied, relatively satisfied, not very satisfied, and very dissatisfied, scored as 100, 75, 50, and 0 points, respectively, and satisfaction assessments were automatically sent to patients' phones the day after the preoperative education session via SMS or WeChat through our hospital's cloud follow-up satisfaction-survey platform, which patients filled out online before the cloud follow-up platform automatically calculated satisfaction based on the number of valid responses. Specifically, satisfaction was calculated as follows: Step 1: Each Patient's Satisfaction Score = Total Score of Items / Number of Items; Step 2: Patient Satisfaction for Implant Surgery = (Total Patient Satisfaction Score / Total Valid Responses) [15].

Patient compliance

The postoperative compliance behaviors of both groups of patients (pain management, medication adherence, avoiding spicy, hot, and hard foods, and proper oral care) were recorded through follow-ups via phone, to assess compliance. Each nursing measure was categorized into 3 levels:

Fully compliant: Patients fully followed the medical advice without any deviations;

Partially compliant: Patients mostly followed the medical advice but had minor deviations (e.g., occasionally forgetting medication or slight dietary deviations);

Not compliant: Patients did not follow the medical advice or had significant deviations (e.g., frequently consuming spicy foods, and not taking medication on time).

Calculation method

Total compliance rate = (Number of Fully Compliant + Number of Partially Compliant) / Total Number × 100%;

Fully-compliant rate = Number of Fully Compliant / Total Number × 100%;

Partially-compliant rate = Number of Partially Compliant / Total Number × 100%;

Not-compliant rate = Number of Not Compliant / Total Number × 100%.

Statistical methods

IBM SPSS Statistics, Version 19.0 (IBM Corp., Armonk, NY, USA) was used for data statistics, and measurement data following a normal distribution were expressed as ($\bar{x} \pm s$),

with inter-group comparisons performed using the independent-samples t-test. Count data were represented as [n (%)] and inter-group comparisons were performed using the χ^2 test. $P < 0.05$ was considered statistically significant.

Ethics: This study was conducted in accordance with the Declaration of Helsinki and approved by the Research Ethics Committee of Shaoxing Stomatology Hospital (No. 2025-2-1, Date: 2025-02-04), and written informed consent was obtained from all participants. All methods were carried out in accordance with relevant guidelines and regulations.

RESULTS

Comparison of general data between the two groups

Before performing the primary outcome analysis, the general data of the two groups of patients were compared to assess the effect of potential confounders on the results, including age, gender, education level, and cognitive level. The statistical analysis suggested no significant difference in age, sex, and education level between the control and observation groups ($p > 0.05$), indicating inter-group comparability (see Table 1).

Comparison of mastery of education content between the two groups

In terms of mastery of education content, the overall mastery of patients in the observation group was significantly higher than that in the control group ($p < 0.001$). Specifically, the overall mastery of the control and observation groups was 154 (64.44%) and 220 (91.29%), respectively, with statistically significant differences ($\chi^2 = 50.281$, $p < 0.001$) (see Table 2).

Comparison of patient satisfaction between the two groups

For patient satisfaction, the observation group reported significantly higher satisfaction than the control group ($p < 0.05$). Specifically, satisfaction in the control and observation groups was 90.35% and 97.57%, respectively, and the differences were statistically significant ($p < 0.05$) (see Table 3).

Comparison of patient compliance between the two groups

When it came to patient compliance, the compliance of patients in the observation group was higher than that in the control group in 4 aspects of pain management (92.53% *vs.* 78.66%, $\chi^2 = 18.755$, $p < 0.001$), medication adherence (83.82% *vs.* 69.87%, $\chi^2 = 13.122$, $p < 0.001$), avoiding spicy, hot, and hard foods (91.29% *vs.* 76.15%, $\chi^2 = 20.199$, $p < 0.001$), and proper oral care (80.50% *vs.*

Table 1. Comparison of general data between the two groups

Item	Control group (n = 239)	Observation group (n = 241)	Statistics	p
Age/years	49.88 ± 14.678	47.61 ± 15.117	1.6681	0.096
Sex; n (%)			0.0002	0.992
M	107 (44.77)	108 (44.81)		
F	132 (55.23)	133 (55.19)		
Education level/n (%)			1.3282	0.249
High school or below	176 (73.64)	166 (68.88)		
College or above	63 (26.36)	75 (31.12)		

[1]t value;
[2]χ^2 value

Table 2. Comparison of mastery of education content between the two groups [n (%)]

Group	n	Not mastered	Partially mastered	Fully mastered	Overall mastery
Control	239	85 (35.56)	113 (47.28)	41 (17.15)	154 (64.44)
Observation	241	21 (8.71)	65 (26.97)	155 (64.32)	220(91.29)
χ^2					50.281
p					< 0.001

Table 3. Comparison of patient satisfaction between the two groups [n (%)]

Group	SMS sent	Valid responses	Patient satisfaction
Control	239	225	90.35
Observation	241	238	97.57

Table 4. Comparison of patient compliance between the two groups [n (%)]

Group		Control	Observation	χ^2	p
n		239	241		
Pain management	Fully compliant	88 (36.82)	131(54.36)		
	Partially compliant	100 (41.84)	92 (38.17)		
	Not compliant	51 (21.34)	18 (7.47)		
	Overall compliance	188 (78.66)	223 (92.53)	18.755	< 0.001
Medication following the doctor's advice	Fully compliant	130 (54.39)	170 (70.54)		
	Partially compliant	37 (15.48)	32 (13.28)		
	Not compliant	72 (30.13)	39 (16.18)		
	Overall compliance	167 (69.87)	202 (83.82)	13.122	< 0.001
Avoiding spicy/hot/ hard foods	Fully compliant	127 (53.14)	158 (65.56)		
	Partially compliant	55 (23.01)	62 (25.73)		
	Not compliant	57 (23.85)	21 (8.71)		
	Overall compliance	182 (76.15)	220 (91.29)	20.199	< 0.001
Proper dental care	Fully compliant	80 (33.47)	134 (55.6)		
	Partially compliant	89 (37.24)	60 (24.9)		
	Not compliant	70 (29.29)	47 (19.5)		
	Overall compliance	169 (70.71)	194 (80.5)	6.235	0.013

70.71%, $\chi^2 = 6.235$, $p = 0.013$), with statistically significant differences ($p < 0.05$) (see Table 4).

DISCUSSION

In this study, the comparison between traditional health-education methods and the TB method combined with multimedia education found significant advantages of the TB model in improving health-knowledge mastery, patient satisfaction, and patient compliance among patients undergoing oral implant surgery.

Oral implant surgery is a complex dental treatment process, in which the lack of periodontal tissue around the implant makes it highly susceptible to pathogenic bacterial colonization and endotoxin invasion, leading to a decrease in local immune-defense capability and triggering peri-implantitis, which directly threatens the short- and long-term survival of the implant [16]. Therefore, postoperative care is of great significance for the success of the surgery and the prognosis of patients. Nursing for dental implants includes strict oral-hygiene maintenance, postoperative medication management, and dietary control. Meanwhile, these oral-care abilities are related to the level of oral-health knowledge, self-care ability, and patient compliance of the patients. Therefore, it is essential to improve prognosis by enhancing the self-awareness of patients.

Additionally, the average age of patients was relatively high (49.88 ± 14.678 *vs.* 47.61 ± 15.117 years) in this study, along with a low educational level (71.25% had a high-school education or lower). In this regard, the traditional preoperative education method for dental implants relied on verbal instruction by nurses, which was monotonous and dull, largely overlooking the understanding and memory challenges faced by elderly patients and those with lower educational levels [17]. Moreover, patients often experience confusion, tension, and anxiety preoperatively, which reduce their ability to receive and master knowledge. In the control group, only 64.4% of patients could grasp the educational content, and their compliance was lower than that of the observation group, indicating the limited effectiveness of traditional health-education methods for elderly and low-education patient populations. This result also highlights the necessity of optimizing educational methods. A more in-depth exploration through customized multimedia approaches can achieve better results in enhancing knowledge acquisition and compliance. Considerations can be made from the following two aspects: First, adjusting the complexity of multimedia content to suit different levels of health literacy – using simple visual presentations for low-literacy groups and providing detailed explanations for high-literacy groups – can improve understanding. Second, address language barriers by using multilingual subtitles or dubbing, and integrate specific contextual scenarios to address cultural differences.

According to Dale's Cone of Experience, people can remember only 10% of knowledge through reading, 20% through listening, 30% through viewing images, 50% when a demonstration is combined with listening, and up to 70% through participating in discussions [18]. In this study, a health-education model was adopted based on the TB method combined with multimedia, utilizing 30–60-second video push notifications that allow patients to acquire health education and self-care knowledge in a short period. Particularly, the videos can be watched repeatedly, helping patients identify gaps in their understanding and continuously enhance their awareness of their condition and care. This intuitive, vivid, and illustrative presentation method effectively increases patient engagement, encouraging them to recite and express the health-education content. Meanwhile, nurses can assess patients' understanding in real time, addressing their questions on-site and providing reinforcement, thereby offering timely and accurate guidance. This increases patients' ability to receive and master knowledge, enabling them to better follow medical advice for self-management postoperatively, ultimately achieving better surgical outcomes and increasing patient satisfaction. Compared to other research findings, this study further confirms the effectiveness of the TB model in specific patient populations. For instance, Chen [19] implemented the TB method for health education in patients with diabetes and periodontal disease, finding significant relief of periodontal issues and marked improvements in disease-related knowledge and self-care abilities. Moreover, Lei et al. [20] used TB health education in patients with chronic periodontal disease, which helped maintain oral hygiene and reduce plaque indices. These findings align with this study, indicating that the TB model significantly enhances patients' mastery of health knowledge and compliance, particularly among elderly and low-education populations.

However, this study also has certain limitations. First, additional time may be required for the implementation of the TB model to ensure patients fully understand all important information, which could be a challenge for busy healthcare institutions, especially in resource-limited settings. Second, this study primarily focused on short-term health-education outcomes, without evaluating the impact on long-term health outcomes. In this regard, the effects of the TB model on long-term health outcomes, such as postoperative quality of life and complications, should be further investigated in future research. Additionally, patient characteristics such as health literacy, educational background, and cultural differences should also be considered in future research, as these factors may impact the effectiveness of the TB model. Finally, the study was conducted at a single center, which limits the universality of the research conclusions. The patient cohort of this study reflects the specific demographic characteristics and clinical background of this single institution, and the availability of resources also differs from other environments. Therefore, the effectiveness of traditional preoperative education observed here may deviate to some extent from the results of centers with diverse patient populations or different resource allocations. Caution is needed when widely expanding these results.

CONCLUSION

The implementation of health education based on the TB model for patients undergoing oral implant surgery can provide more specialized and standardized educational content, helping patients truly understand relevant health knowledge and improving their mastery of health information. This, in turn, enhances their compliance, encourages them to actively cooperate with treatment, increases patient satisfaction, and effectively prevents conflicts between patients and healthcare providers. Therefore, this approach is of significant value in clinical application and is worthy of promotion and wider application.

Conflict of interest: None declared.

Author contributions

Study design: Zhang SY, Zhou YY;
Data acquisition: Zhang SY, Zhou YY;
Data analysis and interpretation: Zhang SY, Zhou YY;
Manuscript preparation: Zhang SY, Zhou YY;
Critical revision of the manuscript for intellectual content: Zhang SY, Zhou YY;
Manuscript review: Zhang SY, Zhou YY.

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Здравствено образовање засновано на моделу *teach-back* код пацијената који се подвргавају хируршкој уградњи оралних имплантата

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САЖЕТАК

Увод/Циљ Циљ рада је био да се испита ефикасност примене здравственог образовања заснованог на моделу *teach-back* код пацијената који се подвргавају хируршкој уградњи оралних имплантата.

Методе У студију је укључено 480 пацијената којима су од јуна до децембра 2023. године у нашој болници хируршки уграђени орални имплантати. Пацијенти су методом случајне нумеричке табеле распоређени у испитивану групу (n = 241) и контролну групу (n = 239). Контролна група је добила традиционалну преоперативну усмену инструкцију, док је испитивана група добила преоперативну едукацију методом *teach-back* у комбинацији са мултимедијалним приступом, са истим садржајем. Поређени су резултати обе групе по питању усвајања здравственог знања, степена задовољства пацијената и њихове сарадљивости.

Резултати Степен усвајања здравственог знања, задовољства пацијената и сарадљивости био је већи у испитиваној групи у односу на контролну групу, при чему је разлика била статистички значајна.

Закључак Здравствено образовање засновано на моделу *teach-back* може ефикасно да побољша усвајање здравственог знања, задовољство пацијената и њихову сарадљивост током хируршке уградње оралних имплантата.

Кључне речи: *teach-back*; орални имплантати; едукација пацијената

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Comparative outcomes of parallel-wire and antegrade wire escalation techniques following single-wire failure in CTO PCI – a long-term follow-up study

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SUMMARY

Introduction/Objective Following the failure of the single-wire technique in percutaneous coronary intervention (PCI) for chronic total occlusions (CTO), two principal antegrade escalation strategies are commonly employed: the parallel-wire technique and antegrade wire escalation (AWE). Despite their widespread use, comparative data on the procedural characteristics and long-term clinical outcomes of these strategies remain scarce. This study aims to compare the procedural parameters and long-term outcomes of the parallel-wire and AWE techniques after single-wire failure in CTO PCI.

Methods This retrospective, single-center study included patients who underwent successful CTO PCI between January 2018 and December 2023 using either the parallel-wire or AWE technique following single-wire failure. The primary endpoint was a composite of cardiac death, myocardial infarction, stroke, or target vessel revascularization (TVR). Secondary outcomes included procedure duration, fluoroscopy time, contrast volume, and total radiation dose. Median follow-up duration was 1222 days (IQR 580–1969 days).

Results Among 270 CTO PCI procedures, 112 (41.5%) required escalation: 90 with AWE and 22 with the parallel-wire technique. Baseline clinical and angiographic characteristics were comparable. The primary composite outcome occurred in 14.4% of the parallel-wire group and 9.1% of the AWE group (p = 0.73). No significant differences were observed in individual clinical events. Procedure duration was longer (95.5 ± 43.6 *vs.* 77.0 ± 30.7 min; p = 0.064) and contrast volume higher (336.4 ± 113.3 *vs.* 271.6 ± 90.6 mL; p = 0.014) in the AWE group, with similar fluoroscopy time and radiation dose. No clinically or angiographically significant complications occurred during the periprocedural period.

Conclusion Both AWE and parallel-wire techniques demonstrate comparable safety and efficacy following single-wire failure in CTO PCI. While procedural efficiency slightly favored the parallel-wire strategy, overall outcomes support either approach, pending further prospective validation.

Keywords: chronic total occlusion; percutaneous coronary intervention; antegrade approach; wire escalation; parallel wire

INTRODUCTION

Chronic total occlusion (CTO) percutaneous coronary intervention (PCI) represents a frontier of interventional cardiology that continues to evolve in both technique and strategy [1, 2]. Despite advances in operator training, wire technology, and algorithmic approaches, procedural success remains highly dependent on the ability to cross the occlusion efficiently and safely [3, 4].

The single-wire technique is typically employed as the initial strategy during antegrade CTO PCI. However, its success is often limited in complex lesion subsets characterized by blunt or ambiguous caps, heavy calcification, or long occlusion length. In such cases, escalation is required. The parallel-wire (PW) technique, which introduces a second wire after the initial wire enters a subintimal space, enables re-engagement of the true lumen with a different trajectory. Alternatively, the antegrade wire escalation (AWE) strategy involves gradual increases in wire penetration power while maintaining the original trajectory, and is often guided by tactile feedback and intravascular imaging [4, 5].

While both approaches are widely used, comparative data on their clinical efficacy –particularly regarding long-term outcomes such as cardiac death, myocardial infarction, stroke, or target vessel revascularization (TVR) – remain limited. Most previous studies have focused on procedural endpoints, without evaluating

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whether differences in technique result in sustained clinical benefits [6–11]. Considering that patient-specific risk factors – particularly diabetes mellitus – as well as anatomical features such as bifurcation involvement, severe calcification, long occlusion length, and ambiguous proximal caps are associated with increased lesion complexity and adverse long-term outcomes following CTO PCI, understanding the interplay between clinical and anatomical variables remains crucial when assessing escalation strategies [12–16].

This study aimed to compare not only the procedural efficiency and safety of the two strategies, but also their impact on long-term clinical outcomes, thereby providing a more comprehensive understanding of how escalation techniques influence both immediate and long-term patient prognosis.

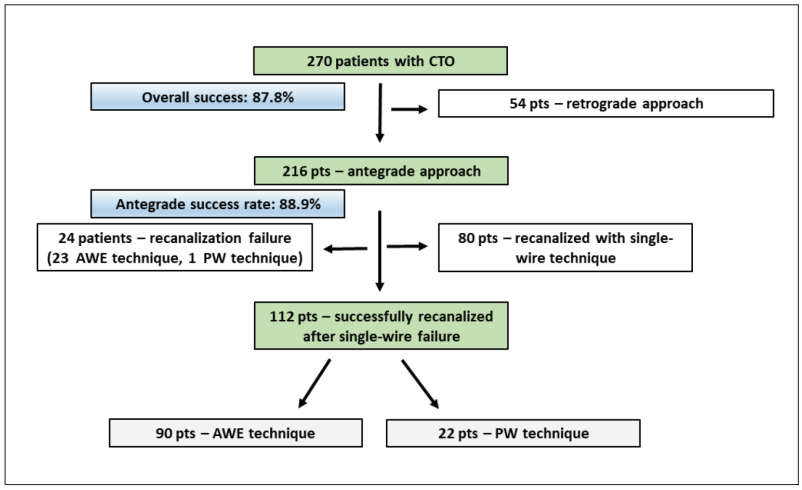

Figure 1. Study flow chart;

CTO – chronic total occlusion; pts – patients; AWE – antegrade wire escalation; PW – parallel wire

METHODS

Study design

This was a retrospective, observational single-center cohort study conducted at the tertiary university Clinical Center of Serbia, approved by the Ethics Committee of the University Clinical Center of Serbia. Patients who underwent CTO PCI between January 2018 and December 2023 were screened. Only those with failure of the initial single-wire antegrade approach, followed by treatment with either a PW or AWE technique, were included. In the analysis, we included procedures that achieved technical success, defined as successful CTO crossing with < 30% residual stenosis and achievement of TIMI 3 flow. All procedures were performed by a senior CTO operator in collaboration with two junior specialists dedicated to CTO interventions, both working under the supervision and proctorship of the senior operator.

Definitions of procedural techniques

Single-wire technique

The single-wire technique refers to the initial approach in PCI for CTO, where a single guidewire is used to attempt lesion crossing in an antegrade fashion. This method typically employs a soft or intermediate-tip wire, guided by angiographic anatomy, without immediate escalation to higher-penetration or multiple-wire strategies. It is considered a low-complexity, first-line technique and often precedes more aggressive methods if unsuccessful.

Antegrade wire escalation (AWE) technique

The AWE technique involves the sequential use of guidewires with increasing tip stiffness and penetration power to cross the occlusion through the true lumen in an antegrade direction. Escalation typically progresses from polymer-jacketed or tapered-tip wires to high-penetration wires, depending on lesion characteristics and operator judgment. This method is generally employed after the failure of the single-wire approach, aiming to overcome resistant proximal caps or ambiguous vessel course without entering the subintimal space [5].

Parallel-wire (PW) technique

The PW technique constitutes a structured escalation approach implemented after the unsuccessful application of the single-wire method. Upon confirmation – or strong suspicion – that the initial guidewire has entered an extraplaque space, a second, usually stiffer or differently tapered wire is advanced in parallel to the first. Employing a microcatheter for enhanced support and directional control, the adjunctive wire is steered along an alternative trajectory, with the explicit aim of re-engaging the true arterial lumen distal to the occlusion. By providing a distinct channel for lesion negotiation and refining torque transmission, this technique has been shown to improve crossing success rates in anatomically challenging CTOs [17].

Endpoints

The primary outcome was the composite of cardiac death, nonfatal myocardial infarction, target vessel revascularization, and stroke. Secondary endpoints included total procedure duration, fluoroscopy time, contrast volume, and the total radiation dose, defined as the cumulative air kerma at the interventional reference point (measured in mGy), recorded at the end of the procedure.

Population and eligibility

The study included patients with angiographically confirmed chronic total occlusion who were initially treated with a single-wire antegrade strategy, followed by escalation to either a PW or antegrade wire technique after failure of the initial attempt. Only patients with complete procedural data and available long-term clinical follow-up were analyzed. Patients treated with retrograde or hybrid techniques, those in whom re-entry devices such as CrossBoss or Stingray were used, as well as individuals with incomplete or unavailable follow-up data, were excluded from the study.

Follow-up

Clinical follow-up data were collected via outpatient visits, electronic medical records, and standardized phone interviews. Median follow-up duration was 1222 days (IQR 580–1969 days).

Statistical analysis

Categorical variables were expressed as counts and percentages, and continuous variables were reported as means ± standard deviations. The χ^2 test or Fisher's exact test was used to compare categorical variables, while continuous variables were compared using the independent-samples t-test or Mann–Whitney U test based on data distribution. A p-value less than 0.05 was considered statistically significant. All statistical analyses were performed using IBM SPSS Statistics version 28.0 (IBM Corp., Armonk, NY, USA).

Ethics: The study received approval from the Ethics Committee of the University Clinical Center of Serbia (Approval No. 30/4).

RESULTS

During the study period, a total of 270 patients underwent percutaneous coronary intervention for chronic total occlusion. Among them, 112 cases (41.5%) necessitated procedural escalation due to unsuccessful single-wire crossing and were subsequently managed with either an AWE strategy (n = 90) or the PW technique (n = 22) (Figure 1). This final study cohort consisted of 112 patients, the majority of whom were male (78.6%).

The mean age was 67.3 ± 10.1 years in the AWE group and 63.1 ± 8 years in the PW group, without a statistically significant difference (p = 0.07).

No significant differences were observed between the AWE and PW groups in terms of diabetes prevalence or family history of coronary artery disease. Baseline demographic, clinical, and procedural characteristics for both groups are detailed in Table 1.

Table 1. Baseline demographic, clinical, and procedural characteristics

Characteristics [n (%)]	Total	AWE	PW	p-value (AWE *vs.* PW)
No of patients	112	90	22	
Age (yrs, mean ± SD)	58.5 ± 9.5	67.3 ± 10.1	63.1 ± 8	0.43
Male (%)	88 (78.6)	70 (77.8)	18 (81.8)	0.68
Family history of CAD (%)	49 (43.8)	37 (41.1)	12 (54.5)	0.25
Diabetes (%)	32 (28.6)	23 (25.6)	9 (40.9)	0.209
– Insulin dependent	7 (6.25)	6 (6.7)	1 (4.5)	
Hypertension (%)	95 (84.8)	75 (83.3)	20 (90.9)	0.38
Hypercholesterolemia (%)	88 (78.6)	69 (76.7)	19 (86.4)	0.32
Smoking status				
– Never	61 (54.5)	46 (51.1)	15 (68.2)	0.11
– Smoker	23 (20.5)	22 (24.2)	1 (4.5)	
– Ex-smoker	28 (25)	22 (24.2)	6 (27.3)	
Previous MI (%)	50 (44.5)	38 (42.2)	12 (54.6)	0.58
– STEMI	33 (29.5)	25 (27.8)	8 (36.4)	
– NSTEMI	17 (15.2)	13 (14.4)	4 (18.2)	
Previous CABG (%)	5 (4.5)	5 (5.6)	0 (0)	0.26
Previous PCI (%)	28 (25)	23 (25.6)	5 (22.7)	0.78
CCS				
– CCS 1	15 (13.4)	13 (14.4)	2 (9.1)	0.76
– CCS 2	80 (71.4)	64 (71.1)	16 (72.7)	
– CCS 3	17 (15.2)	13 (14.4)	4 (18.2)	
CTO artery (n (%))				
– RCA	64 (58.7)	50 (56.6)	14 (66.7)	0.54
– LAD	33 (30.3)	27 (30.7)	6 (28.6)	
– Cx	12 (11)	11 (12.5)	1 (4.8)	
Localization of CTO (n (%))				
– Ostial	1 (0.9)	1 (1.1)	0 (0)	0.37
– Proximal	47 (42)	36 (40)	11 (50)	
– Medial	54 (48.2)	43 (47.8)	11 (50)	
– Distal	10 (8.9)	10 (11.1)	0 (0)	
In-stent CTO (N (%))	10 (8.9)	7 (7.8)	3 (13.6)	0.41
Diameter of CTO vessel (mm, mean ± SD)	3.0 ± 0.4	3.0 ± 0.4	3.2 ± 0.3	0.02
Stump morphology (N (%))				
– Blunt	35 (31.3)	27 (30)	8 (36.4)	0.56
– Tapered	77 (68.8)	63 (70)	14 (63.6)	
J CTO score (mean + SD)	1.69 ± 1.2	1.73 ± 1.1	1.50 ± 1.3	0.38
Side branch (%)	13 (11.6)	12 (13.3)	1 (4.5)	0.25

Data are expressed as the mean ± SD or as the number (percentage); CAD – coronary artery disease; MI – myocardial infarction; STEMI – ST-elevation myocardial infarction; NSTEMI – Non-ST-elevation myocardial infarction; CABG – coronary artery bypass grafting; CCS – Canadian Cardiovascular Society grading of angina pectoris; CTO – chronic total occlusion; LAD – left anterior descending; Cx – circumflex; RCA – right coronary artery; AWE – antegrade wire escalation; PW – parallel wire

Primary composite outcome and secondary endpoints

The primary composite outcome, defined as the occurrence of cardiac death, myocardial infarction, stroke, or TVR was documented in 13.4% of the overall study population. In the parallel wire group, this outcome occurred in 14.4% of patients, while in the AWE group, the incidence was 9.1% (Figure 2). Although the parallel wire group exhibited numerically higher event rates, none of the individual components of the composite outcome reached statistical significance between groups. Moreover, no significant differences were identified in the overall incidence

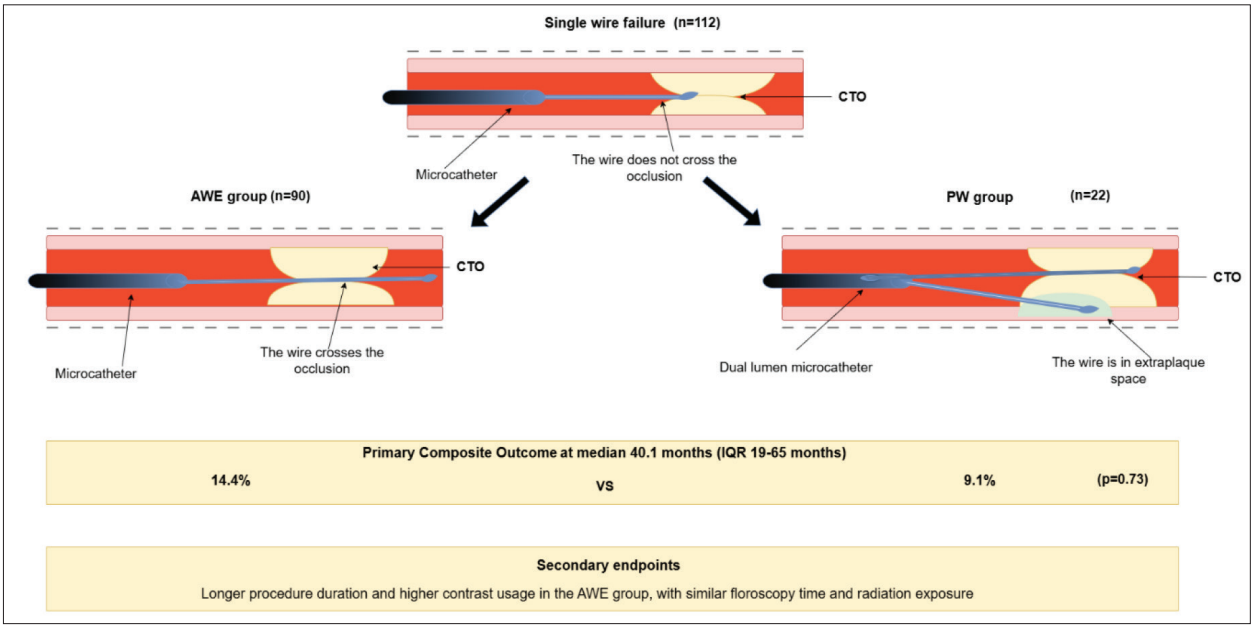

Figure 2. Central illustration;

AWE – antegrade wire escalation; PW – parallel wire; CTO – chronic total occlusion

Table 2. Primary composite outcomes during follow-up

N (%)	AWE	PW	p
Cardiac death	3 (3.3)	0 (0)	1
MI	2 (2.2)	0 (0)	1
TVR	5 (5.6)	1 (4.5)	1
Stroke	3 (3.3)	1 (4.5)	1
Total events	13 (14.4)	2 (9.1)	0.73

The data is numerical;
AWE – antegrade wire escalation technique;
PW – parallel wire techniques; MI – myocardial infarction; TVR – target-vessel revascularization

Table 3. Secondary procedural endpoints: comparison between antergrade-wire escalation and parallel-wire techniques

Parameter	Total	AWE (mean ± SD)	PW (mean ± SD)	p
Procedure time (min.)	91.84 ± 41.9	95.5 ± 43.6	77 ± 30.7	0.06
Fluoroscopy time (min.)	37.57 ± 22.3	38.1 ± 22.8	35.5 ± 21.1	0.62
Contrast volume (mL)	323.71 ± 111.88	336.4 ± 113.3	271.6 ± 90.6	0.01
Air Kerma (mGy)	1582.85 ± 987.32	1596.0 ± 1014.6	1528.9 ± 886.8	0.77

The data is numerical;
AWE – antegrade wire escalation technique; PW – parallel wire technique

of the primary composite endpoint or its constituent events between the two antegrade escalation strategies following failure of the single-wire approach. A detailed distribution of outcome types by group is provided in Table 2.

Secondary procedural endpoints included procedure duration, fluoroscopy time, contrast volume, and radiation dose. The mean procedure time was longer in the AWE group (95.5 ± 43.6 minutes) compared to the PW group (77.0 ± 30.7 minutes), with a trend toward statistical significance (p = 0.064). The contrast volume was significantly greater in the AWE group (336.4 ± 113.3 mL *vs.* 271.6 ± 90.6 mL; p = 0.014). In contrast, no statistically significant differences were observed between the groups in terms of fluoroscopy time and radiation dose (p = 0.624 and p = 0.776, respectively) (Figure 2). A comprehensive overview of these secondary outcomes is provided in Table 3. There were no clinically or angiographically significant complications observed in the periprocedural period.

Specifically, in successfully recanalized patients within the single-wire group, the following guidewires were used: Fielder family in 58 cases (72.5%), Gaia 1st in eight cases (10%), Gaia 2nd in 13 cases (16.25%), and Confianza Pro 9 in one case (1.25%).

In the AWE group, the most frequently selected initial wire was from the Fielder family in 65 cases (72%), followed by Gaia 1st in nine cases (10%), Gaia 2nd in 13 cases (15%), and Gaia 3rd in three cases (3%). Among the wires that ultimately crossed the occlusion in this group, the Gaia family predominated: Gaia 1st in 22 cases (24%), Gaia 2nd in 52 cases (59%), and Gaia 3rd in 10 cases (11%), whereas Confianza Pro – four (4%) and Confianza Pro 12 – two (2%) were used less frequently.

In the PW technique, the first-choice wires were predominantly from the Fielder family in 16 cases (73%), followed by Gaia 1st in 4 cases (18%) and Gaia 2nd in two cases (9%). Wires that successfully entered the distal true lumen included Gaia 1st in four cases (18%), Gaia 2nd in 16 cases (73%), and Gaia 3rd in two cases (9%).

DISCUSSION

While single-wire crossing remains the predominant antegrade strategy in contemporary CTO registries, there is a notable lack of robust data guiding the selection of the most appropriate alternative technique following failure

of the single-wire approach (6). This study offers a comparative analysis of two widely used antegrade escalation strategies – PW technique and AWE – employed following single-wire failure in PCI CTO. Although no statistically significant differences were observed in long-term rates of the primary composite outcome between the groups, both techniques demonstrated high procedural success and low complication rates, underscoring their clinical utility in contemporary CTO practice.

Although the PW group exhibited a numerically higher rate of adverse events, this difference did not reach statistical significance, and the small sample size in this cohort limits the power to draw definitive conclusions. Although the small size of the PW cohort limits statistical power, the absence of baseline imbalances strengthens the internal validity of the findings. The greater contrast use and trend toward longer procedural time in the AWE group may have clinical implications, particularly in patients with renal impairment or complex anatomy. These findings likely reflect the incremental and often repetitive nature of AWE, including multiple wire exchanges and re-engagement attempts. The choice between wire-escalation and PW techniques was largely dictated by procedural circumstances, with longer occlusions being more prone to extra-plaque wiring and thus more often managed by the PW approach, particularly when the initial wire course was close to the distal true lumen. Notably, the relative frequency of both techniques in our cohort is consistent with the proportions reported in major international registries.

Our findings are consistent with prior registry-based observations and expert consensus statements suggesting that both AWE and PW strategies are reasonable and effective options following initial wire failure. While direct comparative data between these two techniques remain limited, some studies comparing PW with dissection and re-entry have suggested procedural trade-offs, with ADR often achieving higher crossing success at the expense of increased contrast and radiation exposure. A comprehensive meta-analysis by Zhao et al. [18] demonstrated that extensive ADR techniques were associated with a significantly increased risk of adverse long-term outcomes – including target vessel revascularization, in-stent restenosis, and the composite of death/myocardial infarction/TVR – when compared with conventional wire escalation strategies. Conversely, limited ADR techniques, particularly those facilitated by dedicated re-entry devices, were shown to have outcomes comparable to those of wire escalation [19]. Supporting this, the PROGRESS-CTO registry analysis compared ADR and PW techniques after failed single-wire attempts and reported that ADR was associated with higher rates of major adverse cardiovascular events (3.7% *vs.* 1.9%, p = 0.029), despite demonstrating slightly higher technical success [20]. This suggests a potential trade-off between technical efficacy and procedural safety, especially in more complex or comorbid patients where ADR tends to be more frequently selected.

Furthermore, findings from the randomized CrossBoss First Trial [21] revealed no significant difference between the CrossBoss-based ADR strategy and standard wire escalation in terms of crossing time, technical or procedural success, or safety outcomes. These results emphasize that while controlled dissection and re-entry techniques may offer utility in specific anatomical scenarios, they do not universally outperform conventional wire-based strategies and should not be considered the default escalation approach.

Our findings, showing no statistically significant differences in primary outcomes between the PW and AWE strategies, are consistent with the results reported by Galassi et al. [22], who demonstrated comparable long-term clinical efficacy between wire-based ADR and conventional antegrade wiring techniques, despite higher lesion complexity in the ADR group. The convergence of clinical outcomes suggests a potential therapeutic equivalence among various wire escalation strategies employed after initial failure, reinforcing the need for prospective investigations utilizing standardized intravascular imaging and adequately powered PW cohorts to refine the decision-making algorithm in this high-risk subset of CTO patients [22].

The choice between wire-escalation and PW techniques was largely dictated by procedural circumstances, with longer occlusions being more prone to extra-plaque wiring and thus more often managed by the PW approach, particularly when the initial wire course was close to the distal true lumen. Notably, the relative frequency of both techniques in our cohort is consistent with the proportions reported in major international registries.

In this context, our data contribute to the growing body of evidence supporting individualized strategy selection based on lesion morphology, operator experience, and patient-specific risk factors. Although no statistically significant difference in long-term clinical outcomes was observed, procedural nuances and patient-related considerations may guide tailored escalation strategy selection. Given that chronic total occlusion represents one of the most complex lesion subsets in interventional cardiology, successful recanalization –despite its technical demands – can enable complete myocardial revascularization, which has been linked to improved long-term prognosis in appropriately selected patients [23, 24]. As the field continues to evolve, further randomized trials are essential to delineate optimal strategy selection and clarify the role of device-assisted techniques within the antegrade escalation hierarchy.

Study limitation

This study has several important limitations that warrant consideration. First, its retrospective and observational design inherently introduces the risk of unmeasured confounding factors, which may have influenced the observed outcomes. Additionally, the single-center nature of the investigation – conducted at a high-volume academic center specializing in CTO interventions – may limit the generalizability of the findings to other clinical settings with differing operator expertise or procedural volume. The choice of escalation strategy was determined by operator

discretion rather than randomization, potentially introducing selection bias.

Furthermore, although intravascular imaging modalities such as IVUS or OCT were utilized in select cases, their use was not standardized across the cohort. This limitation reduces the ability to systematically evaluate procedural decision-making and lesion morphology. Notably, although the PW technique is considered a part of true antegrade crossing (AW-O) according to the ARC-CTO classification, the possibility of partial or complete extra-plaque wire crossing cannot be excluded in the absence of systematic intravascular imaging, which was not implemented in the present study [25].

Another important limitation lies in the relatively small sample size, particularly within the PW group, which not only reduces statistical power but also limits the robustness of subgroup comparisons. Moreover, the sample sizes of the two comparison groups were not homogeneous (90 *vs.* 22), further impacting the reliability of comparative analyses and the generalizability of the findings.

CONCLUSION

No statistically significant differences were observed in primary composite endpoints between the PW and AWE groups; the results suggest comparable clinical efficacy and safety of both strategies in this complex subset of patients.

Further studies with standardized imaging guidance and larger PW cohorts are warranted to better define the optimal strategy after single-wire failure in CTO PCI.

Conflict of interests: None declared.

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Дугорочно праћење упоредних исхода технике паралелних жица и технике ескалације антероградне жице након неуспеха иницијалне жице у перкутаној реканализацији хроничних тоталних оклузија коронарних артерија

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САЖЕТАК

Увод/Циљ Након неуспеха технике једне жице у перкутаној коронарној интервенцији хроничних тоталних оклузија, најчешће се примењују две антероградне ескалационе стратегије: техника паралелних жица и ескалација антероградном жицом (*antegrade wire escalation – AWE*). Иако су широко коришћене, подаци који упоређују процедурне карактеристике и дугорочне клиничке исходе ових техника и даље су ограничени.

Циљ ове студије био је да се упореде процедурни параметри и дугорочни исходи технике паралелних жица и *AWE* након неуспеха технике једне жице у перкутаној коронарној интервенцији хроничних тоталних оклузија.

Методе Једноцентрична ретроспективна студија обухватила је болеснике који су од јануара 2018. до децембра 2023. имали успешну перкутану коронарну интервенцију хроничних тоталних оклузија користећи *AWE* или технику паралелних жица након иницијалног неуспеха. Примарни исход био је композитни – срчана смрт, инфаркт миокарда, мождани удар или реваскуларизација циљног суда. Секундарни исходи обухватали су трајање процедуре, време флуороскопије, количину контраста и дозу зрачења. Медијана трајања праћења болесника износила је 1222 дана, са интерквартилним опсегом од 580 до 1969 дана.

Резултати Од укупно 270 процедура, у 112 (41,5%) примењена је једна од наведених техника: 90 *AWE*, 22 технике паралелне жице. Основне карактеристике биле су сличне. Композитни исход се јавио код 14,4% у групи паралелних жица и 9,1% у *AWE* групи ($p = 0,73$). Примена контрастног средства је била значајно већа у *AWE* групи ($p = 0,014$), док остале разлике нису биле статистички значајне. Током перипроцедуралног периода праћења није било клиничких нити ангиографски значајних компликација.

Закључак Обе технике показују сличну безбедност и ефикасност. Техника паралелних жица нуди нешто већу процедуралну ефикасност, али су потребне даље проспективне студије да би дале коначан одговор.

Кључне речи: хронична тотална оклузија; перкутана коронарна интервенција; антероградни приступ; ескалација жице; паралелна жица

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

The Serbian version of the King's Brief Interstitial Lung Disease questionnaire and its vali-dation in patients with idiopathic pulmonary fibrosis / progressive pulmonary fibrosis

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SUMMARY

Introduction The King's Brief Interstitial Lung Disease (KBILD) is the first health status questionnaire developed for use in patients with interstitial lung diseases (ILD). There is no valid and reliable questionnaire in Serbian to assess the health status of ILD patients. Progressive pulmonary fibrosis (PPF) and idiopathic pulmonary fibrosis (IPF) are ILD diseases with a progressive course and poor prognoses, which have the greatest impact on patients' quality of life among all ILDs. The aim of the study is to validate the Serbian version of the KBILD questionnaire for use in patients with PPF, including IPF.

Methods The KBILD was translated into Serbian language. A total of 35 patients with IPF or PPF completed a translated version of the KBILD questionnaire at the baseline, and 29 of them after one month. Pulmonary lung function tests as well as St. George's Respiratory Questionnaire (SGRQ) were completed at the baseline.

Results Internal consistency was high in the total, psychological, and breathlessness and activities domains, and satisfactory for the chest symptoms domain. The test-retest reliability was good for psychological and breathlessness and activities domains and excellent for chest symptoms and total score. All domains correlated strongly and very strongly with SGRQ, however, we found a weak correlation between the KBILD and lung function, and with Charlson comorbidity index.

Conclusions The Serbian version of the KBILD is valid and reliable for use in patients diagnosed with IPF/PPF.

Keywords: interstitial lung disease; idiopathic pulmonary fibrosis; questionnaire; quality of life

INTRODUCTION

More than 200 interstitial lung diseases (ILDs), ranging from extremely rare to relatively common, have been identified. The majority of ILDs are characterized by inflammation or fibrosis within the interstitial space, leading to impaired gas exchange, which manifests clinically as dyspnea, reduced exercise capacity, and impaired quality of life [1]. Progressive pulmonary fibrosis (PPF) is a subgroup of ILD that is characterized by more frequent exacerbations, a faster decrease in lung function, and earlier death compared to other interstitial illnesses that do not show a progressive-fibrous phenotype [2]. Besides idiopathic pulmonary fibrosis (IPF) being an ideal example of progressive fibrous ILD, other conditions can also manifest similarly. These include connective tissue diseases with pulmonary involvement (such as rheumatoid arthritis, systemic sclerosis, polymyositis/dermatomyositis), hypersensitivity pneumonitis, pneumoconiosis, sarcoidosis, nonspecific interstitial pneumonia, interstitial pneumonia with autoimmune features, unclassified ILDs, and others [2]. PPF is occasionally a consequence of viral pneumonia, as reported in cases during the COVID-19 pandemic [3]. The chronic course of IPF/PPF affects the patients' quality of life (exertional dyspnea, need for oxygen therapy, frequent hospitalizations due to exacerbations, pulmonary controls, etc.).

In daily practice, questionnaires are used to quantify patient complaints. Questionnaires give patients the ability to take an active role in their healthcare by allowing them to describe symptoms and the impact those symptoms have on their day-to-day lives. They provide us with information regarding disease characteristics that are important to a patient but cannot be quantified using physiological tests like lung function [4]. The questionnaires should be administered regularly so that medical professionals can track the evolution of the condition and change their treatment techniques accordingly [5]. However, there is no valid and reliable

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questionnaire in the Serbian language to assess the health status of ILD patients.

The King's Brief Interstitial Lung Disease (KBILD) questionnaire is the first health status questionnaire designed for use in patients with all ILDs. developed by Patel et al. [6] from King's College London in 2012. Since then, it has been translated into various languages (German, French, Italian, Swedish, Dutch, Portuguese, Chinese, etc.) and used in 24 countries [7–10]. The questionnaire consists of 15 items that are categorized into three categories: breathlessness and activities, chest symptoms, and psychological aspects. A seven-point Likert scale was used to conduct the patients' evaluation of the response to each inquiry. The responses are summed up by the scoring algorithm, which then converts them into a range of 0 to 100 for each of the three domains and the total score. Higher scores indicate a better overall health status [6]. The minimal clinically important difference in the KBILD total score is a change of five units [11].

In this paper, we aimed to develop the Serbian adaptation of the KBILD questionnaire, and to verify the reliability and validity of the Serbian adaptation of the KBILD questionnaire for its application in patients with PPF, including IPF. We also assessed the correlation between the KBILD score and patients' quality of life, comorbidities and lung function.

METHODS

The study was conducted at the Institute for Pulmonary Diseases of Vojvodina, Sremska Kamenica, Serbia from October 2023 until March 2024. Every patient was administered antifibrotic medication, either pirfenidone or nintedanib. All patients completed a translated version of the KBILD questionnaire at the beginning of the study, and 29 of them also completed the questionnaire after one month (the other six patients did not complete the second KBILD questionnaire due to exacerbation of the IPF/PPF). At the baseline pulmonary lung function tests (PFT) were done using the Jaeger Master Screen Body (CareFusion, San Diego, CA, USA) spirometer. Measurements included forced vital capacity (FVC), forced expiratory volume in one second (FEV1), total lung capacity (TLC), and diffuse lung capacity (DLCO), expressed as % of the predictive values according to the American Thoracic Society / European Respiratory Society criteria [12, 13]. Additionally, patients completed the St. George's Respiratory Questionnaire (SGRQ), a disease-specific instrument originally developed for chronic obstructive pulmonary disease and asthma, but also utilized for ILDs due to the absence of a specific tool for these conditions [14, 15]. Furthermore, the study documented basic sociodemographic factors such as the patients' sex, age, and any existing comorbidities. The patients' medical histories and records were used to determine the presence of comorbid conditions.

The Charlson comorbidity index (CCI) is utilized as a metric to quantify the burden of comorbidities, as it has been demonstrated to impact outcomes in patients with ILD across multiple studies [16, 17]. The scoring system is based on 19 comorbid conditions, such as cardiac diseases, peripheral vascular diseases, cerebrovascular accident, dementia, chronic obstructive lung disease, connective tissue disease, peptic ulcer disease, liver disease, diabetes mellitus, chronic kidney disease, hematological and solid organ malignancy, and acquired immunodeficiency syndrome. Each condition is assigned a weight based on its potential impact on mortality.

Subjects

The study included 35 adult patients. The inclusion criteria included patients previously diagnosed with either IPF or PPF according to current clinical practice guidelines [2], and patients who were not hospitalized at the time of the study due to IPF/PPF exacerbations.

The exclusion criteria included the unwillingness to take part in the study, and the inability to complete the questionnaire due to cognitive or linguistic limitations, absence of signed informed consent or patients unable to perform or have contraindications for performing spirometry.

Translation of KBILD into the Serbian language

Permission to translate and use KBILD in the Serbian language was obtained from the developer. A multi-stage forward-backward process was used to perform the translation of the KBILD questionnaire. First, it was independently translated into the Serbian language by two different clinicians respectively. There was a discussion with a third doctor about the disagreements that occurred between the two interpreters. The initial translation was then back-translated by a competent multilingual translator who was unaware of the questionnaire's aim. The back-translated version was then compared to the original KBILD version by members of the research team. If there were any misconceptions, the clinicians-translators discussed them. The preliminary version of the translated questionnaire was put through a pilot test on a group of patients consisting of 10 patients with IPF who volunteered to answer the Serbian version of the questionnaire during their regular appointments. After completing the translated questionnaire, patients were interviewed by clinicians-translators to clear up any questions or concerns they had regarding the interpretation of the questions and the answers. The complete translation process is shown schematically in Figure 1.

The reliability and validity

Questionnaire reliability refers to the capacity of a questionnaire to accurately represent the actual value of the assessed qualities. Validity refers to the level to which the examined questionnaire properly evaluates its intended purpose [17].

In our study, for reliability and validation, we tested the following:

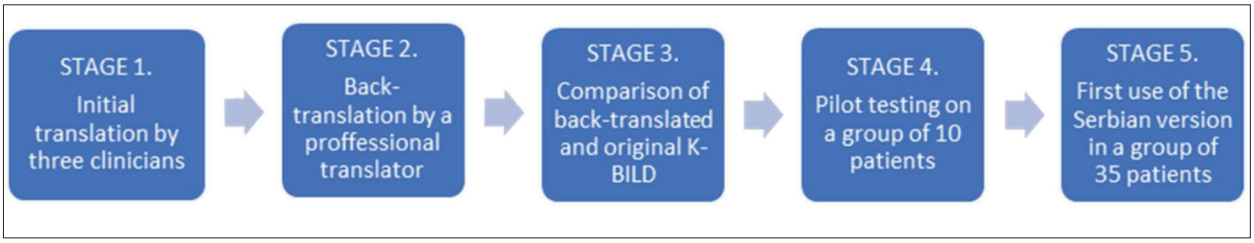

Figure 1. Stages of translating the questionnaire

1) internal consistency that measures the interrelatedness of items

2) test-retest reliability that measures repeatability of KBILD scores at baseline and after one month

3) concurrent validity showing correlations between KBILD scores and SGRQ scores, PFT.

Furthermore, we evaluated whether there are statistical differences between different groups of patients according to sex and smoking status.

Statistical analysis

Nominal level measurement data are described using frequencies. Continuous data are described using the mean and standard deviation or the median and interquartile range as appropriate.

The reliability of internal consistency was examined using Cronbach's α coefficient and Guttman's λ_6. The criteria are as follows: ≤ 0.70 unacceptable, 0.70–0.83 satisfactory, 0.83–0.90 good, and ≥ 0.90 excellent. Test-retest reliability was examined using the intraclass correlation coefficient (ICC3) and Bland-Altman's plots. The interpretation of ICC3 is as follows: ≤ 0.50 is poor, 0.50–0.75 is moderate, 0.75–0.90 is good, and ≥ 0.90 is excellent.

Concurrent validity was evaluated by measuring correlations of KBILD to the SGRQ, PFTs, and CCI. The normality of the univariate distribution of items was tested using the Shapiro–Wilk test. Correlations between variables were examined using Pearson's correlation coefficient or Spearman's rank correlation coefficient.

Differences in the means of two groups for variables with a normal distribution were examined using the independent samples t-test. The assumption of equal variances was checked using Levene's test for equality of variances. If the variances were unequal, the Welch's t-test was used. Differences between groups for variables that do not meet the assumption of normal distribution were examined using the non-parametric alternative to the independent samples t-test, the Mann–Whitney test. The level of statistical significance was set at $p < 0.05$.

Ethics: Before the start of the study, approval was granted by the Ethics Committee of the Institute for Pulmonary Diseases of Vojvodina, Sremska Kamenica, Serbia (No.: 18-I/1).

RESULTS

Characteristics of patients

The study included 35 IPF/PPF patients, with their sex distribution, average age, smoking status, diagnosis, KBILD and SGRQ results, average values of FVC% and DLCO%, and CCI presented in Table 1.

The majority of patients within our cohort were elderly women with moderately impaired lung function and two

Table 1. Characteristics of patients at baseline

Characteristics	Value
Women n (%)	19 (54.29)
Men n (%)	16 (45.71)
Age, years ± SD	62.94 ± 10.9
Smoker (ex or current) n (%)	21 (60)
Non-smoker n (%)	14 (40)
IPF n (%)	24 (68.5)
SSc n (%)	5 (14)
HP n (%)	4 (11.5)
SLE n (%)	1 (3)
NSIP n (%)	1 (3)
FVC, % predicted ± SD	74.74 ± 20.57
DLCO, % predicted ± SD	44.55 ± 14.46
KBILD Total ± SD	50.87 ± 13.05
KBILD Psychological ± SD	52.06 ± 16.54
KBILD Breathlessness and activities ± SD	35.89 ± 22.72
KBILD Chest symptoms ± SD	53.61 ± 29.29
SGRQ Total ± SD	49.33 ± 20.40
SGRQ symptoms ± SD	47.04 ± 20.21
SGRQ activity ± SD	65.11 ± 22.48
SGRQ impacts ± SD	40.86 ± 22.71
CCI ± SD	2.46 ± 1.38

IPF – idiopathic pulmonary fibrosis; SSc – systemic sclerosis; HP – hypersensitive pneumonitis; SLE – systemic lupus erythematosus; NSIP – nonspecific interstitial pneumonia; FVC – forced vital capacity; DLCO – diffusing capacity of the lungs for carbon monoxide; KBILD – King's brief interstitial lung disease; SD – standard deviation; SGRQ – St. George's respiratory questionnaire; CCI – Charlson comorbidity index

Table 2. Internal consistency and test-retest reliability of the KBILD questionnaire

KBILD	Cronbach's α	Guttman's λ6	ICC3
Total	0.956	0.974	0.919
Psychological	0.939	0.942	0.884
Breathlessness and activities	0.913	0.897	0.879
Chest symptoms	0.794	0.782	0.910

KBILD – King's brief interstitial lung disease questionnaire; ICC3 – internal consistency coefficient

Table 3. Correlations between the KBILD and patients' quality of life, lung function, and comorbidities

KBILD	FVC%	DLCO%	FEV1%	TLC%	SGRQ total	SGRQ symptoms	SQRQ activity	SGRQ impacts	CCI
Psychological	-0.086	0.193	0.111	0.062	-0.648**	-0.633**	-0.512*	-0.613*	0.120
Breathlessness and activity	0.110	0.212	0.083	0.220	-0.667**	-0.544*	-0.599*	-0.637**	0.023
Chest symptoms	-0.051	0.125	-0.032	0.015	-0.662**	-0.653**	-0.599*	-0.602*	-0.031
Total	-0.088	0.225	0.073	0.102	-0.742**	-0.686**	-0.651**	-0.695**	0.053

In correlations for domains psychological, total, and Charlson comorbidity index (CCI), due to deviations from normal distribution, we used the Spearman correlation coefficient, while for other parameters, we used the Pearson correlation coefficient;
KBILD – King's brief interstitial lung disease; FVC – forced vital capacity; DLCO – diffusing capacity of the lungs for carbon monoxide; FEV1 – forced expiratory volume in one second; TLC – total lung capacity; SGRQ – St. George's respiratory questionnaire;
*p < 0.05
**p < 0.01

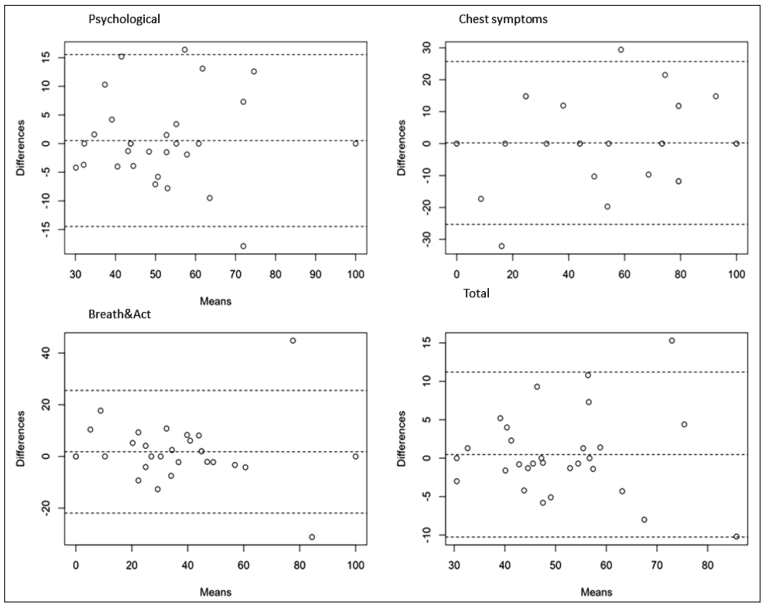

Figure 2. Bland–Altman plots for all domains; "Means" represents the average of measures, while "Differences" shows the difference between the two paired measurements

DISCUSSION

The Serbian version of the KBILD questionnaire is the first disease-specific tool in Serbian that measures health status in IPF and other ILDs. Recently, the King's Sarcoidosis Questionnaire was validated in the Serbian-speaking population. This represents a major advance in analyzing the health status of patients with sarcoidosis, but cannot be used for other ILDs [18].

Most of our patients were elderly with two or more comorbidities, a characteristic commonly observed in individuals with IPF/PPF. In our study, the majority of patients were female, which differs from the literature [19, 20, 21], with the assumption that the small sample size may account for this. There were no significant issues experienced throughout the translation process. The questionnaire was well received by the patients, easily understandable, and required little time for completion. In our study, we demonstrated that the Serbian version of the KBILD has good internal consistency for each domain and total score, as well as good test-retest reliability. The mean (SD) KBILD total score in our cohort was 50.87 (13.05), which is similar to the original KBILD questionnaire [6], as well as in the study of Wapenaar et al. [4] and Szentes et al. [22]. Our patient group showed a reduced health-related quality of life in all domains of KBILD, with breathlessness and activity domain being most impaired, followed by psychological impact and chest symptoms, which is in agreement with previous studies [4, 10, 22]. This gives significant support to the fact that KBILD is applicable and transferable on an international scale [22].

The correlation between KBILD and lung function (DLCO%, FVC%, FEV1%, and TLC%) was weak. Previous studies have indicated that the impact of FVC on quality of life is only partial, whereas the DLCO is more closely associated with the quality of life in patients with ILD [10, 14]. In the original KBILD questionnaire, the greatest correlation was observed between breathlessness and activity score with lung function tests [6]. In other studies, correlations to FVC% and DLCO% were moderate to weak [9, 10]. However, in a study by Wapenaar et al. [4], KBILD showed a weak correlation with the results of pulmonary

or more comorbidities, mainly cardiovascular disease (46%, 16/35). The mean (SD) KBILD Total score in all patients was 50.87 (13.05).

Validation and reliability of the Serbian KBILD

Cronbach's α was high in the total, psychological, and breathlessness and activities domains, and satisfactory for the chest symptoms domain. Guttman's λ_6 was close to Cronbach's α values in all domains. ICC3 were good for psychological and breathlessness and activities domains, and excellent for chest symptoms and total score (Table 2).

All domains of KBILD correlated strongly and very strongly with SGRQ, however, we found a weak correlation between the KBILD and lung function (FVC%, DLCO%, FEV1%, and TLC%), as well with CCI (Table 3).

We examined whether there is a difference in KBILD questionnaire results based on sex (males/females) and smoking status (non-smokers/smokers), and found no statistically significant correlation.

function tests. Further studies and the inclusion of a larger patient cohort will yield a more definitive understanding of the association between KBILD and pulmonary function tests, as well as the divergence between different ILD diseases.

Questionnaires are used in various diseases to measure patients' quality of life [23]. Only a few of them are validated and can be used in patients with IPF [A Tool to Assess Quality of Life in IPF (ATAQ-IPF), Short-Form 36-Item Questionnaire, and SGRQ-I- specific for IPF] while other ILD diseases are generally not considered in the available studies [24, 25, 26]. So far in our everyday clinical practice with ILD patients, we used only SGRQ as well as two measurements for dyspnea – the modified Medical Research scale and Borg's dyspnea scale. The KBILD questionnaire is the first valid, self-completed measurement of the health status of ILD patients and an additional tool to pulmonary function tests in assessing a patient's condition [6]. Concurrent validity of the KBILD domain and total scores were strong compared with the SGRQ domain and total scores ($r = -0,742$, $p < 0.01$), which is comparable to the original version of the questionnaire [6], and to the study by Wapenaar et al. [4]. It is shorter (15 questions) compared to ATAQ-IPF (72 questions), SF-36 (36 questions), and SGRQ-I (50 questions), making it easier for physicians to use during regular visits.

The presence of comorbidities influences the patient's perception of their health status and can influence responses [16, 27]. In our research, most patients (97%) had some of the most important comorbidities that could influence their perception of quality of life. However, our research did not find any association between the CCI and the KBILD outcomes, which is similar to the results of the Asian interstitial disease cohort [9], as well as the results of Szentes et al. [22], in which only depression was negatively associated with the "chest symptoms" domain. These could be explained by the fact that in the process of creating the original KBILD version, certain elements that could impact one's health condition, such as medications, cough, sleep, and sexual health, were excluded, and the KBILD items are specifically designed to evaluate the influence of lung disease on health status [6]. Additionally, the CCI is missing comorbidities that are commonly found in individuals with IPF/PPF, including gastric reflux, pulmonary hypertension, obstructive sleep apnea, and psychiatric disorders. The small size and homogeneity of our study cohort with respect to the CCI score may have contributed to the observed results. Hence, the influence of various comorbidities on the overall health condition of individuals with ILD remains to be determined.

This study has several limitations. This is a single-center observational study in a relatively small group of patients, which is understandable since IPF/PPF are rare diseases. The methodological integration of these and future studies will justify the application of the KBILD in everyday clinical practice. Our study is cross-sectional, and we believe that a more extensive follow-up period is necessary to establish the true link between the KBILD and lung function tests. Our main goal was to validate the Serbian version of KBILD so it could be implemented in everyday medical practice.

CONCLUSIONS

The Serbian version of the KBILD is valid and reliable for use in patients diagnosed with IPF or PPF. There is no correlation between the lung function tests or CCI Score and KBILD, which supports the statement that questionnaires provide us with information that is important to a patient but cannot be measured using physiological tests. The KBILD is easy to use, and its implementation in clinical practice has the potential to provide new information about the quality of life, as well as contribute to a better understanding of the course and prognosis of IPF/PPF. We hope that the validation of the Serbian version of the KBILD will encourage physicians in Serbia to adopt it into their regular medical practice, and that it will become a valuable tool for the longitudinal monitoring and management of patients with IPF/PPF.

Conflict of interest: None declared.

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Српска верзија упитника *King's Brief Interstitial Lung Disease* и његова валидација међу болесницима са прогресивном плућном фиброзом / идиопатском плућном фиброзом

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САЖЕТАК

Увод Упитник *King's Brief Interstitial Lung Disease* (*KBILD*) први је упитник о квалитету живота развијен за болеснике са интерстицијумским болестима плућа. Не постоји валидан и поуздан упитник о квалитету здравља на српском језику намењен болесницима са интерстицијумским болестима плућа. Прогресивна плућна фиброза и идиопатска плућна фиброза су интерстицијумске болести плућа са прогресивним током и лошом прогнозом, које имају највећи утицај на квалитет здравља међу свим интерстицијумским болестима плућа. Циљ ове студије био је да се уради валидација српске верзије упитника *KBILD* међу болесницима са прогресивном плућном фиброзом, укључујући идиопатску плућну фиброзу.

Методе Упитник *KBILD* преведен је на српски језик. Укупно 35 болесника са прогресивном плућном фиброзом или идиопатском плућном фиброзом попунили су српску верзију упитника на почетку, а 29 њих на крају истраживања. На почетку истраживања урађени су и тестови плућне функције, као и попуњавање упитника болнице „Свети Ђорђе" о респираторним тегобама.

Резултати Интерна конзистенција је била висока за укупни скор, психолошки домен, домен недостатка ваздуха и активности, а задовољавајућа за домен о респираторним симптомима. Тест–ретест поузданост је била добра за психолошки домен и домен недостатка ваздуха и активности, и одлична за домене респираторних симптома и укупни скор. Сви домени снажно корелирају са резултатима упитника болнице „Свети Ђорђе", међутим корелација је слаба између резултата упитника *KBILD* и тестова плућне функције, као и Чарлсоновог индекса коморбидитета.

Закључак Српска верзија упитника *KBILD* је валидна и поуздана за примену код болесника са идиопатском плућном фиброзом и прогресивном плућном фиброзом.

Кључне речи: интерстицијумске болести плућа; идиопатска плућна фиброза; упитник; квалитет живота

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Long non-coding RNA signature in beta-thalassemia major associated with prediabetes

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SUMMARY

Introduction/Objective Functional long non-coding RNAs (lncRNAs) and messenger RNAs (mRNAs) were investigated in patients with beta-thalassemia major (β-TM) and prediabetes by analyzing gene chip expression profiles, experimental evidence and bioinformatics.

Methods Total RNA was extracted after taking blood samples from patients. Microarrays were subsequently used for genetic analysis of patients with β-TM/prediabetes (n = 5) compared with healthy individuals (n = 5). Candidate lncRNAs and mRNAs were randomly validated using quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) analysis. Signaling associated with β-TM was identified as being associated with prediabetes based on Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis.

Results Compared with the healthy controls, 1,511 and 1,932 lncRNAs were up- and downregulated, respectively, whereas 1,128 and 752 mRNAs were up- and downregulated, respectively, in the patients with β-TM/prediabetes. Eight dysregulated lncRNA expressions were confirmed using qRT-PCR analysis, which was consistent with the microarray results. The GO and KEGG analyses showed that the intracellular anatomical structure and multicellular organismal process were the most significantly dysregulated, and the most significantly up- and downregulated pathways were herpes simplex virus type 1 infection and vascular smooth muscle contraction, respectively.

Conclusion This study preliminarily determined that the pathophysiology of aberrant glucose homeostasis in β-TM may be linked to the abnormal expression of lncRNAs and mRNAs, deepening the understanding of molecular mechanisms in β-TM complicated by prediabetes.

Keywords: microarray analysis; long non-coding RNAs; beta-thalassemia major; mRNA; prediabetes

INTRODUCTION

Beta-thalassemia (β-TM) is an autosomal recessive hemolytic anemia disorder caused by genetic defects in the regulation of β-globin, leading to impaired β-globin peptide chain synthesis, which in turn causes hemolysis and ineffective hematopoiesis [1]. Patients require regular blood transfusions to sustain life [2, 3]. The extended extravascular hemolysis caused by β-TM enhances the intestinal absorption of iron; however, long-term massive blood transfusions can result in iron overload, leading to insulin resistance, increasing hepatic glucose production and reducing insulin secretion, ultimately causing abnormal glucose metabolism [4, 5]. Recent studies have revealed that long non-coding RNAs (lncRNAs) can mediate iron metabolism and oxidative stress pathways relevant to diabetes. For example, lncRNAs regulate ferroptosis-mediated β-cell injury and complications of diabetes via modulation of reactive oxygen species, Nrf2 and NF-κB signaling pathways. Moreover, several lncRNAs, including *MEG3*, *H19*, *MALAT1* and *GAS5*, have been implicated in insulin resistance and dysregulated glucose or lipid metabolism in metabolic disease contexts. These observations suggest that lncRNAs may have disease-relevant functions in patients with β-TM, potentially linking iron overload, oxidative stress and metabolic dysregulation.

Prediabetes has been identified as a condition lying between normal glycometabolism and diabetes mellitus (DM) [6]. Prediabetes represents a high-risk state for the development of diabetes, with an annual conversion rate of 5–10% [7]. Although prediabetes does not meet the diagnostic criteria for diabetes, it is strongly associated with multiple diseases as well as an elevated risk of patient death [8, 9, 10]. A meta-analysis indicated that the incidence of impaired glucose tolerance (IGT), impaired fasting glycaemia (IFG) and DM in patients with β-TM was 17.21%, 12.46%, and 6.54%, respectively [11]. However, abnormal glucose metabolism in patients with β-TM usually has an insidious onset, and effective biomarkers and diagnostic methods to detect glucose metabolism disorders are lacking [11].

Among gene transcripts, only about 10–20% of transcripts are messenger RNAs (mRNAs) responsible for encoding proteins, whereas 80–90% are non-coding RNAs, including miRNAs and lncRNAs [12, 13]. lncRNAs consist of over 200 bases and lack protein-coding capacity but play key roles in a variety of biological

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processes [12, 13]. Because lncRNAs are stably present in peripheral blood, they are considered potential diagnostic markers [14]. Studies have shown that diabetes and its associated symptoms are closely related to lncRNAs and mRNAs in the peripheral circulation. In addition, lncRNAs have critical roles in regulating pancreatic β-cell function, glucose metabolism and insulin resistance [15, 16]. Recently, genome-wide analyses of pseudogenes have demonstrated the human-specific essentiality of the lncRNA hemoglobin subunit beta pseudogene 1 in erythropoiesis and its implications in β-TM [17].

However, the role of lncRNAs and mRNAs in the molecular mechanism of β-TM associated with prediabetes remains elusive. By analyzing lncRNA compared with mRNA expression profiles in patients with β-TM and prediabetes, potential diagnostic and therapeutic targets may be revealed. In this study, we used microarray technology to detect differentially expressed transcripts in peripheral blood of these patients to explore their potential role in the pathogenesis of prediabetes in patients with β-TM.

METHODS

Patient information

This study was conducted in Hainan Provincial People's Hospital between January and December 2021 and included a total of five patients with β-TM and prediabetes and five healthy controls. All participants signed an informed consent form before the start of the study. The diagnosis of β-TM was based on hemoglobin electrophoresis and genetic analysis, and the diagnosis of prediabetes was based on the American Diabetes Association 2021 guidelines [18] for determining IFG and IGT through the oral glucose tolerance test (OGTT). The inclusion criterion for the healthy controls was the absence of major disease. The clinical and demographic characteristics of the study participants are detailed in Table 1.

Table 1. Clinical characteristics of β-TM patients with prediabetes and healthy controls

Variables	B-TM + prediabetes (n = 5)	Healthy controls (n = 5)	p
Age (years)	10.6 ± 1.7	11 ± 1.4	0.78
Sex (male/female)	3/2	2/3	0.65*
Time of blood transfusion (months)	92 ± 5.7	0 (NA)	<0.001*
Ferritin (ng/ml)	7,377.6 ± 1,021.5	146.9 ± 63.8	<0.001*
BMI	15.3 ± 1.7	20 ± 0.9	0.002*
Liver MRI T2 (ms)*	1.64 ± 0.14	-	<0.001*
ALT (U/L)	72.3 ± 46.22	12.94 ± 4.11	0.00286*
Fasting glucose (mmol/l)	6.8 ± 0.48	4.48 ± 0.49	0.00555*
Two-hour plasma glucose (mmol/l)	9.04 ± 0.79	5.04 ± 0.6	0.00901*
Fasting insulin (pmol/L)	66.23 ± 5.09	55.9 ± 8.44	0.00234*
Fasting C-peptide (nmol/l)	0.82 ± 0.17	0.67 ± 0.24	0.0117
HOMA-IRI	2.72 ± 0.3	1.58 ± 0.16	0.00756*
HOMA-ISI	0.62 (0.58–0.68)	0.51 (0.48–0.7)	0.0157*
HOMA-βFI	75.76 ± 14.14	183.84 ± 92.52	0.00258*
HOMA-SC	28.74 (26.02–31.39)	35.85 (27.13–37.35)	0.0157*

BMI – body mass index; HOMA-IRI – insulin resistance index; HOMA-ISI – insulin sensitivity index; HOMA-βFI – β-cell function index; HOMA-SC – secretory capacity;
notes: data presented as mean ± SD or median (interquartile range);
*p < 0.05 (significant differences)

Sample collection

On the day prior to blood collection, the participants were instructed to fast from 8 PM until the completion of blood collection the following day. Blood draws were scheduled for the following morning to ensure that the participants had fasted for more than eight hours. The median vein or cephalic vein at each participant's elbow was selected as the blood sampling site. The blood collection site was disinfected with a sterile cotton ball with a diameter of approximately 5 cm, starting at the puncture site and moving outwards in concentric circles; the disinfectant was then left to dry naturally. A lancet was inserted into the vein, ensuring that the needle was completely inside the vessel, and an ethylenediaminetetraacetic acid anticoagulant tube was then inserted into the other end of the lancet once blood return was observed. Subsequently, 5 mL of peripheral blood was collected, taking care to avoid blood spillage or bubble generation to ensure smooth blood collection. Following blood collection, the tourniquet was released, gauze was pressed gently onto the site, the lancet was withdrawn and the gauze was fixed with tape to prevent bleeding. The collected blood samples were sent to a laboratory for the following tests: ferritin, fasting plasma glucose, fasting insulin and fasting C-peptide levels. In addition, participants were scheduled for an OGTT, and peripheral blood was collected again two hours after glucose administration to determine two-hour blood glucose levels to complete the OGTT assessment.

RNA extraction

For RNA extraction from blood samples, the blood samples were first treated with TRIzol™ Reagent (Catalogue No. 15596026, Thermo Fisher Scientific, Delaware, DE, USA), mixed thoroughly at a ratio of 5 mL TRIzol™ per 1 mL of blood and left for five minutes to facilitate separation of nucleic acid–protein complexes. Chloroform was subsequently added, and the mixture was shaken vigorously and centrifuged to separate the RNA phase from the upper layer. An equal volume of isopropanol was then added, allowed to stand at room temperature and centrifuged to pellet the RNA. The pellet was washed with 75% ethanol, and finally, RNA was solubilized with RNase-free water. Next, RNA was further purified using RNeasy Mini Kit (74104, Qiagen, Hilden, Germany).

Microarray analysis

Microarray experiments were performed by Kangchen Biotechnology (Biotechnology Co., Ltd., Shanghai, China). Microarray hybridizations were performed according to the

standard protocols of Agilent Technologies Co., Ltd. (Santa Clara, CA, USA). The extracted RNA was first purified and subsequently transcribed into fluorescently labelled cRNA, and complementary DNA hybridization was performed on microarray chips. Following the completion of hybridization, the chips were scanned using an Agilent microarray scanner to obtain raw image data. Agilent feature extraction software (version 11.0.1.1) (Agilent Technologies Co., Ltd.) was used for data extraction and preprocessing. This software can automatically read and process up to 100 raw image files to complete the determination of feature strength and ratio. Subsequently, expression data were normalized using quantile normalization with Agilent software and a robust multi-chip averaging technique.

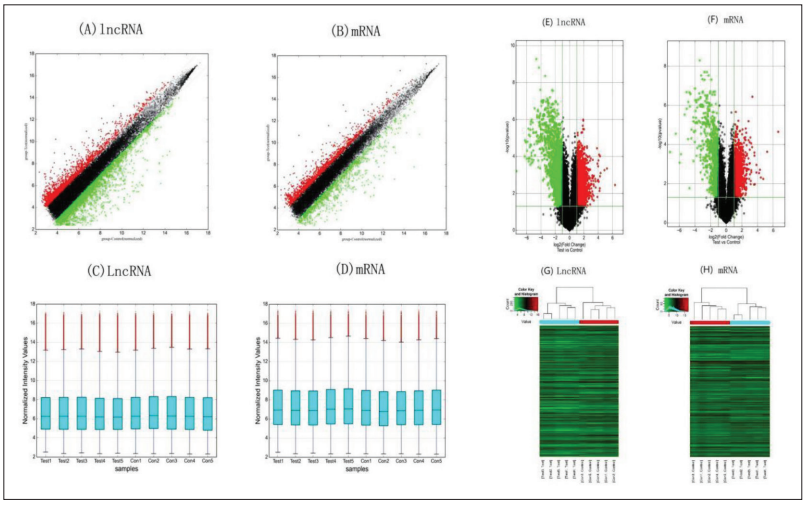


Figure 1. The characteristics of long non-coding RNA (lncRNA) and messenger RNA (mRNA) differential expression and volcano plot and hierarchical clustering of differentially expressed transcripts; a scatter plot shows the differences between lncRNA (A) and mRNA (B) expression in patients with β-thalassemia major with prediabetes (y-axis, group-test) versus healthy controls (x-axis, group-control); red and green represent ≥ 2-fold, $p < 0.05$; the box plots show the lncRNA (C) and mRNA (D) expression levels of each individual sample; (E) volcano plot of differentially expressed long non-coding RNAs (lncRNAs); (F) volcano plot of differentially expressed mRNAs; (G) Hierarchical clustering of lncRNA profiles in patients with β-thalassemia major (β-TM) with prediabetes compared with healthy controls (≥ 2-fold, $p < 0.05$); (H) hierarchical clustering of mRNA profiles in patients with β-TM with prediabetes compared with healthy controls (≥ 2-fold, $p < 0.05$)

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses

GO analysis was used to assess gene enrichment, implemented using the enrich GO function to screen significantly enriched GO terms (set p or corrected $p < 0.05$). Subsequently, KEGG analysis was used to explore the involvement of these genes in specific biological pathways, and pathway enrichment analysis was completed with the help of the enrich KEGG function to select significant metabolic or signaling pathways (threshold at p or corrected $p < 0.05$). Finally, the GO and KEGG analysis results were combined to reveal the potential biological significance of differentially expressed genes at the cellular function and pathway level.

Reverse transcription polymerase chain reaction

In this study, RNA samples were reverse transcribed using the PrimeScript RT kit and gDNA eraser (Takara Bio Inc., Kusatsu, Shiga, Japan) to remove genomic DNA contamination and synthesize complementary DNA. Subsequently, quantitative real-time reverse transcription chain reaction (qRT-PCR) was performed using the CFX96 Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA) and SYBR™ Green PCR kit (Takara Bio Inc.) to detect the expression levels of target genes. All qRT-PCR data were quantitatively analyzed using the $2^{-\Delta\Delta CT}$ method. The primer sequences are presented in Supplementary Table 1.

Statistical analysis

For data analysis, SPSS Statistics for Windows version 25.0 software (IBM Corp., Armonk, NY, USA) was used. The normality of continuous variable data was assessed using the Shapiro–Wilk test, the data were presented as mean ± standard deviation and the t-test was used to compare differences between groups; non-normally distributed data were presented as interquartile ranges and analyzed using the Mann–Whitney U test for non-differences. In all statistical tests, $p < 0.05$ was considered statistically significant.

Ethics: The Helsinki Declaration was followed in the conduct of this investigation. Hainan General Hospital's Ethics Committee gave its approval for this research to be conducted (Approval number: No. 2021-239). All participants gave their permission in writing after being fully informed of the study protocols.

RESULTS

Profile changes of long non-coding RNAs

A total of 26,035 lncRNAs were identified in the lncRNA expression profiles of both the control samples and the samples of β-TM with prediabetes. These were then assessed via a microarray analysis (Figure 1A and C). A total of 3,443 differentially expressed lncRNAs were identified using the criteria of $p < 0.05$ and $|\log2FC| > 2.0$, including 1,511 upregulated and 1,932 downregulated lncRNAs (Figure 1E–H). The 20 lncRNAs with the most significant changes are recorded in Table 2. Among them, the most significant upregulation was in ENST00000496629, which had a fold change (FC) of 82.1860551, whereas the most significant downregulation was in ENST00000581274, which had an FC of 170.1365176. In addition, the taxonomic distribution of dysregulated lncRNAs was summarized. Among the deregulated lncRNAs, there were a total of 1,799 intergenic lncRNAs, 980 antisense lncRNAs, 409 intronic lncRNAs and 255 bidirectional lncRNAs (Supplementary Figure 1).

Table 2. The 20 most differentially expressed lncRNAs in patients with β-TM with prediabetes relative to matched controls

lncRNA ID	p	False discovery rate	Fold change	Regulation	Chromosome	Strand	Relationship	Database
ENST00000496629	0.003526083	0.032526596	82.1860551	up	chr8	-	bidirectional	GENCODE
ENST00000431759	0.000454682	0.010781106	37.6048365	up	chr1	+	natural antisense	GENCODE
ENST00000583516	0.000556435	0.012007394	29.3773871	up	chr3	-	intronic antisense	GENCODE
ENST00000582591	0.000794281	0.014552508	20.1327846	up	chr18	+	natural antisense	GENCODE
T144753	9.72507E-05	0.004628744	19.1867411	up	chr17	-	natural antisense	RNA-seq: Iyer et al 2015
HBMT00001229624	0.001197038	0.018044649	19.0708748	up	chr6	+	intergenic	FANTOM5CAT
T092137	0.005372424	0.041556365	18.7283573	up	chr13	+	natural antisense	RNA-seq: Iyer et al 2015
ENST00000495240	0.007330467	0.049419663	18.2757294	up	chr21	-	bidirectional	GENCODE
ENCT00000417654	8.05354E-05	0.004137151	17.5584774	up	chr7	-	intergenic	FANTOM5CAT
ENST00000523831	0.002313282	0.025628209	16.9052281	up	chr8	+	intergenic	GENCODE
ENST00000581274	0.000623989	0.012711699	170.1365176	down	chr18	-	intergenic	GENCODE
T048514	9.98219E-05	0.004660449	76.2685338	down	chr10	-	intergenic	RNA-seq: Iyer et al 2015
ENST00000421375	1.35499E-05	0.001367337	75.6516897	down	chr3	-	intergenic	GENCODE
ENST00000556546	1.72633E-05	0.001558422	68.9262261	down	chr14	-	intergenic	GENCODE
ENST00000608466	0.000131548	0.005419482	63.0881264	down	chr5	-	bidirectional	GENCODE
ENCT00000192781	4.96226E-05	0.002997505	61.6649287	down	chr18	+	intergenic	FANTOM5CAT
ENST00000586947	1.57655E-05	0.001487151	58.2341391	down	chr18	+	intergenic	GENCODE
ENST00000495493	3.16861E-06	0.000598221	54.343005	down	chr1	-	exon sense-overlapping	GENCODE
AK024231	0.000182406	0.00656838	52.8044804	down	chr17	+	intergenic	NRED
ENST00000511474	0.000135322	0.005506699	52.6697045	down	chr5	+	intergenic	GENCODE

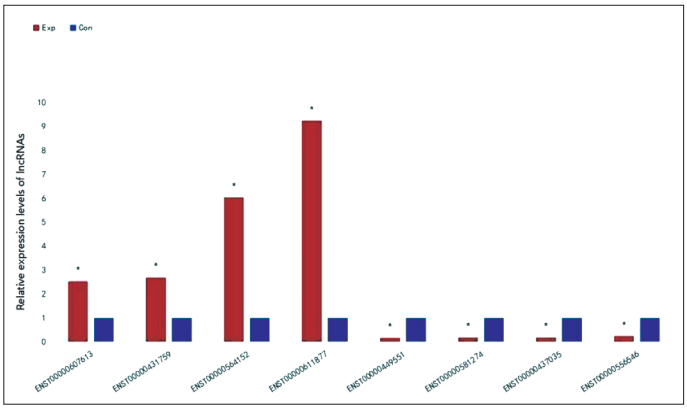


Figure 2. The expression of selected lncRNAs; the expression level detected by qRT-PCR showed that the expression level of lncRNAs ENST00000449551, ENST00000581274, ENST00000437035, and ENST00000556546 was significantly lower and that of lncRNAs ENST00000607613, ENST00000431759, ENST00000564152, and ENST00000611877 was significantly higher in patients with β-TM with prediabetes compared with the controls (*p < 0.05); the qRT-PCR analysis was similar in most respects to the lncRNA microarray analysis

Changes in the mRNA profile in patients with beta-thalassemia/prediabetes

In this study, we examined mRNA expression patterns in patients with β-TM/prediabetes using microarray analysis. Compared with healthy individuals, the results revealed 17,044 mRNAs (Figure 1B and D), and 1,880 differentially expressed mRNAs were identified using the criteria of $p < 0.05$ and $|\log2FC| > 2.0$. In the patients with β-TM and prediabetes, 1,128 mRNAs were upregulated and 752 were downregulated (Figure 1F and 2H). The 20 most dramatically changing mRNAs are presented in Table 3. The most substantially upregulated and downregulated mRNAs were GDF15 (FC = 100.2322219) and GUCA2B (FC = 143.9714162), respectively.

Validation of dysregulated long non-coding RNAs by quantitative real-time reverse transcription polymerase chain reaction

To further confirm the accuracy of the microarray results, we randomly selected eight dysregulated lncRNAs for validation using qRT-PCR. The qRT-PCR results were consistent with the microarray result trends (Figure 2). Among the validated transcripts, ENST00000496629 and AC090912.2 (lncRNAs) and GDF15 (mRNA) exhibited the most pronounced expression changes. These molecules were therefore considered the most biologically relevant candidates for subsequent functional interpretation.

GO and KEGG analyses

To further explore the function of lncRNAs with substantial changes, we performed GO and KEGG analyses. The GO analysis results showed that upregulated mRNAs were involved in 985 terms and 275 terms were enriched in downregulated mRNAs. Compared with the healthy group, significantly changed upregulated lncRNA functions were mainly concentrated in unfolded protein binding, intracellular anatomy and cellular macromolecular metabolic processes, whereas downregulated lncRNA functions were mainly concentrated in neuropeptide receptor binding, cell periphery and multicellular organismal processes (Figure 3A and B).

The KEGG analysis showed that a total of 31 pathways were upregulated, with the herpes simplex virus 1 (HSV-1) infection pathway being the most significantly enriched. Among the 17 downregulated pathways, the vascular smooth muscle contraction pathway was the most significantly enriched (Figure 3C and D). Beyond listing enriched pathways, these findings warrant biological

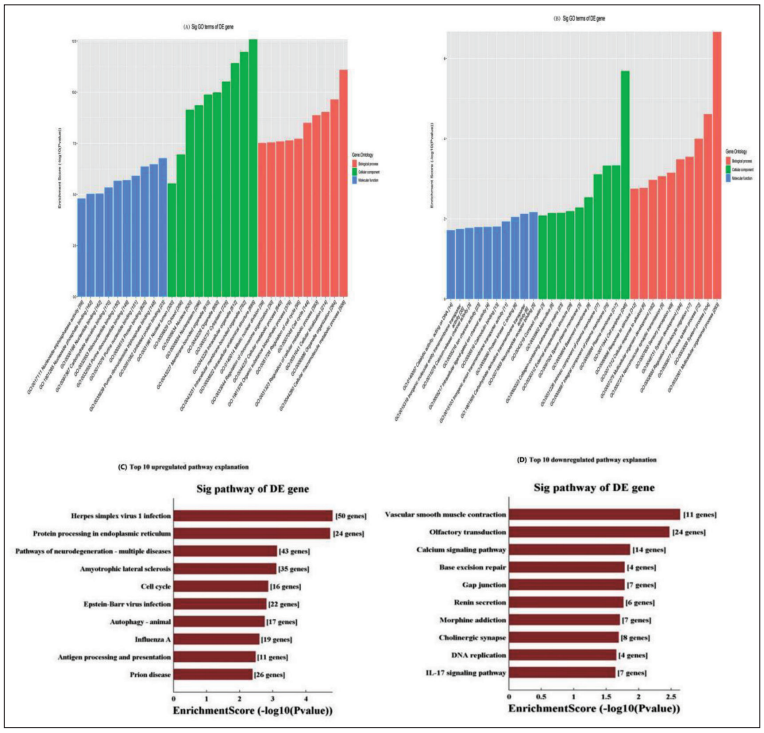
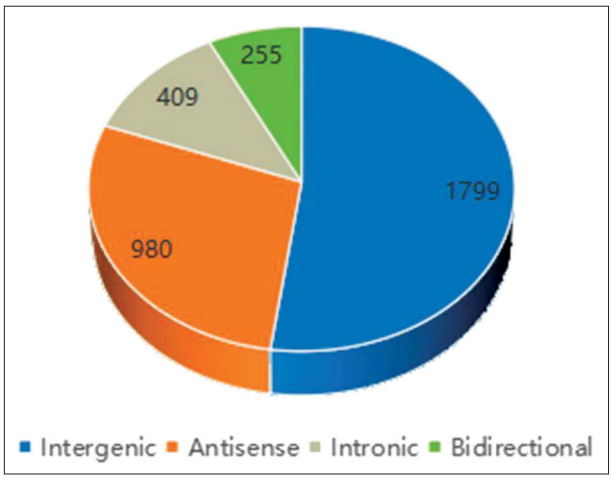


Figure 3. Gene ontology enrichment and genomes pathway analysis of differentially expressed mRNAs



Supplementary Figure 1. Distribution of dysregulated lncRNAs

interpretation. Several KEGG pathways enriched in differentially expressed genes are closely related to abnormal glucose metabolism, including protein processing in the endoplasmic reticulum (hsa04141), the cell cycle (hsa04110), autophagy (hsa04140), the NOD-like receptor signaling pathway (hsa04621), the interleukin (IL)-17 signaling pathway (hsa04657), cortisol synthesis and secretion (hsa04927) and pancreatic secretion (hsa04972). Likewise, GO terms such as positive regulation of insulin secretion (GO:0032024), the glucose metabolic process (GO:0006006), negative regulation of gluconeogenesis (GO:0045721) and positive regulation of the endothelial cell apoptotic process (GO:2000353) have direct implications for glucose homeostasis and diabetes-related vascular complications. Notably, enrichment in the HSV-1 infection and vascular smooth muscle contraction pathways has not been previously reported in β-TM or β-TM-associated dysglycemia. Iron overload in β-TM can induce immune dysregulation, and the HSV-1 infection pathway involves immune-modulatory signaling; our preliminary unpublished data indicate elevated circulating IL-1β, IL-6, IL-8, IL-10 and TNF-α levels in β-TM with prediabetes, suggesting possible immune activation through this pathway. Similarly, vascular smooth muscle contraction is relevant to endothelial dysfunction, a hallmark of diabetes-related vascular injury. Whether iron overload in β-TM drives vascular damage through this pathway remains unknown and warrants further mechanistic investigations. These novel pathway associations provide exploratory targets for future research into the biological mechanisms linking β-TM to early glucose metabolism impairment.

DISCUSSION

Beta-thalassemia is an autosomal recessive inherited disorder characterized by reduced or complete loss of β-globin chain production. At present, the molecular pathogenesis between β-TM and prediabetes has not been clarified. To explore the relationship between the two, it is important to detect lncRNA and mRNA expression in patients with β-TM to reveal the etiology and pathophysiology of β-TM complicated with prediabetes. Previously, Fakhr-Eldee et al. [19] found that lncRNAs, *MALAT1*, *MIAT* and *ANRIL* may be closely related to the pathogenesis of β-TM and are expected to be novel molecular biomarkers of β-TM. On this basis, this study is the first to analyze lncRNA and mRNA expression profiles in patients with β-TM complicated with prediabetes using microarray technology. The analysis results showed that differentially expressed lncRNAs may play a key role in the pathogenesis of β-TM, and this conclusion is basically consistent with the results of previous studies. Notably, ENST00000496629 and AC090912.2 (lncRNAs) together with GDF15 (mRNA) were identified as the most significantly dysregulated transcripts in our study. Moreover, ENST00000496629, which has not been previously reported in β-TM or glucose metabolism, may represent a novel regulatory molecule linking iron overload to metabolic dysregulation. In addition, AC090912.2, although poorly characterized, exhibited marked down-regulation and may be involved in pathways affecting glucose homeostasis, and GDF15, a stress-responsive cytokine elevated in various metabolic and cardiovascular disorders, is known to influence insulin sensitivity and β-cell function [20, 21]. These findings suggest that these three molecules could be promising candidates for further mechanistic studies and potential biomarkers for early detection of glucose metabolism impairment in β-TM.

Table 3. The 20 most differentially expressed mRNAs in patients with β-TM with prediabetes relative to matched controls

Gene symbol	p	False discovery rate	Fold change	Regulation	Chromosome
GDF15	2.26375E-05	0.002411457	100.2322219	up	chr19
HBG1	0.000236675	0.008984146	37.5558847	up	chr11
TMEM269	0.005760896	0.048273699	26.6075395	up	chr1
ST8SIA5	0.004899657	0.045003731	21.4706818	up	chr18
PAQR9	0.001958959	0.028087005	19.8210477	up	chr3
TOMM5	0.002893375	0.034055763	18.4003285	up	chr9
ACSM3	0.001112436	0.020491209	14.4928213	up	chr16
BBS12	0.002714217	0.032809297	14.4565088	up	chr4
SFRP2	0.010723854	0.067903233	12.6215505	up	chr4
IFI44L	0.000374268	0.011411502	12.1742708	up	chr1
GUCA2B	0.000243604	0.009222062	143.9714162	down	chr1
B3GALNT2	2.82567E-05	0.002715737	86.152204	down	chr1
TOX2	0.000854932	0.017757699	62.2958244	down	chr20
FBXL13	0.000314764	0.010403005	54.406973	down	chr7
OR10G4	2.96323E-06	0.000742725	39.4923179	down	chr11
NAA80	2.12895E-07	0.000229889	33.3880965	down	chr3
CLUH	1.51667E-06	0.000470002	32.7656707	down	chr17
PCDHA9	5.28901E-05	0.00411625	29.1615088	down	chr5
BCAP31	6.76081E-07	0.000338915	28.6257891	down	chrX
CATG00000107414.1	0.01698672	0.088837575	25.8281041	down	chr9

To our knowledge, this is the first study to comprehensively profile lncRNA and mRNA expression specifically in patients with β-TM and prediabetes, as distinct from β-TM without abnormal glucose metabolism or from diabetes alone. Previous transcriptomic or non-coding RNA studies of β-TM have primarily focused on erythropoiesis-related pathways, iron metabolism or diabetic complications individually [22]. Our results identify several lncRNAs (e.g. ENST00000496629, AC090912.2) and mRNAs (e.g. GDF15, GUCA2B) that have not been previously associated with β-TM or prediabetes in the existing literature. These molecules reveal potential mechanistic links between iron overload characteristic of β-TM, oxidative stress and early-stage glucose dysregulation. The co-enrichment of HSV-1 infection pathways and vascular smooth muscle contraction among differentially expressed transcripts also extends current understanding by implicating immune modulation and vascular responses much earlier in glucose metabolism impairment in β-TM than previously recognized. This suggests that gene regulation in β-TM-associated prediabetes may share overlapping inflammatory and endothelial dysfunction components with, but also diverging from, established type 2 diabetes pathogenesis.

Diabetes and its associated complications are closely associated with lncRNAs in the peripheral circulation. Studies have shown that specific lncRNAs play a key role in the occurrence, development and complications of diabetes. For example, *MALAT1* showed expression changes associated with insulin resistance in patients with type 2 diabetes, and its expression levels may be affected by resistin and homeostasis model assessment (HOMA-IR) measures [15]; moreover, *MALAT1* expression was significantly reduced in serum samples or exosomes from patients with type 2 diabetes [23]. In addition, lncRNA CASC2 is significantly downregulated in blood and kidney tissues of patients with diabetic chronic renal failure, suggesting that it may be associated with the pathological process of diabetic nephropathy [16]. In the present study, significant changes in lncRNA expression profiles in prediabetes compared with peripheral circulation were further revealed. A large number of significantly up- and downregulated lncRNAs were identified, with *FGFR1* and AC090912.2 being the most significantly up- and downregulated. In another study, *FGFR1* was demonstrated to be associated with diabetes and metabolic diseases, but the relationship between AC090912.2 and diabetes remains to be further investigated [24].

Located on human chromosome 14q32.3 within the imprinted DLK1–*MEG3* locus, *MEG3* is a non-coding transcript with β-cell-specific expression in the islets of individuals without diabetes; however, its expression is reduced in the pancreatic islets of patients with type 2 DM. The imprinted DLK1–*MEG3* region on chromosome 14q32.2 has also been shown to alter susceptibility to type 1 diabetes. In vivo and in vitro studies in mice have demonstrated that *MEG3* expression is enriched in pancreatic islets compared with exocrine tissue in Balb/c mice and is downregulated in the islets of both type 1 and type 2 diabetic mouse models. In MIN6 cells and isolated mouse islets, *MEG3* expression is primarily regulated by glucose levels. Inhibition of *MEG3* expression in vitro impairs insulin synthesis and secretion and increases β-cell apoptosis. Furthermore, in vivo knockout of *MEG3* leads to glucose intolerance, reduced insulin secretion and decreased mRNA as well as the protein levels of *Pdx-1* and *MafA* [25].

A conserved lncRNA located at chr19:5795689-5802671, *MALAT1* shows aberrant expression in diabetic retinopathy. Transcription factors play a critical role in regulating cellular processes and signal transduction in response to external stimuli. Analysis using TRANScription FACtor database predicts transcription factor binding sites within the *MALAT1* sequence, indicating potential interaction with NF-κB as a cis-acting element. Hyperglycemia induces *MALAT1* upregulation in retinal endothelial cells and in the retinas of rats with diabetes. Silencing *MALAT1* expression markedly attenuates diabetes-induced retinal neovascularization, vascular leakage and retinal inflammation. *In vitro* genetic knockout of *MALAT1* affects key endothelial cell functions, including proliferation, migration and tube formation. Collectively, these findings suggest that *MALAT1* may mitigate hyperglycemia-induced retinal degeneration and improve retinal function, highlighting its

broader role in the microvascular complications of diabetes. These dysregulated lncRNAs may influence the pathophysiology of diabetes and its complications by regulating gene expression or participating in signaling pathways. In addition, mRNA expression profiling has revealed significant gene expression changes in patients with prediabetes, with GDF15 and GUCA2B being the most significantly upregulated and downregulated mRNAs [26, 27]. Moreover, GDF15 expression is increased in diabetes and cardiovascular disease and is considered a potential therapeutic target and prognostic indicator, whereas GUCA2B is involved in lipid metabolism and shows protective effects in obesity-associated nonalcoholic fatty liver disease, with increases in its levels associated with improved liver injury [28]. In summary, lncRNA and mRNA expression changes in the peripheral circulation are closely related to the pathophysiological mechanisms of diabetes and its complications, and these molecules may serve as potential biomarkers or therapeutic targets to provide new directions for the early diagnosis and intervention of diabetes.

In patients with β-TM, diabetes and its complications substantially contribute to increased mortality. Understanding the molecular mechanisms driven by hyperglycemia and its related complications is therefore essential for improving clinical strategies and exploring novel therapeutic targets. In recent years, the functional roles of lncRNAs in diabetes and diabetic complications have been increasingly recognized. Notably, a considerable proportion of single nucleotide polymorphisms associated with type 1 and type 2 diabetes are located within lncRNA regions, suggesting their potential involvement in disease pathogenesis. The pancreas senses blood glucose levels and, through pancreatic β cells, secretes insulin to stimulate glucose uptake by peripheral tissues. In type 2 diabetes, hyperglycemia results from β-cell dysfunction combined with insulin resistance, whereas in type 1 diabetes, it arises from the loss of β cells and insulin production. In this study, microarray technology was used to identify differentially expressed lncRNAs and mRNAs in patients with β-TM and prediabetes. Functional enrichment analyses, including pathway, disease and GO annotations, were conducted to investigate their potential roles in disease onset and progression. These findings provide a molecular basis for subsequent functional studies of lncRNAs in β-TM-associated prediabetes and offer novel research directions for biological therapy. As well as potentially serving as early diagnostic biomarkers for patients with β-TM at risk of developing diabetes, the identified lncRNAs may represent promising therapeutic targets for drug development.

In patients with β-TM, iron overload is a common pathological feature, mainly due to abnormal iron absorption resulting from frequent blood transfusions and ineffective erythropoiesis. Accumulation of iron overload in the pancreas may induce oxidative stress in pancreatic β cells, which in turn affects insulin secretion and glucose metabolism homeostasis and increases the risk of prediabetes and diabetes [29]. Studies have shown that lncRNAs play a key role in the regulation of islet function. For example, the lncRNA *MEG3* exhibited downregulation in pancreatic tissue in rat models of type 1 and type 2 diabetes, whereas its silencing further impaired insulin secretion and glucose homeostasis. By contrast, normal *MEG3* expression promotes insulin secretion and inhibits apoptosis of diabetic pancreatic β cells, suggesting it has a protective role in maintaining islet function [30]. In addition, the lncRNA *MALAT1* is closely associated with resistin and HOMA-IR in type 2 diabetes. In cell models, knockdown of *MALAT1* decreased pro-inflammatory cytokine production while improving glucose absorption and intracellular signaling, suggesting it has a potential regulatory role in diabetes pathophysiology [15]. lncRNAs may be an important molecular mechanism of β-TM complicated by prediabetes, providing a new research direction for the early identification of the disease and prompt intervention.

In this study, through GO functional annotation and KEGG pathway analysis, we investigated the potential role of differentially expressed genes in β-TM complicated by prediabetes in depth. GO analysis showed that deregulated mRNAs were mainly involved in metabolic processes, cellular communication and cellular responses to a variety of stimuli, such as inflammatory responses and oxidative stress, and KEGG pathway analysis further revealed multiple significantly enriched pathways, which included protein processing in the endoplasmic reticulum, a process closely associated with glucose metabolism. In addition, key pathways such as the cell cycle, calcium signaling pathway and NOD-like receptor signaling pathway are involved [31]. The results of these analyses suggest that differentially expressed mRNAs are widely involved in inflammation-mediated signaling and oxidative stress responses, reflecting a complex genetic and metabolic regulatory network in prediabetes in patients with β-TM. These dysregulated genes may run through various biological stages of prediabetes development, from metabolic disorders to cell signaling, inflammation and oxidative stress responses, which together drive the development and progression of prediabetes in patients with β-TM.

Summary of findings

In total, 3,443 differentially expressed lncRNAs and 1,880 differentially expressed mRNAs were identified, with ENST00000496629 and AC090912.2 (lncRNAs) and GDF15 (mRNA) emerging as the most significantly dysregulated transcripts. Functional enrichment analyses implicated these transcripts in glucose metabolism, vascular smooth muscle contraction, immune regulation and protein processing in the endoplasmic reticulum. These results suggest novel molecular links between iron overload, oxidative stress and early glucose dysregulation in β-TM.

Interpretation

Previous transcriptomic studies of β-TM have focused on erythropoiesis or iron metabolism. Our findings extend this by identifying transcripts not previously associated with β-TM or prediabetes. In particular, ENST00000496629, upregulated in our cohort, has not been reported in glucose

metabolism, suggesting a novel regulatory role in iron-induced metabolic disturbances. Moreover, AC090912.2, although poorly characterized, showed marked downregulation and may affect glucose homeostasis. Finally, GDF15, a stress-response cytokine, is elevated in metabolic disorders, insulin resistance and cardiovascular disease. Enrichment of HSV-1 infection and vascular smooth muscle contraction pathways – previously unreported in β-TM-related dysglycemia – suggests immune activation and early endothelial dysfunction may contribute to disease. This aligns with our unpublished findings of elevated IL-1β, IL-6, IL-8, IL-10 and TNF-α in β-TM with prediabetes.

Limitations and future directions

This study has several limitations. First, the small sample size (n = 5 per group) limits statistical power and generalizability; these results should be interpreted as exploratory. Second, recruitment was constrained by the rarity of β-TM with prediabetes due to premarital screening and improved management, and the lack of a group of patients with β-TM but without prediabetes prevents separation of thalassemia-related from dysglycemia-specific transcriptomic changes. Finally, age and sex were not strictly matched, potentially influencing gene expression. Future studies should recruit larger, matched cohorts including patients with β-TM but without prediabetes and perform in-depth functional validation of candidate molecules in cellular and animal models to clarify their roles in disease mechanisms.

CONCLUSION

In summary, the present study revealed a possible close link between substantial changes in lncRNA and mRNA expression profiles and β-TM complicated by prediabetes. These differentially expressed molecules may influence the pathophysiological mechanisms of diseases through complex regulatory networks or participate in specific biological processes. The findings of this study provide an important theoretical basis and research direction for future exploration of potential therapeutic targets, diagnostic markers and prognostic evaluation indicators of β-TM complicated by prediabetes.

ACKNOWLEDGMENTS

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Conflict of interest: None declared.

Supplementary Table 1. Reverse transcription-quantitative polymerase chain reaction primers for randomly selected lncRNAs

Transcript ID	Forward primer (5'–3')	Reverse primer (5'–3')
ENST00000431759	CCACGCAAACTCCTTCTGTA	GCCATTTTTTACCCTTTAGTTC
ENST00000611877	CTTCAGAGTGGGTGGTTTCC	CCTTCGCTGTCCTTTGAGTT
ENST00000564152	GTGGTTGGGTTTCTGAGTTTG	TGTCTGGCTTCCCTCTGTTC
ENST00000607613	TGCTGAGAGGGGTTTAGGAA	GGAATCTGGAAAACTGCCCA
ENST00000581274	ATGACATGGGGAAATGGAAGG	GCTGATCGCACTCAACTCTT
ENST00000449551	TTTCAGAGGAGTGGCTGGTA	TGGAGTGGATCACAGGCTTA
ENST00000437035	ACAAATCTGCCACTCAAGCC	TCTACTCCTGGATGTCTCTTCT
ENST00000556546	CTGCCTCCGATCCAAATTGT	CCTTGGAGAAACGCCATTGA

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Потпис дугих некодирајућих РНК код бета-таласемије мајор удружене са предијабетесом

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САЖЕТАК

Увод/Циљ Испитиване су функционалне дуге некодирајуће РНК (*lncRNA*) и матричне РНК (*mRNA*) код болесника са бета-таласемијом мајор (*β-TM*) и предијабетесом, анализом експресионих профила гена добијених помоћу микрочипова, експерименталним доказима и биоинформатиком.

Методе Укупна РНК је изолована након узимања узорака крви од болесника. Микрочипови су затим коришћени за генетичку анализу болесника са *β-TM*/предијабетесом ($n = 5$) у поређењу са здравим особама ($n = 5$). Потенцијалне *lncRNA* и *mRNA* насумично су верификоване применом анализе *qRT-PCR*. Сигнални путеви повезани са *β-TM* идентификовани су као повезани са предијабетесом на основу анализа *Gene Ontology* (*GO*) и *Kyoto Encyclopedia of Genes and Genomes* (*KEGG*).

Резултати У поређењу са контролном групом, 1.511 *lncRNA* било је повећано, а 1.932 смањено, док је код пацијената са *β-TM*/предијабетесом 1.128 *mRNA* било повећано, а 752 смањено. Осам дерегулисаних експресија *lncRNA* потврђено је анализом *qRT-PCR*, што је било у складу са резултатима микрочипова. Анализа *GO* и *KEGG* показала је да су унутарћелијска анатомска структура и процес вишећелијског организма били најзначајније дерегулисани, док су најзначајније повећани и смањени путеви били инфекција вирусом *Herpes simplex 1*, односно контракција глатких мишића крвних судова.

Закључак Ова студија је прелиминарно утврдила да патофизиологија нарушене глукозне хомеостазе у *β-TM* може бити повезана са абнормалном експресијом *lncRNA* и *mRNA*, чиме се продубљује разумевање молекуларних механизама у *β-TM* компликованој предијабетесом.

Кључне речи: анализа микрочипом; дуге некодирајуће РНК; бета-таласемија мајор; *mRNA*; предијабетес

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Unraveling infertility – most frequent causes and essential predictors of assisted reproductive technologies outcomes

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SUMMARY

Introduction/Objective Infertility is a global health concern affecting millions of couples worldwide. The aim of this study was to identify the most common causes of infertility among patients undergoing assisted reproductive technologies procedures and to determine key predictors of a successful outcome.

Methods This retrospective observational study included 164 patients treated at a University Clinical Center. Patients characteristics included age, body mass index, duration of infertility, baseline hormone levels were recorded, along with stimulation protocol, insemination technique, number of retrieved oocytes, fertilization rate, embryo quality, and treatment outcomes. Univariate and multivariate logistic regression analyses were performed to assess predictors of a clinical pregnancy.

Results The mean age of participants was 34.66 ± 3.69 years, and 89% underwent a short stimulation protocol. Unexplained infertility was most frequent (30.5%), followed by male factor (28%). Among the participants, 65.2% had 4–15 retrieved oocytes, while 19.5% had ≤ 3 and 15.2% had > 15. Embryos of quality A were observed in 56.7% of patients. The overall pregnancy rate was 53.7%. Univariate logistic regression identified lower baseline progesterone, higher number of mature oocytes, and better embryo quality as significant predictors of success. In the multivariate model, the number of mature oocytes ($p = 0.014$) and A-quality embryos ($p = 0.004$) remained independent predictors of a positive outcome.

Conclusion This study demonstrates that the number of mature oocytes and top-quality embryos are essential for achieving favorable in vitro fertilization / intracytoplasmic sperm injection results. Recognizing and addressing these predictive factors may enhance the success rate of assisted reproductive treatments, stressing the need for personalized therapeutic strategies.

Keywords: infertility; assisted reproductive technologies procedures; embryo quality; progesterone; IVF outcome

INTRODUCTION

Affecting millions of couples worldwide, infertility becomes a significant global health concern highlighting the need for effective diagnostic and therapeutic strategies [1]. Diverse underlying factors, such as delayed childbearing, baseline levels of reproductive hormones, ovarian reserve, lifestyle habits (e.g., smoking, obesity), and demographic characteristics of the couples have contributed to development of more sophisticated reproductive approaches [2]. According to the report "Women's Health in Serbia – Past, Present and Future," the leading reproductive problems among women in Serbia are menstrual cycle disorders and infertility, alongside a growing need for assisted reproductive technologies (ART) procedures due to the increased prevalence of these conditions [3].

The current increasing evidences about values of body mass index (BMI), serum progesterone (P4) levels and quality of embryos emphasis its potential role on the in ART success [4]. P4, as a key hormone in the luteal phase, plays a crucial role in endometrial preparation and the maintenance of early pregnancy. There are conflicting results about influence of the smoking habits, alcohol consumption, duration of infertility and causes of infertility on in vitro fertilization (IVF) outcomes [2, 5], but one is for sure that the success of each procedure depends on the patient's individual clinical presentation, emphasizing the importance of a comprehensive approach that carefully evaluates all relevant predictors. Regarding stimulation protocols, studies suggest that the choice of protocol (e.g., long, short, or antagonist) can influence both the quantity and quality of retrieved oocytes. Similarly, the selection of the insemination technique – IVF, intracytoplasmic sperm injection (ICSI), or a combined approach – may be critical in cases of severe male factor infertility or varying oocyte quality [6].

Accordingly, the aim of this study was to analyze the characteristics of patients who underwent IVF/ICSI treatment and identifying key predictors of IVF/ICSI success.

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METHODS

A retrospective observational study was conducted at the Clinic for Gynecology and Obstetrics, University Clinical Center of Serbia. All data were analyzed in accordance with ethical principles and research standards. The recruitment of patients was performed in accordance with the Helsinki declaration. All patients were entirely informed about the research and all signed the informed consents for inclusion in the study, as well as the ART itself. The research was approved by the Ethics Committee of the Faculty of Medicine, University of Belgrade.

The inclusion criteria encompassed women aged 18–40 years undergoing first, second, or third ART procedure as independent patients, with a BMI < 30 kg/m², a confirmed infertility diagnosis. Exclusion criteria consisted of azoospermic male partners and female patients who did not meet the inclusion criteria, such as those over 40 years of age or with a BMI exceeding 30 kg/m². Infertility was diagnosed and classified according to the guidelines of the European Society of Human Reproduction and Embryology [7]. For all patients ultimately included in the study, comprehensive medical histories were obtained, covering the following parameters: demographic characteristics [age, BMI, and duration of infertility), baseline hormonal status (follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), and P4], anti-Müllerian hormone (AMH) levels, factor of infertility (male, ovarian, tubal, combined), stimulation protocol (long antagonist or short antagonist protocol), procedure outcomes (number of retrieved oocytes, number of fertilized oocytes, fertilization rate, embryo quality, and pregnancy rate), and pregnancy outcomes (biochemical pregnancy, miscarriage, or live birth).

The stimulation protocols and patient monitoring have been described in detail in a previous study [4]. Briefly, in the long gonadotropin-releasing hormone (GnRH) agonist protocol, Triptorelin (Diphereline, Ipsen Pharma Biotech, Signes France) was administered in the mid-luteal phase of the preceding cycle, followed by ovarian stimulation with recombinant FSH (GONAL-f®, Merck KGaA, Darmstadt, Germany) from cycle day two or three, based on patient age, BMI, and ovarian reserve. In the short GnRH antagonist protocol, ovarian stimulation commenced with rFSH and Cetrorelix (Cetrotide®, Merck KGaA) added when the leading follicle reached 14 mm, continuing until human chorionic gonadotropin (hCG) administration. Ovarian response was monitored through serial transvaginal ultrasound and serum E2 measurements. When at least two follicles reached ≥ 18 mm, hCG (Pregnyl, Organon & Co., Jersey City, NJ, USA) was administered, with oocyte retrieval performed 34–36 hours later. Retrieved oocytes were classified as mature (MII) or immature (MI) based on their developmental stage.

Insemination methods included IVF, ICSI or a combined approach. Fertilization was assessed 16–20 hours post-insemination based on the presence of two pronuclei. Embryo quality was evaluated according to the Istanbul Consensus of Clinical Embryologists [8]. All assessments were conducted jointly by the embryology team. Embryo transfer was performed on day two or three post-oocyte retrieval, with a maximum of three embryos, depending on the patient's age, medical history, embryo quality, and patient's wish. Luteal phase support with intramuscular P4 initiated from the day of oocyte retrieval. Pregnancy was confirmed by a positive serum β-hCG result 14 days post-transfer. Clinical pregnancy was verified via transvaginal ultrasound at six weeks of gestation.

Statistical analysis

Data were analyzed using appropriate statistical tests based on the nature of the examined variables. Categorical variables were assessed using the χ^2 test to examine the distribution of infertility causes and stimulation protocols. Continuous variables were compared between groups using either the independent t-test or the Mann–Whitney U test, depending on the normality of data distribution. To evaluate the association between various predictors and IVF/ICSI success, univariate logistic regression analysis was performed. Variables with a significant association in the univariate analysis were subsequently included in a multivariate logistic regression model to identify independent predictors of a positive outcome. Statistical significance was set at $p < 0.05$. Results were reported as means with standard deviations or medians with interquartile ranges for continuous variables, and as frequencies with percentages for categorical variables. Odds ratios (OR) with 95% confidence intervals were presented for logistic regression analyses. All statistical analyses were conducted using SPSS Statistics for Windows software, version 22.0 (IBM Corp., Armonk, NY, USA).

Ethics: All patients were entirely informed about the research and all signed the informed consents for inclusion in the study in accordance with institutional ethical standards. The investigation approved by the Ethics Committee of the Faculty of Medicine, University of Belgrade, Belgrade, Serbia (Number 29/XI-1).

RESULTS

This study included 164 patients who underwent IVF/ICSI treatment. The mean patient age was 34.66 ± 3.69 years, with a mean BMI of 22.23 ± 2.65 kg/m². The mean duration of infertility was 4.86 ± 2.61 years (range: 1–17 years) (Table 1). The mean FSH level was 7.24 ± 2.47 mIU/mL,

Table 1. Distribution of demographic characteristics

Parameters		Total	%
Age	Up to 29	19	11.6%
	30–35	73	44.5%
	36+	72	43.9%
Smoking	No	131	79.9%
	Yes	33	20.1%
Body mass index	Up to 25	140	85.4%
	Over 25	24	14.6%

while the mean LH level was 5.15 ± 2.66 mIU/mL. The mean E2 concentration was 43.78 ± 19.45 pg/mL, and the mean P4 concentration was 2.64 ± 1.75 ng/mL. The mean AMH level was 2.55 ± 2.59 ng/mL. The total gonadotropin dose administered was 2205.03 ± 538.93 IU. The results are presented in Table 2. The short stimulation protocol was significantly more prevalent than the long protocol (89% *vs*. 11%, p < 0.0001). The distribution of insemination techniques showed no significant difference, with IVF (42.1%), ICSI (29.3%), and a combined approach (28.7%) (p = 0.06) (Table 3). The most common cause of infertility was unexplained infertility (30.5%), followed by male factor (28%), tubal factor (18.9%), ovarian factor (18.3%), and combined infertility (4.3%). Most patients (65.2%) had 4–15 retrieved oocytes, while 19.5% had ≤ 3 oocytes, and 15.2% had > 15 oocytes. Embryo grading showed that 56.7% of embryos were A-quality, 61% were B-quality, 17.1% were AB-quality, and 20.1% were C-quality, shown in Table 4. Among 164 treatment cycles, 88 cycles (53.7%) resulted in pregnancy, while 76 cycles (46.3%) did not lead to conception. Of the pregnancies, 71 (43.3%) progressed to live birth, 12 (7.3%) were biochemical pregnancies, and five (3%) resulted in miscarriage (Table 5).

Univariate logistic regression analysis identified BMI ≤ 25 kg/m² (OR = 2.5, p < 0.05), infertility duration (OR = 1.9, p < 0.05), number of MII oocytes (p = 0.043), and A-quality embryos (OR = 1.87, p = 0.048) as significant predictors of a positive outcome. The following factors that were identified are presented in Table 6. Multivariate logistic regression confirmed that the number of MII oocytes (p = 0.014) and A-quality embryos (p = 0.004) were independent predictors of a successful pregnancy outcome. The results are presented in Table 7.

DISCUSSION

This study highlights the pivotal factors that influence the success of ART procedures, emphasizing the importance of oocyte quantity and quality, embryo morphology, and hormonal balance in optimizing reproductive outcomes. Our results signify that a higher number of MII oocytes and A-quality embryos are independent predictors of a positive pregnancy outcome. Further, patients with a BMI lower than 25 kg/m² and a longer duration of infertility (> 3.5 years) had better treatment results, suggesting that healthy BMI optimizing hormonal balance, egg quality, and overall treatment response, and reproductive history play a significant role in ART success.

In multivariate analysis, BMI stayed as a borderline significant factor that may have an impact, but it was not an independent predictor of success when the other factors were taken into account. The data

Table 2. Average values of demographic characteristics, baseline hormonal status, and gonadotropin (GT) dose

Parameters		Number	Mean	SD	95% CI		Min.	Max.
					Lower	Upper		
Age	Total	164	34.66	3.69	34.10	35.23	21	41
BMI	Total	164	22.23	2.65	21.82	22.64	18	29.40
Duration of infertility	Total	164	4.86	2.61	4.45	5.26	1	17
FSH	Total	160	7.24	2.47	6.86	7.63	2.60	15
LH	Total	156	5.15	2.66	4.73	5.57	0.40	25.20
E2	Total	157	43.78	19.45	40.71	46.85	10	100
AMH	Total	150	2.55	2.59	2.13	2.97	0.10	14.30
GT dosage	Total	159	2205.03	538.93	2120.62	2289.45	900.0	4125.0
P4	Total	164	2.64	1.75	2.37	2.91	0.22	9.31

BMI – body mass index; FSH – follicle-stimulating hormone; LH – luteinizing hormone; E2 – estradiol; AMH – anti-Müllerian hormone; P4 – progesterone

Tabel 3. Stimulation protocol and insemination technique

Procedure		Total	%	χ^2 (df)	p
Stimulation protocol	GnRH agonists	18	11%	99.1 (1)	< 0.0001
	GnRH antagonists	146	89%		
Insemination technique	IVF	69	42.1%	5.65 (2)	0.06
	ICSI	48	29.3%		
	Combined	47	28.7%		
Total		164	100%		

GnRH – gonadotropin-releasing hormone; IVF – in vitro fertilization; ICSI – intracytoplasmic sperm injection

Table 4. Infertility cause disturbance, the number of retrieved oocytes and characteristics of embryos

		Factor		Total	%
Infertility cause		Male		46	28%
		Female	Tubal	31	18.9%
			Ovarian	30	18.3%
		Combined		7	4.3%
		Unknown		50	30.5%
The number of retrieved oocytes		< 3		32	19.5%
		4–15		107	65.2%
		> 15		25	15.2%
Quality of embryos	A quality embryo	No		71	43.3%
		Yes		93	56.7%
	B quality embryo	No		64	39%
		Yes		100	61%
	AB quality embryo	No		136	82.9%
		Yes		28	17.1%
	C quality embryo	No		131	79.9%
		Yes		33	20.1%

Table 5. Assisted reproductive technologies outcomes

Outcomes		Total	%
No pregnancy		76	46.3%
Pregnancy		88	53.7%
Outcome of pregnancy	Delivery	71	43.3%
	Biochemical pregnancy	12	7.3%
	Miscarriage	5	3%

Table 6. Univariate logistic regression analysis – predictive association with outcome

Parameters	B	SE	Wald	Df	Sig.	Exp(B)	95% CI for EXP(B)	
							Lower	Upper
Age	-0.05	0.04	1.17	1	0.280	0.95	0.88	1.04
BMI	-0.09	0.06	2.09	1	0.148	0.92	0.82	1.03
BMI – up to 25, over 25	-0.77	0.45	2.86	1	**0.091**	0.46	0.19	1.13
Smoking	0.04	0.39	0.01	1	0.909	1.05	0.49	2.25
Baseline concentrations of reproductive hormones								
FSH	0.00	0.06	0.00	1	0.995	1.00	0.88	1.13
LH	0.08	0.07	1.40	1	0.237	1.09	0.95	1.24
E2	0.01	0.01	0.90	1	0.344	1.01	0.99	1.02
AMH	0.03	0.06	0.28	1	0.596	1.03	0.91	1.17
GT dosage	0.00	0.00	0.16	1	0.686	1.00	1.00	1.00
P4	-0.28	0.10	8.10	1	**0.004**	0.75	0.62	0.92
Infertility								
Duration of infertility	0.08	0.06	1.51	1	0.219	1.08	0.96	1.22
Infertility cause	-0.04	0.10	0.17	1	0.681	0.96	0.78	1.17
Stimulation protocol	0.61	0.53	1.35	1	0.246	1.84	0.66	5.17
Technique	-0.22	0.19	1.36	1	0.244	0.80	0.55	1.16
Number of oocytes, fertilization rate and quality of embryos								
The number of oocytes	0.04	0.03	2.78	1	**0.096**	1.04	0.99	1.10
Up to 3,4–15, over 15	0.12	0.27	0.22	1	0.640	1.13	0.67	1.91
The number of mature oocytes	0.06	0.03	4.09	1	**0.043**	1.07	1.00	1.13
The number of fertilized oocytes	0.07	0.04	3.25	1	0.062	1.08	0.99	1.16
Fertilization rate	0.00	0.01	0.16	1	0.690	1.00	0.99	1.01
Quality of embryos	-0.51	0.19	7.36	1	**0.007**	0.60	0.42	0.87
A quality embryo	0.51	0.32	2.58	1	**0.048**	1.87	0.89	3.11

BMI – body mass index; FSH – follicle-stimulating hormone; LH – luteinizing hormone; E2 – estradiol; AMH – anti-Müllerian hormone; GT – gonadotropin; P4 – progesterone; B – unstandardized regression coefficient; SE – standard error; Wald – Wald statistic; Df – degrees of freedom; Sig. – statistical significance; Exp(B) – odds ratio

Table 7. Multivariate logistic regression analysis: predictive association with the outcome

Parameters		B	SE	Wald	Df	Sig.	Exp(B)	95% CI for Exp(B)	
								Lower	Upper
Step first	BMI	-0.99	0.50	3.85	1	**0.050**	0.37	0.14	1.00
	Duration of infertility	0.59	0.37	2.58	1	0.108	1.80	0.88	3.69
	The number of oocytes	-0.10	0.10	1.09	1	0.297	0.90	0.75	1.09
	The number of mature oocytes	0.23	0.14	2.90	1	0.088	1.26	0.97	1.64
	The number of fertilized oocytes	-0.03	0.07	0.20	1	0.655	0.97	0.84	1.11
	Quality of embryos	-0.59	0.21	7.66	1	**0.006**	0.56	0.37	0.84
	Constant	3.12	1.15	7.36	1	0.007	22.61		
Step last	BMI	-0.88	0.49	3.25	1	0.072	0.41	0.16	1.08
	The number of mature oocytes	0.10	0.04	6.02	1	**0.014**	1.11	1.02	1.20
	Quality of embryos	-0.60	0.21	8.43	1	**0.004**	0.55	0.37	0.82
	Constant	3.80	1.00	14.57	1	0.000	44.66		

BMI – body mass index; B – unstandardized regression coefficient; SE – standard error; Wald – Wald statistic; Df – degrees of freedom; Sig. – statistical significance; Exp(B) – odds ratio

from available literature is controversial. Some systematic reviews and meta-analyses have shown that women with a BMI over 25 have lower clinical pregnancy and live birth rates, as well as a higher risk of miscarriage compared to women with a BMI below 25 [9, 10], while others reported that BMI is not associated with the outcomes of fresh embryo transfer in women undergoing their first IVF/ICSI treatment [11].

Longer history of infertility is usually related to reduced chance of IVF/ICSI success, possibly due to the progression of underlying reproductive disorders over time [12]. Also, some studies have not found correlation between the duration of infertility and IVF/ICSI outcomes, indicating that other factors, such as maternal age and embryo quality, may have a greater impact on treatment success [13, 14]. Our study showed that a longer duration of infertility (> 3.5 years) was positively associated with IVF/ICSI success in univariate analysis, which is an uncommon finding, and could be explained with couples with longer infertility durations may have undergone more extensive diagnostic workups and previous treatments, and by the time they reached IVF, the underlying issues were better managed.

Also, patients with long-term infertility might be more motivated, and finally this was the group of patients that were less than 40 years old and that might have influenced our results. Still, in multivariate analysis, infertility duration was not retained as an independent predictor of success.

Results did not show correlation between baseline levels of FSH, LH, E2, AMH, dose of gonadotropin and pregnancy rates, what is in accordance with researches suggesting that elevated levels of FSH (> 10 mIU/mL) may weaken ovarian response but do not necessarily correlate with lower clinical pregnancy rates. Elevated baseline E2 (> 60 pg/mL) correlates with weakened ovarian response and lower pregnancy rates, as well and often masks the real level of FSH presenting ovarian reserve better than it actually is [15, 16, 17]. A systematic review and meta-analysis examining P4 levels at different phases of ART concluded that elevated baseline P4 (> 1.5 ng/mL on day 2–3 of stimulation) does not significantly impact live birth or clinical pregnancy rates in fresh IVF cycles [18]. In our study, univariate logistic regression analysis showed that respondents with lower levels of baseline P4 had higher chance to achieve pregnancy.

AMH, as indicator of ovarian reserve, is also a predictor of response to controlled ovarian stimulation. Our results did not show significant association among AMH levels and pregnancy rates, which is in accordance with other studies that suggest that higher AMH is correlated with a greater number of archived oocytes, but not necessarily with higher live birth rates [19]. Further, findings emphasized that very low AMH levels (< 0.5 ng/mL) usually indicate weak ovarian response with low pregnancy chances. It's role as an independent predictor of IVF success has not been fully established [20, 21].

The number of oocytes retrieved during an ART cycle is labeled with a positive correlation with successful outcomes. Studies reported that retrieving among six to 15 oocytes yields the greatest potential for favorable results, then less, maintaining quality of embryos and decreasing the risk of ovarian hyper stimulation syndrome [22], but emphasizing that oocyte quality is more critical for the success of the procedure than the absolute number of retrieved oocytes. The high-quality oocytes are directly correlated with highest fertilization rate, embryo development, implantation rates, and live births rate [23].

In summary, our study underscores the significance of individualized evaluation in ART, with particular attention to the quantity and quality of oocytes and embryos, as well as patient-specific characteristics such as BMI and infertility history. The most consistent predictors of a successful outcome were a higher number of MII oocytes and top-quality embryos, reaffirming the central role of gamete and embryo competence in ART success. While BMI and duration of infertility showed associations in univariate analysis, they did not retain independent predictive value in multivariate models, highlighting the complexity and interplay of contributing factors. Baseline hormonal markers, including AMH and FSH, were not reliable indicators of pregnancy success, supporting the notion that their value lies more in assessing ovarian response than in predicting outcomes. The major limitations of our study, which decrease the strength of our findings, are its retrospective design and the limited sample size.

CONCLUSION

These findings advocate for a multifactorial and individualized approach to patient assessment and treatment planning in ART, rather than relying solely on traditional baseline parameters. In the realm of ongoing innovation and advancement in the area of ART a more precise and comprehensive understanding of the significance and contribution of predictive factors is crucial for optimizing outcomes. Additionally, development of predictive models using patient-specific factors could contribute to increase of ART success and live birth rates.

Conflicts of interest: None declared.

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Испитивање неплодности – најчешћи узроци и кључни предиктори исхода поступака асистираних репродуктивних технологија

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САЖЕТАК

Увод/Циљ Неплодност представља глобални здравствени проблем који погађа милионе парова широм света. Циљ ове студије био је да идентификује најчешће узроке неплодности код пацијенткиња који се подвргавају процедурама асистираних репродуктивних технологија и да одреди кључне предикторе успешног исхода.

Методе Ова ретроспективна опсервациона студија обухватила је 164 пацијенткиње лечене у Универзитетском клиничком центру. Карактеристике пацијенкиња укључивале су старост, индекс телесне масе, трајање неплодности, базални хормонски статус, као и протокол стимулације, технику инсеминације, број добијених јајних ћелија, стопу фертилизације, квалитет ембриона и исход лечења. Спроведене су униваријантна и мултиваријантна логистичка регресиона анализа ради процене предиктора клиничких тешкоћа.

Резултати Просечна старост испитаница била је 34,66 ± 3,69 година, а 89% је било подвргнуто кратком протоколу стимулације. Најчешћи узрок неплодности био је непознат фактор (30,5%), затим мушки фактор (28%). Код 65,2% испитаница добијено је између четири и 15 јајних ћелија, док је 19,5% имало ≤ 3, а 15,2% > 15. Ембриони А-квалитета добијени су код 56,7% пацијенткиња. Укупна стопа трудноће износила је 53,7%. Униваријантна логистичка регресија показала је да су нижи базални ниво прогестерона, већи број зрелих јајних ћелија и бољи квалитет ембриона значајни предиктори успеха. У мултиваријантном моделу, број зрелих јајних ћелија ($p = 0,014$) и ембриони А-квалитета ($p = 0,004$) остали су независни предиктори позитивног исхода.

Закључак Ова студија показује да су број зрелих јајних ћелија и добар квалитет ембриона од кључне важности за постизање добрих резултата вантелесне оплодње. Препознавање и адекватно управљање овим предиктивним факторима може побољшати успешност асистираних репродуктивних третмана, наглашавајући значај персонализованих терапијских стратегија.

Кључне речи: неплодност; вантелесна оплодња; поступци; квалитет ембриона; прогестерон; исход поступка

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Long-term effects of monocular myopic anisometropia correction on uncorrected ocular axial length in minors and its influencing factors

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SUMMARY

Introduction/Objective The objective of the paper was to investigate the long-term effects of orthokeratology (ortho-k) correction for monocular myopia and the factors influencing axial length (AL) changes in the untreated eye of minors.

Methods A total of 81 patients with monocular myopia receiving ortho-k lenses for the first time were enrolled. Eyes corrected with ortho-k lenses were designated as the myopic group and contralateral non-myopic eyes formed the non-myopic group. Changes in AL from baseline to follow-up examinations were recorded. Univariate and multivariate linear regression analyses were performed sequentially to explore the correlation between baseline parameters and AL changes in the non-myopic eyes.

Results After wearing ortho-k lenses for six and 12 months, the AL of the corrected myopic group was 24.48 ± 0.35 and 24.56 ± 0.31 mm, respectively, whereas that of the uncorrected non-myopic group was 23.55 ± 0.24 and 23.7 ± 0.22 mm, respectively. After six and 12 months, the amount of change in the AL was higher in the uncorrected non-myopic group than in the corrected myopic group ($p < 0.001$). Moreover, the difference in AL between the two eyes gradually decreased ($t = 2.376$, $p = 0.018$); the change in AL difference (-0.10 ± 0.08 *vs.* -0.18 ± 0.13, $p < 0.001$) was significant.

Conclusion Orthokeratology lens wear may accelerate myopia progression in contralateral, initially non-myopic eyes. Younger children with monocular myopia and a higher baseline spherical equivalent are likely to experience faster myopia progression in the other, unaffected eye following ortho-k lens correction.

Keywords: orthokeratology; unilateral myopic anisometropia; axial length

INTRODUCTION

Myopia is increasingly prevalent worldwide [1–4]. Epidemiological studies indicate a progressive increase in the prevalence of the condition, with the population prevalence as high as 60% in Asia and 40% in Europe [5, 6]. Children who develop myopia in adolescence are at greater risk of developing pathologic, high myopia in adulthood, with a risk of blindness. Therefore, the prevention and control of myopia, particularly during adolescence, are of great significance.

Anisometropia refers to a spherical equivalent (SE) refractive power difference greater than 1.00 diopters (D) [7, 8, 9]. Due to the disparity in the size of the retinal images of the two eyes of patients with anisometropia, visual fatigue and abnormalities in binocular vision may occur [10, 11]. Research indicates that when the disparity in diopter of the two eyes reaches 2.50 D, the differential in the size of the retinal images is 5% [12]. Patients with binocular visual impairment have a remarkable reduction in their visually guided motor ability. This primarily manifests as a slower pace, inferior accuracy and poor depth judgment [13, 14], which, in turn, impairs the precision of visual–motor abilities [15] and affects learning, work and daily life. If minors exhibit anisometropia during the crucial period of visual development, the inferior eye is prone to retinal defocus, or the eye with the higher refractive diopter may develop monocular inhibition [16]. Moreover, relevant research suggests that the progression rate of myopia in both eyes of minors with anisometropia is faster than that in those without the condition [17]. As the degree of myopia and age increase, the degree of anisometropia will further amplify [9].

To date, the mechanism underlying the formation of myopia has not been fully elucidated. In addition to genetic and environmental factors, visual stimulation of the peripheral retina also plays a significant role in the formation of myopia [18]. Currently, methods proven to effectively control the progression of myopia primarily include the administration of atropine eye drops, orthokeratology (ortho-k) lenses and spectacle lenses, as well as contact lenses with a peripheral myopia defocus design.

Orthokeratology is an optical approach for controlling the progression of myopia in minors. It reduces peripheral astigmatism by reshaping the corneal curvature to better visualise external objects on the retina, effectively

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reducing axial elongation by 43–63% [19, 20]. Several recent studies have demonstrated that ortho-k effectively inhibits the axial elongation and reduces the degree of refractive interocular difference in adolescents with unilateral myopic anisometropia [21, 22]. However, few previous studies have investigated the changes in axial length of the contralateral eye without a lens and the factors affecting these changes.

METHODS

Study design

A total of 81 minors who were first fitted with ortho-k lenses for monocular myopia between January 2022 and June 2023 were selected using the convenience sampling method. The myopic eyes fitted with ortho-k lenses were then allocated to the corrected myopic eye group and the contralateral non-myopic eyes were allocated to the uncorrected non-myopic eye group. The inclusion criteria were as follows: (1) age 8–16 years; (2) unilateral myopic anisometropia with SE diopter of myopia of -6.00 to -0.75 D and corrected visual acuity ≥ 1.0, and the contralateral eye with SE diopter of -0.50 to– +1.50 D and visual acuity of > 1.0; (3) astigmatism with-the-rule in both eyes ≤ 2.00 D; (4) SE difference ≥ 1.00 D in both eyes; (5) first-time fitting of corneal contact lenses, with no contraindications to contact lens fitting; and (6) intraocular pressure (IOP) value = 10–21 mmHg. The exclusion criteria were as follows: patients (1) with presence of medications and history of treatment with myopia prevention tools that may affect refractive outcomes; (2) with presence of binocular vision problems such as dominant strabismus and amblyopia; (3) with past history of ocular trauma or surgery; (4) suffering from corneal diseases, glaucoma, uveitis, cataract or fundus diseases, which may affect vision, diopter or choroidal structure; (5) suffering from systemic diseases such as diabetes, hypertension or autoimmune diseases that may cause eye disease; and (6) unable to understand and cooperate with the examination. The study was approved by the hospital's ethics committee. This was a prospective study and all participants (or their guardians) signed an informed consent form before the start of the study.

Relevant inspection and methods

Visual acuity examination

Distance visual acuity is assessed at 5 m using an international standard chart under good illumination, with the 1.0 line at eye level. Each eye is tested separately: first uncorrected, then corrected, with the fellow eye fully occluded without pressure. Patients identify the gap direction in descending 'E' optotype order. If no optotype is discerned at 5 m, the patient moves closer until identifying the 0.1 line. Failure to see line 0.1 at 1 m prompts finger counting against light at the maximum discernible distance; inability to count fingers necessitates light perception testing. Near acuity is measured at 30 cm with a standard chart; if line 1.0 is unclear, the chart is moved progressively closer.

Axial length examination

In this study, axial length (AL) measurements were obtained using an optical biometer (SW-9000, Suiwei, China). During examination, the chin rest height was adjusted to align the patient's outer canthus with the marking line. The operator then manoeuvred the instrument handle until the pupil was centred within the white dot-shaped aperture and the white dual light rays appeared clear; at this point, the button was pressed. Following this, the examination position was adjusted until a green aperture appeared. The handle was then adjusted to centre the pupil within the green aperture before pressing the button again, completing one measurement. Measurements were performed at least three times per eye, and the average value was used.

Diopter examination

The diopter examination began with an objective refraction test using a fully automatic computerized refractometer (Model: KR-800, Topcon Corporation, Japan). The examinee's chin rest and headrest were adjusted, and they were instructed to focus on the hot air balloon or small house inside the device. The tracking ball was adjusted to position the pupil centre between the inner and outer aligning rings, initiating automatic measurements. Three separate measurements were taken for each eye, with the average value, automatically calculated by the computerized refractometer, then recorded. The standard deviation of the three measurements had to be < 0.05 mm, otherwise the measurement had to be repeated. Next, subjective refraction was assessed using a fully automatic comprehensive optometry instrument (VT-10, Topcon Corporation). Briefly, the objective refractive values obtained from the computerised refractometer were inputted into the comprehensive optometry instrument, followed by the maximum plus to maximum visual acuity (MPMVA) procedure. During MPMVA, a fogging lens (typically +0.75 D) was employed to induce controlled myopia, relaxing accommodation for more accurate visual acuity determination. Under fogging conditions, the optometrist simultaneously evaluated binocular vision, ensuring binocular harmony by adjusting lenses. Binocular function was further assessed using the red–green test, based on accommodative balance. The fogging lens power was then gradually reduced until the patient achieved clear vision of the chart letters; the corresponding lens power was recorded as the endpoint. Cycloplegic refraction was performed after inducing cycloplegia. Participants aged 8–12 years received 1% cyclopentolate hydrochloride eye drops for mydriasis, whereas those aged ≥12 years received compound tropicamide eye drops. Here, 1% cyclopentolate was administered twice, with a five-minute interval between each administration, and compound tropicamide was administered three times, again with a 5-minute interval between each administration. Mydriatic refraction was conducted 30 minutes after

the last instillation, upon disappearance of the pupillary light reflex.

Intraocular pressure examination

Intraocular pressure was measured using a non-contact tonometer (CT-800, Topcon Corporation, Tokyo, Japan). The participant was instructed to place their chin on the chin rest and press their forehead against the headrest, aligning the eye with the examination nozzle at the appropriate height. The instrument's focal length was then aligned with the examined eye. The patient was asked to keep their eyes open and focus on the yellow-green fixation target inside the instrument. When the bright focal point within the pupil was clearly aligned with the instrument's focal point, the nozzle automatically emitted a puff of air. This measurement was repeated three times per eye, and the average value was recorded.

Corneal endothelial cell count examination

Corneal endothelial cell count is considered a reliable indicator of corneal hypoxia. It also serves as a key safety assessment parameter for ortho-k lenses during follow-up examinations. Here, the examination was performed using a non-contact specular microscope (SP-1P, Topcon Corporation). The chin rest was adjusted to position the patient's examined eye within the imaging aperture. The patient was instructed to keep their eyes open and focus on the fixation light within the instrument. The examiner adjusted the working distance to achieve a clear image and then captured it to complete the measurement.

Fitting method and recheck

All patients were fitted with either spherical or aspherical ortho-k lenses designed by Beijing Eyebright (made of fluorosilicone-acrylate material, oxygen permeability Dk value of 125 × 10 – 11 [cm^2/s]*[$mLO_2/(mL \times mmHg)$]), with a central optical thickness of 0.22 mm. All ortho-k lens users wore them continuously at night for 8–10 h and cleansed and soaked their lenses daily with a multifunctional solution (Menicon Co., Ltd, Nagoya, Japan). The lenses were worn continuously for 12 months, without the use of glasses during the day. Regular examinations were conducted at follow-ups after one, three, six, nine, and 12 months. All measurements, record keeping and patient follow-ups were conducted by an ophthalmologist at the time of the initial application of the ortho-k lenses and throughout subsequent follow-up visits.

Outcome measures

Baseline data were collected for analysis, including age at the initiation of ortho-k lens wear, sex, SE refraction at baseline (SE = spherical diopter + 1/2 astigmatism), IOP and cell density (CD). The changes in AL during baseline examination and follow-up (six and 12 months) were recorded.

Statistical analysis

All data were statistically analyzed using IBM SPSS Statistics, Version 26.0 (IBM Corp., Armonk, NY, USA). Measurement data were expressed as mean ± standard deviation (x ± s), and a paired-sample t-test was used for comparing the parameters of both eyes. Enumeration data were expressed as the number of cases (n) and rate (%), and the chi-squared (χ^2) test was used for the intergroup comparison. A univariate analysis was performed on the fellow eyes of all patients to evaluate the correlation between baseline variables and changes in AL in these eyes. Factors showing a $p < 0.05$ in the univariate analysis were then entered into a multivariate regression model, with fellow eye AL change as the dependent variable. The correlation strength was expressed by beta (β) value, 95% confidence interval, corrected R^2 value and p value. The level of significance was set at $\alpha = 0.05$. There were no changes in personnel at baseline and subsequent follow-ups, and there were no participants with refractions < -0.5 D (as myopia is irreversible) and no adverse events.

Ethics: This study was conducted in accordance with the Declaration of Helsinki and approved by the ethics committee. This was a prospective study and all participants (or their guardians) signed an informed consent form before the start of the study.

RESULTS

Baseline data

As shown in Table 1, 81 patients with unilateral myopic anisometropia were recruited, with an average age of 11.08 ± 2.57 years. The corrected myopic eye group had SE, IOP, AL and endothelial CD values of −2.35 ± 0.87 D, 18.13 ± 2.61 mmHg, 24.41 ± 0.29 mm and 3,361.21 ± 35.15 μm^2, respectively, whereas the values for those in the uncorrected non-myopic eye group were −0.04 ± 0.38 D, 18.02 ± 2.79 mmHg, 23.37 ± 0.31 mm and 3,352.53 ± 37.18 μm^2, respectively. Statistically significant differences in SE (-2.35 ± 0.87 *vs.* -0.04 ± 0.38, $p < 0.001$) and AL (24.41 ± 0.29 *vs.* 23.37 ± 0.31, $p < 0.001$) between the two groups.

Table 1. Comparison of baseline data of patients before wearing ortho-k lenses

Item	Corrected myopic eye (n = 81)	Uncorrected non-myopic eye (n = 81)	χ^2/t	p
SE/D	-2.35 ± 0.87	-0.04 ± 0.38	24.453	< 0.001
IOP/mmHg	18.13 ± 2.61	18.02 ± 2.79	0.289	0.773
AL/mm	24.41 ± 0.29	23.37 ± 0.31	24.622	< 0.001
Endothelial CD/(cells/mm^2)	3361.21 ± 35.15	3352.53 ± 37.18	1.705	0.090

SE – spherical equivalent; IOP – intraocular pressure; AL – axial length; CD – cell density

Axial length changes

The change in AL is defined as the difference between the AL at the follow-up time point and the baseline AL. As shown in Table 2, the changes in AL at six and 12 months for the corrected myopic eye group were 0.07 ± 0.04 and 0.15 ± 0.07 mm, respectively. In contrast, the changes in AL for the uncorrected non-myopic eye group at six and 12 months were 0.18 ± 0.07 and 0.33 ± 0.11 mm, respectively. The change in AL in the uncorrected non-myopic eye group was significantly greater than that in the corrected myopic eye group at both the six-month (0.07 ± 0.04 *vs.* 0.18 ± 0.07, p < 0.001) and 12-month (0.15 ± 0.07 *vs.* 0.33 ± 0.11, p < 0.001) follow-ups, with statistically significant differences.

Table 2. Changes of axial length after one year of wearing ortho-k lenses (mm)

Group	Wearing ortho-k lenses for six months	Wearing ortho-k lenses for 12 months
Corrected myopic eye (n = 81)	0.07 ± 0.04	0.15 ± 0.07
Uncorrected non-myopic eye (n = 81)	0.18 ± 0.07	0.33 ± 0.11
t	13.712	13.874
p	< 0.001	< 0.001

The interocular AL difference was defined as the difference in AL between a participant's eyes. The change in this difference over time was defined as a positive change if the difference increased and a negative change if it decreased. Before wearing corrective lenses, the average difference in AL between the eyes was 1.03 ± 0.33 mm (Table 3). After wearing lenses for six and 12 months, the AL of the corrected myopic eye group was 24.48 ± 0.35 and 24.56 ± 0.31 mm, respectively, and that of the uncorrected non-myopic eye group was 23.55 ± 0.24 and 23.7 ± 0.22 mm, respectively. The ocular AL differences in both eyes were 0.94 ± 0.28 and 0.86 ± 0.19 mm, with a change of -0.10 ± 0.08 and -0.18 ± 0.13 mm, respectively. After wearing lenses for six and 12 months, the AL difference between eyes gradually decreased (0.94 ± 0.28 *vs.* 0.86 ± 0.19, t = 2.376, p = 0.018), and the change in AL difference (-0.10 ± 0.08 *vs.* -0.18 ± 0.13, p < 0.001) was statistically significant.

Univariate analysis results

Univariate analysis was conducted, with baseline non-myopic eye SE, non-myopic eye IOP, and non-myopic eye endothelial CD as independent variables, and non-myopic eye AL growth as the dependent variable. As shown in Table 4, a significant statistical correlation was found between baseline SE of non-myopic eyes (p < 0.001) and AL increment in those eyes.

Multivariate analysis results

A multiple regression analysis was performed with baseline age and fellow eye SE as independent variables and fellow eye AL change as the dependent variable. As shown in Table 5, baseline SE was positively correlated with non-myopic eye AL growth (β = 0.073, p = 0.002).

Table 5. Multivariate linear regression analysis between different variables and axial length growth of the uncorrected non-myopic eyes

Variable	β	p	95% CI
SE	0.073	0.002	0.027, 0.122
Final model	R^2 = 0.296	Corrected R^2 = 0.284	

SE – spherical equivalent

DISCUSSION

Recent years have witnessed a high and rising prevalence of myopia among minors in China, and a portion of the myopic population develops anisometropia [8]. As the age increases and the degree of myopia deepens, both the prevalence and severity of myopic anisometropia increase [17]. Anisometropia-induced unequal visual input and blurred vision can impair visual function, potentially leading to monocular suppression, strabismus, impaired stereopsis and amblyopia [23]. Therefore, early correction of anisometropia to control its progression is crucial. Unilateral myopic anisometropia – defined as anisometropia where one eye is myopic and the fellow eye is almost emmetropic – represents a specific form of anisometropia. For managing anisometropia, particularly in eyes with relatively high myopia, ortho-k lenses have demonstrated superior efficacy to low-concentration atropine in controlling myopia

Table 3. Changes of axial length difference between eyes of patients after wearing ortho-k lenses

Item	Wearing ortho-k lenses for six months	Wearing ortho-k lenses for 12 months	t	p
Axial length of the corrected myopic eye group (mm)	24.48 ± 0.35	24.56 ± 0.31		
Axial length of the uncorrected non-myopic eye group (mm)	23.55 ± 0.24	23.70 ± 0.22		
Difference in axial length of both eyes (mm)	0.94 ± 0.28	0.86 ± 0.19	2.376	0.018
Change in axial length difference (mm)	-0.10 ± 0.08	-0.18 ± 0.13	5.267	< 0.001

Note: '-' means that the difference has decreased.

Table 4. Univariate linear regression analysis between different variables and axial length growth of the uncorrected non-myopic eyes

Variable	Mean value	β	R^2	Corrected R^2	p	95% CI
SE/D	2.32 ± 0.84	0.074	0.18	0.16	< 0.001	0.027, 0.129
IOP/mmHg	18.74 ± 2.66	0.013	0.097	0.082	0.577	0.003, 0.023
Endothelial CD/μm²	3375.24 ± 35.86	-0.002	0.061	0.044	0.524	-0.005, 0.000

SE – spherical equivalent; IOP – intraocular pressure; AL – axial length; CD – cell density

progression [24]. Therefore, for minors with monocular myopia, ortho-k lenses are usually chosen as a means to correct and control myopia.

Orthokeratology lenses are rigid gas-permeable corneal contact lenses with a reverse-geometry design. When worn overnight, they induce changes in corneal morphology. This induces myopic defocus in the peripheral retina, thereby potentially slowing axial elongation and myopia progression. Additionally, ortho-k lenses can improve accommodative function and reduce accommodative lag [25]. The improvement of accommodation lag in myopic eyes following the use of the lenses also plays a role in delaying the growth of AL.

This study showed that the AL of the eyes increased after wearing lenses for six months and 12 months compared with baseline, and that the AL of non-myopic eyes increased more obviously than that of corrected myopic eyes (six months: 0.07 0.04 *vs.* 0.18 0.07, t = 13.712, p < 0.001) and (12 months: 0.15 ± 0.07 *vs.* 0.33 ± 0.11, t = 13.874, p = < 0.001). However, the AL difference of both eyes gradually decreased (0.94 ± 0.28 *vs.* 0.86 ± 0.19, t = 2.376, p = 0.018). While ortho-k lenses effectively controlled myopia progression in the treated eyes, the refractive error of the non-myopic eye progressed rapidly towards myopia. This suggests that the reduction in interocular refractive difference following ortho-k lens wear may result not only from slowed myopia progression in the treated eye but also from accelerated myopia development in the fellow eye. A controlled study discovered that the AL of minors with myopia wearing single-vision frame glasses increased by 0.63 ± 0.26 mm on average in two years, whereas that of minors with myopia wearing ortho-k lenses increased by an average of 0.36 ± 0.24 mm in the same period, with a statistically significant difference (p < 0.01) [26]. Compared with single-vision spectacles, ortho-k lenses slowed AL progression by 43% [27]. Given individual variations in genetics and visual behavior, researchers employed a self-controlled design in patients with unilateral myopia to evaluate the efficacy of ortho-k lenses in controlling axial elongation. Related research [28] found that the eyes treated with ortho-k experienced an average axial growth of 0.08 ± 0.15 mm over a year, whereas the contralateral eye showed a substantially faster average axial growth of 0.39 ± 0.32 mm (p < 0.001). It has been demonstrated that with one year of ortho-k treatment, the AL growth (0.05 ± 0.19 mm) of the myopic eye in minors with unilateral myopia was significantly less than that of the non-myopic eye (0.34 ± 0.12 mm) [28].

Univariate linear regression analyses were performed to assess the association between the change in AL of the fellow eye and the following independent variables: sex, baseline age, baseline fellow eye SE, baseline fellow eye IOP and baseline fellow eye corneal endothelial CD. It was found that there was a significant statistical correlation between baseline age (p = 0.033), baseline non-myopic eye SE (p < 0.001) and non-myopic eye AL growth. Further multivariate linear regression analysis showed that the baseline age was negatively correlated with the growth of non-myopic eye AL (β = -0.014, p = 0.031), and that baseline non-myopic eye SE was positively correlated with the growth of non-myopic eye AL (β = 0.073, p = 0.002). Age is a predictive factor of axial elongation in minors with myopia wearing ortho-k lenses [29]. In a related study observing 31 minors wearing these lenses, it was concluded from single-variable and multivariable analyses that the greater the age, the lower the increase in AL [30]. In the present study, a larger baseline SE was significantly associated with less AL elongation. This association may be explained by the greater degree of peripheral retinal myopic defocus induced by ortho-k lenses in patients with high myopia, which more effectively impedes myopia progression [31].

This study has certain limitations. First, there was selection bias in the collection of the participants, limiting the applicability of the results. Second, a small sample was included, and larger samples and multicentre studies are still needed to provide a stronger basis. Finally, a limited number of independent variables were included in the process of exploring the factors influencing AL, and the impact of indices such as corneal curvature, astigmatism and central corneal thickness was not considered.

CONCLUSION

While ortho-k lenses function well in controlling the growth of AL of the myopic eye in minors with unilateral myopic anisometropia, they accelerate the progression of myopia in the contralateral non-myopic eye. Minors with monocular myopia who are of a younger age and have greater SE diopter at baseline will develop myopia faster in the contralateral eye following correction with ortho-k lenses.

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Authors' contributions: WTT conceived of the study, WLJ and GH participated in its design and data analysis and statistics, and XYX helped to draft the manuscript. All authors read and approved the final manuscript.

Conflict of interest: None declared.

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Дугорочни ефекти корекције монокуларне миопичне анизометропије на некориговану аксијалну дужину ока код малолетника и фактори њеног утицаја

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САЖЕТАК

Увод/Циљ Циљ овог рада био је да испита дугорочне ефекте корекције монокуларне миопије ортокератолошким (орто-к) сочивима и факторе који утичу на промене аксијалне дужине (АД) у некоригованом оку код малолетника.

Методе У студију је укључен 81 пацијент са монокуларном миопијом који је први пут користио орто-к сочива. Очи које су кориговане орто-к сочивима сврстане су у миопичну групу, док су контралатералне некориговане очи сврстане у немиопичну групу.

Забележене су промене АД од почетног мерења до контролних прегледа, а униваријантне и мултиваријантне линеарне регресионе анализе вршене су секвенцијално да би се истражила корелација између почетних параметара и промена АД у некоригованим очима.

Резултати Након ношења орто-к сочива 6 и 12 месеци, АД у коригованој групи са миопијом износила је 24,48 ± 0,35 и 24,56 ± 0,31 *mm*, док је АД код некориговане немиопне групе била 23,55 ± 0,24 и 23,7 ± 0,22 *mm*. Након 6 и 12 месеци, промена АД била је већа у некоригованој немиопичној групи него у коригованој миопичној групи ($p < 0,001$), а разлика у АД између два ока се постепено смањивала ($t = 2,376$, $p = 0,018$). Промена разлике АД (-0,10 ± 0,08 *vs.* -0,18 ± 0,13, $p < 0,001$) била је статистички значајна.

Закључак Ортокератологија убрзава прогресију миопије код контралатералних немиопичних очију. Млађа деца са монокуларном миопијом и већом сферном еквивалентном диоптријом брже ће развити миопију у контралатералном оку након корекције орто-к сочивима.

Кључне речи: ортокератологија; једнострана миопична анизометропија; аксијална дужина очне јабучице

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Breast masses in children and adolescents – expect infrequent but possible diagnosis

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SUMMARY

Introduction/Objective Breast masses are uncommon in children and adolescents. Fortunately, most of these breast lesions are benign, self-limiting changes, although malignant diseases have also been described. The largest proportion of young patients are diagnosed with fibroadenoma. However, biopsy and histopathological examination are necessary in all suspected cases to exclude malignancy.
This study aims to highlight the importance of the diagnosis and treatment of breast masses, as well as the possibility of developing malignant breast disease at this age, by presenting our case.

Methods We analyzed data from 27 patients who presented to our clinic in the two-year period. Breast ultrasound was performed on all patients presenting with pain and breast oedema. The breast masses were classified using the Breast Imaging Reporting and Data System (BI-RADS).

Results The median age of the 27 female patients was 15.44 ± 1.39 years, with breast masses mostly located on the right side in 16 (59.26%) patients, and the prevalence of BI-RADS III in 18 (66.66%) patients. Only two patients (7.4%) were reported as BI-RADS IV and seven (25.92%) as BI-RADS II. The most common mass lesions were fibroadenoma (20/27, 74.07%), benign phyllodes tumour (3/27, 11.11%), and hematoma, abscess, and juvenile papillomatosis (1/27, 3.7%), respectively. Breast cancer was diagnosed in one case (3.7%).

Conclusion Primary breast cancer is relatively uncommon in adolescents. However, clinicians should consider breast cancer in the differential diagnosis of a breast mass in adolescence.

Keywords: breast mass; carcinoma; puberty; adolescents

INTRODUCTION

With a prevalence of 3.2%, breast masses are uncommon in children and adolescents. Fortunately, most breast lesions that arise in this age group are benign, self-limiting changes, although malignant diseases have also been described. Ninety-five per cent of surgically removed breast masses in children are benign fibroadenomas, and only 0.02% are malignancies [1, 2, 3]. The differential diagnosis of breast masses includes fibroadenoma, phyllodes tumor, hemangioma, abscess, and primary breast cancer. Early clinical evaluation and careful follow-up are necessary to rule out malignancy. A detailed history, clinical examination, and meticulous breast palpation are crucial in the follow-up for girls with breast masses.

The best screening tool for characterizing the breast lesion and detecting the presence of solid and cystic masses in adolescent girls and teenagers without exposing them to radiation is ultrasound (US). A fixed solid mass with nipple discharge and nipple retraction raises significant concern for cancer. Mammography is rarely used in adolescents due to the dense nature of breasts, which significantly reduces the sensitivity of this examination. Fine-needle aspiration (FNA) can provide a cytopathological diagnosis. Magnetic resonance imaging (MRI) is the most reliable method for suspected malignancy or disseminated disease cases [4].

There have been 39 published cases of primary breast cancer in pediatric patients [2, 5]. Primary breast cancer is sporadic in children and adolescents, with a frequency of one in 1,000,000 [6]. Younger patients are more likely to have a large mass at the time of breast cancer diagnosis, characterized as a firm, fixed, poorly defined lump. Breast retraction and axillary metastases are uncommon.

In patients with breast malignancy, an individualized therapeutic plan is required. This principle is based on considering hereditary factors, future fertility, tumor type, and the presence of axillary and distant metastases. Surgical excision of a breast mass is recommended in the case of fast-growing masses with altered architecture of the breast parenchyma, as well as in tumors larger than 5 cm, even if they have fibroadenoma characteristics on US [6].

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Aim

The study aimed to highlight the importance of timely diagnosis of breast masses in puberty and adolescence, as well as to describe the frequency of malignant breast disease at this age. Consequently, the goal was to define the sequence of diagnostic procedures and therapeutic options.

METHODS

The 41 patients were identified by searching electronic records in the local medical information system using the terms "breast," "mass," and "female" within a two-year interval (January 2021 – December 2023), according to the requested terms, and all of them had at least 12 months of follow-up. Of these, 14 individuals were excluded from the study: 10 were diagnosed through clinical examination and US, but there was no increase in breast mass over the 12-month follow-up period; therefore, biopsy and histopathological (HP) examinations were not performed, and four patients rejected the biopsy. Finally, 27 participants were enrolled in the study and analyzed.

An expert ultrasonographer performed all US examinations. Patients were in the supine position, using high-resolution linear probes (16 MHz) (ACUSON NX3, Siemens, Healthineers, Mountain View, CA, USA). The radiologist examined the breast in four quadrants, the nipple, and the axilla. When suspicious changes were detected, several images were taken in different planes, and the exact location and size of the lesions were defined. After the completion of bilateral examinations, the images were archived on the hard drive. The breast masses were classified using the Breast Imaging Reporting and Data System (BI-RADS) criteria established by the American College of Radiology (BI-RADS 0: incomplete; BI-RADS 1: negative; BI-RADS 2: benign; BI-RADS 3: probably benign; BI-RADS 4: suspicious for malignancy; BI-RADS 5: highly suggestive of malignancy; BI-RADS 6: known biopsy-proven malignancy).

Statistical analysis

A database was generated in MS Office Excel (Microsoft, Redmond, WA, USA) and processed using IBM SPSS Statistics software (IBM Corp., Armonk, NY, USA). Continuous variables are shown as means ± standard deviation (SD). Categorical variables were reported as simple numbers and percentages (n, %), with p-values ≤ 0.05 indicating statistical significance.

Ethics: The study received approval from the Review Board of the Kosovska Mitrovica Clinical-Hospital Centre Ethics Committee, Kosovska Mitrovica, Serbia, on February 22, 2024 (No. 1361).

RESULTS

The median age of 27 female patients was 15.44 ± 1.39 years. The breast masses were mostly on the right side in 16 (59.26%), with statistical significance (p ≤ 0.05), and a prevalence of BI-RADS III in 18 (66.66%) (p ≤ 0.05). Additionally, only two patients (7.4%) were reported as BI-RADS IV and seven (25.92%) as BI-RADS II (Table 1).

Table 1. Distribution of breast masses by age, side, and BI-RADS classification

Age			Side		BI-RADS classification N (%)		
Years	N	%	Left N (%)	Right N (%)			
					II	III	IV
13	3	11.1	1 (3.7)	2 (7.4)	2 (7.4)	1 (3.7)	0
14	5	18.51	2 (7.4)	3 (11.11)	2 (7.4)	3 (11.11)	0
15	5	18.51	2 (7.4)	3 (11.11)	1 (3.7)	4 (14.81)	0
16	5	18.51	3 (11.11)	2 (7.4)	2 (7.4)	2 (7.4)	1 (3.7)
17	9	33.33	3 (11.11)	6 (29.62)	0	8 (29.62)	1 (3.7)
Σ 27,100			**11 (40.74)**	**16 (59.26)***	**7 (25.92)**	**18 (66.66)***	**2 (7.4)**
X ± SD 15.44 ± 1.39			p-values ≤ 0.05*		p-values ≤ 0.05*		

BI-RADS – Breast Imaging Reporting and Data System

A total of 27 patients underwent breast mass excision, and excised tissue samples were sent for HP examination. Table 2 summarizes the breast mass distribution based on age, size, side, HP examination, and tumor type (benign or malignant). Fibroadenomas (FA) were found in 20 (74.07%) patients, benign phyllodes tumor in 3 (11.11%), and hematoma, abscess, and juvenile papillomatosis in one patient (3.7%), respectively, which was statistically significant (p ≤ 0.05) (Table 3). Breast cancer was diagnosed in only one case (3.7%), in a 16-year-old girl.

A 16-year-old girl was admitted to the Department of Pediatric Surgery due to a palpable mass, swelling, and redness in the enlarged right breast. A huge breast mass was observed in the upper outer quadrant, with a prominent venous pattern and lobulated surface, without nipple discharge from the nipple (Figure 1). The girl was diagnosed with schizoaffective disorder, according to the International Classification of Diseases (ICD) ICD-10 F25.9, and had a very poor interaction with her guardians in the foster family. Because of this, she did not report a mass in her breast for months until it became visible to the people around her.

The skin above the tumor and lymph nodes in the anterior part of the right axilla were fixed. There was no family history of breast or ovarian cancer, nor had there been any previous radiation exposure. Secondary sexual features were normally developed. The findings in the left breast and axilla were normal. The ultrasound assessment of the abdomen, chest, cranium, and spine revealed no abnormalities.

Ultrasound examination revealed a hypoechogenic tumorous formation 81 × 98 mm, with irregular morphology and shape, unclear margins, increased rim vascularization, and no calcifications (BI-RADS IV). Ipsilateral axillary lymph nodes were enlarged up to 25 mm (Figure 2).

Table 2. The age, side, size, classification, HP diagnosis, and type of malignancy of all breast lesions in 27 female patients

No.	Age (years)	Side L/R	BI-RADS classification	Size (mm)	HP diagnosis	Tumor type
1	16	L	III	27 × 36	Fibroadenoma complex mammae	Benign
2	17	R	III	33 × 35	Fibroadenoma juvenile mammae	Benign
3	14	L	II	42 × 28	Fibroadenoma mammae	Benign
4	15	R	III	29 × 31	Fibroadenoma complex mammae	Benign
5	14	L	III	44 × 52	Haemathoma mammae	Benign
6	17	R	III	59 × 48	Tu phyllodes mammae benignum	Benign
7	17	R	III	53 × 32	Tu phyllodes mammae benignum	Benign
8	17	R	III	44 × 41	Fibroadenoma complex mammae	Benign
9	17	R	III	35 × 39	Fibroadenoma mammae	Benign
10	13	R	II	29 × 40	Fibroadenoma juvenile mammae	Benign
11	**16**	**R**	**IV**	**81 × 98**	**Carcinoma ductale mammae**	**Malignant**
12	15	R	III	21 × 23	Fibroadenoma juvenile mammae	Benign
13	14	R	III	19 × 22	Fibroadenoma juvenile mammae	Benign
14	16	L	II	18 × 23	Fibroadenoma juvenile mammae	Benign
15	14	R	II	21 × 22	Fibroadenoma mammae	Benign
16	13	R	III	30 × 32	Fibroadenoma mammae	Benign
17	15	L	III	18 × 22	Fibroadenoma mammae	Benign
18	17	L	III	34 × 24	Fibroadenoma mammae	Benign
19	17	L	IV	55 × 48	Juvenile papillomatosis	Benign
20	16	L	III	44 × 39	Fibroadenoma complex mammae	Benign
21	15	R	II	40 × 35	Fibroadenoma juvenile mammae	Benign
22	14	R	III	19 × 23	Fibroadenoma juvenile mammae	Benign
23	13	L	II	22 × 36	Abscessus mamme	Benign
24	15	L	III	32 × 26	Tu phyllodes mammae benignum	Benign
25	17	D	III	44 × 39	Fibroadenoma mammae	Benign
26	16	D	II	43 × 40	Fibroadenoma mammae	Benign
27	17	L	III	34 × 32	Fibroadenoma mammae	Benign

L – left; R – right; BI-RADS – Breast Imaging-Reporting and Data System; Ph – pathophysiological

Table 3. Distribution of the surgical patients according to tumor type

Tumor type	N	%
Fibroadenoma	20	74.07*
Phyllodes tumor	3	11.11
Hematoma	1	3.7
Abscess	1	3.7
Juvenile papillomatosis	1	3.7
Breast cancer	1	3.7
Σ	27	100

*$p \leq 0.05$

US examination of the contralateral breast, as well as the entire abdomen, was normal. Due to the high suspicion of malignancy on clinical and US examination, a core-needle biopsy was performed. The specimen was sent for HP examination. The result of the pathohistological finding was ductal adenocarcinoma.

Immunohistochemistry was used to evaluate the expression of estrogen (ER) and progesterone receptors (PR). The tumor was ER- and PR-negative. Considering the type of tumor, age, and negative family history, a segmental mastectomy with axillary dissection was performed. The resection margins were clean, without malignant cells.

Histopathological examination confirmed ductal carcinoma with metastases in all 22 excised lymph nodes (Figure 3). On the seventh postoperative day, the patient was discharged home and referred to the Breast Cancer Oncology Council, and chemotherapy was started. The patient was in good condition three months after the operation, but she had only one follow-up with the Pediatric Surgery Department, with no evidence of recurrence. Fifteen months after the surgery, by which time she had reached adulthood, the patient was admitted to General Surgery in a very poor condition with recurrence of the breast tumor. She died after a few days.

DISCUSSION

Breast changes are uncommon in young children and pubertal girls, but up to 3% of adolescents may develop a breast lesion before adulthood. When they occur, they are associated with tremendous stress for patients and family members.

FA is a benign lump formed by the proliferation of connective tissue stroma in the breast lobules. It accounts for 91% of all solid breast masses in girls under 19 [3, 4, 6], which completely correlates with the results of our study. Usually, these estrogen-sensitive tumors are not detected before puberty. FA can be simple or complex. Complex FA include cysts, sclerosing adenosis, and epithelial calcifications in the papillary regions. They are more common among older teenagers and adolescents with a slightly higher chance of developing breast cancer [7]. Usually, FA is clinically "silent" and manifested by accidental palpation of the tumor mass that is most often first noticed by the patient. If a clinical diagnosis of FA cannot be made, a US examination is required for additional study. It is a precise, non-invasive tool that does not require ionizing radiation exposure. FA is a fast-developing tumor that distorts the surrounding skin. The presence of the breast mass can be extremely frustrating for children and parents, and the most common reason to insist on its surgical removal. Children's breast lesions are treated differently depending on the type of lesion, its size, location, features, and whether it is benign or malignant. If FA and other benign breast lesions in children are small, asymptomatic, and not expanding, ultrasound follow-up is sufficient [8]. When benign breast lesions are growing, causing pain or discomfort, or creating cosmetic issues, surgery is necessary, favoring excisional biopsy as a minimally invasive surgical approach that can be used to remove the lesion while protecting breast tissue [9]. According to recently released American Pediatric Surgical Association guidelines, low-risk breast lesions < 5 cm that show ultrasound evidence of FA should be monitored only 6–12 months, since there is no chance of recurrence. Postoperative surveillance does not raise the risk of developing breast cancer again after complete excision [8].

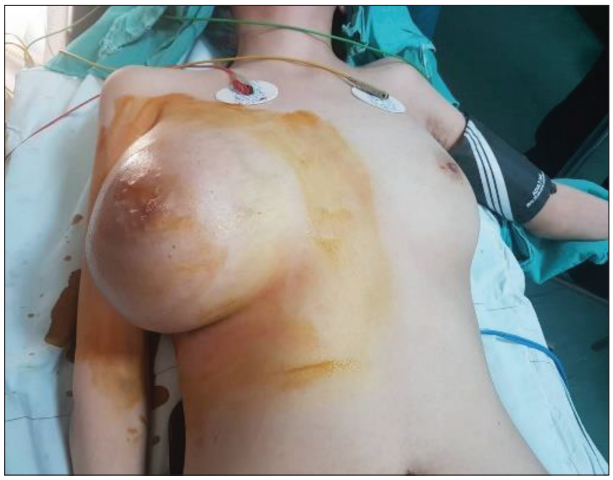

Figure 1. Clinical presentation of the breast mass in the right breast in a 16-year-old girl

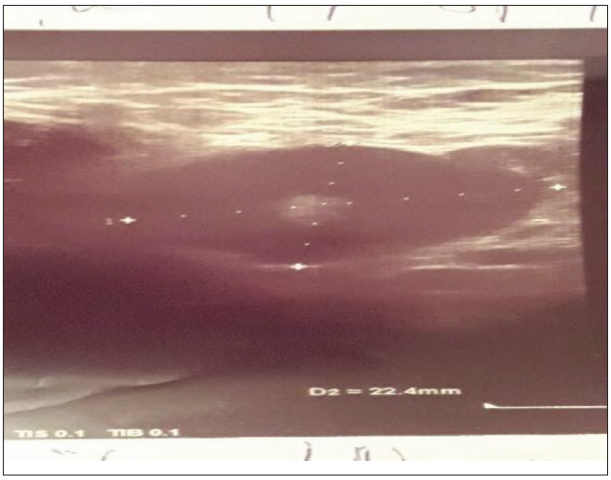

Figure 2. Ultrasound findings of the axilla – enlarged axillary lymph node

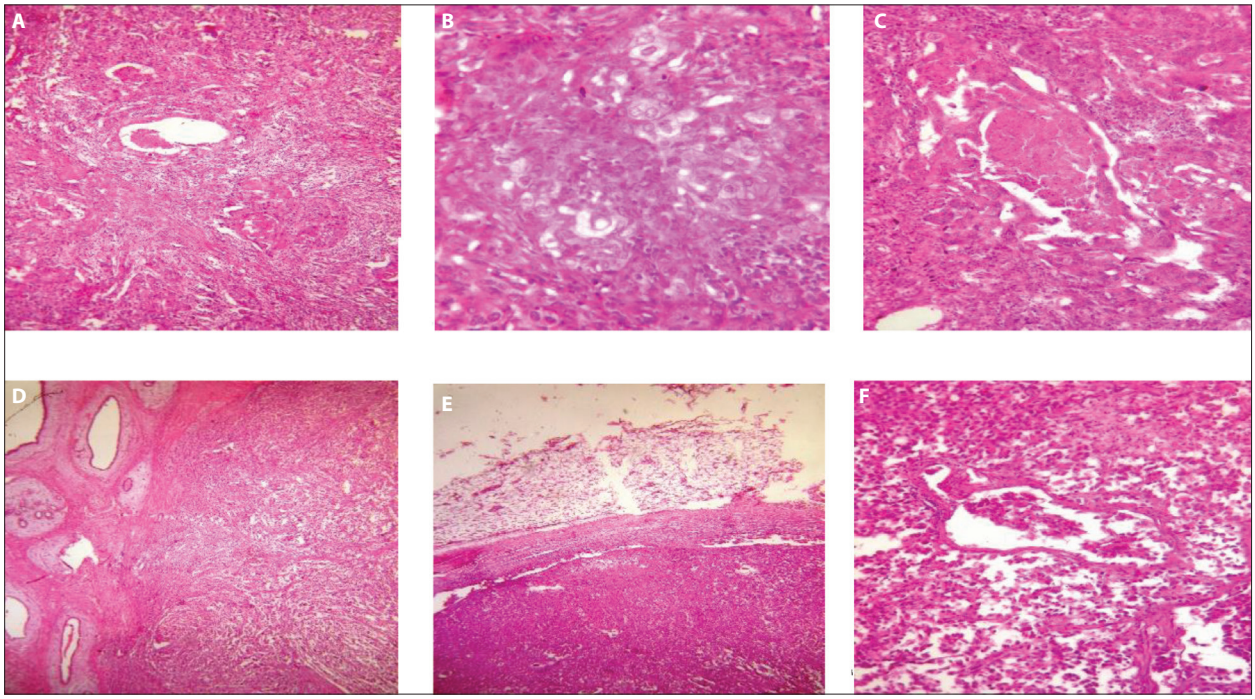

Figure 3. Histopathological findings; **A**: clusters of tumor cells in the stroma showing retraction, surrounding edema, and moderate mixed inflammatory infiltrate (H&E, 100 ×); **B**: marked pleomorphism of cells, irregular distribution of chromatin with prominent nucleoli (H&E, 200 ×); **C**: extensive zone of necrosis in the tumor parenchyma (H&E, 400 ×); **D**: border between preserved breast tissue and tumor-altered tissue (H&E, 400 ×); **E**: lymph node parenchyma almost completely replaced by metastatic tumor tissue with spread into the node capsule, without perinodal tissue invasion (H&E, 200 ×); **F**: lymphovascular invasion (H&E, 200 ×)

Primary breast cancer under the age of 18 is extremely uncommon in girls, with only cases in boys also reported in the literature [4].

Until the year 2000, Murphy et al. [11] described 38 cases of primary breast cancer in girls aged up to 19 years, while in the period 2000–2015, a total of 18 patients were recorded, of which the most common type was ductal breast cancer. The literature has proven the existence of several risk factors that influence the development of breast cancer: a strong family predisposition (diagnosed breast cancer in a close female relative, mother, daughter, or sister), genetic mutations (*BRCA1*/*BRCA2*), or earlier radiation [12, 13]. Patients who receive radiation therapy for pediatric chest cancers are more likely to get breast cancer in the future. Breast tissue is most severely damaged by radiation therapy between the ages of 10 and 16, when it is developing to its fullest. After 20 years, about 40% of girls who received radiation therapy for Hodgkin's lymphoma and thyroid cancer may develop breast cancer. It has been proven that all types of ionizing radiation, regardless of origin (both as part of radiation treatment for primary carcinoma and during wartime activities), can cause the development of secondary tumors [1, 14–17].

The most prevalent primary breast cancer in children, secretory adenocarcinoma, was found in 31 out of 39 cases (84%) [18]. Secretory adenocarcinoma is presented as a

well-defined cystic formation with a thickened capsule during ultrasound examination. The clinical characteristics of this tumor are significantly different in children compared to the adult population. Namely, the tumor shows benign clinical characteristics and prolonged growth, which, combined with the ultrasound findings, can mislead the clinician into believing it is benign [12].

Medullary and inflammatory carcinomas are far less common malignancies in children, but they are much more aggressive and have a higher mortality rate. Children with breast cancer rarely develop axillary metastases, as was the case with our patient. If axillary metastases are found, they seldom involve more than three lymph nodes in the axilla [19, 20].

Similar to adult breast pathologies, benign cysts, phyllodes tumors, sarcoma, lymphangioma, hemangioma, intraductal papilloma, fibroadenoma, abscesses, metastatic malignancy, or galactoceles in young boys are all included in the differential diagnosis for pediatric breast alterations [21, 22]. In our study, two patients had breast masses unrelated to breast epithelium and fibrous tissue. One girl had a hematoma with a US presentation consistent with BI-RADS III, while a 13-year-old girl with a breast mass on the left side presented in the US as BI-RADS II.

The diagnosis of breast cancer in children is usually delayed due to the non-specific clinical picture and the low degree of suspicion of malignancy. Therefore, medical history and clinical evaluation are essential. As the first diagnostic tool, the US provides the best image for pediatric patients. According to the Expert Consensus Recommendations of the APSA Cancer Committee, FNA is essential for pathological diagnosis, for masses larger than 3 cm in diameter, and in all suspected cases [8], although most parents insist on removing tumor alterations even if the HP diagnosis is benign [23], as was in our case series. Mammography is not a sufficiently specific or reliable method in children, due to the different composition of the breast parenchyma compared to the adult population [24]. MRI avoids ionizing radiation in children, but the effectiveness and accuracy of breast MRI assessment in children have not yet been validated [25].

Wide local excision should be the initial treatment for all breast masses in prepubescent girls, and mastectomy should only be used if and when the cancer is diagnosed or has progressed. A modified radical mastectomy, followed by radiation and chemotherapy, is required in patients with advanced cancer and axillary metastases. Additionally, a sentinel lymph node biopsy must be performed. Postoperative radiation therapy reduces the risk of local recurrences. Consideration should be given to the advantages and disadvantages of radiation and chemotherapy in the context of the tumor type and the stage of the disease [14].

A lack of required screening, more severe disease, and a delayed diagnosis may all contribute to a poor prognosis in children and adolescents. A multidisciplinary team approach is recommended in order to optimize patient care for the rare malignant lesions [9].

CONCLUSION

Breast cancer should always be considered when making a differential diagnosis of breast nodules in prepubertal girls, teenagers, and adolescents. The most common imaging modality to determine the features of breast swelling is the US. Excisional biopsy and HP evaluation are mandatory to rule out atypical but possibly malignant lesions. The primary goal of surgical treatment is the complete excision of the tumor mass with the preservation of normal breast tissue if possible. The management of breast masses and breast cancer is still controversial, based on the limited data available in the literature, and larger series are required to standardize the treatment protocol in children. A multidisciplinary team approach is recommended in order to optimize patient care for the rare malignant lesions.

Conflicts of interest: None declared.

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Туморске масе дојке код деце и адолесцената – страх од ретке али могуће дијагнозе

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САЖЕТАК

Увод/Циљ Туморске промене у дојкама су изузетно ретке код деце и адолесцената. Срећом, већина ових промена су бенигне и самолимитирајуће, иако су у литератури описани и случајеви малигних промена. У највећем проценту дијагностикује се фиброаденом, али је у свим сумњивим случајевима неопходна биопсија и хистопатолошка анализа ради искључивања малигнитета.

Циљ студије је указати на значај дијагностике и третмана туморских маса у дојкама, имајући у виду могућност развоја малигнитета у овој узрастној групи, као што је приказано у нашем случају.

Методе Анализирани су подаци 27 болесница које су хоспитализоване на Клиници и лечене у периоду од две године. Код свих болесница са болом и отоком у пределу дојке урађен је ултразвучни преглед. Класификовање промена у ткиву дојке вршено је коришћењем система радиолошке класификације промена на дојци – *Breast Imaging Reporting and Data Sistem* (*BI-RADS*).

Резултати Средња старосна доб 27 болесница износила је 15,44 ± 1,39 година. Десностране промене дијагностиковане су код 16 (59,26%) болесница, а промене класификоване као *BI-RADS* III код 18 (66,66%) болесница. Код две болеснице (7,4%) промене су одговарале стадијуму *BI-RADS* IV, а код седам (25,92%) стадијуму *BI-RADS* II. Патохистолошком анализом утврђено је да је највећа учесталост фиброаденома (20/27, 74,07%), следи бенигни филоидни тумор (3/27, 11,11%), а потом хематом, апсцес и јувенилна папиломатоза код по једне болеснице (1/27, 3,7%). Карцином дојке описан је у једном случају (3,7%).

Закључак Примарни карцином дојке је веома редак код деце и адолесцената. Ипак, увек се мора размишљати о његовом постојању у оквиру диференцијалне дијагнозе туморских маса у ткиву дојке код болесница овог узраста.

Кључне речи: туморске промене; карцином; пубертет; адолесценти

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Rhodococcus equi infections in HIV late presenters – a case series and therapeutic challenges

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SUMMARY

Introduction *Rhodococcus equi* is a rare but clinically relevant opportunistic pathogen, primarily affecting individuals with compromised cellular immunity. In people living with HIV/AIDS, it typically manifests as severe pulmonary disease. The objective of this case series is to describe the clinical features, management, and outcomes of *Rhodococcus equi* infection in three men with advanced HIV/AIDS.

Outlines of cases We retrospectively analyzed three cases of *Rhodococcus equi* infection treated between 2004 and 2011 at the Clinic for Infectious and Tropical Diseases, University Clinical Center of Serbia, in Belgrade. All patients were men with CD4 counts below 50 cells/mL at the time of presentation. Clinical symptoms included prolonged fever, productive cough, weight loss, and malaise. Pulmonary involvement was universal, with radiological findings of necrotizing pneumonia or cavitary lung abscess. One patient developed cerebritis as an extrapulmonary manifestation. *Rhodococcus equi* was isolated from sputum in all three cases and from blood cultures in two cases. All patients required prolonged hospitalization and combination antibiotic therapy, including macrolides, carbapenems, rifampicin, and trimethoprim-sulfamethoxazole, with antiretroviral therapy introduction. Two patients achieved long-term clinical stability, while one had persistently low CD4 count and detectable viral load due to adherence issues.

Conclusion *Rhodococcus equi* in patients with advanced HIV/AIDS may cause severe pulmonary or disseminated disease. Early recognition, tailored antimicrobial regimens, and careful timing of antiretroviral therapy initiation are critical to improve outcomes in this population.

Keywords: *Rhodococcus equi*; HIV; AIDS; opportunistic infections; pulmonary infection; antimicrobial therapy

INTRODUCTION

Rhodococcus equi is an aerobic, Gram-positive, partially acid-fast, non-spore-forming, facultatively intracellular, pleomorphic coccobacillus which was previously classified within the genus *Corynebacterium* [1]. Originally described as a veterinary pathogen in foals in the 1920s, *Rhodococcus equi* has since been identified in a variety of environmental reservoirs and has been implicated in zoonotic transmission [2]. Human infections are rare but have been increasingly reported among immunocompromised individuals with the capacity of causing life-threatening conditions [1]. This pathogen was reported in human for the first time in 1967, but since then has gained wider recognition during HIV/AIDS epidemics in 1980s, primarily due to its pulmonary manifestations in patients with advanced immunosuppression [3]. In people living with HIV/AIDS, *Rhodococcus equi* typically presents as a subacute or chronic pulmonary infection, often mimicking tuberculosis (TB) or *Nocardia spp*, with radiological findings that include cavitary lesions, consolidations, and abscess formation [3]. In immunocompromised hosts, pulmonary involvement occurs in up to 95% of cases, while extrapulmonary dissemination – including central nervous system (CNS) and soft tissue involvement – can occur via hematogenous spread [4]. The organism's ability to survive and replicate within macrophages contributes to its pathogenicity and complicates treatment, often requiring prolonged multidrug regimens [2]. The diagnosis is frequently delayed due to the slow growth of the organism in cultures and resemblance to diphteroids on Gram stain which is sometimes dismissed as commensal flora and leads to misidentification, contributing to underdiagnosis [3]. Moreover, treatment guidelines were developed in the pre-ART era and still pose challenges due to intrinsic resistance to many antibiotics, lack of standard regimens, and complex interactions with antiretroviral therapy (ART) [3].

CASE REPORTS

In this case series, we describe three male individuals living with HIV and advanced immunodeficiency who developed *Rhodococcus equi* infections between 2004 and 2011. We highlight their clinical presentations, microbiological findings, therapeutic management, and complications – including immune reconstitution inflammatory syndrome (IRIS) and neurologic involvement – as well as long-term outcomes. These cases emphasize the importance

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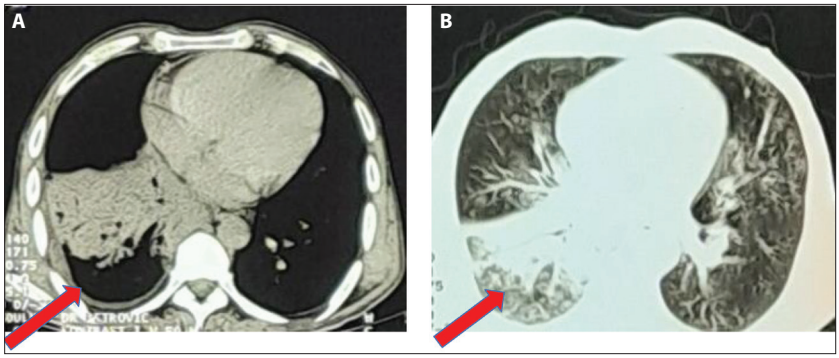

Figure 1. Case 1;
thoracic computed tomography scan: (A, B) – approximately 3 cm below the tracheal bifurcation, posteriorly on the right, involving the posterior half of the right hemithorax – an irregular, infiltrative mass with heterogeneous structure and central necrosis; in the posterior caudal supradiaphragmatic region, the pleural space is filled with fluid

Table 1. Antibiotic susceptibility testing results

Antibiotic	Case 1 Sputum	Case 1 Blood (sterile)	Case 2 Sputum	Case 2 Blood	Case 3 Sputum	Case 3 Blood
Penicillin G	R	-	R	R	R	R
Ampicillin	R	-	-	-	R	R
Amoxicillin	R	-	-	-	R	R
Amox + β-lactamase inhibitor	R	-	-	-	-	-
Piperacillin	R	-	-	R	R	R
Piperacillin-tazobactam	-	-	-	-	-	R
Cefotaxime	R	-	R	R	R	R
Ceftazidime	R	-	R	-	R	R
Cefepime	R	-	-	-	-	R
Ceftriaxone	R	-	S	S	R	R
Cefuroxime	-	-	-	R	-	-
Cefaclor	-	-	-	-	R	R
Meropenem	S	-	S	S	S	S
Imipenem	S	-	S	S	S	S
Erythromycin	S	-	S	S	S	S
Azithromycin	R	-	-	-	-	-
Clindamycin	-	-	S	R	R	R
Chloramphenicol	S	-	-	-	S	S
Vancomycin	S	-	S	S	S	S
Ciprofloxacin	S	-	S	S	S	S
Gentamicin	-	-	-	-	S	S
Amikacin	R	-	S	R	S	S
Trimethoprim-Sulfamethoxazole	R	-	S	R	S	S
Rifampicin	R	-	S	R	S	S

S – susceptible; R – resistant; - – not tested or not reported; sample types: sputum – respiratory sample; blood – blood culture result

of early recognition, tailored antimicrobial strategies, and careful timing of ART initiation in the management of *Rhodococcus equi* infections in people living with HIV.

Case 1

A 40-year-old male was diagnosed with HIV in 1994 but did not seek medical care or initiate ART until 2004, when he presented with advanced immunosuppression: CD4 cell count was cells/mm^3, viral load unavailable), reporting 12-week-long symptoms of progressive weight loss, dysphagia, productive cough, and malaise. Physical examination revealed oropharyngeal candidiasis, generalized lymphadenopathy, bilateral basal crackles, splenomegaly, seborrheic dermatitis, and hairy leukoplakia. Thoracic computed tomography (CT) scan demonstrated necrotizing pneumonia in the posterior right hemithorax (Figure 1). *Rhodococcus equi* was isolated from sputum (growth at 4–6 days) while blood cultures remained sterile (Table 1). The patient was hospitalized twice. During the first hospitalization (21 days), he received erythromycin, ciprofloxacin, trimethoprim-sulfamethoxazole, and terbinafine, resulting in partial pneumonia resolution. He was discharged afebrile and clinically stable, but ART was not initiated due to unresolved insurance status. Twenty days later, ART was started. After 15 days, the patient developed fever, respiratory symptoms, and fatigue, requiring a second hospitalization. He received dual macrolide–fluoroquinolone therapy for 53 days, with gradual improvement. Pulmonary TB was excluded, ART was temporarily interrupted and later resumed, and IRIS was suspected but not confirmed. The patient was discharged in good clinical condition. At follow-up, he remained alive, with CD4 count of 190 and suppressed viral load at the most recent check-up in 2024. The patient resided in an urban area and reported no known occupational or environmental exposure risks, nor any contact with domestic or farm animals.

Case 2

A 43-year-old male was diagnosed with HIV and *Rhodococcus equi infection* concurrently in 2004. At presentation, he was ART-naïve with profound immunosuppression (CD4: 4 cells/mm^3, viral load not available). He reported 12-week-long history of fever higher than 38°C, hemoptysis, weight loss, and malaise. Clinical examination revealed hepatomegaly and tachycardia. Imaging revealed right-sided pulmonary infiltrates. *Rhodococcus equi* was isolated both from blood cultures and sputum (Table 1). During hospitalization, the patient developed sudden-onset right-sided hemiparesis.

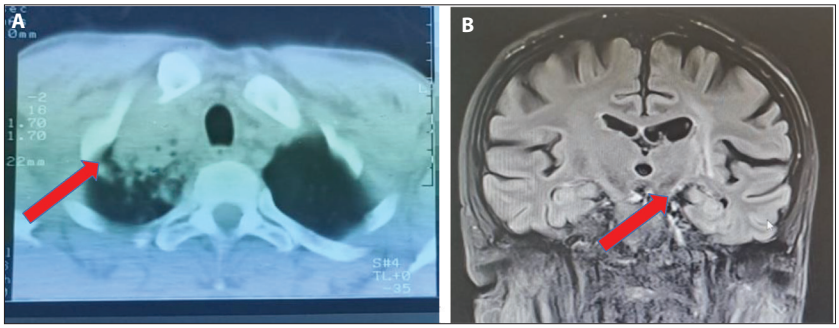

Figure 2. Case 2;
A – thoracic computed tomography: right-sided pulmonary infiltrates; B – brain magnetic resonance imaging (MRI): coronal T2-weighted fluid-attenuated inversion recovery MRI showing a hyperintense lesion in the left basal ganglia, consistent with cerebritis

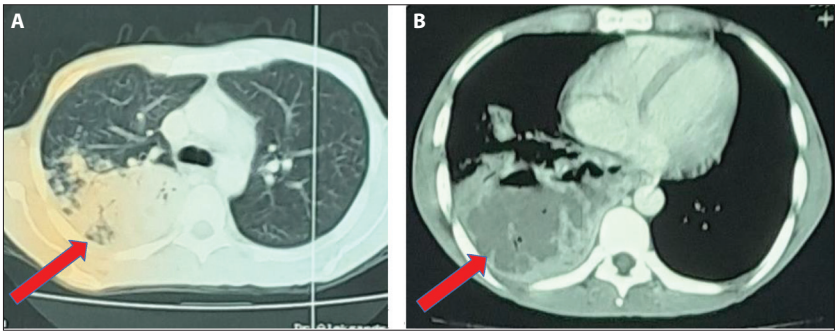

Figure 3. Case 3;
thoracic computed tomography: (A, B) – the entire lower lobe of the right lung is occupied by a large liquefied lesion containing air, measuring approximately 10 × 20 cm in cross-section – a lung abscess; a small pleural effusion is visible in the right basal pleura

Brain magnetic resonance imaging (MRI) revealed a T2-weighted imaging with a fluid-attenuated inversion recovery hyperintense lesion in the left basal ganglia, without mass effect, restricted diffusion, or contrast enhancement, findings consistent with cerebritis, presumed to result from septic embolic dissemination of *Rhodococcus equi*. Initial antimicrobial therapy consisted of erythromycin, rifampicin, and trimethoprim-sulfamethoxazole for 30 days, with modifications according to clinical response and susceptibility. ART was initiated during hospitalization. Lumbar puncture revealed normal cerebrospinal fluid (CSF) findings, and *Rhodococcus equi* was not isolated from CSF. A follow-up brain MRI was not performed, as the patient achieved complete neurological recovery without sequelae**.** At follow-up, viral load remains undetectable, and the latest CD4 count was 348 cells/mm^3. Considering socio-epidemiological data, patient resided in an urban area and reported no occupational or environmental exposure risks, although had regular contact with domestic animals (poultry, swine, cats, dogs).

Case 3

A 32-year-old male was diagnosed with HIV in 2003 and initiated ART which he discontinued in 2008. In 2011, he was presented at our Clinic due to eight weeks of progressive cough, dyspnea, diarrhea, weight loss, and fever up to 39°C. At that moment, CD4 count was 5 cells/mm^3, and HIV viral load was 138,531 copies/mL. Examination revealed tachycardia, hepatosplenomegaly, and crepitations in the right thorax. Chest CT revealed complete involvement of the lower lobe of the right lung with a massive cavitating abscess (10 × 20 cm), consistent with necrotizing infection, with air-fluid levels and associated right pleural effusion. Bilateral zones of consolidation were noted in the middle right lobe and left lingula. Both sputum and blood cultures yielded *Rhodococcus equi* (Table 1). Time to culture positivity was three days from sputum and six days from blood. Initial empiric antibiotics were escalated over time due to lack of response and complications, ultimately including meropenem, imipenem, rifampicin, trimethoprim-sulfamethoxazole, amikacin, tigecycline, and metronidazole, alongside intravenous immunoglobulins. ART was reinitiated during hospitalization, and the total duration of antibiotic therapy was 85 days. The patient required pleural drainage for two months. A pleural pneumothorax and subcutaneous emphysema developed following active drainage, complicated by secondary infection with *Pseudomonas aeruginosa* and *Klebsiella spp.* Despite multiple complications, the patient fully recovered after three months of hospitalization. The latest follow-up in 2024 showed CD4 51 cells/mm^3 and viral load was 15,500 copies/mL, emphasizing adherence issues. Similar to Case 1, the patient denied any contact with animals or known environmental exposure associated with *Rhodococcus equi* infection.

Ethics: This study was performed in line with the principles of the Declaration of Helsinki and good clinical practice. Approval was granted by the patients who signed an informed consent for participation in this case report series. Signed form available upon request.

DISCUSSION

Here we present three cases of *Rhodococcus equi* infection in people living with HIV/AIDS with severe immunosuppression, treated at the Clinic for Infectious and Tropical Diseases, University Clinical Centre of Serbia in Belgrade, between 2004 and 2011. All three are men; the youngest is 33 and the oldest is 43, all Caucasian from Serbia. Without other significant comorbidities, they have in common that they had terminal immunodeficiency caused by HIV at the time of clinical presentation in our clinic. Other data in the literature also show that *Rhodococcus equi* manifests in the stage of advanced immunodeficiency in people

living with HIV/AIDS and that it is most common in men [5]. Several case series, including an extensive review by Yamshchikov et al. [6], have shown a higher incidence of *Rhodococcus equi* infections in males, especially in people living with HIV and transplant recipients. To our knowledge, no study has systematically examined the potential behavioral, biological, or healthcare-related factors that could impact this sex distribution. Epidemiological data are significant because they relate to contact with animals that serve as the natural reservoir for *Rhodococcus equi* [7, 8]. Except for one, the other two of our patients did not have a clear epidemiological risk when it comes to contact with domestic/wild animals, occupational risk, or living in a rural environment. Regarding the clinical presentation, in addition to AIDS-indicative conditions such as oral candidiasis, seborrheic dermatitis, and oral hairy leucoplakia, our patients exhibited severe pulmonary involvement: one developed necrotizing pneumonia with pleural effusion, another presented with a lung abscess, and one patient experienced an extrapulmonary neurological manifestation of *Rhodococcus equi* infection. The most common manifestations of *Rhodococcus equi* in patients with AIDS, as described in the literature, are pulmonary [5]. Radiographic findings in our three patients – necrotizing pneumonia with pleural effusion, with/or cavitation and abscess – are consistent with common radiological features of *Rhodococcus equi* pulmonary infection in people living with HIV, as reported in the literature [5]. Although rare, extrapulmonary manifestations of *Rhodococcus equi* in humans can be very divergent, and various organ and tissue involvement has been reported in the literature, such as osteomyelitis, pericarditis, brain abscesses, spleen, kidney and liver abscesses, mesenteric lymphadenitis and colitis, among others [4, 5]. One of the patients presented in our paper had cerebritis as an extrapulmonary manifestation of *Rhodococcus equi* infection. As far as neurological manifestations of *Rhodococcus equi* infection are concerned, they are rare, with only a few cases described in the literature to date, including purulent meningitis and brain abscess [4]. The differential diagnosis in such cases is broad, covering cerebral toxoplasmosis, tuberculous or pyogenic abscess, cryptococcal or cytomegalovirus encephalitis, progressive multifocal leukoencephalopathy (PML), primary CNS lymphoma, neurosyphilis, and bacterial cerebritis. A prospective study by Sawardekar et al. [9] with 150 HIV-positive patients demonstrating CNS space-occupying lesions demonstrated that the most prevalent etiologies were tuberculomas (29.3%), toxoplasmosis (22.7%), PML (17.3%), primary CNS lymphoma (15.3%), and brain abscess (10%). The findings correspond with broader clinical experience and are supported by a review by Sheybani et al. [10], emphasizing the necessity of timely imaging and empirical therapy due to the overlapping presentations of CNS infections among people living with HIV. In such situations, empiric antimicrobial and antiparasitic therapy with adequate CNS penetration is typically necessary until a definitive diagnosis is confirmed.

IRIS can be unmasking and paradoxical and can develop during the initial period of antiretroviral treatment more often in deeply immunosuppressed individuals. Typical IRIS diagnoses include TB, cryptococcosis, and PML but practically any opportunistic infection can manifest in the context of IRIS. To the best of our knowledge, there have been no reports of *Rhodococcus* infection in the context of IRIS, as described in one of our patients.

Isolation of *Rhodococcus equi* in our three patients was predominantly based on sputum samples and blood cultures. Depending on the clinical manifestations, cultivation of samples from bronchoscopy, CSF, pleural punctate, ascitic fluid, as well as abscess aspirates, is also considered [11].

The antibiotic treatment for the patients presented in this paper consisted of trimethoprim-sulfamethoxazole, macrolides, rifampicin and carbapenems, following initial use of antimicrobial agents from other antibiotic classes. The reason for polypharmacy in these cases was mainly the use of initial empiric antibiotic therapy for patients with severe pulmonary manifestations of AIDS, which was subsequently adjusted based on culture and susceptibility results (antibiogram). *Rhodococcus equi* can show resistance to various antibiotics and can develop resistance during treatment with only one drug [2]. To date, multiple case reports and case series of *Rhodococcus equi* infection in people living with HIV/AIDS have been published, describing various antibiotic regimens used in the therapeutic approach [11].

The optimal drug regimen and duration of treatment for *Rhodococcus equi* pneumonia have not been clearly defined. For now, recommendations are usually based on two-drug regimens, according to susceptibility testing. Recommended choices usually include vancomycin, meropenem, imipenem, macrolides, rifampicin, and levofloxacin [11]. Ranganath et al. [3] reported over 95% susceptibility of *Rhodococcus equi* to imipenem, vancomycin, linezolid, rifampin, and clarithromycin. The majority of patients described in their paper received two- or three-drug combination therapy for 2–6 months with favorable clinical response, and they concluded that imipenem and vancomycin remain appropriate empiric treatment options for *Rhodococcus equi*. [3]. Torres-Tortosa et al. [12] reported susceptibility rates of *Rhodococcus equi* as high as 100% for vancomycin and amikacin, followed by 97.9% and 97.6% for rifampicin and imipenem, respectively. Less than half of isolates of *Rhodococcus equi* in the same study (44.7%) were susceptible to trimethoprim-sulfamethoxasole (co-trimoxazole) [12]. Considering the small number of cases globally and the different geographic and clinical settings in which diagnosis and treatment are carried out, it is not surprising that a standard therapy for *Rhodococcus equi* has not yet been established. Judging by the data from the literature, a clearly defined consensus has not yet been reached regarding the recommended antibiotic therapy for *Rhodococcus equi* infection, nor its duration. Prolonged use of combination antibiotic therapy, particularly regimens containing macrolides, fluoroquinolones, and rifampicin, has been associated with hepatotoxicity, QT interval prolongation, and gastrointestinal complications [13]. Among these, *Clostridioides difficile* infection (CDI) represents a

significant concern, especially in immunocompromised patients. A recent systematic review noted antibiotic exposure as the primary risk factor for CDI in diverse populations [14]. This potential complication requires awareness during extended treatment, particularly in immunocompromised individuals. In addition, rifampicin is a potent inducer of cytochrome P450 enzymes and can reduce plasma levels of several antiretroviral agents, including non-nucleoside reverse transcriptase and integrase inhibitors [15]. Adjustments to ART may therefore be necessary during concomitant use.

Rhodococcus equi is an emerging human pathogen, especially in immunocompromised individuals. Our cases complement existing literature data on *Rhodococcus equi* infections in people living with HIV, highlighting the importance of early recognition, tailored antimicrobial strategies, careful timing of ART initiation, as well as the need for standardized antibiotic therapy guidelines. Clinicians should take precautions regarding the diagnosis and treatment of *Rhodococcus equi* in people living with HIV, considering it can be clinically similar to other opportunistic infections and can be susceptible to some antimicrobial agents that are often used as first-line options in those with AIDS but often for a shorter course than what is required for successful treatment of *Rhodococcus equi*.

Conflict of interest: None declared.

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Инфекције изазване *Rhodococcus equi* код касних презентера са ХИВ инфекцијом – прикази болесникâ и терапијски изазови

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САЖЕТАК

Увод *Rhodococcus equi* је редак али клинички значајан опортунистички патоген који се првенствено јавља код особа са компромитованим ћелијским имунитетом. Код особа које живе са ХИВ-ом/сидом, *Rhodococcus equi* обично изазива тешке инфекције плућа. Циљ овог рада је да прикаже клиничке карактеристике, терапијске приступе и исходе инфекције изазване *Rhodococcus equi* код тројице мушкараца са узнапредовалом ХИВ инфекцијом.

Приказ болесникâ Ретроспективно су анализирана три случаја инфекције *Rhodococcus equi*, лечена у периоду од 2004. до 2011. године на Клиници за инфективне и тропске болести Универзитетског клиничког центра Србије у Београду. Сви пацијенти били су мушкарци, са бројем *CD4* ћелија испод 50/*μL* у тренутку пријема. Доминантне клиничке манифестације укључивале су продужену фебрилност, продуктиван кашаљ, губитак телесне масе и изражену малаксалост. Код свих је инфекција имала тешку плућну презентацију, уз радиолошке налазе типичне за некротизујућу пнеумонију или кавитационе апсцесе. Код једног пацијента регистрована је и екстрапулмонална манифестација у виду церебритиса. *Rhodococcus equi* је изолован из спутума код сва три пацијента, а из хемокултуре код два. Лечење је захтевало продужену хоспитализацију и комбиновану антимикробну терапију, укључујући макролиде, карбапенеме, рифампицин и триметоприм-сулфаметоксазол, уз антиретровирусну терапију. Два пацијента су постигла дугорочну клиничку и имунолошку стабилност, док је код једног забележен трајан пад *CD4* ћелија и перзистентна виремија услед лоше адхеренције на антиретровирусну терапију.

Закључак Инфекција изазвана *Rhodococcus equi* код особа са узнапредовалом ХИВ инфекцијом може довести до тешке плућне и дисеминоване болести. Рано препознавање, адекватна антимикробна терапија и правовремено започињање антиретровирусне терапије кључни су за повољан исход.

Кључне речи: *Rhodococcus equi*; ХИВ; сида; опортунистичке инфекције; плућна инфекција; антимикробна терапија

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Successful replantation after avulsion amputation of the thumb

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SUMMARY
Introduction Degloving injuries to the fingers, especially the thumb, present a significant challenge for reconstructive surgeons. Several classifications have been proposed to assess the extent of injury. Latest approach is now that microsurgical repair is the method of choice in all types of ischemic injuries, including type III ring avulsion.
Case outline We present a case of a successful microvascular replantation of the thumb amputated by an avulsion mechanism caused by a drill in the 19-year-old male. The thumb was almost completely degloved about 2 cm distal to the metacarpophalangeal joint. According to the Urbaniak classification, it was a type III degree of injury (Kay type IV). Surgery was performed three hours after the injury. The ulnar digital artery was anastomosed end-to-end. Two dorsal veins were drained and anastomosed. After six months, the appearance of the replanted finger was aesthetically pleasing. The patient was followed up again 30 months after surgery. Grip strength measured with Jamar's dynamometer is 5% less compared to the uninjured hand. According to the total arc of motion scale, almost full range of motion was obtained in the metacarpophalangeal joint with full opposition and good grip. According to the Medical Research Council scale, the sensitive recovery has reached S3. The disabilities of the arm, shoulder and hand score is 0 point. This represents a complete and unlimited function even though the thumb is contracted in the interphalangeal joint.
Conclusion The patient returned to his previous workplace and he remains satisfied with the function of the replanted thumb for daily and work activities.
Keywords: avulsion amputation; replantation; hand

INTRODUCTION

Degloving injuries to the fingers, especially the thumb, present a significant challenge for reconstructive surgeons, as it is extremely difficult to revascularize the amputated finger and restore its function and appearance. Such injuries can range from soft tissue injury to complete amputation [1]. Several classifications have been proposed to assess the extent of injury. Urbaniak's [2] classification is widely accepted. According to this classification, type I represents a circumferential laceration of the skin, without damage to the circulation. Type II represents incomplete annular avulsion, with impairment of arterial or venous blood flow and requires repair of the artery or vein, while type III represents complete avulsion. Urbaniak's classification later had two modifications. Kay et al. [3] expanded the classification into four categories and Sturzenegger et al. [4] proposed a modification based on the level of injury to the neurovascular elements [5, 6] (Figure 1).

Urbaniak Classification

Class	*Description*	*Treatment*
Class I	Circulation adequate	Standard bone and soft tissue care
Class II	Circulation inadequate	Vessel repair
Class III	Complete degloving or complete amputation	Amputation

Kay, Werntz and Wolff Classification

Class	*Description*	*Treatment*
Class I	Circulation adequate	Standard bone and soft tissue repair
Class II	Arterial compromise only	Vessel repair
Class III	Inadequate circulation with bone, tendon, or nerve injury.	Amputation
Class IV	Complete degloving or complete amputation.	Amputation

Figure 1. Urbaniak and Kay classification

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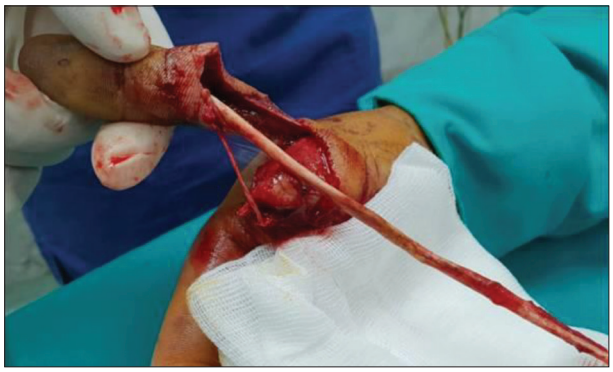

Figure 2. Finger injury

According to the referred classification, in type III avulsions, replantation is not indicated and some authors prefer primary amputation or ray resection [2, 3, 4]. On the other hand, type II is considered an absolute indication for microvascular repair, because almost complete function of the finger can be expected [5]. Latest approach is now that microsurgical repair is the method of choice in all types of ischemic injuries, including type III ring avulsion [7, 8, 9].

We present a case of a successful microvascular replantation of the thumb amputated by an avulsion mechanism caused by a drill.

CASE REPORT

A 19-year-old male, a mechanic by trade, injured his thumb while on the job when a drill caught his glove. The thumb was almost completely torn off, degloved about 2 cm distal to the metacarpophalangeal joint. According to the Urbaniak classification, it was a type III degree of injury (Kay type IV). The amputated part was in contact via a 3 mm wide skin flap and contained part of the proximal and the entire distal phalanx, skin, subcutaneous tissue, and nail. The long flexor of the thumb was completely torn from the forearm along with the muscular part. He had signs of a major soft tissue contusion. The proximal phalanx, tendons of the thumb and part of the thenar muscle were exposed. Both severed neurovascular pedicles were distended with significant elements of contusion (Figure 2). The proximal phalanx of the thumb had a comminuted fracture with a bony defect (Figure 3).

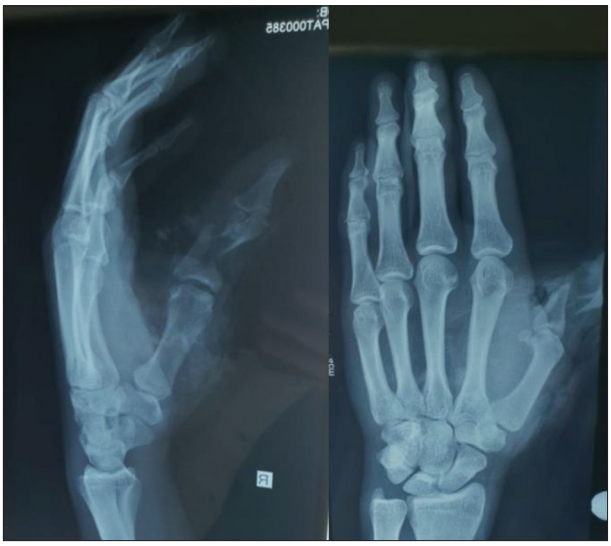

Figure 3. Preoperative X-ray scans of the affected hand and finger

The surgery was performed three hours after the injury. The operation was done under regional anesthesia. After copious irrigation, a careful excision of the contused edges of the wound was performed. Then the preparation of neurovascular structures was done under a microscope. It was decided to attempt microvascular reconstruction when the ulnar digital artery was observed to function well enough for successful microvascular anastomosis. The proximal phalanx was repositioned and fixed with two 1.5 mm Kirschner pins (Figure 4). The ulnar digital artery was anastomosed end-to-end with a 9-0 nylon suture. After removing the microvascular clip, the replanted thumb received circulation. Two dorsal veins were drained and anastomosed with 10-0 nylon thread. The digital artery on the radial side had severe elements of tearing, so its reconstruction was not possible. Both digital nerves were sutured, but both were severely damaged and distended (Figure 5). The surgery took three hours, and the duration of ischemia was around five hours. After the operation, the patient was given anticoagulant, antiplatelet, and triple antibiotic therapy, as well as antitetanus protection. Diuresis was monitored, blood counts were checked, and the circulation of the replanted

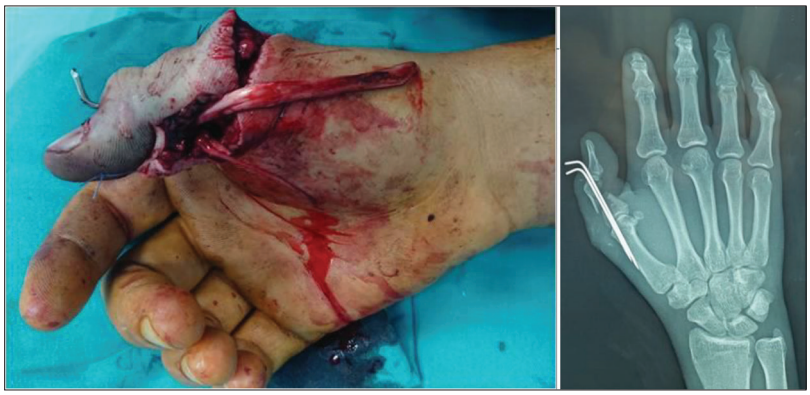

Figure 4. Finger and X-ray with K-needles

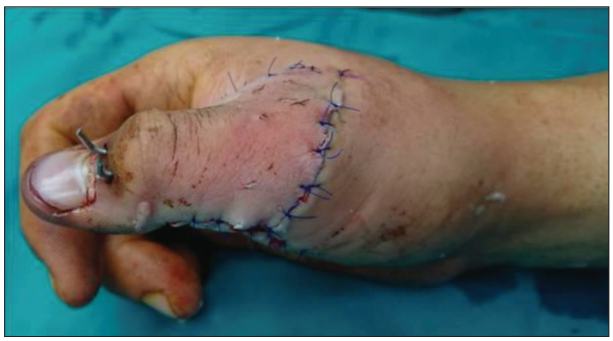

Figure 5. Finger immediately after surgery

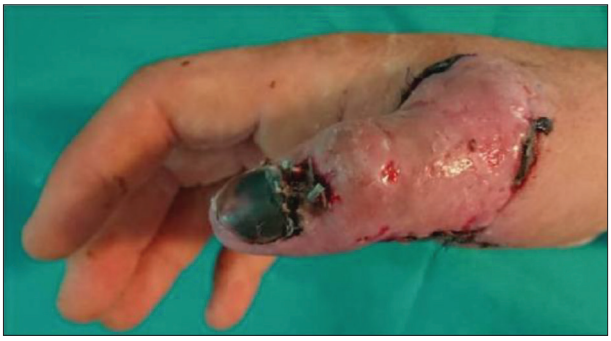

Figure 6. Thumb without K-needles

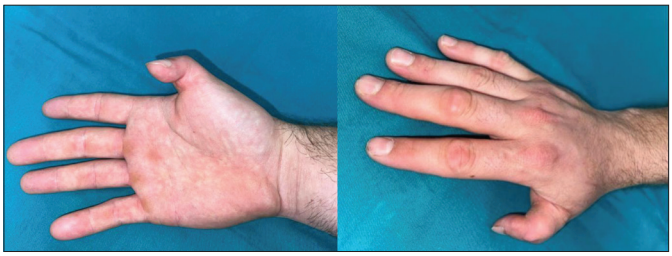

Figure 7. Final results

finger was observed frequently. The wound healed without infection. The sutures were removed after two weeks, and the patient was discharged from the hospital three weeks after surgery. The Kirschner pins were removed ten weeks after surgery (Figure 6). After six months, the appearance of the replanted finger was aesthetically pleasing.

The patient was followed up again 30 months after surgery.

The nail was growing, but the sensation in the thumb was poor. Grip strength measured with Jamar's dynamometer is 5% less compared to the uninjured hand.

The range of motion was measured according to the total arc of motion (TAM) scale and almost full range of motion was obtained in the metacarpophalangeal joint with full opposition and good grip. The interphalangeal joint remained contracted in the physiological position. The patient returned to his previous workplace and he remains satisfied with the function of the replanted thumb for daily and work activities. (Figure 7). According to the Medical Research Council scale, the sensitive recovery has reached S3. The disabilities of the arm, shoulder and hand score is 0 point. This represents a complete and unlimited function even though the thumb is contracted in the interphalangeal joint.

Ethics: All procedures performed in the studies were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Written consent to publish all shown material was obtained from the patient.

DISCUSSION

Avulsion amputations are most often caused by rotating machinery, transmission belts for transport or traction or a ring when a classic ring avulsion occurs. Digital nerves and blood vessels are often severed beyond the level of the skin wound [10, 11, 12]. The amputated part usually has additional contusions, which can also damage the capillary network [13]. The microsurgeon must carefully assess the degree of soft tissue contusion and the degree of blood vessel damage [14]. The presented case is classified as a complete Urbaniak III or Kay IV amputation and as such is an indication for amputation according to earlier recommendations. According to the new standards, it is recommended that an arterial anastomosis is to be performed first, so that the surgeon can assess the ability of the repaired artery to supply blood to the finger. If the blood flow through the soft tissue is not satisfactory, it indicates that the blood vessel and capillary network have suffered severe injury. This order avoids long-term replantation attempts in futile cases [15, 16]. A method for identifying the dorsal veins is to look for a spot of bleeding or coagulum at the dorsal edge of the amputated part, after the arterial anastomoses have been completed. Venous bleeding suggests that satisfactory venous flow can be maintained after replantation. If no veins are found or the veins are too damaged to have adequate venous flow, a vein graft can be taken [17, 18]. Medical leeches can be used for venous congestion in the postoperative period [19]. In their work, Kurata et al. [20] showed that the artery is more important than the vein in final survival. Microvascular replantation of degloved fingers has functional and aesthetic advantages over alternative reconstructive techniques and the authors recommend that replantation efforts should be pursued. However, the probability of achieving successful replantation is much lower than with straight amputation and according to the authors, in 83% of cases patients with type III avulsion require additional surgery [21]. When vessel damage is such that an anastomosis is impossible, alternative reconstructive options are considered. One option is a vascularized skin flap of the first dorsal metacarpal artery arising from the radial artery just distal to the extensor pollicis longus tendon [22, 23]. Preservation of the thumb can also be achieved with a reverse radial skin flap from the forearm, which is "wrapped" around the completely degloved finger and thus a high-quality skin cover is obtained [24]. Another alternative method is the pedicled abdominal flap, but due to the patient's discomfort, the need for subsequent flap separation, the impossibility of elevating the injured hand and the length of time this method is more often used in complex injuries involving the entire hand [25, 26, 27]. Patients should be informed preoperatively about all options and that replantation may not be technically possible and all alternative methods of reconstruction should be considered during surgery.

Treatment results are good in type I and II injuries, but in type III injuries, where there is severe damage to neurovascular structures, successful replantation can be done in very rare cases. Other adequate solutions are resorted to in order to preserve the length of the finger.

Conflict of interest: None declared.

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Успешна реплантација након авулзионе ампутације прста

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САЖЕТАК

Увод *Degloving* повреде прстију, посебно палца, представљају значајан изазов за реконструктивног хирурга. Предложено је неколико класификација како би се проценио обим повреде.

Савремени ставови указују да је микрохируршка репарација метода избора код свих типова исхемијских повреда, укључујући и тип III *ring avulsion* повреда.

Приказ болесника Приказујемо случај успешне микроваскуларне реплантације ампутираног палца услед авулзионе повреде задобијене бушилицом. Деветнаестогодишњи мушкарац, механичар по занимању, задобио је повреду палца на радном месту када је бушилица закачила његову рукавицу. Палац је био скоро потпуно откинут, дегловиран, око 2 *cm* дистално од метакарпофалангеалног зглоба. Према Урбаниак класификацији радило се о повреди типа III (*Kay* тип IV). Пацијент је оперисан три сата након повреде. Улнарна дигитална артерија анастомозирана је *end-to-end* анастомозом. Након уклањања микроваскуларне клеме, реплантирани палац је добио циркулацију. Дренирале су га две аностомозиране дорзалне вене. Након шест месеци изглед реплантираног прста био је естетски задовољавајући. Пацијент је дошао на контролу 30 месеци након операције. Снага стиска мерена Јамаровим динамометром износила је 5% мање у односу на неповређену руку. Обим покрета мерен је према скали *Total arc of motion* и добијен је скоро пун обим покрета у метакарпофалангеалном зглобу са потпуном опозицијом и добрим хватом. Према скали *MRC* (*Medical Research Council* – Савет за медицинска истраживања) сензитивни опоравак достигао је ниво *S3*. Скор на упитнику о инвалидитету руке, рамена и шаке (*DASH*) износио је 0 поена. Постигнута је одлична функција иако је палац остао у контрактури у интерфалнгеалном зглобу.

Закључак Пацијент се вратио на претходно радно место и задовољан је функцијом реплантираног палца за свакодневне и радне активности.

Кључне речи: авулзиона ампутација; реплантација; шака

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Challenges in the treatment of hypotension in an extremely preterm neonate

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SUMMARY
Introduction Hypotension is often seen in preterm neonates, as a result of various factors such as immature myocardium, transitional circulation, perinatal hypoxia, positive-pressure ventilation, and relative adrenal insufficiency. The leading causes of hypotension in preterm neonates are sepsis and septic shock, patent ductus arteriosus (PDA), and necrotizing enterocolitis.
Case outline The female preterm neonate was delivered at $24^{1/7}$ weeks of gestation with a birth weight of 710 g and an Apgar score of 3 in the first minute after birth. Hypotension was noted from the first day of life, so dopamine was administered. The neonate was admitted to the Institute of Neonatology on the fourth day of life and we continued dopamine and dobutamine. Despite increasing the dose, the hypotension remained persistent, and we started epinephrine. Echocardiography showed PDA and signs of heart failure. The neonate's condition was complicated by acute renal injury. Therefore, ibuprofen was not used to close the PDA, so we continued its conservative treatment. In order to correct the hypotension, hydrocortisone was added to the therapy. Despite conservative treatment of the PDA, hypotension was persistent and severe, and its treatment lasted for four weeks of hospitalization. After achieving hemodynamic and respiratory stability, surgical ligation of the PDA was performed.
Conclusion Hypotension is a complication in the early neonatal period in an extremely preterm neonate. Knowing the cause, as well as the pathophysiology, allows for the selection of the appropriate drug and proper treatment of hypotension in preterm neonates, which is very challenging for neonatologists.
Keywords: premature newborn; ductus arteriosus; hypotension; cardiovascular agents

INTRODUCTION

Hypotension is often encountered in preterm neonates. Various factors are associated with hypotension in preterm neonates such as immature myocardium, transitional circulation, perinatal hypoxia, positive-pressure ventilation (PPV) after birth and transient or relative adrenal insufficiency. The leading causes of hypotension in preterm neonates are sepsis and septic shock, patent ductus arteriosus PDA and necrotizing enterocolitis [1, 2, 3]. Patent ductus arteriosus is a common cause of low diastolic blood pressure (BP) as a result of the diastolic outflow of blood into the pulmonary circulation from the left-to-right (L–R) shunt, which makes it difficult to treat [4]. Therefore, pharmacotherapy should be adjusted according to the pathophysiology of the condition that caused hypotension [1, 5, 6]. We report a case of an extremely preterm neonate with severe and persistent hypotension caused by hemodynamically significant PDA.

CASE REPORT

The preterm female neonate was delivered vaginally at $24^{1/7}$ weeks of gestation with a birth weight of 710 g and an Apgar score of 3 in the first minute after birth. The pregnancy was properly controlled, complicated by the mother's vaginal bleeding one month before delivery. There was no infection during pregnancy. Immediately after birth, the neonate was intubated. In the Neonatal Intensive Care Unit (NICU) in the Maternity Hospital, the neonate was placed on conventional mechanical ventilation (MV) and surfactant was administered. The neonate received dual antibiotic therapy (ampicillin and gentamicin) and total parenteral nutrition. In order to stimulate breathing, a preparation of methylxanthine, caffeine, was used. Because of hypotension, the neonate received a fluid bolus and during the first three days of life (DOL), inotropic support with dopamine was applied. Also, the neonate received a red blood cell transfusion in order to correct anemia.

The neonate was admitted to the Institute of Neonatology on the fourth DOL, in severe condition, orotracheally intubated, with a weight of 590 g, heart rate (HR) of 126 beats/min, BP of 61/28/41 mmHg and respiratory rate of 52 breaths/min. Oxygen saturation (SpO_2) was 94% on MV with a fraction of inspired oxygen (FiO_2) of 0.4. In our NICU, conventional MV was continued. Blood gas analysis showed metabolic acidosis. Chest X-ray showed signs of respiratory distress syndrome with moderate cardiomegaly. In the following hours after admission to the Institute, hypotension, predominantly diastolic hypotension, and oliguria were noted in the neonate, so inotropic support with dopamine and dobutamine in minimal

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doses was started. At the same time, cardiac auscultation was normal, without heart murmurs. Despite increasing the dose of dopamine and dobutamine, the hypotension was persistent (minimal diastolic BP was 10 mmHg), so on the second day of hospitalization, the administration of epinephrine was started. Echocardiography showed PDA measuring 2 mm with a L–R shunt of blood flow, as well as signs of heart failure. There was massive regurgitation of both AV valves. Also, patent foramen ovale was present, with an L–R shunt of blood flow. Because of acute kidney injury (stage 3 KDIGO classification) and green gastric residuals, ibuprofen was not used to close PDA. Restriction of total fluid intake (120 ml/kg per day) was applied. In accordance with BP values and diuresis, the dose of epinephrine was gradually increased to the maximum. After a short phase of maintaining stable BP values and diuresis at maximum doses of epinephrine, a gradual reduction in the dose of epinephrine was started. Diastolic hypotension then occurred again, so at the end of the first week of hospitalization, in addition to epinephrine, hydrocortisone was introduced for the therapy of hypotension. In the following days, the doses of epinephrine and hydrocortisone were gradually titrated. Control echocardiography was performed four days after the first examination and the findings showed no significant changes. Because of heart failure, intermittent administration of loop diuretics such as furosemide was started. In order to treat hypotension and stimulate diuresis, the neonate still required very high doses of inotropes and hydrocortisone. Control echocardiography findings were without significant changes. At the end of the third week of hospitalization, we began gradually reducing the doses of inotropes based on BP values and diuresis. In order to maintain normal BP values, hydrocortisone was administered until the end of the fourth week of hospitalization in minimal daily doses. BP values, as well as the inotropic and/or vasopressor therapy applied in our case, are shown in Table 1. When the neonate was hemodynamically stabilized, a cardiosurgical evaluation was performed. Surgical ligation of PDA was performed on the 43rd DOL without complications.

The neonate was on conventional MV for 46 days, then on non-invasive respiratory support (nasal continuous positive airway pressure) for 10 days and further on oxygen therapy until the age of two months (corrected 36 weeks of gestation). Sepsis screening was positive for late-onset sepsis for *Staphylococcus haemolyticus*. The head ultrasound on the seventh DOL showed cerebellar hemorrhage. During a control head ultrasound, a gradual reduction of cerebellar hemorrhage was observed. Because of neonatal seizures, the neonate was treated with phenobarbital and midazolam. Electroencephalography (EEG) showed irregularity and depression of basic activity with epileptic discharges. On control EEG examinations, there were no epileptic discharges, the neonate had no neonatal seizures, so she was discharged home without antiseizure medications. During hospitalization, the neonate received five red blood cell transfusions and one platelet transfusion. Minimal enteral nutrition was started at the end of the second week of life. Full enteral nutrition was achieved in the 6th week of hospitalization. At the age of two months and 10 days, due to retinopathy of prematurity (ROP) intravitreal application of anti-VEGF was performed. The neonate was discharged home in the fourth month of life (corrected 40 weeks of gestation) with a weight of 2700 g (less than the 10th percentile for the gestational age).

Ethics: The manuscript has been written in accordance with the ethical standards of the Declaration of Helsinki. The publication of the data in the manuscript was approved by the Ethics Committee of the Institute of Neonatology (No. 2398/3).

DISCUSSION

After birth, there are complex and sudden changes that mostly affect the cardiovascular and respiratory system. In healthy full-term neonates, rapid closure of the ductus arteriosus occurs at birth, which is one of the key changes during the transition from fetal to neonatal circulation. However, in preterm neonates, constriction and permanent closure of the ductus arteriosus at birth are delayed, which may be associated with neonatal morbidity and mortality. The risk for PDA is inversely proportional to the gestational age. It is estimated that about 87% of neonates born at 24 weeks of gestation have a symptomatic PDA [3, 7, 8].

Hypotension is especially common in preterm neonates during the first 72 hours of life, as a result of large transient adaptive changes in the respiratory, cardiovascular and neuroendocrine systems. There is wide variation in the definition of hypotension in preterm neonates. One of the most common definitions of hypotension in preterm

Table 1. Blood pressure values and inotropic and/or vasopressor therapy in our patient

First week of hospitalization	BP	37/12	37/11	43/15	42/15	32/10	41/16	40/17
	Th	dop + dob	dop + dob	epi	epi	epi	epi	epi + hydr
Second week of hospitalization	BP	36/17	46/20	58/22	37/18	46/23	49/26	44/15
	Th	epi + hydr	epi + hydr	epi + hydr	epi + hydr	epi + hydr	epi + hydr	dop + dob + hydr
Third week of hospitalization	BP	42/19	50/25	63/32	56/21	44/22	51/29	43/16
	Th	dop + dob + hydr	dop + dob + hydr	dop + dob + hydr	dop + dob + hydr	dop + dob + hydr	dop + dob + hydr	dop + dob + hydr
Fourth week of hospitalization	BP	56/20	52/30	56/24	51/20	59/27	64/35	64/34
	Th	dop + dob + hydr	dop + dob + hydr	dop + dob + hydr	dop + dob + hydr	hydr	hydr	

BP – blood pressure; Th – therapy; dop – dopamine; dob – dobutamine; epi – epinephrine; hydr – hydrocortisone

neonates used in clinical practice is given by the British Association of Perinatal Medicine. According to this association, hypotension is defined as a value of mean BP that is less than the weeks of gestation. According to another definition, hypotension in preterm neonates is defined as mean BP below the 5th or 10th percentile for the weeks of gestation and postnatal age. Also, hypotension in preterm neonates is defined as a mean BP value below 30 mmHg. This definition is particularly useful for neonates after 72 hours of life [1, 6]. Hypotension associated with PDA can be challenging and difficult to treat [4, 9].

Preterm neonates are usually treated for hypotension in the first 24 hours of life. Batton et al. [10] in a prospective observational study showed that 55% of extremely preterm neonates received therapy for hypotension and 28% received a vasoactive medication. Our patient was an extremely preterm female neonate and had hypotension within 24 hours of life, so she received a fluid bolus and dopamine in the Maternity Hospital. The first-line therapy for hypotension in preterm neonates in many NICUs is the administration of a fluid bolus followed by inotropes such as dopamine. However, in neonates with PDA and heart failure a fluid bolus in hypotension therapy might be harmful. Also, dopamine, although it increases BP, can have a negative effect on cerebral perfusion and cerebral autoregulation in very low birth weight neonates (birth weight < 1500 g), which can increase the risk of intraventricular hemorrhage [6, 9, 11].

In accordance with the guidelines and recommendations, if hypotension is caused by myocardial dysfunction, the first-line therapy is dobutamine, which achieves its effect by stimulating β-1 adrenergic receptors [12]. Dobutamine increases myocardial contractility, HR, right and left ventricular output and mean BP. As a second-line therapy, we should consider dopamine, whose effect on BP and different organs is dose-dependent. In doses that are most often used in NICUs, 3–10 μg/kg/min, by stimulating adrenergic and dopaminergic receptors, dopamine increases HR, myocardial contractility and BP [1, 6].

Epinephrine is the third-line therapy for severe and persistent hypotension caused by myocardial dysfunction. Its hemodynamic effects, achieved through alpha and beta adrenergic receptors, are dose-dependent. In low doses (0.01–0.1) μg/kg/min epinephrine stimulates the α-2, β-1, and β-2 adrenergic receptors, which causes peripheral vasodilatation, increased myocardial contractility and HR, and finally increases BP. At higher doses (> 0.1) μg/kg/min epinephrine achieves effects through α-1 adrenergic receptors whose activation causes peripheral vasoconstriction and increased HR [1, 6, 12]. Also, corticosteroids, such as hydrocortisone, are used as adjunctive or rescue therapy for refractory hypotension in preterm neonates, i.e., neonates who are hypotensive despite the administration of two inotropes. Their short-term use seems safe; however, a question is raised about their impact on neurodevelopmental outcome in preterm neonates [13, 14].

In addition to dopamine, dobutamine and epinephrine, norepinephrine is also used in the therapy of hypotension in neonates. Norepinephrine is an endogenous sympathomimetic that is a potent alpha-1 agonist, with moderate to weak effects on beta-1 and beta-2 adrenergic receptors. Because the effect on β-2 adrenergic receptors is minimal, norepinephrine has combined inotropic and peripheral vasoconstrictive effects. Norepinephrine is recommended as first-line treatment for septic shock in adults, children, and full-term neonates [15]. It is used in less than 5% of preterm neonates with shock as second- or third-line therapy [16, 17]. Preterm neonates have been shown to have higher levels of catecholamines, which may account for a weaker response to norepinephrine [18]. Although the effect of norepinephrine on BP in preterm and full-term neonates with septic shock, pulmonary hypertension or isolated systemic hypotension has been confirmed in several retrospective studies with significant improvement in diuresis, arterial blood gas values and tissue perfusion, in clinical practice it is traditionally used as second- or third-line therapy or as an adjuvant anti-hypotensive drug [19].

Milrinone is a type III phosphodiesterase inhibitor that acts on the myocardium by increasing intracellular concentrations of cyclic AMP and calcium by inhibiting the breakdown of cAMP. It has positive inotropic and lusitropic effects on the myocardium, while reducing systemic and pulmonary vascular resistance [20]. Clinical studies on the use of milrinone in neonates have mainly focused on the treatment of persistent pulmonary hypertension of the newborn, postoperative use after corrective cardiac surgery, and prevention and treatment of low left ventricular output syndrome after PDA ligation. However, the use of this drug also carries a risk of severe hypotension [21, 22].

Vasopressin is also used in the treatment of hypotension. It is synthesized as a prohormone, primarily in hypothalamic neurons. It plays a key role in the control of BP, osmotic balance, kidney function and sodium homeostasis. More recent clinical studies have confirmed the efficacy of vasopressin as rescue therapy in neonates with persistent pulmonary hypertension of the newborn and catecholamine-refractory hypotension [23].

Levosimendan, a pyridazinone-dinitrile derivative, is a calcium sensitizer with positive inotropic and vasodilatory properties. It achieves its inotropic effect by selectively binding to cardiac troponin, improving myocardial contractility by increasing the sensitivity of contractile myofilaments to intracellular calcium. Data on the use of levosimendan in neonates and children are limited. Relevant systematic reviews have reported good effects on cardiac functions, but there is no significant difference in hemodynamic effects compared to standard inotropic therapy (including dopamine, dobutamine and milrinone). Some studies have shown a potentially positive effect on cardiovascular function in preterm neonates with pulmonary hypertension and its safety. Although in some studies it has shown promising effects in improving hemodynamics in neonates, its specific use for PDA-induced hypotension is not well established. Additional research is needed to determine its effectiveness and safety in the treatment of hypotension associated with PDA. For now, levosimendan remains an innovative therapeutic option for the treatment of severe cardiac dysfunction and pulmonary hypertension in preterm neonates [24, 25].

Clinical and laboratory findings in our case showed hemodynamically significant PDA, which was also confirmed by echocardiographic examination. There were contraindications for the use of ibuprofen in order to close the PDA. At that time, acetaminophen was not introduced in the Institute as a therapy for closing the PDA, so conservative treatment was applied. First of all, along with the previously started antihypotension therapy, fluid restriction was implemented with optimal ventilation, i.e., increasing positive end-expiratory pressure. Hemodynamic assessment of the patient was performed daily based on the clinical picture, blood gas analysis, serum lactate concentration, BP values and diuresis, with occasional echocardiographic examinations. Despite early recognition and conservative treatment of the PDA in our case, hypotension was persistent and severe, and its treatment lasted for four weeks of hospitalization. There was no effect on closing the PDA by conservative treatment, so after achieving hemodynamic and respiratory stability, surgical ligation of the PDA was performed.

With advances in technology and neonatal intensive therapy and care, the survival of preterm neonates, especially extremely preterm neonates, has increased. Along with their survival, as a consequence of immaturity, neonatologists are increasingly encountering complications of premature birth in the early neonatal period, among which is hypotension. Knowing the cause, as well as the pathophysiology, allows the selection of the appropriate drug and proper treatment of hypotension in preterm neonates. In order to reduce the adverse effects of drugs, a gradual titration of the dose is required along with monitoring of the hemodynamic state of the neonate.

Conflict of interest: None declared.

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Изазови у лечењу хипотензије код екстремно незрелог новорођенчета

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САЖЕТАК

Увод Хипотензија је честа код претерминске новорођенчади и последица је различитих фактора, као што су незрели миокард, транзиторна циркулација, перинатална хипоксија, вентилација позитивним притиском и релативна адренална инсуфицијенција. Водећи узроци хипотензије код претерминске новорођенчади су сепса и септични шок, отворен артеријски канал и некротизујући ентероколитис.

Приказ болесника Женско претерминско новорођенче рођено је у $24^{1/7}$ недељи гестације, са телесном масом на рођењу од 710 *g* и Апгар скором 3 у првом минуту после рођења. Хипотензија је регистрована од првог дана живота, па је примењиван допамин. Новорођенче је примљено у Институт за неонатологију у четвртом дану живота и настављена је терапија допамином и добутамином. Упркос повећању дозе, хипотензија је и даље била упорна, због чега је примењен адреналин. Ехокардиографски преглед је показао постојање отвореног артеријског канала и знакова срчане инсуфицијенције. Стање новорођенчета било је компликовано акутним оштећењем бубрега. Због тога ибупрофен није коришћен у затварању отвореног артеријског канала, па је настављено његово конзервативно лечење. У циљу корекције хипотензије у терапију је додат хидрокортизон. Упркос конзервативном лечењу отвореног артеријског канала, хипотензија је била упорна и тешка, а њена терапија је трајала током четири недеље хоспитализације. Након постизања хемодинамске и респираторне стабилности, урађена је хируршка лигација отвореног артеријског канала.

Закључак Хипотензија је компликација у раном неонаталном периоду код екстремно незреле новорођенчади. Познавање узрока, као и патофизиологије, омогућава одабир одговарајућег лека и правилно лечење хипотензије код претерминске новорођенчади, што је за неонатологе веома изазовно.

Кључне речи: претерминско новорођенче; артеријски канал; хипотензија; кардиоваскуларни агенси

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Analysis of treatment options for Merkel cell carcinoma of the eyelid

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SUMMARY

Introduction Merkel cell carcinoma (MCC) is a clinically rare primary neuroendocrine carcinoma of the skin, which is more prevalent in the head and neck but rare in the eyelid. In clinical practice, it is characterized by a high misdiagnosis rate, high degree of malignancy and extremely poor prognosis. At present, there is no matured and effective treatment plan for MCC.

Case outline This case study retrospectively reports a patient experiencing MCC of the eyelid in Tangshan Ophthalmology Hospital. One month after tumor resection, the patient experienced cervical lymph node spread on the same side. Following chemotherapy, no abnormal lesions were found during a follow-up of two years and three months.

Conclusion The case study demonstrates that MCC is diagnosed mainly based on pathological examination and treated with surgical resection as the preferred option. In addition, postoperative adjuvant systemic chemotherapy and local radiotherapy have an inhibitory effect on the disease's metastasis and recurrence. Immunotherapy and molecular targeted drugs are the new development trends.

Keywords: Merkel cell carcinoma; malignant tumor; eyelid; surgery combined with chemoradiotherapy

INTRODUCTION

Merkel cell carcinoma (MCC) is a rare primary neuroendocrine carcinoma of the skin [1]. Malignant neuroendocrine tumors of the skin originating from epidermal stem cells are more prevalent in areas exposed to sunlight, mainly occurring in the head, neck, and limbs of the elderly. In one study, head and neck cases accounted for 50%, with unique ultrastructural changes and immunohistochemical staining characteristics [1]. Immunohistochemistry often shows chromogranin A (CgA [+]) and synapsin (Syn [+]). A primary lesion located in the eyelid region is rare (5–10% of cases), and may easily lead to misdiagnosis [2, 3]. To enhance the understanding of MCC, this case study reports a patient experiencing MCC of the eyelid, including her clinical manifestations and treatment, as well as a review of the relevant literature.

CASE REPORT

Case data

A 62-year-old female patient was admitted to Tangshan Ophthalmology Hospital with a protuberant mass on the left upper eyelid. Ophthalmic examination showed a purplish-red mass-like protrusion of 5 × 5 × 3 mm (Figure 1A). Fundus examination revealed transparent cornea, clear anterior chamber, normal depth, turbid lens, and no obvious congestion on the surface of the palpebral conjunctiva or obvious abnormalities. One month after local hot compress and application of erythromycin eye ointment, the mass exhibited progressive growth. In January 2021, the patient was hospitalized for mass resection. The resected mass was submitted to Tangshan Union Medical College Hospital for pathological examination.

Surgical method

First, chest and orbital computed tomography (CT) scans and abdominal, and neck color Doppler ultrasound were performed. On January 28, 2021, the patient underwent extended resection on the left upper eyelid combined with blepharoplasty. The entire upper eyelid was intraoperatively resected and the hard palate mucosa was taken as a substitute for the tarsus (Figure 1B), as per the National Comprehensive Cancer Network clinical practice guidelines (version 1.2020). Eyelid reconstruction was achieved by transplanting the excised hard palate mucosa's inferior border to the lower eyelid's tarsal sulcus and transferring the upper eyelid skin flap (Figure 1C).

Pathological and immunohistochemical results

Postoperative pathological results suggested a malignant small round-cell tumor with diffuse tumor cells and abundant interstitial blood vessels (Figure 2A). Immunohistochemical results

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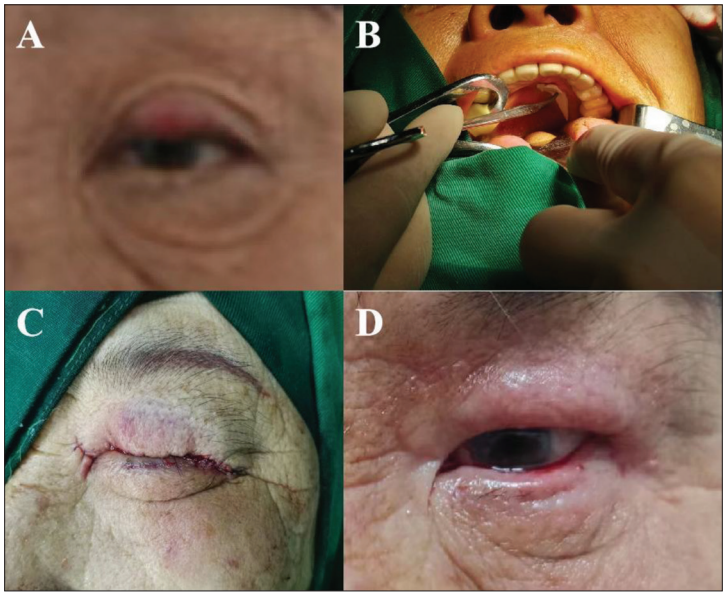

Figure 1. Photographs of a left eyelid mass, surgical process and postoperative outcome; A – a red mass-like protrusion was observed in the center of the left upper eyelid, with a size of 1 mm × 0.8 mm × 0.5 mm; B – the hard palate mucosa was taken; C – the eyelid was reconstructed; D – three months after hard palate mucosa transplantation, the palpebral fissure was incised; the size, opening and closing of the left palpebral fissure were all normal

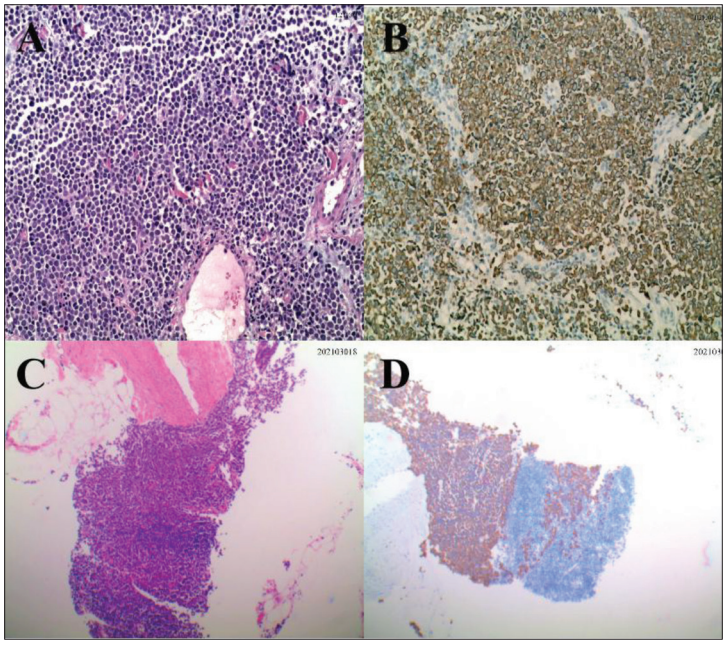

Figure 2. Pathological and immunohistochemical results; A – left upper eyelid, 20 × 10 H&E; B – left upper eyelid, immunohistochemistry, cytokeratin 20 (CK20), 20 × 10; C – left supra-clavicular lymph nodes, 10 × 10 H&E; D – left supra-clavicular lymph nodes, CK20, 10 × 10

(Figure 2B) showed cytokeratin (CK) (+), vimentin (–), S-100 (–), cluster of differentiation (CD)56 (+), Syn (+), neuron-specific enolase (–), CD34 (+) vessels, CD7 (+) T cells, TDT (–), Pax-5 (+) B cells, CD20 (+) B cells, CD79a (+) B cells, CD3 (+) T cells, CD45Ro (+) T cells, CD10 (–), CD21 (–), *BCL6* (–), *MUM1* (–), CD5 (+) T cells, cyclind1 (–), Ki-67 (approximately 60% +) and *BCL2* (+). The patient was diagnosed with MCC of the left eyelid.

Postoperative treatment plan

On March 1, 2021, the patient was readmitted for further postoperative treatment. Plain and contrast-enhanced CT scan of the neck displayed multiple lymph nodes. Ultrasound-guided lymph node biopsy revealed metastatic MCC (Figure 2C). Immunohistochemistry showed vimentin (–), CK (–), CD56 (weakly +) scattered cells, CK20 (+) and Ki-67 (30% +) (Figure 2D).

The radiotherapy regimen included electron beam radiotherapy for the left eyelid using fractionated doses of 200 cGy for a total of 3000 cGy, and conformal radiotherapy for left periclavicular lymph nodes using fractionated doses of 200 cGy for a total of 5000 cGy five times weekly. A total of six courses of chemotherapy treatment were conducted based on etoposide combined with carboplatin regimen (20 days per course). Three months after hard palate mucosa transplantation, the palpebral fissure was incised. The size, opening and closing of the left palpebral fissure were all normal (Figure 1D). Following chemotherapy, whole-body physical examination was performed once quarterly, and then once every six months. There were no significant complications observed on the eyeball following electron beam radiotherapy.

Ethics: This study was conducted in accordance with the Declaration of Helsinki and approved by the Research Ethics Committee of Tangshan Peoples Hospital (No. rmyy-IIks-151). All methods were carried out in accordance with relevant guidelines and regulations.

DISCUSSION

MCC, also known as cylindrical cell carcinoma, is aggressive and mainly affects the elderly, with an average onset age of 68 years [4]. It has a local recurrence rate of 25%, lymph node metastasis incidence of 52%, distant metastasis proportion of 34% and mortality rate of 14–52%. Due to its low incidence, a unified and matured treatment plan is yet to be developed. Generally, tumors < 2 cm have better prognosis than those > 2 cm [5].

Extended tumor resection and lymph node dissection are the mainstay of treatment, and the optimal efficacy can be achieved when combined with chemoradiotherapy. However, there is no unified standard for the specific range of surgical resection, as the tumor is prone to recurrence and metastasis due to lymph node spread. Currently, most scholars advocate for active surgical resection and adjuvant radiotherapy, as their combination is significantly more effective than radiotherapy alone.

In the case reported here, the postoperative pathological report indicated total resection and no tumor cells at the tumor edge. First, the patient was treated with complete resection under a microscope and then intraoperatively. The entire upper eyelid was resected at a distance of 1.2 cm from the outer margin of the mass, and the hard palate mucosa was taken as a substitute for the tarsus.

The most typical clinical manifestation of MCC is a rapidly enlarging red or purplish-red nodule in the short term, which is also non-specific and may be accompanied by pain or ulcers. Early diagnosis of MCC is challenging and prone to misdiagnosis, based on histopathological and immunohistochemical examinations. Under microscope examination, the majority of MCCs are found within the dermis, revealing a round or oval nuclei, clear nuclear membranes, thin and scattered chromatin, unclear nucleoli, few endochylema and visible karyokinesis. On immunohistochemistry, MCCs mostly display neuroendocrine features. CK20 is a sensitive and specific biomarker of MCC, which is usually located near the nucleus in a comma or cap-like shape. In addition, tumor cells are diffusely positive for synaptophysin and CgA, and constantly positive for CD56. Komatsu et al. [6] reported a case of MCC of the lower eyelid in a 37-year-old non-immunocompromised female patient. The patient presented with normal vision at initial presentation with an eyelid tumor measuring 12 × 10 mm with a violaceous surface that was firm and immobile. Pathological results showed that the tumor cells exhibited small round-cell infiltration. Immunohistochemical examination indicated that the tumor cells exhibited a positive reaction to synaptophysin, CgA and CK20, whereas thyroid transcription factor-1 showed a negative reaction. The final diagnosis of MCC was confirmed. This article highlights the importance of considering MCC as a differential diagnosis of eyelid tumors in younger patients, even in the absence of immune compromise.

In recent years, advances in immunotherapies, particularly immunotherapies involving the programmed cell death protein 1 and programmed cell death ligand 1 pathways have greatly prolonged the survival of patients with metastatic diseases. Immunotherapy agents such as avelumab and famotidine have shown great efficacy in treating metastatic MCC. Research has shown that patients with surgically incurable locally advanced MCC can be treated with intratumoral injection of talimogene laherparepvec [7]. Several MCC treatment alternatives, such as MLN0128 and pazopanib, continue to be researched.

MCC is mainly treated with surgical resection as the preferred option; however, postoperative adjuvant systemic chemotherapy and local radiotherapy inhibit its metastasis and recurrence. The application of immunotherapy and molecular targeted drugs remains critical for effective treatment of MCC.

MCC is a rare and highly aggressive neuroendocrine carcinoma, often presenting with non-specific clinical manifestations, such as a rapidly enlarging red or purplish-red nodule. This non-specificity, combined with its rarity, frequently leads to misdiagnosis in clinical practice. Early diagnosis relies heavily on pathological examination and immunohistochemical staining. Immunotherapy and molecular targeted drugs represent emerging treatment trends, offering hope for further improving the prognosis of MCC. However, the high misdiagnosis rate of eyelid MCC highlights the need for increased awareness among clinicians and pathologists, as well as the development of more specific diagnostic markers and standardized treatment protocols.

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Availability of data and materials: The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest: None declared.

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Анализа терапијских приступа код Меркеловог карцинома капка

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САЖЕТАК

Увод Карцином Меркелових ћелија је клинички редак примарни неуроендокрини карцином коже, који се чешће јавља на глави и врату, али се ретко јавља на капку. У клиничкој пракси одликује се веома честом погрешном иницијалном дијагнозом, високим степеном малигнитета и изузетно лошом прогнозом. Тренутно не постоји дефинитиван и ефикасан план лечења карцинома Меркелових ћелија.

Приказ болесника Ова ретроспективна студија приказује пацијента који је у Офталмолошкој болници у Тангшану лечен због карцинома Меркелових ћелија очног капка. Месец дана након ресекције тумора дошло је до ширења тумора у лимфне чворове врата на истој страни. Након хемиотерапије, током праћења од две године и три месеца, нису пронађене патолошке лезије.

Закључак Приказ болесника показује да се карцином Меркелових ћелија дијагностикује углавном на основу патохистолошке анализе и лечи се хируршком ресекцијом као пожељном опцијом. Постоперативна адјувантна системска хемиотерапија и локална радиотерапија имају инхибиторни утицај на настанак метастаза и рецидиве. Имунотерапија и молекуларно циљани лекови су нови трендови у третману овог карцинома.

Кључне речи: карцином Меркелових ћелија; малигни тумор; очни капак; хирургија комбинована са хемиотерапијом

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REVIEW ARTICLE / ПРЕГЛЕДНИ РАД

Application of fiberoptic bronchoscopy in the diagnosis and treatment of pneumonia in children

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SUMMARY
Pneumonia is a leading cause of morbidity and mortality in children, caused by various pathogens such as bacteria, viruses, mycoplasma, and fungi, each with distinct clinical manifestations. Fiberoptic bronchoscopy (FOB) is an invaluable tool for visualizing the airway, collecting lower respiratory tract specimens, and diagnosing and treating pediatric pneumonia. This review explores the role of FOB in managing pneumonia caused by different etiologies, including mycoplasma, adenovirus, and aspiration pneumonia. It also addresses the complications and precautions associated with FOB, emphasizing the importance of careful patient selection and the need for timely referrals to specialized centers, particularly in primary healthcare settings where access to FOB may be limited. The paper concludes with a discussion on the future development of FOB, focusing on advancements in technology, such as improved imaging, multi-modal functions, and artificial intelligence integration, which will enhance diagnostic accuracy and treatment efficacy for pneumonia.
Keywords: fiberoptic bronchoscopy; pneumonia; diagnosis; treatment; children

INTRODUCTION

Pneumonia is an inflammation of the pulmonary alveoli and interstitium, commonly seen in pediatric population – defined as individuals from birth to 18 years of age – and a leading cause of childhood mortality. The World Health Organization estimates that nearly two million children under the age of five die from pneumonia annually [1]. The disease is caused by various pathogens, including bacteria, viruses, mycoplasma, and fungi, with symptoms such as fever, cough, dyspnea, and chest pain [2, 3]. Epidemiological studies indicate that viruses are the most common cause of pediatric pneumonia, accounting for approximately 60–70% of cases in young children, with respiratory syncytial virus and influenza viruses being predominant [4]. Bacterial pathogens, particularly Streptococcus pneumoniae, are responsible for about 20–30% of cases, often in conjunction with or secondary to viral infections [5]. *Mycoplasma pneumoniae* (MPP) is increasingly recognized as a significant pathogen in school-aged children, contributing to 10–40% of community-acquired pneumonia cases in this age group [5]. Diagnosing and treating pneumonia requires a comprehensive approach, including medical history, clinical signs, laboratory tests, and imaging. However, diagnosis can be challenging, and treatment failures are frequent, particularly in primary healthcare settings, where limited resources hinder early detection and effective management [6–9].

Fiberoptic bronchoscopy (FOB) is a crucial tool for visualizing the airway and collecting specimens from the lower respiratory tract, playing a vital role in diagnosing and treating pediatric respiratory diseases [10, 11]. Developed by a Japanese thoracic surgeon Shigeto Ikeda, FOB has become a cornerstone of modern pulmonary medicine [12]. In pediatric care, FOB is primarily used for airway inspection, removal of secretions or foreign bodies, sample collection, and placement of devices or drugs [13]. In primary healthcare settings, particularly in community hospitals with limited access to FOB, clinicians must understand its indications and recognize when referral to specialized centers is necessary. Timely referral can significantly improve the diagnosis and management of conditions like pneumonia. While several reviews have addressed bronchoscopy in pediatric respiratory diseases, this review is distinct in its focused evaluation of FOB's role across a broad pediatric age range, its emphasis on specific pneumonia etiologies such as mycoplasma, adenovirus, and aspiration, and its integration of recent technological advancements – including improved imaging, multi-modal functions, and AI-driven diagnostics – offering updated insights for both clinical practice and future research. This review

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highlights the importance of FOB in pediatric care and emphasizes its value in primary care settings. Understanding when to refer patients for FOB is essential for clinicians to improve the care of pediatric respiratory patients.

DIAGNOSTIC VALUE OF FOB IN PEDIATRIC PNEUMONIA

FOB allows direct observation of airway structure, identifying abnormalities such as stenosis, inflammation, and tumors. It also facilitates lower respiratory tract sample collection through bronchial irrigation, bronchoalveolar lavage (BAL), and biopsy. These samples support microbiological testing for bacteria, viruses, mycoplasma, fungi, and tuberculosis, enhancing diagnostic accuracy for pediatric pneumonia. In primary care, FOB is often used for repeated or unresolved pneumonia, unclear etiology, and suspected foreign body aspiration, prompting referrals for specialized care.

Evaluation of inflammatory response and immune status of the lungs

FOB, through BAL fluid (BALF), is essential for evaluating the inflammatory response and immune status of the lungs. Indicators such as cellular components, cytokines, and immunoglobulins in BALF reflect the degree of inflammation and immune responses. For example, an increased proportion of neutrophils suggests bacterial pneumonia, while lymphocyte dominance points to viral or tuberculous pneumonia. Elevated eosinophil counts indicate allergic or fungal pneumonia, and reduced pulmonary surfactant proteins are associated with alveolar surfactant deficiency, contributing to atelectasis and respiratory failure [14, 15, 16].

Assessment of lung structure and function

FOB also provides a detailed assessment of lung structure and function through bronchoscopic imaging. Key parameters, such as airway diameter, length, branching, angle, and wall thickness, are used to evaluate airway integrity and detect abnormalities like stenosis, deformity, inflammation, bleeding, or tumors. These findings are crucial for determining the location, extent, and severity of pneumonia, as well as airway damage. For instance, a reduction in airway diameter and length may suggest stenosis or collapse, while changes in branching or angle could indicate deformities. An increase in wall thickness may point to inflammation or tumors, and the presence of secretions or foreign bodies suggests obstruction or infection [17, 18, 19].

IDENTIFYING DIFFERENT TYPES OF PNEUMONIA

Unresolved or recurrent pneumonia

In pediatric pneumonia, cases that do not improve with antibiotics or where the etiology remains unclear, FOB is essential for identifying rare causes, such as atypical pathogens or complications like pleural effusion or lung abscesses. Early referral to a bronchoscopy-equipped facility ensures timely diagnosis and better outcomes.

Mycoplasma pneumonia, caused by MPP, accounts for 10–40% of pediatric pneumonia cases [20]. It typically presents with low fever, cough, sore throat, and headache, with patchy infiltrates on chest X-rays. Extrapulmonary symptoms, including erythema multiforme and myocarditis, are common [21]. Diagnosis relies on serology and molecular tests, though serology may suffer from cross-reactivity and molecular tests may yield false results [22]. FOB with BALF improves specificity, avoiding contamination. Cytological and pathological analysis of BALF enhances detection, and bronchoscopic imaging can identify mucous emboli, characteristic of severe mycoplasma pneumonia [23, 24].

Adenovirus pneumonia, responsible for 10–20% of pediatric pneumonia, particularly in children under five, presents with high fever, cough, dyspnea, and chest pain, with patchy infiltrates and pleural effusion on chest X-rays [25, 26]. Diagnosis depends on virological and molecular tests, but these can produce false results [27]. FOB enhances diagnostic accuracy by reducing upper respiratory contamination through BALF, enabling better immune response assessment. Bronchoscopic imaging can reveal airway abnormalities such as inflammation and necrosis [28, 29].

Aspiration pneumonia

Aspiration pneumonia, caused by inhaling gastric contents, vomit, or foreign bodies, is common in children with neurological impairments or swallowing difficulties, accounting for 5–15% of pediatric cases, particularly in newborns and infants [30]. It presents with wheezing, shortness of breath, and cyanosis, with chest X-rays showing patchy infiltrates and pleural effusion [31]. Diagnosis typically involves medical history, clinical signs, chest X-rays, and bronchoscopy, although sample quality may affect accuracy [32]. FOB is essential for diagnosis and management, as BALF helps identify aspirated material, improving specificity. Cytological and pathological analyses of BALF assess inflammatory responses, and bronchoscopy can directly visualize aspirated substances, characteristic of aspiration pneumonia [27, 33].

Timing of FOB in pediatric pneumonia

The timing of FOB is crucial for maximizing diagnostic yield and therapeutic benefit in pediatric pneumonia. For MPP pneumonia, particularly severe or refractory cases, FOB is recommended within 7–10 days of symptom onset,

especially if there is no clinical improvement after 48–72 hours of appropriate antimicrobial therapy. Early bronchoscopy during this window allows for effective removal of mucus plugs and mucous casts, which are prone to form during the peak inflammatory phase, thereby preventing bronchiolar obstruction and atelectasis [23, 24]. In adenovirus pneumonia, which often progresses rapidly and may lead to airway necrosis and post-infectious bronchiolitis obliterans, FOB should be performed earlier – ideally within the first 5–7 days of hospitalization – if there is clinical deterioration, persistent hypoxia, or radiological progression despite supportive care. This enables timely pathogen confirmation via BALF and intervention to clear necrotic debris [28, 29]. For aspiration pneumonia, FOB is most beneficial when performed acutely, within 24–48 hours of the suspected aspiration event, to directly visualize and remove aspirated material, reduce bacterial load, and prevent secondary chemical pneumonitis or infection. In chronically aspirating children, such as those with neurodevelopmental disorders, semi-elective FOB may be scheduled to evaluate recurrent pulmonary infiltrates or persistent symptoms, even in the absence of an acute event [32, 33]. Overall, the decision to perform FOB should balance the urgency of intervention with procedural risks, and close monitoring during the initial phase of illness is essential to identify the optimal time window for intervention.

THERAPEUTIC VALUE OF FOB IN PEDIATRIC PNEUMONIA

The therapeutic application of FOB in pediatric pneumonia should be guided by the stage of disease progression. Interventions differ significantly between the acute and recovery phases, with distinct priorities in each.

Acute phase interventions (first 1–2 weeks of illness)

During the acute phase, the primary goals of FOB are to restore airway patency, manage life-threatening obstructions, and support gas exchange. This phase is characterized by intense inflammation, mucus hypersecretion, and potential airway plugging – particularly in severe mycoplasma, adenovirus, and aspiration pneumonias. FOB is indicated for the immediate removal of viscous secretions, mucus plugs, necrotic debris, or aspirated material via suction, irrigation, or forceps extraction [34, 35]. In children with respiratory distress, hypoxemia, or atelectasis unresponsive to conventional therapy, such interventions can rapidly improve ventilation and prevent progression to respiratory failure [36]. Additionally, in cases of bronchial obstruction due to inflammatory casts or foreign bodies, timely FOB-guided clearance is critical to prevent irreversible lung damage. Direct instillation of saline lavage or mucolytic agents during this phase may further aid in breaking down tenacious secretions [37].

Recovery phase interventions (beyond two weeks or in persistent / recurrent cases)

In the recovery phase, FOB shifts from emergency intervention to diagnostic and rehabilitative roles. Persistent radiological infiltrates, prolonged oxygen dependence, or failure to thrive may indicate unresolved atelectasis, bronchial stenosis, or evolving airway complications such as bronchiectasis or bronchiolitis obliterans – particularly following severe adenovirus or mycoplasma infections [28, 29]. FOB during this phase allows for reassessment of airway integrity, clearance of residual secretions, and targeted delivery of anti-inflammatory agents (e.g., corticosteroids) or antibiotics directly to affected segments, enhancing local efficacy while minimizing systemic exposure [36, 37]. Furthermore, FOB enables the placement of airway stents or balloon dilation in cases of acquired bronchial stenosis, thereby improving long-term pulmonary function and reducing the risk of recurrent infections [38, 39]. Serial BAL in the recovery phase may also be used to monitor inflammatory markers and microbiological clearance, guiding the duration of therapy.

COMPLICATIONS AND PRECAUTIONS OF FOB

Despite the advantages of FOB in the diagnosis and treatment of pneumonia in children, FOB is associated with risks and complications. FOB-related complications are described below.

Anesthesia and sedation-related complications

Anesthesia and sedation are required in FOB, which may lead to anesthesia and sedation-related complications such as respiratory depression, arrhythmia, hypotension, and allergic reactions [36]. In order to reduce the incidence of these complications, it is necessary to fully evaluate the patients before the procedure, select appropriate anesthesia and sedatives, monitor the vital signs of patients, and promptly find out and handle abnormal conditions [40].

Airway-related complications

FOB is operated through the airway and may lead to airway-related complications, such as laryngospasm, bronchospasm, airway bleeding, airway injury, and pneumothorax [36]. It is important to select an appropriate bronchoscope model, avoid excessive operation, control the pressure of the airway, keep the airway moist, and promptly find out and handle abnormal conditions to reduce the incidence of these complications [35].

Infection-related complications

The bronchoscope may contact with the secretions or tissues of the lower respiratory tract during the process of FOB, and may consequently cause infection-related

complications, such as the spread of bacteria, viruses, mycoplasmas, and fungi, leading to cross-infection and nosocomial infections [36]. In order to reduce the incidence of these complications, it is essential that aseptic operation is strictly followed, bronchoscope and necessary instruments are thoroughly cleaned and disinfected, children are pre-treated with appropriate antibiotics for preventive purposes, and abnormal conditions are detected and handled promptly [41].

CONSIDERATIONS FOR FOB IN PEDIATRIC PNEUMONIA

Several issues need to be noted when FOB is used in the diagnosis and treatment of pneumonia in children, as described below.

Indications and contraindications of FOB

It is essential to understand the indications and contraindications of FOB when diagnosing and treating pneumonia in children to minimize risks. Indications include pneumonia with unclear etiology or treatment failure; severe pneumonia with complications like respiratory failure, difficult weaning, or organ dysfunction; pneumonia with airway blockages due to secretions or foreign bodies; pneumonia with airway abnormalities such as stenosis, deformities, inflammation, bleeding, or tumors; and cases requiring drug instillation or device placement in the airway [42]. Contraindications include severe systemic conditions like shock, coma, and massive bleeding; cardiovascular diseases such as heart failure, arrhythmia, and pericardial tamponade; coagulation dysfunction like thrombocytopenia; severe airway or lung abnormalities, including laryngeal edema, tracheal or esophageal fistulas, pleural effusion, and pneumothorax; and serious anesthesia or sedation risks, such as allergies and drug interactions [43].

Referral recommendations for FOB in primary healthcare settings

Given the limited availability of FOB in primary healthcare facilities, clinicians must recognize when referral to a specialized center is necessary. Referral indications include the following [15, 20, 21, 44]: if a child's pneumonia does not improve despite appropriate antimicrobial therapy or if the etiology remains unclear, FOB should be considered to identify atypical pathogens, foreign body aspiration, or underlying airway abnormalities not detected by routine methods. For severe pneumonia requiring intensive care or mechanical ventilation, FOB allows for a comprehensive airway assessment and pathogen identification, facilitating targeted treatment and better management of complications such as pleural effusion or bronchial obstruction. In cases of suspected aspiration pneumonia, especially in children with neurological conditions or feeding difficulties, FOB helps identify and remove aspirated material, improving outcomes and preventing further complications. Additionally, when structural airway abnormalities like stenosis or tumors are suspected, FOB provides direct visualization to evaluate the extent of these issues and guide appropriate treatment, including surgical interventions. Finally, for cases requiring therapeutic interventions such as secretion removal, lavage, or device insertion, FOB offers the advantage of simultaneously providing diagnostic and therapeutic procedures, thereby optimizing patient management and outcomes.

Selection of appropriate FOB model and operation mode

When FOB is used in the diagnosis and treatment of pneumonia in children, the selection of an appropriate model and operation mode is required. Appropriate diameter, length, and curvature of fiberoptic bronchoscope should be selected based on factors such as the age, body weight, airway size, and conditions of children to avoid airway injury and operation difficulties caused by the inappropriate size of the bronchoscope [45]. At the same time, appropriate operation modes, including spontaneous breathing, mechanical ventilation, and high-frequency oscillatory ventilation are selected according to the factors of the children, such as respiratory pattern, airway pressure, and oxygenation level to avoid the incidence of intraoperative complications such as dyspnea, hypoxemia and high airway pressure [46].

Age-specific considerations in FOB application

The application of FOB varies significantly across pediatric age groups due to differences in airway anatomy, size, and physiological resilience. Neonates and infants have smaller airway diameters, increased airway compliance, and higher risks of hypoxia, requiring ultra-thin bronchoscopes (2–2.8 mm in diameter) and meticulous sedation management [47]. In contrast, older children and adolescents can tolerate larger scopes (3–4 mm) and may undergo procedures under moderate sedation with spontaneous breathing. Premature infants and those with underlying lung disease (e.g., bronchopulmonary dysplasia) are at increased risk for complications such as bronchospasm and desaturation. Furthermore, the indications for FOB may differ by age: viral and aspiration pneumonias are more common in infants and toddlers, while MPP predominantly affects school-aged children and adolescents [47]. Therefore, age-stratified approaches to patient selection, procedural planning, and post-procedure monitoring are essential to optimize safety and efficacy.

Combination with other examination and treatment methods

The diagnostic and therapeutic efficacy of FOB is significantly enhanced when integrated into a multimodal clinical strategy. FOB should be used in conjunction with

advanced imaging modalities such as chest computed tomography and point-of-care ultrasound, which provide complementary structural and functional information about the extent of pulmonary consolidation, pleural involvement, and airway dynamics [48]. BALF obtained via FOB can be analyzed using rapid molecular techniques – including multiplex polymerase chain reaction and next-generation sequencing – to identify pathogens with high sensitivity and specificity, particularly in cases of culture-negative or atypical pneumonia [49]. Furthermore, BALF enables host immune response profiling (e.g., cytokine and cellular analysis), which, when combined with serum biomarkers such as procalcitonin and C-reactive protein, supports differentiation between bacterial, viral, and inflammatory etiologies. Physiological assessments, including pulmonary function tests (when feasible in older children) and pulse oximetry monitoring during and after the procedure, further refine clinical decision-making. This integrative approach – combining FOB with imaging, molecular diagnostics, and systemic biomarkers – facilitates precise etiological diagnosis, monitors treatment response, and personalizes management strategies in children with complex or refractory pneumonia [50].

FUTURE DEVELOPMENT OF FOB

Remarkable achievements have been made for FOB in the diagnosis and treatment of pneumonia in children. However, there are still limitations and deficiencies in this technique, and further development and improvement are needed. Future developments of FOB are proposed and described below.

Pediatric applicability of advanced imaging technologies

While optical coherence tomography (OCT), endoscopic ultrasound (EUS), fluorescent bronchoscopy (FBS), and magnetic resonance bronchoscopy (MRBS) offer enhanced airway and parenchymal visualization, their application in children remains limited and largely investigational. OCT, which provides high-resolution cross-sectional imaging of airway walls, has shown potential in assessing bronchial inflammation and remodeling in adult chronic lung diseases, but its use in pediatric pneumonia is constrained by the small diameter of pediatric airways and the lack of suitably miniaturized probes compatible with ultra-thin bronchoscopes (2–2.8 mm) used in infants and young children [51]. Similarly, EUS-guided bronchoscopy, valuable for evaluating peribronchial lymphadenopathy or parenchymal consolidation, is rarely performed in children due to the size of current echo-bronchoscopes and the complexity of the procedure, which often requires general anesthesia and advanced expertise not widely available in pediatric centers [52]. MRBS, though non-invasive and radiation-free, is currently theoretical and faces significant technical hurdles in real-time airway navigation. Future development must prioritize the design of pediatric-specific probes, rigorous safety studies, and clinical trials in pediatric populations to determine the diagnostic and therapeutic value of these technologies in childhood pneumonia and other respiratory conditions.

Improving the performance and functions of FOB

The performance and functions of future FOB should be enhanced by increasing the adjustability of related parameters such as the diameter, length, and curvature of the bronchoscope for children of different ages, body weights, and airway sizes, improving the quality of airway observation and diagnosis via increasing the clarity, resolution, and color of the bronchoscope, and enhancing the efficiency of airway operation and treatment via increasing the operability, flexibility, and stability of FOB [53].

Adding functions and accessories to FOB

More functions, such as optical, acoustic, electronic, and magnetic functions, will be added to future FOB for multimodality imaging and examination, including OCT, EUS, FBS, and MRBS, to improve the structural and functional evaluation of the airways [51]. Meanwhile, accessories for fiberoptic bronchoscopes, such as various pliers, brushes, wires, catheters, stents, and balloons, will be added for multiple airway operations and treatments, including airway dilation, resection, biopsy, perfusion, and placement, to improve airway patency and stability [54].

Combining FOB with artificial intelligence and robotics

Artificial intelligence and robotics will be combined with FOB in the future. For instance, artificial intelligence and machine learning are used to analyze and identify the image and data obtained by FOB to improve the accuracy and objectivity of airway diagnosis and evaluation, for example, the identification of airway stenosis, deformity, inflammation, bleeding, and tumors, and evaluation of airway parameters, including diameter, length, branch, angle, and wall thickness, which can predict the prognosis and therapeutic effect of airway diseases [55]. In addition, robotic technology is used to optimize and automate the operation and control of fiberoptic bronchoscopes to improve the efficiency and safety of airway operation and treatment, including the accurate positioning, navigation, tracking, operation, and feedback for the airways [56].

In summary, while FOB has proven valuable in diagnosing and managing pediatric pneumonia, its application in primary healthcare settings remains limited due to equipment and expertise constraints. Therefore, it is crucial for clinicians to recognize when referral to specialized centers is necessary. Key indications for referral include unresolved pneumonia despite appropriate treatment, suspected aspiration pneumonia, or when structural airway abnormalities are suspected. Timely referral enables accurate

diagnosis, identification of atypical pathogens, and better management of complications, ultimately improving patient outcomes. Further advancements in FOB technology and training are essential to enhance its accessibility and effectiveness in pediatric care.

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Author Contributions

Zhang H conception, analysis of the data, and writing of the manuscript;

Wang DX critical review of significant intellectual value, final revision of the manuscript being prepared for publication;

Yu HM obtaining of results or analysis and interpretation of results;

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Примена фиброптичке бронхоскопије у дијагностици и лечењу пнеумоније код деце

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САЖЕТАК

Пнеумонија је водећи узрок морбидитета и морталитета код деце, а изазивају је разни патогени, као што су бактерије, вируси, микоплазме и гљивице, од којих сваки има специфичне клиничке манифестације. Фиброптичка бронхоскопија (ФОБ) представља непроцењив алат за визуализацију дисајних путева, прикупљање узорака из доњих дисајних путева и дијагностиковање и лечење педијатријске пнеумоније. Овај преглед истражује улогу ФОБ-а у управљању пнеумонијом узрокованом различитим етиологијама, укључујући микоплазму, аденовирус и аспирациону пнеумонију. Такође се бави компликацијама и мерама опреза повезаним са применом ФОБ-а, наглашавајући значај пажљивог избора пацијената и потребу за правовременим упућивањем у специјализоване центре, посебно у примарној здравственој заштити, где приступ ФОБ-у може бити ограничен. Рад се завршава дискусијом о будућем развоју ФОБ-а, са фокусом на напретку у технологији, попут побољшаног сликања, мултимодалних функција и интеграције вештачке интелигенције, што ће побољшати дијагностичку тачност и ефикасност лечења пнеумоније.

Кључне речи: фиброптичка бронхоскопија; пнеумонија; дијагностика; лечење; деца

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REVIEW ARTICLE / ПРЕГЛЕДНИ РАД

The importance of optimal balance of calcium, phosphorus and vitamin D and adequate physical activity during the period of growth and development for bone health

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SUMMARY
Bones are mineralized connective tissue that provides to body anti-gravity support, enables movement with the help of muscles, and protects internal organs. Individual skeletal quality, as a result of genetic, hormonal, and external factors, such as nutrition, physical activity and others, is achieved during the period of growth and development. Peak bone mass is attained by the end of the second decade of life and is maintained until age 40–50, then gradually decreases without the possibility of rebuilt. It is therefore clear that the lack of adequate bone mass building during the development period, in addition to the immediate consequences, represents a high risk of osteoporosis and its complications in later life, especially in old age. The purpose of this article is to review the importance of optimal calcium, phosphorus, and vitamin D balance and adequate physical activity during growth and development in achieving maximizing peak bone mass.
Keywords: bone health; children; adolescents

INTRODUCTION

Bones are specialized mineralized and multifunctional connective tissue composed of minerals (60–70%), primarily hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$], collagenous and non-collagenous proteins (20–40%), cells (osteoblasts, osteoclasts, and osteocytes) and a small portion of water and lipids [1]. Osteoblasts induce the formation of new bone by secreting collagen fibers, which is then mineralized, while osteoclasts dissolve bone by secreting enzymes and acids. Osteocytes arise from osteoblasts after finished secreting matrix. They secrete soluble factors that influence osteoclastic and osteoblastic activity and play a central role in bone remodeling in response to mechanical stress.

According to their formation, structure, and function, bones are divided into long, flat, short, irregular, and sesamoid. Long bones include bones in the upper and lower limbs, while flat bones form the skull, face, thoracic cage, and the pelvis. Short bones are present in the wrist and ankle, and the patella is a sesamoid bone within the ligament of the quadriceps femoris muscle.

Long bones, along with short bones and the patella, support the body's weight and enable movement, while flat bones protect internal organs of the head, face, thorax, and pelvis [2]. In addition, within the flat bones is red bone marrow [2]. The vertebrae, the irregular bones of the spinal column, support the head, neck, and body, allowing them to move while protecting the spinal cord at the same time [2]. In addition, bones are an endocrine organ, reservoir of calcium and phosphorus, and a significant participant in the regulation of acid-base homeostasis of the body [2]. The hormonal role of bone is reflected in the production of fibroblast growth factor-23 (FGF-23), osteocalcin, and sclerostin. FGF-23, which originates from osteocytes and osteoblasts, enhances renal phosphate excretion directly through inactivation of sodium/phosphate cotransporter (NaPi)-2a and NaPi-2c in the proximal tubules and indirectly by suppressing [$1,25(OH)_2D_3$] synthesis and promoting [$1,25(OH)_2D_3$] conversion to inactive [$24,25(OH)_2D_3$] [2, 3, 4]. Osteocalcin, produced by osteoblasts, participates in the regulation of energy metabolism, glucose tolerance, testosterone production, and bone resorption, while sclerostin, a product of osteocytes, is a suppressor of osteoblast differentiation [2].

Skeletal quality, as a result of both genetic, hormonal, and external factors, such as diet, physical activity, and others, is attained by the end of the second decade of life. Peak bone mass achieved during that period is maintained until age 40–50, then bone density gradually irreversibly decreases leading, if not at an adequate level, to various consequences both during that period and later [5–11]. Also, in numerous diseases, both hereditary and acquired, various

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Table 1. Dietary reference intakes of calcium (mg/d) [15]

Age (male & female)	Estimated average requirement	Estimated average requirement	Recommended dietary allowance	Upper level
0–6 months	–	–	200 (AI)	1.000
7–12 months	–	–	260 (AI)	1.500
1–3 years	500	500	700	2.500
4–8 years	800	800	1.000	2.500
9–18 years	1.100	1.100	1.300	3.000

AI – adequate intake

Table 2. Dietary calcium content, percentage absorption, and net absorbed amount [24]

Type of food	Serving size	Calcium content (mg)	Estimated absorption (%)	Net calcium absorbed (mg)
Cow milk	250 ml	310	32	100
White beans (cooked)	125 ml	85	22	18
Red beans (cooked)	125 ml	26	24	6
Whole wheat bread slice	35 g	26	82	21
Broccoli (cooked)	125 ml	33	20	20
Spinach (cooked)	125 ml	129	5	7
Almonds (raw, roasted)	60 g	97	21	21
Soy "milk" (unfortified)	125 ml	5	31	2

pathogenetic mechanisms lead to serious disruption of the integrity of the skeletal bone system [3, 12, 13, 14].

The aim of this review is to highlight the importance of meeting optimal calcium, phosphorus, and vitamin D needs, as well as maintaining adequate physical activity during growth and development to achieve maximized peak bone mass as essential components of health both during this period and in later life.

CALCIUM

Calcium is the fifth most abundant element in the human body. It is predominantly (99%) found in the hydroxyapatite of bones and teeth, and only 1% in other tissues [15, 16, 17]. It is present in serum at concentrations of 2.12–2.62 mmol/L, both in ionized and non-ionized form, and in intracellular fluid at 100–200 nmol/L [15, 18]. Calcium homeostasis is carried out at the level of the gastrointestinal tract, kidneys and skeleton under the influence of $[1,25(OH)_2D]$ and parathyroid hormone (PTH) [15, 16, 19, 20]. A standard diet, with an optimal balance of vitamin D, meets the body's calcium needs [15, 16]. It is most abundant in milk and dairy products, as well as in green leafy vegetables [15, 16]. Calcium is absorbed in the small intestine, mainly by active transport and partly by passive diffusion [15, 16, 20]. The main stimulator of intestinal absorption is $[1,25(OH)_2D]$, which induces enterocyte expression of calcium channels, calbindin, Ca^{2+}ATPase (calcium pump), and $3Na^+/Ca^{2+}$ ion exchangers [16]. The utilization of calcium from food is favorably affected by low chyme pH, lactose, lactic, and citric acids, some amino acids PTH, growth hormone, prolactin, estrogen, and insulin-like growth factor, while luminal phosphates, oxalate, phytate, tannin, iron, rapid intestinal transit, glucocorticoids, calcitonin, and FGF-23 have an unfavorable effect [15, 16, 21]. The main route of elimination of calcium from the body is the kidney. The majority of calcium filtered by the glomerulus is reabsorbed along the nephron, mostly (> 60%) from the proximal tubule, so daily calciuria in a healthy child does not exceed 4 mg/kg [16, 20]. A precise insight into the calcium balance in the body is obtained by determining the ratio of calcium to creatinine in the urine (CaUmg/CrUmg), which normally is 0.1–0.2 [15]. Due to low creatinuria, the upper reference value of CaUmg/CrUmg in a child in the first year is higher (up to six months 0.8, and 6–12 months 0.6) [16, 22]. The main stimulators of tubular calcium reabsorption are $[1,25(OH)_2D]$ and PTH, and the inhibitors are hyperphosphaturia, metabolic acidosis, and polyuria [16, 23]. In a state of negative calcium balance, due to insufficient intestinal reabsorption and/or tubular reabsorption, maintenance of its serum and intracellular concentrations is provided by the skeleton [20]. The inducers of this process are PTH and $[1,25(OH)_2D]$ [2].

Table 1 shows the recommendation of the Institute of Medicine of the United States of America (IOM) Committee [15] for reference intakes of calcium in the diet during the period of growth and development, and Table 2 shows the calcium content in the diet, the percentage of absorption and the net amount absorbed [24].

For children aged 0–12 months, the adequate intake is equivalent to the average calcium intake of a healthy and optimally nourished breastfed infant, whereas at age 7–12 months, the adequate intake assumes 120 mg calcium from human milk plus 140 mg calcium from complementary food, while the recommended dietary allowances for older children and adolescents are based on intakes associated with bone accumulation and positive calcium balance [15].

As can be seen in Table 2, the calcium content in food and the degree of its absorption are highly variable. Milk, yogurt, and cheese are the best sources of calcium [15, 24]. The degree of calcium absorption from breast milk compared to cow's milk, due to the better ratio of calcium to phosphorus (1.7:1 *vs.* 1:1), as well as the higher lactose content (7 g/dl *vs.* 4.7 g/dl), is 2.5 times higher [25, 26]. Phytates and oxalates, present in spinach, cucumber, potatoes, and beans, inhibit calcium absorption by forming insoluble calcium salts in the gastrointestinal tract [15]. On the other hand, the bioavailability of calcium from broccoli, kale, and cabbage, which do not contain these compounds, is relatively high [15]. However, due to the low utilization in the former case and the low content in the latter, these foods are much weaker sources of calcium compared to milk and dairy products [15, 24]. Therefore, people with lactose intolerance, milk protein allergy, and those who avoid dairy products (including vegans) are at high risk of inadequate calcium intake [27, 28]. The prerequisite for covering the need for calcium, regardless of its origin, is an optimal status of vitamin D [6, 15]. In general, the degree of absorption of calcium is relatively high if its content in food is low, as well as during the period of

growth and development, especially in the phases when it is the most intense, i.e., in the first three years after birth and puberty [15]. The recommended daily amount of plain milk, yogurt or sour milk that, along with other standard nutrition, provides an optimal calcium balance for a child in their second year is 500 ml, 2–8 years 625 ml, and 9–18 years 750 ml [29].

PHODPHORUS

Phosphorus is the sixth most abundant element in the human body. It is mostly found in the hydroxyapatite of bones and teeth, 15–20% in the intracellular space of soft tissues and 1% extracellularly [30, 31]. The intracellular concentration of organic phosphates varies from 5 to 70 mmol/L, and inorganic from 0.7 to > 2 mmol/L [30]. Its concentrations in the extracellular space are 0.8–1.4 mmol/L, about 85% in free form, 10% bound to proteins and 5% in complex with calcium or magnesium [30].

A normal diet for a healthy person meets their phosphorus needs [31, 32]. It is most abundant in protein-rich foods, such as meat, eggs, milk and dairy products, and legumes [31]. Phosphorus absorption is carried out in the small intestine by the active pathway via the $2Na^{+}/HPO42^{-}$ cotransporter (NaPi-2b), whose expression depends on the current needs of the organism and the stimulation of $[1,25(OH)_2D]$ [4, 31]. It is also partly absorbed paracellularly (passively) [4, 31]. The main regulator of phosphorus homeostasis is the kidney. About 80% of filtered phosphorus at the glomerular level is actively reabsorbed at the proximal tubule level via NaPi-2a and NaPi-2c cotransporters, so its renal clearance is normally only 10.8 ± 2.7 ml/min [4, 30, 31]. The most important stimulator of tubular phosphorus reabsorption is $[1,25(OH)_2D]$, and the inhibitors are FGF-23 and PTH [4, 31, 33].

Considering its high abundance in food, both plant and animal, and the efficient intestinal absorption (50–90%), phosphorus deficiency due to negative nutritional balance as a primary cause of skeletal hypomineralization is rare [32]. It is seen in hyperphosphaturia caused by excess FGF-25, such as X-linked, autosomal dominant, and autosomal recessive hypophosphatemic rickets and tumor-induced osteomalacia or due to genetic defects of renal tubular phosphate reabsorption, such as hypophosphatemic rickets with hypercalciuria [3, 4, 30, 34]. On the other hand, in calcipenic states, either due to insufficient intake and/or vitamin D deficiency, negative phosphorus balance and accompanying skeletal hypomineralization as a consequence of hyperphosphaturia caused by secondary hyperparathyroidism is a common associated phenomenon [35, 36].

VITAMIN D

Vitamin D, i.e., its active metabolite calcitriol $[1,25(OH)_2D]$, is an important factor in calcium and phosphorus homeostasis and bone mineralization. In addition to calcium and phosphorus intestinal absorption and renal reabsorption, calcitriol, by activating the nuclear vitamin D receptor, induces the transition of mesenchymal stem cells into mature osteoblasts and their production of type I collagen, osteopontin, osteocalcin, and other extracellular bone matrix proteins necessary for the formation and maintenance of bone strength [6, 37]. In parallel, calcitriol, through the nuclear vitamin D receptor, exerts a suppressive effect on the differentiation and activation of osteoclasts – osteolytic cells that play a crucial role in skeletal modeling and in the removal of old or damaged bone tissue [16, 37]. Thus, the endocrine function of vitamin D is primarily directed to calcium and phosphorus intestinal absorption and renal reabsorption and skeletal mineralization, except in conditions of hypocalcemia when, together with PTH, it induces osteoclasts in the mobilization of calcium from bones [6, 15, 37, 38].

Most of our vitamin D needs are met by cutaneous photolysis of 7-dehydrocholesterol under the action of sun ultraviolet-B rays of wavelengths 290–315 nm, while foods, excluding fish oil, fatty fish, liver, egg yolks, edible mushrooms treated with UV light, and fortified foods, such as milk formulas, are poor sources of vitamin D [15, 38]. Serum 25(OH)D levels, whose half-life in circulation is about 15 days, represent a reliable indicator of vitamin D status in the body [1 = 15; 15]. According to criteria of the IOM, optimal serum [25(OH)D] levels considered adequate for bone and overall health in healthy individuals range from 20 ng/ml (50 nmol/L) to 50 ng/ml (125 nmol/L) [15]. A value lower than this causes numerous negative consequences, including optimal bone integrity, while a value over 125 nmol/L, apart from the risk of a toxic effect of vitamin D, has no evidence to support additional health benefits. Associations of the Nordic and DACH countries (Germany, Austria, and Switzerland), Australia and New Zealand, as well as the American Academy of Pediatrics and the Endocrine Society agree with the IOM guidelines regarding the lower limit of vitamin D adequacy based on serum [25(OH)D] concentration [6, 39].

The required and upper levels of vitamin D intake in the absence of optimal sun exposure according to the IOM recommendations are given in Table 3 [15]. The European Food Safety Authority and the Endocrine Society agree with the IOM recommendations, as do the European Society of Pediatric Gastroenterology, Hepatology and Nutrition and the American Academy of Pediatrics [6, 40, 41].

PHYSICAL ACTIVITY

It is well known that physical activity with its stimulating effect during childhood and early adulthood plays a vital role in the development and achieved pick bone mass and thus the prevention of osteoporosis and its complications in later life [5, 7–11, 42]. Certainly, in order to preserve the acquired quality of the skeletal system, this practice, along with optimal intake of calcium, phosphorus, protein and other nutritional factors, as well as ensuring an adequate balance of vitamin D, should be continued in other stages of life [43–46].

Table 3. Dietary reference intakes of vitamin D for children and adolescents [15]

Life stage group (years)	Recommended dietary allowance (IU/day)	Upper-level intake (IU/day)
0–6 months	400*	1.000
6–12 months	400*	1.500
1–3 years	600	2.500
4–8 years	600	3.000
9–18 years	600	4.000

*adequate intake for infants 0–12 months of age is 400 IU/day

Table 4. World Health Organization guidelines on physical activity of children and adolescents [47, 48]

• Children 1–2 years of age should spend at least 180 minutes in a variety of types of physical activities at any intensity, including moderate-to vigorous-intensity physical activity, spread throughout the day; more is better.
• Children 3–4 years of age should spend at least 180 minutes in a variety of types of physical activities at any intensity, of which at least 60 minutes is moderate- to vigorous- intensity physical activity, spread throughout the day; more is better.
• Children and adolescents (aged 5–17 years) should do at least an average of 60 minutes per day of moderate- to vigorous-intensity, mostly aerobic, physical activity, across the week. Vigorous-intensity aerobic activities, as well as those that strengthen muscle and bone, should be incorporated at least three days a week.

The World Health Organization guidelines on physical activity of children and adolescents are given in the Table 4 [47, 48]. Identical recommendations also come from the U.S. Department of Health and Human Services, 2018 [46].

CONCLUSION

Bone mass attained during growth and development is one of the most important determinants of lifelong skeletal health. Peak bone mass, which is attained by the end of the second decade of life, is maintained until the age of 40–50, and then gradually decreases without the possibility of being rebuilt. Optimal coverage of calcium, phosphorus and vitamin D needs and adequate physical activity during the developmental period are crucial for good bone health both at that age and in later life stages.

Ethics: The authors declare that the article was written in accordance with ethical standards of the Serbian Archives of Medicine as well as ethical standards of medical facilities for each author involved.

Conflict of interest: None declared.

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Значај оптималног баланса калцијума, фосфора и витамина Д и адекватне физичке активности током периода раста и развоја за коштано здравље

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САЖЕТАК

Кости су минерализовано везивно ткиво које телу пружа антигравитациони ослонац, омогућава кретање уз помоћ мишића и штити унутрашње органе. Индивидуални квалитет скелета, као резултат генетских, хормоналних и спољашњих фактора, као што су исхрана, физичка активност и други, постиже се у периоду раста и развоја. Врхунац коштане масе достиже се крајем друге деценије живота и одржава се до 40. или 50. године, а затим постепено опада без могућности обнављања. Стога је јасно да изостанак адекватне изградње коштане масе у периоду развоја, поред непосредних последица, представља висок ризик од остеопорозе и њених компликација у каснијем животном добу, посебно позном. Сврха овог чланка је да се сагледа значај оптималног баланса калцијума, фосфора и витамина Д и адекватне физичке активности током раста и развоја у постизању максималне вршне коштане масе.

Кључне речи: коштано здравље; деца; адолесценти

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REVIEW ARTICLE / ПРЕГЛЕДНИ РАД

Constipation in childhood and adolescent age

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SUMMARY
Constipation is a common problem in childhood and adolescence. It occurs as a functional (primary or idiopathic) disorder or as part of various pathological conditions that compromise intestinal emptying. In 90–95% of cases, constipation in childhood and adolescence is of a functional nature.
Given the seriousness of the problem, as well as potential complications – sometimes very severe – constipation requires prompt diagnostic and therapeutic intervention.
The therapy of functional constipation is based on dietary modification to normalize the consistency of the stool and facilitate defecation, as well as establishing a normal rhythm of intestinal emptying, and, during the first two months – sometimes longer – the use of laxatives; therapy for secondary constipation targets the underlying cause.
Keywords: constipation; children and adolescents; clinical manifestations; therapy

INTRODUCTION

The term 'constipation' implies difficult, incomplete, and irregular elimination of excessively consistent fecal content [1, 2]. Defecation is often painful and sometimes accompanied by traces of light blood in the stool [2, 3]. According to the literature data, it is registered in 3–14% of children and adolescents [2, 4–8]. From the etiological aspect, it is classified into functional (primary or idiopathic) and secondary [1, 2]. Functional constipation, unlike the secondary one, is characterized by the absence of a pathological background accompanied by difficult intestinal emptying [1]. Constipation in childhood and adolescence is in 90–95% of cases of a functional nature [1, 2].

This article provides a brief overview of the etiopathology, clinical characteristics, and treatment of constipation in childhood and adolescence.

FUNCTIONAL CONSTIPATION

Functional constipation is, along with recurrent functional abdominal pain and irritable bowel syndrome, the most common functional gastrointestinal disorder in childhood and adolescence [4, 9, 10, 11]. It occurs between the ages of 2–4 years, less often earlier or later, until the onset of puberty with equal frequency in both sexes, and then somewhat more commonly in girls [1, 3, 4, 12, 13, 14].

From an etiopathogenetic perspective, functional constipation is a multifactorial disorder, i.e. it occurs as a consequence of hereditary predisposition, consumption of a diet low in fiber content, including fruits and vegetables, neglect of normal toilet rhythm, poor fluid intake, insufficient physical activity, and psychological problems (stress, anxiety, depression), or a combination of these factors [1, 2, 7, 15–19]. In order to prevent constipation, the diet of children and adolescents must be complete and optimally balanced. It is known that adequate intake of fruits, vegetables, and less-refined cereals favors regular bowel movements, while excessive consumption of animal-based foods, especially milk, cheese, and eggs, as well as chocolate sweets, has the opposite effect [1, 17, 18]. Optimal intake of fermented dairy products in liquid form, such as yogurt and sour milk, compared to regular milk, has a less negative impact on intestinal motility [19, 20]. An extremely important place in the prevention of constipation is not to neglect the gastrocolic reflex, i.e., the urge to go to the toilet, which occurs naturally 15–30 minutes after a meal, especially in the morning [21, 22].

In addition to immediate discomfort, constipation is often accompanied by additional manifestations, such as poor appetite, nausea, abdominal pain, anal fissures, rectal prolapse, and fecal impaction [1, 3, 15, 17, 23]. Anal fissures and prolapse of the rectal mucosa cause very painful defecation and bleeding, while the decomposition of intestinal contents above the fecal plug, which is seen in more severe and neglected cases of constipation, leads to pseudodiarrhea and encopresis [1, 3, 15, 16, 17, 23]. In addition, it should be noted that "lazy bowels" are often accompanied by "lazy bladders," so these children, especially girls, show an increased tendency to urinary tract infections [2, 17, 19, 23]. Extremely rarely, mainly in

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adolescence, neglected chronic constipation can be complicated by hemorrhoids, secondary megacolon, and even stercoral perforation accompanied by peritonitis, sepsis, and a potentially fatal outcome [24–27]. It has been proven that long-term chronic functional constipation can also have negative repercussions on the longitudinal growth of a child [17, 28].

Considering the numerous complications and possible consequences, often very serious, functional constipation requires timely diagnosis and appropriate treatment [4, 29].

The diagnosis of functional constipation is based on the exclusion of a pathological background of the disorder and the presence of two or more of the following criteria occurring at least once a week for at least one month: ≤ 2 defecations per week in a child ≥ 4 years of age, ≥ 1 episode of fecal incontinence per week, evidence of retentive posturing or excessive volitional stool retention, evidence of hard or painful bowel movements, presence of large fecal mass in rectum and large diameter stools that obstruct the toilet [4]. Digital rectal examination should not be performed routinely in functional constipation, i.e., it is indicated only in conditions suspected of fecal impaction, Hirschsprung disease, and anorectal anomaly [1, 29]. Also, in the absence of anamnestic and/or clinical indicators that would indicate constipation as a secondary manifestation, radiographic or ultrasound examinations of the abdomen, as well as contrast enemas and various laboratory and other tests, are not necessary [1, 18, 29]. Anorectal manometry offers a more detailed assessment of anorectal function and sensation in patients with functional constipation accompanied by fecal incontinence, as well as in those who do not respond to standard therapy or those suspected of having dyssynergic defecation [7, 18, 30].

The therapy of functional constipation is based on dietary modification to normalize the consistency of the stool and facilitate defecation, as well as establishing a normal rhythm of intestinal emptying (toilet training) and during the first two months – sometimes longer – the use of laxatives [1, 7, 17, 29, 31, 32]. Physical activity and optimal fluid and fiber intake stimulate intestinal peristalsis, while excessive fiber intake has a counterproductive effect and is not recommended [1, 2, 17, 18, 19, 29, 32]. The use of glycerin suppositories and enemas is only justified in cases of fecal impaction [1, 16, 29]. Due to possible colon perforation, digital disimpaction should be avoided [1]. According to the results of a large number of studies, there is no evidence that the use of probiotics, symbiotics and prebiotics significantly contributes to resolving functional constipation [29, 33]. Table 1 provides the doses of the most commonly used laxatives intended for the treatment of constipation in children and adolescents [1, 16, 17, 29, 32, 34, 35].

Polyethylene glycol (PEG) is the first-choice laxative in the treatment of functional constipation. It is characterized by good solubility in water, negligible intestinal absorption (0.1–0.2%) and, accordingly, high effectiveness in treating constipation in all ages [1, 16, 29]. In addition, it is successfully used in the treatment of fecal impaction [1, 18, 29].

Table 1. Dosages of most-frequently used laxatives

Laxatives	Age (years)	Dosages
Polyethylene glycol	All ages	Maintenance: 0.2–0.8 g/kg/day; Fecal disimpaction: 1–1.5 g/kg/day (max. six consecutive days)
Lactulose (70% solution)	All ages	1–2 mL/kg/day in one or two doses
Sorbitol (70% solution)	1–11 > 12	1 mL/kg/day in one or two doses 15–30 mL/kg/day in one or two doses
Lactitol	1–6 6–12 12–18	0.5–1 g/kg/day in two or three doses 10–30 g/day in two or three doses 20–60 g/day in two or three doses
Glycerin suppository	< 1 > 1	0.5 pediatric suppositories once daily 1 pediatric suppository once daily

Table 2. Causes of secondary constipation in childhood and adolescence

Hirschsprung disease	Anorexia nervosa
Anorectal malformations	Psychological stress
Spina bifida	Depression
Hypothyroidism	Autism
Diabetes mellitus	Cerebral palsy
Celiac disease	Meningomyelocele
Cow's milk protein allergy	Chronic intestinal pseudo-obstruction
Cystic fibrosis	Visceral/autonomic neuropathy
Diabetes insipidus	Diuretics
Vitamin D intoxication	Anticholinergics
Hypokalemia	Anticonvulsants
Uremia	Calcium channel blockers
Porphyria	Antidepressants
Down syndrome	Chemotherapy
Ehlers-Danlos syndrome	Methylphenidate
Scleroderma	Heavy metal poisoning (Pb, Hg, Cd, As)

Like other oral laxatives, it is contraindicated in diseases accompanied by intestinal obstruction [18].

Lactulose (4-0-β-D-galactopyranosyl-D-fructofuranose) is a synthetic disaccharide composed of galactose and fructose molecules linked by a β1→4 glycosidic bond. Like PEG, it is used in the treatment of functional constipation at all ages [1, 16, 29]. It achieves its laxative effect by not breaking down in the small intestine and, together with the accompanying water fraction, reaches the colon making fecal contents less consistent and easier to eliminate [18, 35]. The osmotic laxative effect is further enhanced by lactic and acetic acids, which are produced by the fermentation of a portion of lactulose by colonic bacteria. The use of lactulose in the treatment of functional constipation, thanks to its high efficiency and safety, has lasted for more than 50 years. Due to the presence of free galactose, it is contraindicated in patients with galactosemia.

Sorbitol and lactitol are polyhydroxy alcohols (polyols) with a sweet taste. The osmotic laxative effect is a result of the low degree of intestinal absorption.

There are other medications for the same purpose, such as magnesium hydroxide, magnesium citrate, bisacodyl, sodium phosphate, sodium picosulfate, senna, and mineral oils, but their use in the treatment of functional constipation in children and adolescents is less common [17, 29, 32, 35].

SECONDARY CONSTIPATION

Secondary constipation is a consequence of pathological conditions that compromise intestinal emptying. It occurs as part of various anatomical, neuromuscular, inflammatory, endocrine, metabolic and neoplastic diseases, the use of some drugs and heavy metal poisoning (Table 2) [1, 2, 4, 5, 36–48].

With the exception of Hirschsprung disease and anorectal malformations, where constipation may be the only sign of the disease, in the other pathological conditions listed it is only one of the manifestations of the underlying disorder [44]. Therefore, a detailed history and complete physical examination play an important role in identifying the underlying disorder and planning its confirmation. Accordingly, it should be noted that the failure to pass meconium within 48 hours after birth suggests Hirschsprung disease, the presence of persistent constipation with onset in the neonatal period suggests Hirschsprung disease, cystic fibrosis, hypothyroidism, congenital anomalies of the anorectal or spinal region, the absence of anal and/or cremasteric reflex suggests a spinal cord anomaly, verification of spasm, and lack of rectal contents on digital examination, and explosive elimination of liquid stool and gases upon finger withdrawal suggests Hirschsprung disease, hypertrichosis, fovea, lipoma or hemangioma in the lumbar region suggests spinal dysraphism, and the like [1, 17, 23].

Treatment of secondary constipation is primarily causal in nature.

CONCLUSION

Constipation is a common disorder in childhood and adolescence. Functional constipation, which accounts for 90–95% of this disorder, occurs in the absence of a pathological background, while secondary constipation is a consequence of various pathological conditions accompanied by the inability to have normal intestinal emptying. Considering the importance of the problem, as well as the potential complications, sometimes very serious, chronic constipation requires a prompt diagnostic and therapeutic approach. The therapy of functional constipation is based on dietary modification, normalizing the rhythm of intestinal emptying, and adjuvant use of laxatives, while the treatment of secondary constipation is causal in nature.

Ethics: The authors declare that the article was written in accordance with the ethical standards of the Serbian Archives of Medicine as well as the ethical standards of medical facilities for each author involved.

Conflict of interest: None declared.

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Опстипација у дечјем и адолесцентном добу

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САЖЕТАК

Опстипација је чест проблем у дечјем и адолесцентном добу. Јавља се као функционални (примарни или идиопатски) поремећај или у оквиру различитих патолошких стања која компромитују интестинално пражњење. У 90–95% случајева опстипација у дечјем и адолесцентном добу је функционалне природе. Имајући у виду озбиљност проблема, као и могуће компликације, некад и веома озбиљне, опстипација захтева неодложан дијагностичко-терапијски приступ. Терапија функционалне опстипације заснива се на модификацији исхране ради нормализације конзистенције столице и олакшања акта дефекације, као и на успостављању нормалног ритма интестиналног пражњења. Током прва два месеца, а понекад и дуже, примењују се лаксативи, док је терапија секундарне опстипације каузалног карактера.

Кључне речи: опстипација; деца и адолесценти; клиничке манифестације; терапија

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CURRENT TOPIC / АКТУЕЛНА ТЕМА

An anesthesiologist's perspective on advancements in perioperative analgesia

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SUMMARY

Introduction Effective perioperative pain management remains a cornerstone of modern anesthesiology, directly influencing patient outcomes, recovery times, and overall satisfaction. This paper aims to explore advancements in perioperative analgesia, focusing on updated guidelines, innovative strategies for pain management, and the role of multimodal anesthesia in optimizing outcomes. The paper highlights the anesthesiologist's pivotal role in implementing these strategies while addressing challenges such as opioid overuse and inadequate pain control.

Methods A comprehensive study was conducted, analyzing recent studies, clinical trials, and guideline updates published over the past decade. The paper examines the evolution of multimodal anesthesia, which integrates non-opioid analgesics, regional techniques, and non-pharmacologic interventions to reduce opioid reliance and enhance recovery. Special attention is given to the management of pain in vulnerable populations, including pediatric and geriatric patients, and those with chronic conditions.

Results Findings reveal that adherence to evidence-based guidelines, combined with a multimodal approach, significantly improves pain management and patient outcomes. Recent innovations, including the use of enhanced recovery after surgery protocols, perioperative nerve blocks, and adjunct therapies such as ketamine and gabapentinoids, are reshaping clinical practice. The paper also emphasizes the importance of personalized pain management plans and continuous education for anesthesiologists.

Conclusion This paper concludes that the integration of updated guidelines, multimodal strategies, and patient-centric care models is essential for achieving optimal analgesic outcomes. Future research should focus on refining multimodal protocols, exploring novel analgesics, and addressing barriers to implementation in diverse clinical settings.

Keywords: perioperative analgesia; multimodal anesthesia; enhanced recovery after surgery

INTRODUCTION

Effective perioperative pain management is a critical aspect of modern anesthesia. Poorly controlled pain during and after surgery not only delays recovery but can also lead to short- and long-term complications, including chronic post-surgical pain (CPSP) and opioid dependency [1]. Current clinical practice often relies heavily on opioids as the cornerstone of analgesia, despite the well-documented risks of opioid-related side effects, including respiratory depression, nausea, and the potential for long-term dependency. Pain management remains inconsistent across different healthcare settings, with significant variability in the adoption of multimodal analgesic strategies and adherence to evidence-based guidelines [2].

New guidelines and research emphasize the need for multimodal anesthesia (MMA) – an approach that combines various analgesic techniques and pharmacologic agents that act synergistically to target different pain pathways and reduce the reliance on any single drug, particularly opioids. Techniques such as regional anesthesia and the use of adjunctive medications (e.g., ketamine, dexmedetomidine, and gabapentinoids) are gaining interest in the last decade. The integration of these strategies into routine clinical practice is still slow, due to lack of training, resource constraints, and variability in institutional protocols [1].

The aim of this review is to address updates to guidelines, novel perioperative pain management strategies, and the role of MMA in optimizing surgery outcomes, as well as improving and enhancing patient-centered care.

METHODS

Literature search was conducted using PubMed, MEDLINE, and Google Scholar database for published articles in the past 30 years (1995–2025). Terms used in the search were "perioperative analgesia," "multimodal analgesia," "MMA," "enhanced recovery after surgery," "ERAS," "perioperative pain," "multimodal anesthesia," "anesthesia." Further articles were found through cross-referencing. Only articles

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that included human studies and were published in English were included in the review. Primary studies (e.g., retrospective studies, prospective studies, observational studies, randomized controlled trials, etc.), basic science research, metanalyses, and systematic reviews were included, while case reports were excluded from the review.

Current challenges in pain management

Reliance on opioids as the primary analgesic has contributed to the opioid crisis, indicating a need for safer, multimodal approaches. Perioperative pain management is particularly challenging in special populations, such as elderly patients, pediatric patients, and individuals with significant comorbidities. A change in clinical practice is required, which focuses on individualized, evidence-based strategies that decrease opioid use, but still offer efficient analgesia.

Inadequate perioperative pain control

Perioperative pain control remains inadequate, despite advances in anesthetic techniques and pain management protocols. Many surgical patients continue to experience moderate to severe postoperative pain. Poorly managed pain can lead to immediate postoperative complications, prolonged hospital stays, and even long-term health consequences such as CPSP [3].

CPSP is defined as pain persisting beyond three months after surgery and affects approximately 10–50% of patients, depending on the surgical procedure [4]. Surgeries with the highest CPSP incidence include thoracotomy, mastectomy, total knee arthroplasty, hernia repair, and amputation. Risk factors for CPSP include poorly controlled acute pain, repeated surgical trauma, nerve damage, psychological stress, and pre-existing chronic pain conditions. Effective perioperative pain management, particularly through regional anesthesia and multimodal analgesia, can significantly reduce the risk of CPSP [4].

Severe postoperative pain can lead delayed recovery and increased morbidity, including the risk of deep vein thrombosis (DVT), pulmonary embolism, pneumonia, and muscle deconditioning. Inadequate analgesia prolongs hospital stays and increases the likelihood of unplanned readmissions, raising healthcare costs. Poorly controlled pain can impair gastrointestinal function, leading to postoperative ileus, nausea, and vomiting, further delaying recovery [5]. It can also have significant psychological and emotional impact, contributing to postoperative anxiety, depression, and sleep disturbances [6].

Opioid use and associated risks

Opioids have traditionally been the cornerstone of perioperative pain management due to their potent analgesic effects. Overreliance on opioids and their widespread use has led to a host of complications, including dependency, addiction, and a growing healthcare burden. Studies indicate that 5–10% of opioid-naïve patients who receive opioids postoperatively develop long-term dependence [7]. A significant proportion of opioid-related deaths stem from prescriptions initially provided for postoperative pain management [8]. Adverse effects of opioids include acute opioid tolerance, iatrogenic withdrawal syndrome, persistent opioid use, chronic pain, and opioid-induced hyperalgesia [9].

MMA PROTOCOL IN PAIN MANAGEMENT

Pharmacological combinations of analgesics and non-pharmacological therapies and minimize opioid use are the fundamental components of MMA protocol. Adjuvant therapies should be administrated before surgery to optimize intraoperative and postoperative analgesia, to reduce opioid-related adverse effects in the recovery period [10].

Multimodal approach is more focused on non-opioid analgesics (nonsteroidal anti-inflammatories, acetaminophen, gabapentinoids, N-methyl D-aspartate (NMDA) antagonists, alpha-2-agonists), regional anesthesia techniques, and non-pharmacological strategies to decrease the use of opioids and associated side effects [10]. It may be more effective as a pain control strategy, decreasing the complications associated with suboptimal pain control, such as pneumonia, DVT, and postoperative cognitive dysfunction. Nevertheless, opioids still have a critical role in acute postoperative pain management especially for procedures where a primary regional, neuraxial, or local infiltration is not possible [11].

Acetaminophen remains the recommended first-line treatment for mild-to-moderate acute pain in general population, especially in vulnerable populations. It can be used in combination with non-steroidal anti-inflammatory drugs (NSAIDs) with synergistic effect, which is desirable in multimodal protocols for optimal pain control [12]. There is evidence that acetaminophen achieves analgesic effect through multiple pathways. One mechanism of action is through cyclooxygenase (COX)-dependent inhibitory effect and by the formation of the bioactive AM404 metabolite as a potent activator of transient receptor potential vanilloid 1, a major contributor to neuronal response to pain in the brain and dorsal horn. Acetaminophen also decreases central oxidative stress and prostaglandin release, which effects descending pain inhibitory pathways. The side effects are generally mild and include nausea, headache, stomach pain, and a rash, while the most serious side effect is liver toxicity which does not occur unless taken in large quantities [13].

NSAIDs reduce pain by reducing the activity of cyclooxygenase (or COX) enzymes and inhibiting peripheral prostaglandin synthesis. Non-selective NSAIDs such as ibuprofen, diclofenac, and naproxen inhibit both COX-1 and COX-2 enzymes. Selective NSAIDs (COX-2 inhibitors, coxibs) such as celecoxib and etoricoxib selectively inhibit COX-2, which plays a greater role in prostaglandin mediated pain and inflammation [14]. NSAIDs are routinely used in perioperative and postoperative analgesia, as opioid-sparing analgesics, for treatment moderate to severe

Table 1. Non-opioid analgesics used in multimodal approach of pain control

Analgesics	Analgesic Mechanism of action	Application	Dosage	Adverse effects
Acetaminophen	• Inhibiting COX-1 and COX-2 enyzmes • Activating the TRPV1 and CB-1	Oral, rectal, and intravenous route	• In children > 12 years and adults 500–1000mg every 4–6 hours (maximum 4000 mg daily) • In children one month to 12 years 15 mg/kg per dose every six hours	• Potentiate warfarin anticoagulation • Chronic alcohol misuse increases the risk of toxicity • Contraindicated in cases of active liver disease or severe hepatic impairment. • Other: skin rash, hypersensitivity reactions, nephrotoxicity
NSAIDs	• Non selective inhibition of both COX-1 and COX-2 or selective inhibition of COX-2	Oral, intramuscular, and intravenous route	Diclofenac: 50–75mg mg 2–3 times/daily or (max 150 mg) PO or IM. Ibuprofen: Initial dose 400 mg IV, then 100–200 mg 4–6 hours IV 1200-3200m/daily in 3–4 doses PO Ketrolac: 15–30 mg every 4–6 hours IM or IV or 10 mg 4–6 hours PO Meloxicam: 7.5–15 mg daily PO Celecoxib: 50–200 mg (daily in one or two doses PO	• Prolonged use can lead to gastric, renal and cardiovascular adverse effects • Hepatic adverse effects are less common • Possible hematologic adverse effects in hemophilia, thrombocytopenia, von Willebrand, etc.) • Other: anaphylactoid reactions that involve urticaria and aspirin-exacerbated respiratory disease.
Gabapentinoids	• Inhibition of calcium-mediated neurotransmitter release through effects on α2δ-1 subunits	Oral route	Gabapentine: 900–3600 mg/day in three doses PO Pregabaline:150–600 mg/day in two to three doses PO	• CNS adverse effects (dizziness, senescence, gait disturbances) • Gastrointestinal adverse effects (abdominal distension, abnormal appetite, constipation, dry mouth and nausea) • Weight gain • Respiratory depression when used in combination with opioids
NMDA antagonist (ketamine)	• NMDA and glutamate receptor antagonist	Intranasal, intravenous intramuscular route	• 0.3–0.5 kg/kg IV bolus • 0.1–0.2 mg / kg / h IV infusion • 1 mg/kg intranasally	• Cardiovascular, gastrointestinal, respiratory, neurologic and psychiatric side effects • Coadministration with opioid analgesics, benzodiazepines, CNS depressants, and alcohol, may induce profound sedation, respiratory depression, coma, and potentially fatal outcomes. • Coadministration with sympathomimetic medications may increase sympathomimetic effects • Coadministration with theophylline or aminophylline could potentially reduce the seizure threshold. • Contraindicated in patients with aortic dissection, uncontrolled hypertension, myocardial infarction, or aneurysms
Magnesium	•NMDA receptor antagonist	Oral, and intravenous route	Post-surgery: 30 mg/kg bolus followed by an infusion of 10 mg / kg / h Oral intake Children 2.5–5 mg/kg daily Adults 310–420 mg/daily	Contraindicated in renal dysfunction, pregnancy, and neuromuscular disease. Hypermagnesemia (levels greater than 2.6 mg/dL and above) can lead to cardiovascular complications, neurological disorder, cardiorespiratory arrest (serum values exceeding 15 mg/dL)
Alpha-2 agonists	Agonist of presynaptic and postsynaptic α_2-adrenergic receptors nociceptive	Oral, IV, transdermal, epidural, perineural	Clonidine Oral premed: 2–4 μg/kg; IV bolus: 1–2 μg/kg; Epidural: 150–300 μg Dexmedetomidine IV bolus: 0.5–1 μg/kg over 10 min Infusion: 0.2–0.7 μg/kg/h	Hypotension, bradycardia, sedation, dry mouth Contraindicated in severe bradyarrhythmia, hemodynamic instability, severe ventricular dysfunction, uncontrolled cerebrovascular disease
Intravenous lidocaine	Inhibition of VGSCs inhibition of G protein-coupled receptors Inhibition of NMDA receptors	Intravenous route	1.5 mg/kg IV bolus 1–2 mg / kg / h IV infusion	Cardiovascular (bradycardia, hypotension) and neurological side effects (dizziness, seizures)

COX – cyclooxygenase; TRPV – transient receptor potential vanilloid; CB – cannabinoid; IM – intramuscular; IV – intravenously; PO – per os; NMDA – N-methyl-D-aspartate; CNS – central nervous system; VGSC – voltage-gated sodium channel

pain in children and adults. Regarding adverse effects and safety of NSAIDs, most patients are not at any increased risk of developing adverse central, gastrointestinal, renal and respiratory adverse events. The recent introduction of celecoxib, a selective COX-2 inhibitor, has significantly decreased the risk of postoperative gastrointestinal bleeding and anastomotic leak that are traditionally associated with non-selective NSAIDs. Despite their overall favorable safety profile, NSAIDs should be used with caution in patients with non-erosive reflux disease, patients with prior myocardial infarction on antithrombotic therapy, patients with asthma, and patients with a history of renal disease [15].

Gabapentinoids (gabapentin and pregabalin) are antiepileptic agents which can be used as perioperative analgesics. Mechanism of actions involves inhibition of presynaptic calcium-mediated neurotransmitter release through effects on α2δ-1 subunits in the dorsal root ganglia and spinal cord which are upregulated in the event of surgical trauma, resulting in non-distribution excitatory neurotransmitters [16]. Gabapentinoids are effective in gynecologic, breast, orthopedic, and spine surgery, when administered two hours before surgery in smaller doses due to concern for respiratory depression [17].

NMDA antagonist. Ketamine has been shown to effectively alleviate pain as antagonists of central NMDA receptors. Its effects are dose dependent: at anesthetic doses it produces dissociative anesthesia, whereas at subanesthetic doses (typically 0.25–0.5 mg/kg bolus followed by 0.1–0.3 mg/kg/h infusion) it exerts strong analgesic and anti-hyperalgesic effect without loss of consciousness [18]. The most common adverse events associated with ketamine/s-ketamine are dissociation, anxiety, nausea, increased blood pressure, and headache. Most side effects are mild, transient, dose dependent, and attenuate with subsequent treatments. When compared to ketamine, use of subanesthetic S-ketamine in MMA protocols is associated with fewer psychomimetic adverse effects, less emergence delirium, and overall better tolerability [19].

Magnesium is a NMDA receptor antagonist, causing central nervous system (CNS) depression through calcium channels. Magnesium has analgesic effect, reduces postoperative pain and opioid consumption in surgical patients and can be successfully used as a part of multimodal perioperative analgesia [20].

Alpha-2 agonists (clonidine, dexmedetomidine) are often used perioperatively to enhance analgesia, stabilize hemodynamics, and reduce opioid consumption [2]. They act as antagonists of presynaptic and postsynaptic α_2-adrenergic receptors in the CNS, leading to inhibition of norepinephrine release, decreased sympathetic outflow, and modulation of spinal and supraspinal nociceptive transmission. This results in sedative, anxiolytic, and analgesic effects, along with an opioid-sparing action valuable in perioperative pain management [11]. Adverse effects are generally dose dependent and include bradycardia, hypotension, sedation, and, less frequently, dry mouth and delayed recovery if overdosed.

Intravenous local anesthetics (lidocaine). These medications are currently not routinely used for perioperative pain control, because of limited evidence available in the literature [13]. There are data that indicate that lidocaine infusions reduce postoperative pain scores, nausea, and vomiting, and accelerate bowel function and reduce opioid consumption after abdominal surgery [21]. Lidocaine inhibits of sodium channels, G protein-coupled receptors, and NDMA receptors, leading to suppression of local and systemic inflammation, nociceptive transmission, and central sensitization. Side effects from perioperative intravenous lidocaine infusion include drowsiness and blunted response to tracheal extubation, as well as symptoms commonly associated with local anesthetic systemic toxicity such as tinnitus, perioral numbness, lightheadedness, dizziness, visual changes, and more seriously arrhythmias and seizures [22].

REGIONAL AND LOCAL ANESTHESIA TECHNIQUES

Regional anesthesia includes both neuraxial (spinal and epidural) anesthesia and peripheral nerve blocks. These techniques have resulted in better control of perioperative pain, decreased opioid use and time spent in the post-anesthesia care unit after various surgical procedures [23]. There is evidence that show that application of regional anesthesia can prevent persistent postoperative pain and chronic postsurgical pain in postoperative settings. Regional anesthesia techniques carry a certain risk of complications like nerve injury, bleeding, infection, rebound pain, and potential local anesthetic toxicity. In the last decade, use of ultrasound guidance has mostly contributed to wider use and the safety of regional anesthesia, resulting in reducing these complications in clinical settings [24].

Traditionally, bupivacaine was mostly used in regional anesthesia. Levobupivacaine and ropivacaine were developed as isomers with nearly identical effects in onset, quality, and duration of sensory block, but better safety profile comparing to bupivacaine [24]. For better pain management, both proved to be effective in combination with fentanyl [23].

Epidural anesthesia and spinal anesthesia are one of the oldest forms of regional anesthesia. They are often used in thoracic surgery, orthopedic surgery, and obstetrical procedures. The paravertebral block involves the delivery of local anesthetics to the paravertebral space near the spinal nerves emerging from the intervertebral foramen which is a reliable technique for reducing pain in the immediate postoperative period after breast surgery. Upper limb nerve blocks are employed due to the high incidence of postoperative pain in upper limb surgeries. The transverse abdominis plane block involves application of local anesthetics to the fascial plane between the internal oblique and transverse abdominus muscles and it is used for colorectal surgeries and obstetrical procedures. Quadratus lumborum block involves application of local anesthetic around the quadratus lumborum muscle and covers which is effective for abdominal, obstetric, pelvic, and renal surgeries. Erector spinae block is a fascial plane technique used to treat both acute and chronic pain, with many applications

ranging from head and neck, thoracic, abdominal, and lower extremity surgical procedures. These techniques are considered safe, result in excellent perioperative analgesia and decrease use of opioids postoperatively [25].

One major drawback of regional anesthesia is its limited duration of effective analgesia. This depends on the type, volume, centration of applied local anesthetic, and patients' comorbidities, but could result in intense postoperative pain and rebound pain. These effects can be more easily managed for patients in hospital settings, than for those who had one-day surgery which usually require for post-discharge medical attention. As a result of efforts to increase the length of analgesia in pre-existing local anesthetics led to development of new formulations of local anesthetics like EXPAREL® (Pacira BioSciences, Inc., Brisbane, CA, USA) liposomal bupivacaine and SABER®-Bupivacaine (DURECT Corp., Cupertino, CA, USA). These medications have significantly reduced postoperative pain levels up to 72 hours, opioid consumption and hospital stay, while increasing the time to first rescue opioid medication use [23].

ROLE OF MMA PROTOCOL IN ENHANCED RECOVERY AFTER SURGERY (ERAS)

ERAS protocols are multimodal perioperative care pathways designed to achieve fast recovery in patients after surgical procedures by defining and maintaining preoperative organ function and minimizing the stress response following surgery. Unlike traditional perioperative management, which often focuses on isolated interventions, ERAS integrates multimodal protocols across various perioperative stages to create multidisciplinary, evidence-based approach designed to improve surgical outcomes, minimize reliance on opioids while enhancing pain control and reduce postoperative complications by optimizing perioperative care [11].

A multi-society consensus statement [26] establishes seven guiding principles for acute perioperative pain management to help institutions enhance surgical patient care. These principles emphasize the need for preoperative evaluation of medical and psychological conditions, screening for potential substance use disorders, a focus on multimodal analgesia incorporating nonpharmacologic interventions, and the use of validated pain assessment tools to guide and adjust treatment. The consensus highlights that clinicians should conduct a thorough preoperative evaluation, assessing medical and psychological conditions, medication history, chronic pain, substance use disorder, and prior postoperative pain treatment responses to develop a personalized pain management plan [11].

Preoperative phase in ERAS protocol involves comprehensive preoperative preparation, where MMA plays a vital role in effective perioperative analgesia. Psychological preparation of patients, including cognitive behavioral strategies and anxiety reduction techniques, minimizes the stress response to surgery. Preoperative counseling also addresses expectations regarding postoperative pain control and early mobilization [24]. Preemptive administration of non-opioid analgesics, such as acetaminophen or NSAIDs, is an essential part of ERAS. Preoperative carbohydrate loading reduces insulin resistance and enhances metabolic recovery, indirectly contributing to improved analgesia and reduced catabolism [24]. Patients at risk of chronic pain or opioid use disorder may receive gabapentinoids or NMDA receptor antagonists as part of a preemptive multimodal approach [16].

The intraoperative phase of ERAS focuses on maintaining hemodynamic stability, minimizing surgical stress, and ensuring effective analgesia while avoiding excessive sedation or opioid burden. MMA within ERAS emphasizes a balance between analgesia and hemodynamic stability. Planned application of fluid therapy prevents fluid overload, which reduces tissue edema, optimizes oxygen delivery, and enhances postoperative recovery. Maintaining normothermia through warming devices reduces surgical stress and supports hemodynamic stability [26]. A balanced anesthesia approach prioritizes opioid-sparing approach prioritize integration of regional techniques and patient-specific sedation. Total intravenous anesthesia with added dexmedetomidine or lidocaine may improve postoperative pain control while avoiding opioid-related side effects such as nausea and ileus [11]. The use of short-acting anesthetic agents facilitates early emergence and immediate postoperative recovery. Suppressing the surgical stress response using multimodal strategies reduces postoperative catabolism, immune dysfunction, and pain hypersensitivity. Techniques such as regional blocks or neuraxial anesthesia mitigate the neuroendocrine stress response to surgery. Anti-inflammatory agents, such as corticosteroids, further enhance recovery by modulating the surgical inflammatory response [14].

The postoperative phase of ERAS focuses on optimizing analgesia to supports rapid emergence and allow patients to regain consciousness and protective airway reflexes faster. Avoiding long-acting opioids and benzodiazepines facilitates early extubation, reducing the risk of respiratory complications and postoperative delirium [26]. Multimodal analgesia also minimizes postoperative shivering, nausea, and excessive sedation, improving time spent in post-anesthesia care unit. It facilitates early return of bowel function and early oral intake [10]. Early mobility accelerates muscle recovery, shortens hospital length of stay, and decreases the incidence of postoperative complications such as pneumonia and DVT [11].

FUTURE DIRECTIONS AND IMPLEMENTATION OF MULTIMODAL APPROACHES IN SERBIA

The adoption of multimodal analgesia in perioperative protocols in Serbia faces several clinical challenges, although studies were already published on the subject [27]. One of the primary concerns is the existing training gap among anesthesiologists and surgical teams. Despite the evident benefits of multimodal analgesia, many healthcare professionals lack adequate exposure and hands-on experience in administering regional anesthesia techniques

effectively. Additionally, resistance to change among medical practitioners remains a significant obstacle. Many anesthesiologists and surgeons continue to rely on conventional management strategies due to familiarity and institutional inertia. Another critical challenge is the variability in hospital protocols across different healthcare institutions. While some hospitals have adopted comprehensive multimodal analgesia protocols, others continue to follow outdated pain management strategies, leading to inconsistent patient outcomes [28].

Limited access to resources slows down implementation of multimodal analgesia in Serbia. The availability of regional anesthesia techniques and multimodal drug combinations is inconsistent across hospitals, particularly in smaller healthcare facilities. To overcome these challenges, primary goal should be establishing structured educational programs that focus on multimodal analgesia. These programs should be integrated into anesthesiology training curricula and include hands-on workshops to ensure competency in regional anesthesia techniques. Efforts should be made to promote adherence to national and international guidelines and standardize perioperative pain management protocols across healthcare institutions. Interdisciplinary collaboration between anesthesiologists, surgeons, and pain specialists is essential for the successful adoption of multimodal analgesia. Establishing acute pain service units in hospitals can also facilitate coordinated pain management efforts and ensure optimal patient care [29].

CONCLUSION

Advancements in perioperative analgesia like importance of MMA, adherence to evolving guidelines, and a personalized approach have significantly transformed pain management and improve patient recovery and satisfaction. By integrating opioid-sparing techniques and regional anesthesia, anesthesiologists can optimize pain control while minimizing adverse effects. Recognizing the variability in patient responses emphasize the need for a personalized approach, where treatment is adapted to factors such as surgical type, comorbidities, and patient preferences. Anesthesiologists and healthcare providers must remain committed to continuous learning and guideline adherence to ensure optimal patient outcomes. Institutional policies should support the widespread implementation of evidence-based analgesic protocols, promoting interdisciplinary collaboration in perioperative care. By prioritizing these efforts, the medical community can continue to advance perioperative analgesia and improving patient safety and quality of life.

Ethics: The authors hereby declare that this article was prepared in full compliance with the ethical standards set forth by the journal Serbian Archives of Medicine, as well as the ethical guidelines and institutional policies adhered to by each author involved in the research and writing process.

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Перспектива анестезиолога о новинама на пољу периоперативне аналгезије

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САЖЕТАК

Увод Ефикасна периоперативна терапија бола остаје основа модерне анестезиологије, јер директно утиче на исходе оперативног лечења пацијената, време опоравка и укупно задовољство пацијената. Овај рад има за циљ да истражи новине у периоперативној аналгезији, фокусирајући се на нове смернице и стратегије за терапију бола, као и улогу мултимодалне анестезије у оптимизацији исхода лечења. Битно је истаћи улогу анестезиолога у имплементацији ових стратегија, али и изазове у клиничкој пракси, као што су прекомерна употреба опиоида и неадекватна контрола бола.

Методе Спроведено је свеобухватно истраживање које анализира најновије студије, клиничка испитивања и ажуриране смернице објављене током претходне деценије. Рад испитује еволуцију мултимодалне анестезије, која обједињује употребу неопиоидних аналгетика, регионалних техника и нефармаколошких интервенција како би се смањило ослањање на опиоиде и побољшао опоравак. Посебна пажња се посвећује управљању болом у угроженим популацијама, укључујући педијатријске и геријатријске пацијенте и оне са хроничним стањима.

Резултати Налази истраживања откривају да праћење смерница заснованих на доказима, у комбинацији са мултимодалним приступом, значајно побољшава терапију бола и исходе оперативног лечења пацијената. Савремене иновације, укључујући спровођење протокола побољшаног опоравка после операције, употребу периоперативних нервних блокова и помоћних медикамената као што су кетамин и габапентиноиди, значајно мењају клиничку праксу. Овај рад наглашава и важност персонализованих планова за терапију бола и спровођења континуиране едукације за анестезиологе.

Закључак Треба нагласити да је интеграција нових смерница, мултимодалних стратегија и модела неге усмерених на пацијента од суштинског значаја за постизање оптималних исхода аналгезије. Будућа истраживања треба да се фокусирају на усавршавање мултимодалних протокола, истраживање нових аналгетика и решавање препрека за имплементацију ових метода у различитим клиничким окружењима.

Кључне речи: периоперативна аналгезија; мултимодална анестезија; побољшан опоравак након операције

Пре подношења рукописа Уредништву часописа „Српски архив за целокупно лекарство“ (СА) сви аутори треба да прочитају Упутство за ауторе (*Instructions for Authors*), где ће пронаћи све потребне информације о писању и припреми рада у складу са стандардима часописа. Веома је важно да аутори припреме рад према датим пропозицијама, јер уколико рукопис не буде усклађен с овим захтевима, Уредништво ће одложити или одбити његово публиковање. Радови објављени у СА се не хонорарашу. За чланке који ће се објавити у СА, самом понудом рада Српском архиву сви аутори рада преносе своја ауторска права на издавача часописа – Српско лекарско друштво.

ОПШТА УПУТСТВА. СА објављује радове који до сада нису нигде објављени, у целости или делом, нити прихваћени за објављивање. СА објављује радове на енглеском и српском језику. Због боље доступности и веће цитираности препоручује се ауторима да радове свих облика предају на енглеском језику. У СА се објављују следеће категорије радова: уводници, оригинални радови, претходна и кратка саопштења, прикази болесника и случајева, видео-чланци, слике из клиничке медицине, прегледни радови, актуелне теме, радови за праксу, радови из историје медицине и језика медицине, медицинске етике, регулаторних стандарда у медицини, извештаји са конгреса и научних скупова, лични ставови, коментари по позиви, писма уреднику, прикази књига, стручне вести, *In memoriam* и други прилози. Оригинални радови, претходна и кратка саопштења, прикази болесника и случајева, видео-чланци, слике из клиничке медицине, прегледни радови и актуелне теме, публикују се искључиво на енглеском језику, а остале врсте радова се могу публиковати и на српском језику само по одлуци Уредништва. Радови се увек достављају са сажетком на енглеском и српском језику (у склопу самог рукописа). Текст рада куцати у програму за обраду текста *Word*, фонтом *Times New Roman* и величином слова 12 тачака (12 *pt*). Све четири маргине подесити на 25 *mm*, величину странице на формат А4, а текст куцати с двоструким проредом, левим поравнањем и увлачењем сваког пасуса за 10 *mm*, без дељења речи (хифенације). Не користити табулаторе и узастопне празне карактере (спејсове) ради поравнања текста, већ алатке за контролу поравнања на лењиру и *Toolbars*. За прелазак на нову страну документа не користити низ „ентера“, већ искључиво опцију *Page Break*. После сваког знака интерпункције ставити само један празан карактер. Ако се у тексту користе специјални знаци (симболи), користити фонт *Symbol*. Подаци о коришћеној литератури у тексту означавају се арапским бројевима у угластим заградама – нпр. [1, 2], и то редоследом којим се појављују у тексту. Странице нумерисати редом у доњем десном углу, почев од насловне стране.

При писању текста на енглеском језику треба се придржавати језичког стандарда *American English* и користити кратке и јасне реченице. За називе лекова користити искључиво генеричка имена. Уређаји (апарати) се означавају фабричким називима, а име и место произвођача треба навести у облим заградама. Уколико се у тексту користе ознаке које су спој слова и бројева, прецизно написати број који се јавља у суперскрипту или супскрипту (нпр. ^{99}Tc, *IL*-6, O_2, *CD*8). Уколико се нешто уобичајено пише курзивом (*italic*), тако се и наводи, нпр. гени (*BRCA1*).

Уколико је рад део магистарске тезе, односно докторске дисертације, или је урађен у оквиру научног пројекта, то треба посебно назначити у Напомени на крају текста. Такође, уколико је рад претходно саопштен на неком стручном састанку, навести званичан назив скупа, место и време одржавања, да ли је рад и како публикован (нпр. исти или другачији наслов или сажетак).

КЛИНИЧКА ИСТРАЖИВАЊА. Клиничка истраживања се дефинишу као истраживања утицаја једног или више средстава или мера на исход здравља. Регистарски број истраживања се наводи у последњем реду сажетка.

ЕТИЧКА САГЛАСНОСТ. Рукописи о истраживањима на људима треба да садрже изјаву у виду писаног пристанка испитиваних особа у складу с Хелсиншком декларацијом и одобрење надлежног етичког одбора да се истраживање може извести и да је оно у складу с правним стандардима. Експериментална истраживања на хуманом материјалу и испитивања вршена на животињама треба да садрже изјаву етичког одбора установе и треба да су у сагласности с правним стандардима.

ИЗЈАВА О СУКОБУ ИНТЕРЕСА. Уз рукопис се прилаже потписана изјава у оквиру обрасца *Submission Letter* којом се аутори изјашњавају о сваком могућем сукобу интереса или његовом одсуству. За додатне информације о различитим врстама сукоба интереса посетити интернет-страницу Светског удружења уредника медицинских часописа (*World Association of Medical Editors – WAME; http://www.wame.org*) под називом „Политика изјаве о сукобу интереса“.

АУТОРСТВО. Све особе које су наведене као аутори рада треба да се квалификују за ауторство. Сваки аутор треба да је учествовао довољно у раду на рукопису како би могао да преузме одговорност за целокупан текст и резултате изнесене у раду. Ауторство се заснива само на: битном доприносу концепцији рада, добијању резултата или анализи и тумачењу резултата; планирању рукописа или његовој критичкој ревизији од знатног интелектуалног значаја; завршном дотеривању верзије рукописа који се припрема за штампање.

Аутори треба да приложе опис доприноса појединачно за сваког коаутора у оквиру обрасца *Submission Letter*. Финансирање, сакупљање података или генерално надгледање истраживачке групе сами по себи не могу оправдати ауторство. Сви други који су допринели изради рада, а који нису аутори рукописа, требало би да буду наведени у Захвалници с описом њиховог доприноса раду, наравно, уз писани пристанак.

ПЛАГИЈАРИЗАМ. Од 1. јануара 2019. године сви рукописи подвргавају се провери на плагијаризам/аутоплагијаризам преко *SCIndeks Assistant – Cross Check* (*iThenticate*). Радови код којих се докаже плагијаризам/ аутоплагијаризам биће одбијени, а аутори санкционисани.

НАСЛОВНА СТРАНА. На првој страници рукописа треба навести следеће: наслов рада без скраћеница; предлог кратког наслова рада, пуна имена и презимена аутора (без титула) индексирана бројевима; званичан назив установа у којима аутори раде, место и државу (редоследом који одговара индексираним бројевима аутора); на дну странице навести име и презиме, адресу за контакт, број телефона, факса и имејл адресу аутора задуженог за кореспонденцију.

САЖЕТАК. Уз оригинални рад, претходно и кратко саопштење, преглед литературе, приказ случаја (болесника), рад из историје медицине, актуелну тему, рад за рубрику језик медицине и рад за праксу, на другој по реду страници документа треба приложити сажетак рада обима 100–250 речи. За оригиналне радове, претходно и кратко саопштење сажетак треба да има следећу структуру: Увод/Циљ рада, Методе рада, Резултати, Закључак; сваки од наведених сегмената писати као посебан пасус који почиње болдованом речи. Навести најважније резултате (нумеричке вредности) статистичке анализе и ниво значајности. Закључак не сме бити уопштен, већ мора бити директно повезан са резултатима рада. За приказе болесника сажетак треба да има следеће делове: Увод (у последњој реченици навести циљ), Приказ болесника, Закључак; сегменте такође писати као посебан пасус који почиње болдованом речи. За остале типове радова сажетак нема посебну структуру.

КЉУЧНЕ РЕЧИ. Испод Сажетка навести од три до шест кључних речи или израза. Не треба да се понављају речи из наслова, а кључне речи треба да буду релевантне или описне. У избору кључних речи користити *Medical Subject Headings – MeSH (https://www.nlm.nih.gov/mesh/meshhome.html)*.

ПРЕВОД НА СРПСКИ ЈЕЗИК. На трећој по реду страници документа приложити наслов рада на српском језику, пуна имена и презимена аутора (без титула) индексирана бројевима, званичан назив установа у којима аутори раде, место и државу. На следећој – четвртој по реду – страници документа приложити сажетак (100–250 речи) с кључним речима (3–6), и то за радове у којима је обавезан сажетак на енглеском језику. Превод појмова из стране литературе треба да буде у духу српског језика. Све стране речи или синтагме за које постоји одговарајуће име у нашем језику заменити тим називом. Уколико је рад у целости на српском језику, потребно је превести називе прилога (табела, графикона, слика, схема) уколико их има, целокупни текст у њима и легенду на енглески језик.

СТРУКТУРА РАДА. Сви поднаслови се пишу великим масним словима (болд). Оригинални рад и претходно и кратко саопштење обавезно треба да имају следеће поднаслове: Увод (Циљ рада навести као последњи пасус Увода), Методе рада, Резултати, Дискусија, Закључак, Литература. Преглед литературе и актуелну тему чине: Увод, одговарајући поднаслови, Закључак, Литература. Првоименовани аутор прегледног рада мора да наведе бар пет аутоцитата (као аутор или коаутор) радова публикованих у часописима с рецензијом. Коаутори, уколико их има, морају да наведу бар један аутоцитат радова такође публикованих у часописима с рецензијом. Приказ случаја или болесника чине: Увод (Циљ рада навести као последњи пасус Увода), Приказ болесника, Дискусија, Литература. Не треба користити имена болесника, иницијале, нити бројеве историја болести, нарочито у илустрацијама. Прикази болесника не смеју имати више од пет аутора.

Прилоге (табеле, графиконе, слике итд.) поставити на крај рукописа, а у самом телу текста јасно назначити место које се односи на дати прилог. Крајња позиција прилога биће одређена у току припреме рада за публиковање.

СКРАЋЕНИЦЕ. Користити само када је неопходно, и то за веома дугачке називе хемијских једињења, односно називе који су као скраћенице већ препознатљиви (стандардне скраћенице, као нпр. ДНК, сида, ХИВ, АТП). За сваку скраћеницу пун термин треба навести при првом навођењу у тексту, сем ако није стандардна јединица мере. Не користити скраћенице у наслову. Избегавати коришћење скраћеница у сажетку, али ако су неопходне, сваку скраћеницу објаснити при првом навођењу у тексту.

ДЕЦИМАЛНИ БРОЈЕВИ. У тексту рада на енглеском језику, у табелама, на графиконима и другим прилозима децималне бројеве писати са тачком (нпр. 12.5 ± 3.8), а у тексту на српском језику са зарезом (нпр. 12,5 ± 3,8). Кад год је то могуће, број заокружити на једну децималу.

ЈЕДИНИЦЕ МЕРА. Дужину, висину, тежину и запремину изражавати у метричким јединицама (метар – *m*, килограм (грам) – *kg* (*g*), литар – *l*) или њиховим деловима. Температуру изражавати у степенима Целзијуса (°C), количину супстанце у молима (*mol*), а притисак крви у милиметрима живиног стуба (*mm Hg*). Све резултате хематолошких, клиничких и биохемијских мерења наводити у метричком систему према Међународном систему јединица (*SI*).

ОБИМ РАДОВА. Целокупни рукопис рада који чине – насловна страна, сажетак, текст рада, списак литературе, сви прилози, односно потписи за њих и легенда (табеле, слике, графикони, схеме, цртежи), насловна страна и сажетак на српском језику – мора износити за оригинални рад, рад из историје медицине и преглед литературе до 5000 речи, а за претходно и кратко саопштење, приказ болесника, актуелну тему, рад за праксу, едукативни чланак и рад за рубрику „Језик медицине“ до 3000 речи; радови за остале рубрике могу имати највише 1500 речи.

Видео-радови могу трајати 5–7 минута и бити у формату *avi, mp4* (*flv*). У првом кадру филма мора се навести: у

наднаслову Српски архив за целокупно лекарство, наслов рада, презимена и иницијали имена и средњег слова свих аутора рада (не филма), година израде. У другом кадру мора бити уснимљен текст рада у виду апстракта до 350 речи. У последњем кадру филма могу се навести имена техничког особља (режија, сниматељ, светло, тон, фотографија и сл.). Уз видео-радове доставити: посебно текст у виду апстракта (до 350 речи), једну фотографију као илустрацију приказа, изјаву потписану од свег техничког особља да се одричу ауторских права у корист аутора рада.

ПРИЛОЗИ РАДУ су табеле, слике (фотографије, цртежи, схеме, графикони) и видео-прилози.

Свака табела треба да буде сама по себи лако разумљива. Наслов треба откуцати изнад табеле, а објашњења испод ње. Табеле се означавају арапским бројевима према редоследу навођења у тексту. Табеле цртати искључиво у програму *Word*, кроз мени *Table–Insert–Table*, уз дефинисање тачног броја колона и редова који ће чинити мрежу табеле. Десним кликом на мишу – помоћу опција *Merge Cells* и *Split Cells* – спајати, односно делити ћелије. Куцати фонтом *Times New Roman*, величином слова 12 *pt*, с једноструким проредом и без увлачења текста. Коришћене скраћенице у табели треба објаснити у легенди испод табеле. Уколико је рукопис на српском језику, приложити називе табела и легенду на оба језика. Такође, у једну табелу, у оквиру исте ћелије, унети и текст на српском и текст на енглеском језику (никако не правити две табеле са два језика!).

Слике су сви облици графичких прилога и као „слике" у СА се објављују фотографије, цртежи, схеме и графикони. Слике се означавају арапским бројевима према редоследу навођења у тексту. Примају се искључиво дигиталне фотографије (црно-беле или у боји) резолуције најмање 300 *dpi* и формата записа *tiff* или *jpg* (мале, мутне и слике лошег квалитета неће се прихватати за штампање!). Уколико аутори не поседују или нису у могућности да доставе дигиталне фотографије, онда оригиналне слике треба скенирати у резолуцији 300 *dpi* и у оригиналној величини. Уколико је рад неопходно илустровати са више слика, у раду ће их бити објављено неколико, а остале ће бити у е-верзији чланка као *PowerPoint* презентација (свака слика мора бити нумерисана и имати легенду).

Видео-прилози (илустрације рада) могу трајати 1–3 минута и бити у формату *avi, mp4*(*flv*). Уз видео доставити посебно слику која би била илустрација видео-приказа у е-издању и објављена у штампаном издању. Уколико је рукопис на српском језику, приложити називе слика и легенду на оба језика.

Слике се у свесци могу штампати у боји, али додатне трошкове штампе сносе аутори.

Графикони треба да буду урађени и достављени у програму *Excel*, да би се виделе пратеће вредности распоређене по ћелијама. Исте графиконе прекопирати и у *Word*-ов документ, где се графикони означавају арапским бројевима према редоследу навођења у тексту. Сви подаци на графикону куцају се у фонту *Times New Roman*. Коришћене скраћенице на графикону треба објаснити у легенди испод графикона. У штампаној верзији чланка вероватније је да графикон неће бити штампан у боји, те је боље избегавати коришћење боја у графиконима, или их користити различитог интензитета. Уколико је рукопис на српском језику, приложити називе графикона и легенду на оба језика.

Цртежи и схеме се достављају у *jpg* или *tiff* формату. Схеме се могу цртати и у програму *CorelDraw* или *Adobe Illustrator* (програми за рад са векторима, кривама). Сви подаци на схеми куцају се у фонту *Times New Roman*, величина слова 10 *pt*. Коришћене скраћенице на схеми треба објаснити у легенди испод схеме. Уколико је рукопис на српском језику, приложити називе схема и легенду на оба језика.

ЗАХВАЛНИЦА. Навести све сараднике који су допринели стварању рада а не испуњавају мерила за ауторство, као што су особе које обезбеђују техничку помоћ, помоћ у писању рада или руководе одељењем које обезбеђује општу подршку. Финансијска и материјална помоћ, у облику спонзорства, стипендија, поклона, опреме, лекова и друго, треба такође да буде наведена.

ЛИТЕРАТУРА. Списак референци је одговорност аутора, а цитирани чланци треба да буду лако приступачни читаоцима часописа. Стога уз сваку референцу обавезно треба навести *DOI* број чланка (јединствену ниску карактера која му је додељена) и *PMID* број уколико је чланак индексиран у бази *PubMed/MEDLINE*.

Референце нумерисати редним арапским бројевима према редоследу навођења у тексту. Број референци не би требало да буде већи од 30, осим у прегледу литературе, у којем је дозвољено да их буде до 50, и у метаанализи, где их је дозвољено до 100. Број цитираних оригиналних радова мора бити најмање 80% од укупног броја референци, односно број цитираних књига, поглавља у књигама и прегледних чланака мањи од 20%. Уколико се домаће монографске публикације и чланци могу уврстити у референце, аутори су дужни да их цитирају. Већина цитираних научних чланака не би требало да буде старија од пет година. Није дозвољено цитирање апстраката. Уколико је битно коментарисати резултате који су публиковани само у виду апстракта, неопходно је то навести у самом тексту рада. Референце чланака који су прихваћени за штампу, али још нису објављени, треба означити са *in press* и приложити доказ о прихватању рада за објављивање.

Референце се цитирају према Ванкуверском стилу (униформисаним захтевима за рукописе који се предају биомедицинским часописима), који је успоставио Међународни комитет уредника медицинских часописа (*http://www.icmje.org*), чији формат користе *U.S. National Library of Medicine* и базе научних публикација. Примери навођења публикација (чланака, књига и других монографија, електронског, необјављеног и другог објављеног материјала) могу се пронаћи на интернет-страници *https://www.nlm.*

nih.gov/bsd/uniform_requirements.html. Приликом навођења литературе веома је важно придржавати се поменутог стандарда, јер је то један од најбитнијих фактора за индексирање приликом класификације научних часописа.

ПРОПРАТНО ПИСМО (*SUBMISSION LETTER*). Уз рукопис обавезно приложити образац који су потписали сви аутори, а који садржи: 1) изјаву да рад претходно није публикован и да није истовремено поднет за објављивање у неком другом часопису, 2) изјаву да су рукопис прочитали и одобрили сви аутори који испуњавају мерила ауторства, и 3) контакт податке свих аутора у раду (адресе, имејл адресе, телефоне итд.). Бланко образац треба преузети са интернет-странице часописа (*http://www.srpskiarhiv.rs/en/submission-letter/SubmissionLetterForm2023.pdf*).

Такође је потребно доставити копије свих дозвола за: репродуковање претходно објављеног материјала, употребу илустрација и објављивање информација о познатим људима или именовање људи који су допринели изради рада.

ЧЛАНАРИНА И НАКНАДЕ ЗА ОБРАДУ И ОБЈАВЉИВАЊЕ ЧЛАНКА. Да би рад био разматран за објављивање у часопису *Српски архив за целокупно лекарство*, сви аутори који су лекари или стоматолози из Србије морају бити чланови Српског лекарског друштва (у складу са чланом 9 Статута Друштва) у години у којој рад предају на разматрање.

Следеће накнаде су обавезне како би рад био прегледан, обрађен и потенцијално објављен у *Српском архиву за целокупно лекарство*:

- накнада за преглед сваког примљеног рада домаћих аутора: 6.000 динара по раду;
- накнада за прихваћен рад, односно накнада за објављивање рада домаћих аутора: 12.000 динара по раду;
- накнада за преглед сваког примљеног рада страних аутора: 75 евра (или 9000 динара) по раду;
- накнада за прихваћен рад, односно накнада за објављивање рада страних аутора: 150 евра (или 18000 динара) по раду.

Накнаде се плаћају пре прегледања, односно пре објављивања рада. Радови за које нису плаћене накнаде неће бити прегледани, односно објављени.

Треба напоменути да уплата накнаде за преглед рада није гаранција да ће рад бити прихваћен и објављен у *Српском архиву за целокупно лекарство*.

Установе (правна лица) не могу преко своје претплате да испуне овај услов аутора (физичког лица). Уз рукопис рада треба доставити копије уплатница за чланарину и накнаду за преглед чланка, као доказ о уплатама. Часопис прихвата донације од спонзора који сносе део трошкова или трошкове у целини оних аутора који нису у могућности да измире накнаду за преглед чланка (у таквим случајевима потребно је часопису ставити на увид оправданост таквог спонзорства).

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