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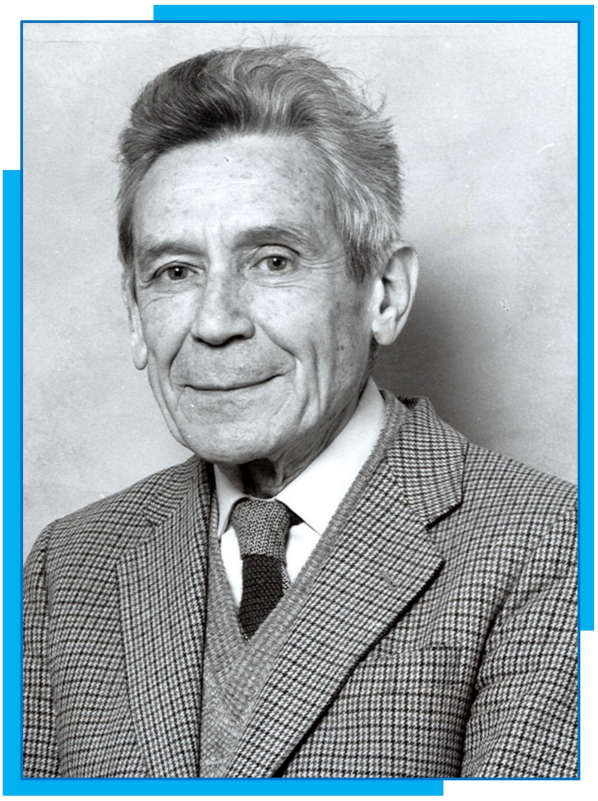
"Najveće bogatstvo nije ono što imamo, već ono što smo spremni da damo."

Vladeta Jerotić



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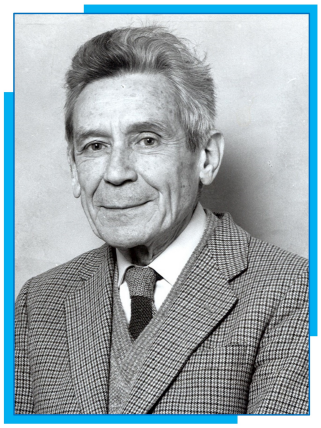

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Academician Vladeta Jerotić (August 2, 1924 – September 4, 2018), a member of the Serbian Academy of Sciences and Arts, was a prominent Serbian physician, neuropsychiatrist, psychotherapist, writer, and erudite. He expressed interest in numerous fields (psychiatry, philosophy, sociology, religion), which he transmuted into over 70 books and hundreds of scientific articles. In a great many public appearances and lectures he gave, he spread the ideas of tolerance, understanding, and love among people based on moral values. He received numerous awards for his scientific work and social contribution. In August of this year, we commemorate the 100th anniversary of his birth.

Akademik Vladeta Jerotić (02.08.1924 – 04.09.2018), član Srpske akademije nauka i umetnosti, bio je istaknuti srpski lekar, neuropsihijatar, psihoterapeut, književnik i erudita. Imao je interesovanja iz mnogobrojnih oblasti (psihijatrije, filozofije, sociologije, religije) koje je pretočio u preko 70 knjiga i više stotina naučnih članaka. U mnogobrojnim javnim nastupima i predavanjima koje je držao širio je ideje tolerancije, razumevanja i ljubavi među ljudima na temeljima moralnih vrednosti. Dobitnik je brojnih priznanja za naučni rad i društveni doprinos. U avgustu ove godine obeležavamo 100 godina od njegovog rođenja.

ORIGINAL ARTICLES

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Application of internal fixator system for anterior pelvic ring with simultaneous application of sacroiliac screw internal fixation of the posterior pelvic ring in Tile C-type unstable pelvic fractures

Primena sistema unutrašnjeg fiksatora za prednji prsten karlice uz istovremenu unutrašnju fiksaciju zadnjeg prstena karlice sakroilijačnim zavrtnjem kod nestabilnih preloma karlice tipa C po Tile-u

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Abstract

Background/Aim. The number of pelvic traumas is increasing globally, mostly due to car accidents but also due to an increasing number of sports and recreational traumas. Tile C-type unstable pelvic trauma (TCUPT) is a kind of high-energy trauma that occurs during traffic accidents or accidents when falling from big heights. The aim of our study was to explore the application of the internal fixation (INFIX) system for the anterior pelvic ring (APR) with simultaneous application of sacroiliac screw (SS) INFIX of the posterior pelvic ring (PPR) in TCUPT. **Methods.** The subjects (89 of them in total) were recruited among patients with TCUPT from December 2020 to December 2023. A retrospective analysis of the subjects' data was performed, after which the subjects were divided into two groups based on different therapeutic regimens applied: group A (INFIX system for the APR + SS INFIX of the PPR, n = 46) and group B (external fixator for the APR + SS INFIX of the PPR, n = 43). **Results.** In comparison with group B, group A had a shorter period before the commencement of the first activity after surgery, shorter fracture healing time and joint function recovery time, as well as length of hospital stay (t = 6.623, 4.796, 7.992, and 5.227, respectively, $p < 0.05$). The surgery duration and bleeding volume showed no significant differences between the two groups (t = 1.433, 1.123, respectively, $p > 0.05$). The fracture reduction outcomes were better in group A than in group B (Z = 2.058, $p < 0.05$). The incidence rate of complications was lower in group A than in group B (2.17% vs. 18.60%) (χ^2 = 4.917, $p < 0.05$). **Conclusion.** For patients with TCUPT, the INFIX system for the APR with simultaneous application of SS INFIX of the PPR achieves good fracture reduction outcomes.

Key words:
bone screws; fractures, bone; fracture fixation, internal; open fracture reduction; pelvic bones; orthopedic procedures; treatment outcome.

Apstrakt

Uvod/Cilj. Broj slučajeva povrede karlice je u porastu na globalnom nivou, najviše zbog saobraćajnih nezgoda, ali i zbog sve većeg broja sportsko-rekreativnih povreda. Nestabilna povreda karlice tipa C po Tile-u (*Tile C-type unstable pelvic trauma*-TCUPT) je povreda koja se dešava tokom saobraćajnih nezgoda ili prilikom pada sa velike visine. Cilj rada bio je da se ispita primena sistema unutrašnje fiksacije (*internal fixation*-INFIX) za prednji karlični prsten (*anterior pelvic ring*-APR) uz istovremenu primenu INFIX zadnjeg karličnog prstena (*posterior pelvic ring*-PPR) primenom sakroilijačnog zavrtnja (*sacroiliac screw*-SS) (INFIX PPR SS) kod TCUPT. **Metode.** Ispitanici (ukupno 89) selektovani su među bolesnicima sa TCUPT u periodu od decembra 2020. do decembra 2023. godine. Izvršena je retrospektivna analiza podataka ispitanika, nakon čega su ispitanici podeljeni na dve grupe na osnovu različitih terapijskih protokola kojima su podvrgnuti: grupa A (INFIX sistem za APR + INFIX PPR SS, n = 46) i grupa B (spoljni fiksator za APR + INFIX PPR SS, n = 43). **Rezultati.** U poređenju sa grupom B, grupa A imala je kraće vreme: do početka prve aktivnosti nakon operacije, zarastanja preloma i oporavka funkcije zgloba, kao i kraću dužinu boravka u bolnici (t = 6,623, 4,796, 7,992 i 5,227, redom, $p < 0,05$). Nije bilo značajne razlike između dve grupe

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po pitanju dužine trajanja operacije i obima krvarenja (t = 1,433, 1,123, redom, p > 0,05). Rezultati redukcije preloma bili su bolji u grupi A nego u grupi B (Z = 2,058, p < 0,05). Stopa incidencije komplikacija bila je niža u grupi A nego u grupi B (2,17% vs. 18,60%) (χ^2 = 4,917; p < 0,05). **Zaključak.** Kod bolesnika sa TCUPT, primenom INFIX sistema za APR sa istovremenom primenom INFIX PRR SS, postižu se dobri rezultati redukcije preloma.

Ključne reči:
zavrtnji za kost; prelomi; prelomi, fiksacija, unutrašnja; prelom, otvorena redukcija; karlične kosti; ortopedske procedure; lečenje, ishod.

Introduction

As a kind of high-energy pelvic trauma, Tile C-type pelvic fractures (TCPF) are unstable fractures and are mostly attributed to traffic accidents and fall accidents from a high place, belonging to the fracture types of a high mortality rate [1]. In the case of TCPF, the structure of the anterior pelvic ring (APR) and posterior pelvic ring (PPR) is completely destroyed, giving rise to rotational and vertical instability of pelvic rings, which will result in pain, unbalanced sitting posture, and abnormal gait. TCPF are mainly treated by surgery in clinical practice. Traditional surgical methods include posterior trans-bilateral sacroiliac joint fixation with reconstruction plates plus external fixation (EXFIX) of the APR using the Phannenstiel approach and fixation of the PPR using the iliac approach [2, 3]. However, these surgical methods have many shortcomings. For instance, as to open reduction and combined anterior and posterior fixation, the mechanical stability of the posterior plate is poor, and the patient's position needs to be changed during surgery, which is detrimental to postoperative recovery. For APR EXFIX, the time needed for getting out of bed is long, the recovery of joint function is slow, and the incidence rate of postoperative delirium screw track infection is high. Anterior open reduction and internal fixation (INFIX) will cause a relatively large operative wound to patients, as well as more blood loss, which can easily result in various postoperative complications [4, 5]. In recent years, imaging and minimally invasive technologies have developed rapidly, and sacroiliac screw (SS) INFIX has been widely applied in injuries of sacroiliac joint complex, with good reduction outcomes [6]. APR INFIX and EXFIX systems are minimally invasive fixation methods commonly used for APR fractures, but there are still some disputes about their comparative effects in China and abroad.

In this study, the data of 89 patients with TCPF treated with EXFIX or INFIX system for the APR plus SS INFIX of the PPR were retrospectively analyzed, and the effects of applying the two methods were compared.

Methods

Collection of general data

A retrospective study was approved by the local Ethics Committee and performed on the data of 89 patients with TCPF in our hospital from December 2020 to December 2023. These patients were then assigned to group A (INFIX system for the APR + SS INFIX of the PPR, n = 46) and group B (EXFIX for the APR + SS INFIX of the PPR, n = 43) based on different therapeutic regimens. The sex, age, body mass index, time from injury to surgery, hemoglobin, platelet count, white blood cell count, causes of injury, and complications were comparable between the two groups (p > 0.05) (Table 1).

Table 1

General data

Parameters	Group A (n = 46)	Group B (n = 43)	Statistical value	*p*-value
Sex				
male	27 (58.70)	24 (55.81)	χ^2 = 0.075	0.784
female	19 (41.30)	19 (44.19)		
Age, year	42.16 ± 6.57	41.82 ± 7.54	t = 0.227	0.821
Body mass index, kg/m^2	24.97 ± 2.03	25.10 ± 2.12	t = 0.296	0.768
Time from injury to surgery, hrs	9.43 ± 2.79	9.24 ± 3.06	t = 0.306	0.761
Hemoglobin, g/L (RR: 110–175)	119.42 ± 15.36	120.37 ± 16.18	t = 0.284	0.777
Platelet count, ×10^9/L (RR: 90–320)	186.54 ± 31.52	188.25 ± 34.69	t = 0.244	0.808
White blood cell count, ×10^9/L (RR: 3.9–9.1)	7.41 ± 2.06	7.62 ± 1.94	t = 0.494	0.622
Cause of injury				
traffic accident	24 (52.17)	22 (51.16)	χ^2 = 0.278	0.964
fall accident from a high place	13 (28.26)	14 (32.56)		
bruise by heavy objects	5 (10.87)	4 (9.30)		
others	4 (8.70)	3 (6.98)		
Complications				
chest injury	5 (10.87)	4 (9.30)	χ^2 = 0.011	0.915
craniocerebral injury	6 (13.04)	5 (11.63)	χ^2 = 0.041	0.839
limb fracture	9 (19.57)	7 (16.28)	χ^2 = 0.163	0.687
urethral injury	3 (6.52)	3 (6.98)	χ^2 = 0.114	0.736
others	5 (10.87)	3 (6.98)	χ^2 = 0.073	0.787

RR – reference range. Results are shown as numbers (percentages) or mean ± standard deviation.

Inclusion and exclusion criteria

The following inclusion criteria were used for this study: 1) patients diagnosed with TCPF based on X-ray examinations; 2) patients with basically normal coagulation function; 3) patients who/whose family members signed the informed consent; 4) patients with displacement corrected by pre-operative traction/manual reduction or without obvious displacement. The exclusion criteria involved: 1) patient status complicated by severe osteoporosis; 2) patients with open pelvic fractures; 3) patients with unstable hemodynamics; 4) patients with soft tissue infection at the screw implantation site; 5) patients with pubic symphysis separation; 6) patients with pathological fractures; 7) patient status complicated by severe internal diseases.

Treatment

General anesthesia with tracheal intubation was implemented in both groups. Patients lay on the fluoroscopic operating table in the supine position, with a 2 cm-thick cushion under the sacrum. Thereafter, a pelvic reduction frame was installed, and the reduction of pelvic fractures was carried out through bone traction and Scan screws.

The therapeutic regimen of the INFIX system for the APR plus SS INFIX of the PPR was adopted in group A. In brief, an INFIX system was employed to fix the APR, and 1-2 SS (7.3 mm or 6.5 mm, Shandong Wego Orthopedic Materials Co., Ltd., China) was/were used for the fixation of the PPR. Before surgery, a fluoroscopy of the pelvis was carried out to mark the rotation angle and position of the C-arm. With the axis of the femoral shaft and the vertical line of the anterior superior iliac spine as the insertion points of SS, a 1.5 mm Kirschner wire was first placed. The distance and direction were then adjusted according to the position of the guide wire, followed by the insertion of a 2.5 mm guide wire by tapping with a bone hammer. When the guide wire passed through the sacroiliac joint and sacral foramina, the outlet, entrance, and lateral fluoroscopy of the pelvis were conducted many times to ensure that the guide wire was in an ideal position. Next, an opening was drilled by a hollow drill, in which a hollow screw was inserted. Thereafter, an incision (about 3 cm) was made on the skin 1 cm outside the body surface projection of the anterior inferior iliac spine for fixing the INFIX system for the APR, followed by blunt dissection to the anterior inferior iliac spine, during which attention should be paid to avoid damaging or straining peripheral nerves and blood vessels. After that, a pedicle screw opener was utilized to make an incision at the position 5 mm outside the apex of the anterior inferior iliac spine, with the awl inclined outward by 30° and toward the tail end by 20°, and the tissue was cut apart along the inner and outer sides of the iliac bone. After confirming that the four walls of the channel were complete through probes, the exit position and oblique position of the iliac bone were observed by fluoroscopy to ensure that the position of the guide wire was ideal. Afterward, iliac screws with a length of 75–85 mm and a diameter of 7.5–8.0 mm were inserted. A transverse titanium rod arc with a diameter of 5.5 mm manufactured according to the body shape of patients in advance was then used to connect and fix the iliac screws on both sides. Then, the spreading or hugging device of the foresaid screw-rod system was locked onto the femoral neck with small screws, the screw cap was tightened to complete the fixation of the INFIX system and fluoroscopy of the C-arm was implemented to confirm that the fixation was firm.

Patients in group B were treated with EXFIX for the APR plus SS INFIX of the PPR. Specifically, with the area between the bilateral anterior inferior iliac spine and iliac crest as the insertion point, an incision with a length of about 1 cm was made along the insertion point while trying not to expose the lateral femoral nerve, followed by blunt dissection of the tissue to the bone cortex. As to insertion into the anterior inferior iliac spine, the anterior superior iliac spine, posterior superior iliac spine, and posterior inferior iliac spine were taken as the insertion direction of Schanz screws, with an angle of 30° to the sagittal plane. For insertion into the iliac crest, the insertion direction of the Schanz screws was the iliac tubercle above the acetabulum, with an angle of 15–20° to the sagittal plane. After completing the fixation, the signs of bilateral iliac oblique position and pelvic outlet position were observed by fluoroscopy to confirm that screws were inserted accurately. An EXFIX (Tianjin Xinzhong Medical Devices Co., Ltd., China) was adopted to fix the APR, which was installed in an inverted splay pattern, and the connecting rod and the fixation clamp were fixed together. The proximal end was connected to the abdomen at an angle of 140° by two connecting rods or a single transverse rod, and the distal end was connected by a single transverse rod. The fixation clamp was tightened after a distraction or compression reduction of the pelvis.

Postoperative management

Simultaneous or second-stage surgery was conducted in both groups to treat complications. After surgery, cefazolin (0.5g 2–3 times/day) was administrated for three consecutive days to prevent infections. About two weeks later, stitches were taken out according to the healing of incisions. Patients took such functional exercises as bed exercises, off-bed activities, and weight-bearing training step by step based on their tolerance degree and fracture healing, and received re-examinations in the hospital regularly. At three months after surgery, the INFIX system for the APR was taken out.

Observation of indicators

General surgery indicators and rehabilitation indicators recorded were: surgery duration, bleeding volume, time of the first activity, length of hospital stay, fracture healing time, and joint function recovery time in the two groups.

The fracture reduction outcomes in the two groups were assessed using the Majeed pelvic fracture scoring system, which consisted of five items: pain, sitting, standing, intercourse, and work, with a total score of 100 points. The scores ≥ 85 points, 70–84 points, 55–69 points, and < 55 points

suggested excellent, good, medium, and poor outcomes, respectively [7].

Complications recorded were: the incidence rates of screw track infection, SS withdrawal, fixator breakage, lateral femoral cutaneous nerve injury, and deep vein thrombosis of lower extremities in the two groups.

The Visual Analog Scale was utilized to evaluate pain degree at 1 and 12 weeks after surgery [8], with a score of 0–10 points (10 points denoting severe and unbearable pain).

Statistical analysis

Statistical analysis was done with SPSS 23.0 software. Continuous value data (surgery indicators and rehabilitation indicators) were expressed by mean ± standard deviation and subjected to the *t*-test. Discreet value data (reduction outcomes, complications, and pain degree) were expressed by numbers (percentages) and subjected to the Chi-squared (χ^2) test. For ranked data, the rank sum test was performed. The value of $p < 0.05$ was considered a statistically significant difference.

Results

Preoperative and postoperative images of patients

The preoperative and postoperative images of a patient from group A and a patient from group B are shown in Figure 1.

Surgery and rehabilitation indicators

Group A displayed a shorter period till the first activity, fracture healing time, joint function recovery time, and length of hospital stay ($t = 6.623$, 4.796, 7.992, and 5.227, respectively, $p < 0.05$), but similar surgery duration and bleeding volume ($t = 1.433$, 1.123, respectively, $p > 0.05$) in comparison with group B (Table 2).

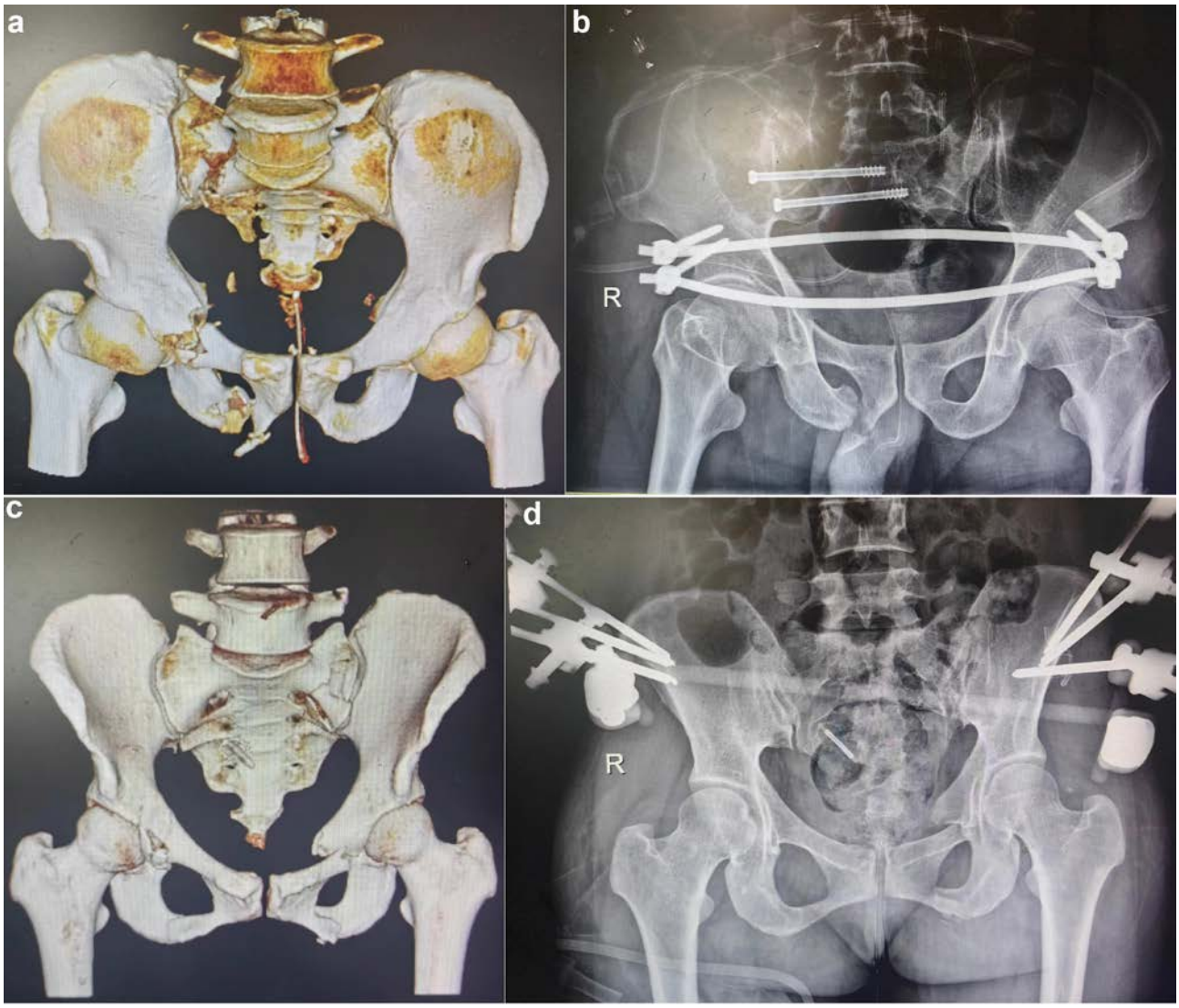

Fig. 1 – Preoperative and postoperative images of the pelvic bone ring: a) Preoperative computed tomography (CT) image of a patient from group A; b) postoperative X-ray image of a patient from group A; c) preoperative CT image of a patient from group B; d) postoperative X-ray image of a patient from group B.

Fracture reduction outcomes

The fracture reduction outcomes in group A were superior to those in group B ($Z = 2.058$, $p < 0.05$) (Table 3).

Postoperative complications

Group A displayed a lower incidence rate of complications than group B (2.17% vs. 18.60%; $p < 0.05$) (Table 4).

Pain degree after surgery

The pain in group A was milder than that in group B at 1 and 12 weeks after surgery ($p < 0.05$) (Table 5).

Discussion

As a kind of fracture relatively difficult to treat, pelvic fractures are accompanied by severe trauma, more complications, and a lower survival rate. According to the Tile classification, they can be classified into three types: Tile A, Tile B, and Tile C, based on the fracture stability, differences in radiological manifestations, and injury mechanisms. TCPF are mainly characterized by vertical and rotational instability, hence the reconstruction of pelvic ring stability is the key to treating patients with TCPF [9].

A study denoted that 40% of stability comes from the APR, and the remaining 60% relies on the PPR when people are in a standing position, thus reduction and fixation of the

Table 2

Surgery and rehabilitation indicators

Parameters	Group A (n = 46)	Group B (n = 43)	*t*	*p*-value
Surgery duration (min)	62.57 ± 12.96	58.82 ± 11.64	1.433	0.156
Bleeding volume (mL)	50.13 ± 7.41	48.39 ± 7.19	1.123	0.265
Elapsed time until first activity (day)	6.93 ± 1.54	9.71 ± 2.36	6.623	< 0.001
Hospital stay length (day)	12.84 ± 2.17	15.23 ± 2.14	5.227	< 0.001
Fracture healing time (week)	10.98 ± 1.32	12.43 ± 1.53	4.796	< 0.001
Joint function recovery time (week)	12.17 ± 1.60	14.85 ± 1.56	7.992	< 0.001

***t* – statistical value. Results are shown as mean ± standard deviation.**

Table 3

Fracture reduction outcomes

Characteristic	Group A (n = 46)	Group B (n = 43)	Z	*p*-value
Excellent	31 (67.39)	21 (48.84)	2.058	0.040
Good	12 (26.09)	13 (30.23)		
Medium	3 (6.52)	7 (16.28)		
Poor	0 (0.00)	2 (4.65)		

Z – statistical value. Results are shown as numbers (percentages).

Table 4

Postoperative complications

Parameter	Group A (n = 46)	Group B (n = 43)	χ^2	*p*-value
Screw track infection	0 (0.00)	2 (4.65)		
Sacroiliac screw withdrawal	0 (0.00)	3 (6.98)		
Fixator breakage	0 (0.00)	1 (2.33)		
Lateral femoral cutaneous nerve injury	1 (2.17)	0 (0.00)		
Deep vein thrombosis of lower extremities	0 (0.00)	2 (4.65)		
Total	1 (2.17)	8 (18.60)	4.917	0.027

χ^2 – Chi-square. Results are shown as numbers (percentages).

Table 5

Pain degree after surgery

Parameter	1 week after surgery		12 weeks after surgery	
	group A (n = 46)	group B (n = 43)	group A (n = 46)	group B (n = 43)
Painless	0 (0.00)	0 (0.00)	39 (84.78)	27 (62.79)
Mild	15 (32.61)	8 (18.60)	5 (10.87)	11 (25.58)
Moderate	29 (63.04)	26 (60.47)	2 (4.35)	5 (11.63)
Severe	2 (4.35)	9 (20.93)	0 (0.00)	0 (0.00)
Z	2.312		2.349	
p-value	0.021		0.019	

Z – statistical value. Results are shown as numbers (percentages).

PPR are more important [10]. Currently, percutaneous SS fixation, splay steel plate fixation in front of the sacral joint, and posterior sacral rod fixation are common fixation methods for pelvic fractures. Related research suggests that percutaneous SS fixation is more stable and less invasive than transsacral plate fixation and sacral rod fixation, and it is also less traumatic with similar stability in contrast with splay steel plate fixation in front of the sacral joint [11, 12]. Since the APR and PPR of patients with TCPF have been seriously damaged, simultaneous fixation of both the APR and PPR is required. There are many approaches to fixing the APR and PPR in clinical practice at present. However, no conclusion has yet been reached on the best fixation method, and some researchers believe that minimally invasive fixation is the tendency in treating these kinds of fractures [13]. In this study, patients with TCPF were treated with EXFIX or INFIX system for the APR plus SS INFIX of the PPR. It was found that group A exhibited shorter time until first activity, fracture healing time, joint function recovery time, length of hospital stay, better reduction outcomes, and a lower incidence rate of complications than group B (2.17% vs. 18.60%). These results indicate that compared with the surgical scheme of EXFIX for the APR, the surgical scheme of the INFIX system for the APR achieves better reduction outcomes, which can promote fracture healing, shorten length of hospital stay and joint function recovery time, and reduce complications.

SS INFIX of the PPR is a central fixation method with biological stability similar to that of steel plate fixation, and intramedullary fixation can shorten fracture healing time, facilitate early activities of patients, and avoid deep vein thrombosis of lower extremities and other complications, improving the quality of life of patients [14]. It is worth noting that in SS INFIX of the PPR, fluoroscopy should be conducted with patience, and the angle and position of the C-arm should be marked to ensure the correct direction of the guide wire and reduce the damage to peripheral blood vessels and nerves [15]. The INFIX system, a novel INFIX method for treating unstable APR fractures, is minimally invasive and safe, which is conducive to the postoperative rehabilitation of patients [16, 17]. Because the ischial groove between the anterior inferior iliac spine and the posterior superior iliac spine is relatively wide, fixation of iliac screws can be realized by the pedicle screw technique. To ensure the stability of pedicle screws, the length of iliac screws in bone should not be less than 50 mm. The bottom of the iliac screw groove should be slightly higher than the deep fascia layer to reduce the pressure on nerves and blood vessels, and both ends of the connecting rod should exceed the fixed screws to avoid damage to the lateral femoral cutaneous nerve [18, 19]. The EXFIX for the APR has many shortcomings. First of all, it has poor biomechanical stability and only offers limited marginal fixation without internal fixation effects. Second, the reduction loss of the APR is likely to cause the SS withdrawal in the posterior ring, leading to delayed healing or even non-union of fractures. Last, the screw loosening of the EXFIX may lead to further displacement of fractures, thus affecting the healing of fractures [20, 21]. Biomechanical research results denoted that the INFIX system has an advantage in overall axial mechanics compared with EXFIX in the process of APR fixation for patients with unstable pelvic ring injuries, which can reduce discomfort and the incidence rate of complications [22]. Moreover, it was discovered in this study that the pain in group A was milder than that in group B at 1 and 12 weeks after surgery, signifying that the INFIX system for the APR plus SS INFIX of the PPR is capable of relieving the postoperative pain of patients with TCPF. This may be ascribed to the fact that intramedullary fixation enables the compression fixation of the broken ends of a fractured bone, increasing the stability of the APR and PPR and thereby avoiding the postoperative pain caused by pelvic instability. Not only does the INFIX system have the advantages of traditional EXFIX, but it also achieves the strength of steel plate fixation, which is helpful for early functional exercise and postoperative body recovery.

Conclusion

Internal fixation system for the anterior pelvic ring plus sacroiliac screw internal fixation of the posterior pelvic ring achieves good reduction outcomes for patients with Tile C-type pelvic fractures, which promotes fracture healing, reduces the recovery time of joint function, alleviates pain, and decreases complications.

Conflict of interest

The authors declare no conflict of interest.

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Clinical outcomes of LeFort colpocleisis: a single-center experience from Turkey

Klinički ishodi Lefortove kolpokleize: iskustvo centra iz Turske

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Abstract

Background/Aim. LeFort colpocleisis (LFC) is a procedure for treating pelvic organ prolapse (POP) in women. The aim of the study was to assess the sociodemographic characteristics, anatomical outcomes, satisfaction, and clinical outcomes of patients who underwent LFC for POP. **Methods.** The study retrospectively and consecutively included 103 patients who underwent LFC for stage III and stage IV POP between January 2010 and December 2022. The participants' sociodemographic characteristics and clinical outcomes were documented. The Turkish version of the Pelvic Floor Distress Inventory (PFDI-20) questionnaire was used to determine quality of life. **Results.** The patients' mean age was 73.1 ± 26.7 years, mean body mass index 27.4 ± 3.8 kg/m^2, parity 4.8 ± 1.5, smoking rate 12.6%, POP quantification (POP-Q) stage III 30.1%, and POP-Q stage IV 69.9%. Their satisfaction rate results were 93.3%. Significant differences were observed in the preoperative period compared to the postoperative period in constipation (40.7% vs. 26.2%; p = 0.038), difficult defecation (22.3% vs. 8.7; p = 0.012), fecal incontinence (18.4% vs. 7.7%; p = 0.039), stress urinary incontinence (25.2% vs. 4.8%; p < 0.001), urge incontinence (49.5% vs. 27.1%, p = 0.001), voiding dysfunction (37.8% vs. 23.3%; p = 0.002), and urinary retention (42.7% vs. 12.6%; p < 0.001). Postoperative PFDI-20 scores were also significantly lower compared to the preoperative period (57.19 ± 16.57 vs. 21.62 ± 6.96; p < 0.001). **Conclusion.** This study showed that LFC has been established as a surgical procedure with high anatomical success, high patient satisfaction rates, and minimal complications, especially in advanced POP with age-related comorbidities.

Key words:
gynecologic surgical procedures; pelvic organ prolapse; quality of life; surveys and questionnaires; women.

Apstrakt

Uvod/Cilj. Lefortova kolpokleiza (LFK) je procedura za lečenje prolapsa karličnih organa (PKO) kod žena. Cilj rada bio je da se procene socio-demografske karakteristike, anatomski ishodi, zadovoljstvo i klinički ishodi kod bolesnica kojima je zbog PKO urađena LFK. **Metode.** U studiju su uključene 103 bolesnice koje su retrospektivno i uzastopno, od januara 2010. do decembra 2022. godine, bile podvrgnute LFK sa PKO III i IV stadijuma. Analizirani su socio-demografske karakteristike i klinički ishodi učesnica studije. Za određivanje kvaliteta života korišćena je turska verzija upitnika *Pelvic Floor Distress Inventory* (PFDI-20). **Rezultati.** Prosečna starost bolesnica bila je 73,1 ± 26,7 godina, srednja vrednost indeksa telesne mase 27,4 ± 3,8 kg/m^2, paritet 4,8 ± 1,5, stopa pušenja 12,6%, kvantifikacija PKO (PKO-K) stadijum III 30,1% i PKO-K stadijum IV 69,9%. Stopa zadovoljstva rezultatima intervencije iznosila je 93,3%. Zapažena je značajna razlika u preoperativnom periodu, u poređenju sa postoperativnim periodom, u konstipaciji (40,7% vs. 26,2%; p = 0,038), otežanoj defekaciji (22,3% vs. 8,7%; p = 0,012), fekalnoj inkontinenciji (18,4% vs. 7,7%; p = 0,039), fizičkim naporom-indukovanoj urinarnoj inkontinenciji (25,2% vs. 4,8%; p < 0,001), urgentnoj inkontinenciji (49,5% vs. 27,1%; p = 0,001), disfunkciji mokrenja (37,8% vs. 23,3%; p = 0.002) i retenciji urina (42,7% vs. 12,6%; p < 0,001). Rezultati PFDI-20 u postoperativnom periodu, u poređenju sa rezultatima u preoperativnom periodu, takođe su bili značajno niži (57,19 ± 16,57 vs. 21.62 ± 6.96; p < 0,001). **Zaključak.** Ova studija je pokazala da je LFK hirurška procedura sa visokim anatomskim uspehom i stepenom zadovoljstva bolesnica, minimalnim komplikacijama, posebno kod uznapredovalog PKO sa komorbiditetima povezanim sa životnim dobom.

Ključne reči:
hirurgija, ginekološka, procedure; karlični organi, prolaps; kvalitet života; ankete i upitnici; žene.

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Introduction

Surgical procedures may be required with advancing age in 20% of women with pelvic organ prolapse (POP), a condition seen in approximately 6% of women over 70 years of age [1, 2]. Moreover, due to the increasing age of the world population, these rates will inevitably rise further. POP may reduce women's quality of life and cause various adverse outcomes, such as recurrent urinary tract infections (UTIs) [3]. The risk of mortality in patients requiring surgical procedures is approximately 14 times higher due to comorbidities such as hypertension, diabetes mellitus, and chronic pulmonary disease [4].

Although some corrective procedures, such as abdominal or vaginal hysterectomy, anterior or posterior colporrhaphy, sacrocolpopexy, and sacrospinous fixation have been described for POP, these may entail high morbidity and complication rates [5–7]. Additionally, POP frequently recurs following such procedures [8–9]. LeFort colpocleisis (LFC) is a highly effective procedure with low morbidity rates, especially in elderly women who do not wish to engage in vaginal intercourse [1]. Studies have reported a patient satisfaction rate of over 90% in the first two postoperative years [1, 10].

LFC, a vaginal obliterative surgical procedure, was first described by Leon LeFort in 1877 and is a good surgical option, particularly for older women with POP with comorbidities because it can be performed using spinal anesthesia, has a shorter operative time than other operations, and involves less blood loss, faster recovery, and has anatomically good results [11]. However, although LFC is a good surgical option because of its low morbidity and mortality, it should also be remembered that it may lead to functional losses, such as impaired sexual function.

The aim of this study was to assess the sociodemographic characteristics, anatomical results, satisfaction levels, early and late postoperative complications, and functional outcomes of patients who underwent LFC for POP.

Methods

The study covered 103 patients who underwent LFC for stage III and stage IV POP according to POP quantification (POP-Q) [8] between January 2010 and December 2022. The participants were retrospectively and consecutively included in the study. Approval was obtained from the Health Sciences University Antalya Training and Research Hospital Ethics Committee, Turkey (No. 8-4, from 2023). Exclusion criteria were the following: a history of anterior or apical POP surgery, suspicious adnexal masses or other factors capable of indicating pelvic malignancy, incomplete data in the records, and the presence of a mental disorder.

Papanikolau test and pelvic ultrasonography were performed before surgery to exclude potential pathologies. Endometrial biopsy was also performed to exclude endometrial malignancy in case of increased endometrial thickness.

Preoperative preparation

Patients prepared for LFC were admitted to the hospital one day before their scheduled operation and underwent a standard preoperative assessment (cell blood count, coagulation tests, and electrocardiography), together with vaginal ultrasonography, for a final control examination. Prophylactic antibiotics (cefazolin 2 g) were administered intravenously (i.v.) as premedication by a gynecologist in all cases, approximately 30 min before surgery. A bladder catheter was inserted before the surgical procedure and was withdrawn 8–12 hrs after mobilization. Antithrombotic prophylaxis was performed in line with the recommendations of the American College of Obstetricians and Gynecologists and the American College of Chest Physicians. Compression banding was also used.

Surgical procedure for LeFort colpocleisis

All patients underwent spinal anesthesia. A fluid bolus of at least 500 mL of Ringer's lactate solution was given before the procedure. The patient was placed in a flexed sitting or lateral decubitus position, a 27-gauge Sprotte® needle was introduced into the lumbar (L)2-L3, L3-L4, or L4-L5 intervertebral space, and 10–12 mg of 0.5% hyperbaric bupivacaine and 15 µg of fentanyl were then injected. After the procedure, the patient was positioned in a moderate Trendelenburg position to accelerate the spread of the local anesthetic agents in the cephalic direction and to provide a sufficient level of anesthesia.

The prolapsed anterior and posterior vaginal mucosa was drawn in rectangular form using a sterile pen. These rectangular vaginal epithelial areas were separated from the underlying fascia using blunt and sharp dissection. These de-epithelized anterior and posterior surfaces and the borders of the quadrilateral were sutured one by one with overlapping sutures. This suture technique resulted in a natural tunnel being formed on the lateral edges of the vagina, which provided drainage of the external cervical os. The urinary catheter was removed 24 hrs after the operation, and residual bladder urine was measured after voiding. Postoperative voiding dysfunction was defined as residual urine exceeding 50 mL.

The participants' sociodemographic characteristics, comorbidities, early and late postoperative complications, functional outcomes, and Pelvic Floor Distress Inventory (PFDI-20) Questionnaire scores were recorded. Body mass index (BMI) was calculated as body weight in kilograms divided by body height in meters squared. Patients were re-evaluated after three, six, and 12 months and two and three years. Urinary and bowel symptoms were also recorded. Complications occurring within the first postoperative week were classified as early, and those developing between one week and three months as late. The data were retrieved from the hospital database and patient files.

Postoperative patients were asked to choose one of the following options to describe their status: "completely healed", "partially healed", "slightly healed", "unchanged",

and "worsened". The Turkish version of PFDI-20 was completed pre- and post-operatively by all participants. The PFDI-20 includes 20 items and is divided into three inventories, the Pelvic Organ Prolapse Distress Inventory-short form 6 (POPDI-6), the Colon Rectal Anal Distress Inventory-short form 8 (CRADI-8), and the Urinary Distress Inventory-short form 6 (UDI-6). Anatomical and subjective assessments were performed at least 12 months after surgery. Anatomical success was defined as POP-Q sites Ba, C, and Bp above the hymenal ring at least one year after surgery. The presence of a mass beyond the hymen was regarded as an anatomical failure.

Statistical analysis

Data were analyzed on Statistical Package for the Social Sciences (SPSS) version 15.0 for Windows software (SPSS, Chicago, IL, USA). The Kolmogorov-Smirnov test was used to determine the normality of the distribution of all continuous variables. Normally distributed variables were compared between the groups using the paired *t*-test, while the Wilcoxon test was applied in the case of non-normally distributed variables. Categorical data were analyzed using Pearson's Chi-square or Fisher's exact test, as appropriate, and were presented as numbers and percentages. A *p*-value lower than 0.05 was regarded as statistically significant.

Results

A total of 103 participants were included in the final analysis. The participants' sociodemographic characteristics and associated comorbidities are presented in Table 1. Their mean age was 73.1 ± 26.7 years, mean BMI 27.4 ± 3.8, parity 4.8 ± 1.5, smoking rate 12.6%, POP-Q stage III 30.1%, POP-Q stage IV 69. 9%, mean blood loss 78.8 ± 36.5, mean operative time 91.5 ± 23.8, mean hospital stay 2.1 ± 1.2 days, and mean follow-up time 36 (18–84) months. The most common comorbidity was hypertension at 53.3%, followed by diabetes mellitus at 34.9%.

The most common complication in the early and late postoperative period was UTI (Table 2).

The participants' functional and anatomical outcomes are shown in Table 3. Significant decreases occurred after the procedure in the following: constipation (40.7% preoperatively vs. 26.2% postoperatively; $p = 0.038$), difficult defe-

Table 1

Participants' sociodemographic characteristics and associated comorbidities

Parameter	Values
Age (years)	73.1 ± 26.72
BMI (kg/m^2)	27.4 ± 3.8
Parity	4.8 ± 1.54
Smoking rate, n (%)	13 (12.6)
POP-Q stage	
III	31 (30.1)
IV	72 (69.9)
Blood loss (mL)	78.8 ± 36.5
Operative time (minutes)	91.5 ± 23.8
Hospital stay (days)	2.1 ± 1.2
Follow-up (months), mean (min-max)	36 (18–84)
Hypertension	57 (55.3)
Diabetes mellitus	36 (34.9)
Heart disease	32 (31.1)
Chronic pulmonary disease	17 (16.5)
Neurological disorder	13 (12.6)
Cerebrovascular disorder	5 (4.8)
Psychiatric disorder	17 (16.5)

BMI – body mass index; POP-Q – pelvic organ prolapse quantification.
All values are given as median, 25th–75th (percentages) or mean ± standard deviation, except smoking rate and follow-up.

Table 2

Early and late postoperative complications

Parameters	Early	Late
Urinary tract infection	3 (2.9)	8 (7.7)
Urinary retention	#	1 (0.9)
Pelvic hematoma	1 (0.9)	#
Gluteal or perineal pain	1 (0.9)	#
Atrial fibrillation	2 (1.8)	#

All values are given as numbers (percentages).
Note: # means that the specified complication did not exist.

cation (22.3% vs. 8.7%; $p = 0.012$), fecal incontinence (18.4% vs. 7.7%; $p = 0.039$), stress urinary incontinence (25.2% vs. 4.8%; $p < 0.001$), urge incontinence (49.5% vs. 27.1%; $p = 0.001$), voiding dysfunction (37.8% vs. 23.3%; $p = 0.002$), and urinary retention (42.7% vs. 12.6%; $p < 0.001$).

The participants' satisfaction after LFC is summarized in Table 4. Analysis showed that 64.1% of participants regarded their status as very much improved, 24.3% as improved, 4.9% as little improved, and 6.7% as unchanged, while none described it as worse. None of the participants had a recurrence of POP nor did they require re-surgical intervention during the follow-up period.

PFDI-20 scores (57.19 ± 16.57 vs. 21.62 ± 6.96, in the pre- and post-operative periods, respectively; $p < 0.001$), POPDI-6 (28.16 ± 9.41 vs. 10.17 ± 4.15; $p < 0.001$), UDI-6 (22.41 ± 7.21 vs. 7.12 ± 3.24; $p < 0.001$), and CRADI-8 (8.24 ± 5.32 vs. 5.58 ± 3.21; $p = 0.041$) all decreased significantly compared to baseline (Table 5).

Discussion

This study was planned to evaluate the sociodemographic characteristics, anatomical outcomes, patient satisfaction, both early and late postoperative complications, and functional outcomes of patients who underwent LFC for POP in our clinic from January 2010 to December 2022. The results showed that postoperative anatomical and functional outcomes improved compared to the preoperative period and that patient satisfaction also increased.

The incidence of POP increases with age. Studies have shown that this can rise up to 50% at 80 years and above and that comorbid disorders such as hypertension and diabetes mellitus accompany more than half of patients undergoing LFC, especially in that age group [1, 5, 11–14]. Good postoperative pain control and caution in terms of embolism and medical applications are of life-saving importance, particularly for patients with cardiovascular disorders [5, 11]. In

Table 3

Participants' functional and anatomical pre- and post-operative outcomes

Parameter	Preoperative	Postoperative	*p*-value
Constipation	42 (40.7)	27 (26.2)	0.038
Difficult defecation	23 (22.3)	9 (8.7)	0.012
Fecal incontinence	19 (18.4)	8 (7.7)	0.039
Stress urinary incontinence	26 (25.2)	5 (4.8)	< 0.001
Urge incontinence	51 (49.5)	28 (27.1)	0.001
Voiding dysfunction	39 (37.8)	24 (23.3)	0.002
Urinary retention	44 (42.7)	13 (12.6)	< 0.001
Vaginal length	#	2.8 ± 1.4	#
Perineal body	#	4.6 ± 1.2	#
Genital hiatus	#	2.2 ± 0.8	#

All values are given as numbers (percentages) and mean ± standard deviation.
Note: # means that patients had total pelvic prolapse.

Table 4

Participants' satisfaction after LeFort colpocleisis

Parameters	Values
Very much improved	66 (64.1)
Improved	25 (24.3)
Little improved	5 (4.9)
No change	7 (6.7)
Worse	0 (0)

All values are given as numbers (percentages).

Table 5

Pelvic Floor Distress Inventory questionnaire results

Parameters	Preoperative	Postoperative	*p*-values
PFDI-20	57.19 ± 16.57	21.62 ± 6.96	< 0.001
POPDI-6	28.16 ± 9.41	10.17 ± 4.15	< 0.001
UDI-6	22.41 ± 7.21	7.12 ± 3.24	< 0.001
CRADI-8	8.24 ± 5.32	5.58 ± 3.21	0.041

PFDI – Pelvic Floor Distress Inventory; POPDI – Pelvic Organ Prolapse Distress Inventory; UDI – Urinary Distress Inventory; CRADI – Colon Rectal Anal Distress Inventory.
All values are given as mean ± standard deviation.

accordance with the previous literature, more than half of the patients in the present study had comorbid disorders.

LFC seems to be a highly effective surgical procedure with few complications in older women with POP and comorbidities. However, it should be remembered that there is a risk of subsequent recurrence and that this may be exacerbated by advanced age, obesity, genetic predisposition, pelvic floor weakness, and poor surgical technique [5, 12, 15]. Corrective surgical procedures with or without mesh are particularly performed for women with POP who wish to maintain their sex lives. However, it should be remembered that these surgical procedures entail high complication and recurrence rates [1, 12, 15–17].

The reported operative time in LFC in previous studies was between 30 and 135 min, and expected blood loss was between 30 and 450 mL [1, 5, 11]. Operative times and expected blood loss in the present study were compatible with the previous literature at 91.5 ± 23.8 min and 78.8 ± 36.5 mL, respectively. Preoperative and postoperative data in the previous literature are not as extensive or comprehensive as those in the present study. Reported complication rates after LFC are approximately 5% [5, 18, 19]. Our anatomical success rate was close to 100%, and our patient satisfaction rate was 93.3%, findings apparently compatible with the existing literature [11, 20, 21].

Intraoperative and early and late postoperative complications of LFC are very rare [1, 11]. A previous retrospective study evaluated 325 cases of LFC. While UTI was observed most frequently in the early and late postoperative periods, the rate of severe complications in the two postoperative periods was below 3% [11]. The most common both early and late complications in the postoperative period in the present study was UTI. In terms of functional outcomes after LFC, significant improvements have been reported in bowel disorders such as constipation and fecal incontinence, urinary and voiding symptoms such as stress incontinence, urge incontinence, voiding dysfunction, and urinary retention [10, 22]. However, it should also be remembered that sexual functions will be lost after LFC, an obliterative surgical procedure.

The advantages of the present research over other studies evaluating the results of LFC include the fact that all surgical procedures were performed by a single gynecologist and that the interobserver error margin was, therefore, low. A standard surgical procedure was applied in all cases rather than different techniques, postoperative complications were reported separately for the early and late periods, and the cases were followed up for a minimum of three years. The limitations of this study include the fact that it was conducted in a tertiary care institution, that it was a single-center study, and that it involved a retrospective study design.

Conclusion

This study shows that LeFort colpocleisis has proved itself to be a surgical procedure with high anatomical success, high patient satisfaction rates, and minimal complications, one that especially improves bowel and urinary symptoms and quality of life in women with advanced pelvic organ prolapse with age-related comorbidities. Further studies with larger cohorts are now needed to confirm our results.

Conflict of interest

The authors declare no conflict of interest.

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Improvement of blood glucose control and reduction of hypoglycemia, body weight, and C-reactive protein in type 1 diabetic patients treated with intensive insulin therapy with insulin analogs

Poboljšanje glikoregulacije, snižavanje broja hipoglikemija, telesne mase i nivoa C-reaktivnog proteina kod bolesnika sa dijabetesom melitusom tipa 1 na intenziviranoj insulinskoj terapiji analozima insulina

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Abstract

Background/Aim. Good metabolic control can delay the onset and progression of chronic complications of diabetes mellitus (DM). Intensified insulin therapy (IIT) is the cornerstone of good metabolic control in the treatment of type 1 DM (T1DM) while avoiding hypoglycemia and body weight (BW) gain in those patients. The aim of the study was to assess the effects of IIT with insulin analogs (aspart and glargine) in T1DM patients. **Methods.** This prospective clinical study included 49 patients with at least one year of T1DM duration, who were on IIT with human insulin at that moment. They commenced therapy with insulin aspart for three months, followed by insulin glargine for another three months. An analysis of blood glucose (BG) control (glycated hemoglobin – HbA1c, mean BG, fasting BG, postprandial BG, and glycemic variability) and analysis of BW, hypoglycemia, and C-reactive protein (CRP) levels were performed. **Results.** The HbA1c level decreased slightly (non-significantly) after three months of insulin aspart therapy (from 9.28% to 8.83%) and decreased significantly after the aspart/glargine combination (to 8.08%; $p < 0.001$). After the first three months with aspart therapy, a significant reduction in postprandial BG was noted after all three main meals. The mean postprandial rise of BG was significantly reduced. The variability of daily BG was significantly reduced (standard deviation of BG fell from 2.28 mmol/L to 1.90 mmol/L; $p < 0.05$). The mean BG value in the profiles decreased (from 9.11 mmol/L to 8.31 mmol/L; $p < 0.05$). All BG values in the profiles after six months were statistically significantly lower compared to the initial values, as well as the mean BG (6.88 mmol/L; $p < 0.001$) and the variability of daily BG (1.49 mmol/L; $p < 0.01$). Our results showed a significant reduction in the number of hypoglycemias after three months, especially after the introduction of insulin glargine therapy (significant reduction in the number of symptomatic, asymptomatic, and nocturnal hypoglycemias). The results showed a discrete but significant reduction in BW and a significant reduction in CRP levels (from 3.43 mg/L to 2.25 mg/L; $p < 0.001$). **Conclusion.** Treatment of patients with T1DM with insulin analogs (insulin aspart and insulin glargine) in IIT leads to improved BG control with a reduction in the number of hypoglycemia, BW, and CRP levels.

Key words:
c-reactive protein; diabetes mellitus, type 1; glycated hemoglobin; glycemic control; hypoglycemia; insulin, long-acting; insulin, short-acting.

Apstrakt

Uvod/Cilj. Dobra metabolička kontrola može odložiti nastanak i napredovanje hroničnih komplikacija dijabetesa melitusa (DM). Intenzivirana insulinska terapija (IIT) je kamen temeljac dobre metaboličke kontrole u lečenju bolesnika sa tipom 1 DM (T1DM), uz izbegavanje hipoglikemije i povećanja telesne mase (TM) kod tih bolesnika. Cilj rada bio je da se procene efekti primene IIT analozima insulina (aspart i glargin) na obolele od T1DM. **Metode.** Prospektivnim kliničkim istraživanjem obuhvaćeno je 49 bolesnika sa T1DM u trajanju od najkraće jedne godine, koji su tada bili na IIT humanim insulinima. Uveden im je insulin aspart u trajanju od tri meseca, a zatim insulin glargin takođe u trajanju od tri meseca. Urađena im je analiza nivoa glukoze u krvi (GK) (glikoziliranog hemoglobina – HbA1c, srednjeg nivoa GK, GK natašte, postprandijalne GK i glikemijske varijabilnosti) i analiza

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TM, broja hipoglikemija i nivoa C-reaktivnog proteina (CRP). **Rezultati.** Nivo HbA1c je posle tri meseca terapije insulinom aspart bio neznatno (bez značajnosti) snižen (sa 9,28% na 8,83%), a posle kombinacije aspart/glargin bio je značajno snižen (na 8,08%; $p < 0,001$). Posle prva tri meseca, zabeleženo je značajno ublažavanje nivoa postprandijalne GK nakon sva tri glavna obroka. Srednji postprandijalni porast GK bio je značajno smanjen. Varijabilnost dnevne GK je bila značajno smanjena (standardna devijacija dnevne GK smanjena je sa 2,28 mmol/L na 1,90 mmol/L; $p < 0,05$). Srednja vrednost GK u profilima je opala (sa 9,11 mmol/L na 8,31 mmol/L; $p < 0,05$). Sve vrednosti GK u profilima posle šest meseci bile su statistički značajno niže u odnosu na početne vrednosti, kao i srednje vrednosti GK (6,88 mmol/L; $p < 0,001$) i varijabilnost dnevne GK (1,49 mmol/L; $p < 0,01$). Naši rezultati pokazali su značajno smanjenje broja hipoglikemija posle tri meseca, a posebno nakon uvođenja terapije insulinskim analogom glargin (značajno smanjenje broja simptomatskih, asimptomatskih i noćnih hipoglikemija). Rezultati su pokazali diskretno, ali značajno smanjenje TM i značajno sniženje nivoa CRP (sa 3,43 mg/L na 2,25 mg/L; $p < 0,001$). **Zaključak.** Terapija obolelih od T1DM analozima insulina (insulin aspart i insulin glargin) u IIT dovodi do poboljšanja kontrole GK sa smanjenjem broja hipoglikemija, TM i nivoa CRP.

Ključne reči:
c-reaktivni protein; dijabetes melitus, tip 1; hemoglobin a, glukozilovan; glukoza u krvi, kontrola; hipoglikemija; insulin, dugodelujući; insulin, kratkodelujući.

Introduction

Intensified insulin therapy (IIT) is the cornerstone of good glycemic control and provides a reduction of risk for developing chronic vascular complications of type 1 diabetes mellitus (DM) – T1DM, as documented in the Diabetes Control and Complications Trial (DCCT) [1].

The beginning of the 21st century in modern diabetology is the decade of insulin analogs. By minor changes in the sequence of amino acids in the peptide chains of insulin, an attempt is made to ensure a change in pharmacokinetics, thus providing better control of glycemia both during and after meals [2, 3]. IIT has two permanent goals: to achieve as good blood glucose (BG) control as possible while reducing the frequency and severity of hypoglycemia.

Although many metabolic abnormalities contribute to the overall risk of developing chronic diabetic complications, hyperglycemia remains the hallmark of DM [4]. Glycated hemoglobin (HbA1c) provides an integrated measure of glucose exposure during the day, where its absolute level is contributed by the level of both fasting BG (FBG) and postprandial glycemia (PPG) [5–8]. PPG contributes significantly to the mean glycemic value, which is a key indicator of glycemic control measured through the HbA1c level. Such findings represent the basis for the formation of the glucose triad model in the treatment of DM. Treatment goals are represented with three glycemic parameters: HbA1c, PPG, and FBG. They are related to each other and represent necessary goals for therapy that try to optimize glycemic control [9].

One of the possibilities is that the risk of complications depends on the magnitude of postprandial spikes in glycemia and the effect of counterregulatory hormones released in hypoglycemia. This is how we came to the concept of variability of daily glycemia and the discussion about its importance [10, 11].

The aim of this study was to assess the effectiveness of IIT with analogs of human insulin through the analysis of glycemic control parameters (levels of HbA1c, FBG, PPG, the mean level of BG, and glycemic variability) and to test the safety of IIT with analogs of human insulin through analysis of the number and severity of hypoglycemia and analysis of changes in body weight (BW).

Methods

This prospective study included patients with T1DM from the University Clinical Center Niš, Serbia, Faculty of Medicine, Clinic for Endocrinology, Diabetes, and Metabolic Diseases. The study was approved by the Institutional Ethical Committee (No. 10413/17), and written consent was obtained from all subjects. Data analysis was performed on 49 patients with a clear diagnosis of T1DM and at least one year of duration of the disease and insulin therapy and who were currently on IIT with human insulins. They were first examined on the existing therapy in a monthly period with the aim of achieving the target glycemic control by correcting the therapy. During that period, patients had weekly check-ups. In addition to regular daily self-monitoring of BG, patients performed glycemic profiles regularly once a week by self-monitoring of BG immediately before and two hours after the main meals (six measurements in total) with measurements before bedtime and at night (at 3 a.m.). The patients were re-educated about the dietary regime, the importance of self-monitoring of BG and the method of correcting the bolus dose, about hypoglycemia, and the way of recording them. Patients had the possibility of 24-hour telephone contact, 7 days a week, with a doctor, a nurse educator, and members of the team who followed them. During the last week of that period, patients performed two daily glycemic profiles.

After that, patients started therapy with a fast-acting analog of human insulin, insulin aspart, instead of human soluble insulin. On the day of the introduction into the therapy of a fast-acting analog of human insulin, the patients underwent a detailed anamnestic, clinical, and biochemical examination (visit 1 – V1). In the following period of three months, regular controls continued, which included correction of insulin therapy according to the results of self-monitoring of BG and control at the Clinic once a month. In

the last week of this period, patients performed two all-day glycemic profiles.

Then, patients started therapy with a long-acting insulin analog, insulin glargine, instead of intermediate-acting human insulin. On the day of the introduction of long-acting human insulin analog therapy, the patients underwent a detailed examination (visit 2 – V2). In the following period of three months, regular controls continued, which included correction of insulin therapy according to the results of self-monitoring of BG and control at the Clinic once a month. In the last week of this period, patients performed two all-day glycemic profiles. At the end of this period, the patients underwent a detailed examination (visit 3 – V3).

The insulin dose was corrected (both basal and boluses) in order to achieve fasting BG levels of 4 to 6 mmol/L and preprandial levels of 5 to 7 mmol/L, i.e., postprandial levels of 5 to 10 mmol/L, bedtime levels of 6 to 8 mmol/L, and at night from 5 to 8 mmol/L. Doses of prandial boluses (both human insulin and insulin aspart) were adjusted depending on BG and according to the level of education of the patients, the size of the meal, and the level of physical activity.

Detailed anamnestic and clinical workup at each check-up included the following: recording anamnestic data on the duration of DM, previous therapy, complications, complaints, and the number and degree of severity of hypoglycemia in the period before control; taking anthropometric measurements [BW and body height (BH) from which the body mass index (BMI) is calculated]. BW was measured in a standing position, using a decimal scale, in patients with light clothing and without shoes and rounded to the nearest 100 g. BH was measured by a standard height meter in a standing position without shoes, with a normal shoulder position. BMI was determined by dividing the BW in kg by the square of BH in m (kg/m^2).

The American Diabetes Association (ADA) Workgroup proposed the following classification of hypoglycemia in diabetes: mild and severe, symptomatic and asymptomatic, and nocturnal hypoglycemia [12].

Hypoglycemias were recorded and collected from patient diaries. Mild hypoglycemia was defined as symptoms indicating hypoglycemia that resolved after taking a meal, with measured glycemic values less than 3.9 mmol/L, which patients could manage on their own, without the help of other people. Asymptomatic hypoglycemias are all measured glycemic values less than 3.9 mmol/L during regular self-monitoring, which are not accompanied by characteristic symptoms. Severe hypoglycemias were defined as hypoglycemias in which the patient had symptoms of hypoglycemia that required the assistance of another person (either a family member or medical staff) and recovery after administration of oral or intravenous (i.v.) glucose or glucagon. Nocturnal hypoglycemias were defined as all hypoglycemias occurring between bedtime and morning (from around 11 p.m. to 7 a.m.).

Biochemical measurements were done in all patients at the beginning of the study (before the introduction of therapy with a fast-acting insulin analog), after three months, and after six months (V1, V2 and V3).

Blood samples were taken in the morning after a twelve-hour overnight fasting from the antecubital vein of patients in a sitting position. Blood centrifugation was performed 30 to 45 min after collection. Biochemical analyses were performed at the University Clinical Center Niš, Center for Medical Biochemistry on the day of blood collection.

Assessment of glycoregulation was performed by analyzing the HbA1c level and analyzing the glycemic profile at 8 points (BG before the main meals and two hours after the main meals, before going to bed, and at 3 a.m.). HbA1c was determined by the immunochemical method with Olympus reagents on the Olympus AU 680 automatic analyzer [reference range (RR) 4.2–6.2%]. The patients performed glycemic profiles regularly by self-monitoring of BG using a calibrated memory glucose meter (RR 3.9–6.1 mmol/L). Preprandial BG, PPG, nighttime BG value (at 3 a.m.), mean daily BG value, and variability of mean daily BG were analyzed separately.

Hematological parameters were determined on a hematological analyzer AL 816 (Graz, Austria) [RR for erythrocytes (Er) 3.8–6.0 $\times 10^{12}$/L; leukocytes (Le) 4.0–10.0 $\times 10^{9}$/L; thrombocytes (Tr) 150–450 $\times 10^{9}$/L; hemoglobin (Hb) 110–170 g/L], while Er sedimentation rate (ESR) was done in vacuum test tubes from Terumo (Leuven, Belgium) according to the modified Westergreen method (RR < 12 mm/h). Determination of C-reactive protein (CRP) was done by immunoturbidimetric method on multi-channel analyzer Olympus AU 400, expressed in mg/L (RR 0.0–5.0 mg/L), and fibrinogen – by the immunoturbidimetric method using saturated Parfontier solution, on the Beckman DU 650 apparatus, expressed in g/L (RR 2.0–4.0 g/L).

Statistical analysis

Data entry, tabular and graphical presentation were performed using MS Office Excel, and statistical calculations were performed using SPSS, version 22.0. Attributive parameters are represented by frequencies and percentages, and continuous (measurable) parameters are represented by means and standard deviations (SD) and median (Md). The normality of the distribution of parameters was determined by the Shapiro-Wilk test. Student's *t*-test of dependent (paired) samples (for normal distributions) and Wilcoxon Signed Ranks Test (for distributions deviating from normal) were used to test the statistical significance of the difference in the values of continuous parameters between visits. The value of p less than 0.05 was considered statistically significant.

Results

The prospective study included 49 subjects with T1DM, of which 27 (55.10%) were male; average age was 29.94 ± 4.98 years, with a median of 29 years; average duration of T1DM was 13.94 ± 7.70 years, with a median of 13 years; average BH was 172.51 ± 7.21 cm, with a median of 171 cm.

The results of monitoring glycoregulation parameters, HbA1c and BG values in daily profiles, the mean value of daily BG, and variability of daily BG level of SD and coefficient of variation (CV) of BG in daily profiles, are shown in Table 1.

The HbA1c level shows a decrease after the third month of therapy [non-significant (ns)], and it is significantly lower after the sixth month of therapy ($p < 0.001$), compared to the beginning of therapy and to V2 ($p < 0.01$) (Wilcoxon test).

The mean value of BG in the profile was statistically significantly higher at V1 than at V2 ($p < 0.05$) (Student's *t*-test) and also at V3 ($p < 0.001$) (Wilcoxon test). The value was higher at V2 than at V3 ($p < 0.001$) (Wilcoxon test).

Changes in the level of HbA1c and the mean value of daily BG in the daily profiles are shown in Figure 1.

The level of FBG did not change significantly after the third month of therapy compared to the level at the beginning of therapy, but it was statistically significantly lower after the sixth month of therapy compared to the level at V1 and also at V2 ($p < 0.001$) (Wilcoxon test).

All other values of BG measured in the mentioned periods, as well as mean values of BG in the profile, SD of MBG in the profile, and CV of MBG in the profile, were the highest at the beginning of therapy. The values were lower after the third month of therapy, and the lowest after the sixth month of therapy. Relationships and differences in BG levels in the profiles are shown in Figures 2 and 3.

Table 1

Blood glucose values in daily profiles and glycated hemoglobin before and after insulin analog therapy

Parameter	V1		V2		V3	
	mean ± SD	median	mean ± SD	median	mean ± SD	median
Breakfast						
before	8.14 ± 2.91	^{g***} 7.90	8.68 ± 3.07	^{g***} 8.30	6.19 ± 1.84	5.60
after	10.42 ± 2.78	^{a**g***} 10.60	9.01 ± 2.25	^{g***} 8.90	7.41 ± 1.73	7.30
Lunch						
before	8.32 ± 2.43	^{g**} 8.70	7.75 ± 2.51	^{g*} 7.80	6.77 ± 1.98	6.60
after	10.84 ± 4.45	^{a**g***} 10.40	8.64 ± 2.95	^{g**} 8.60	6.96 ± 1.92	6.70
Dinner						
before	8.09 ± 2.91	^{g***} 7.80	7.89 ± 2.40	^{g***} 7.60	6.33 ± 1.58	6.30
after	9.80 ± 3.36	^{a*g***} 9.10	8.27 ± 2.99	7.40	7.22 ± 2.38	6.90
Before bed	9.03 ± 2.33	^{g***} 8.50	8.34 ± 2.53	^{g**} 8.10	7.01 ± 1.87	6.70
Night (03 a.m.)	8.23 ± 2.40	^{g*} 8.40	7.88 ± 2.50	7.50	7.16 ± 1.95	6.50
MBG	9.11 ± 2.24	^{a*g***} 9.23	8.31 ± 1.86	^{g***} 8.30	6.88 ± 1.27	6.61
SD of BG	2.28 ± 0.78	^{a*g***} 2.30	1.90 ± 0.84	^{g**} 1.85	1.49 ± 0.52	1.50
CV of BG	25.30 ± 7.73	^{g*} 23.48	23.17 ± 9.68	21.22	21.74 ± 6.50	20.78
HbA1c	9.28 ± 1.50	^{g***} 9.20	8.83 ± 1.61	^{g**} 8.50	8.08 ± 1.46	7.80

V1 – visit 1; V2 – visit 2; V3 – visit 3; BG – blood glucose (mmol/L); MBG – mean BG; SD – standard deviation; CV – coefficient of variation; HbA1c – glycated hemoglobin (%).
Statistical significance: a – vs. V2; g – vs. V3; * $p < 0.05$ (Student's *t*-test); ** $p < 0.01$; *** $p < 0.001$ (Wilcoxon test).

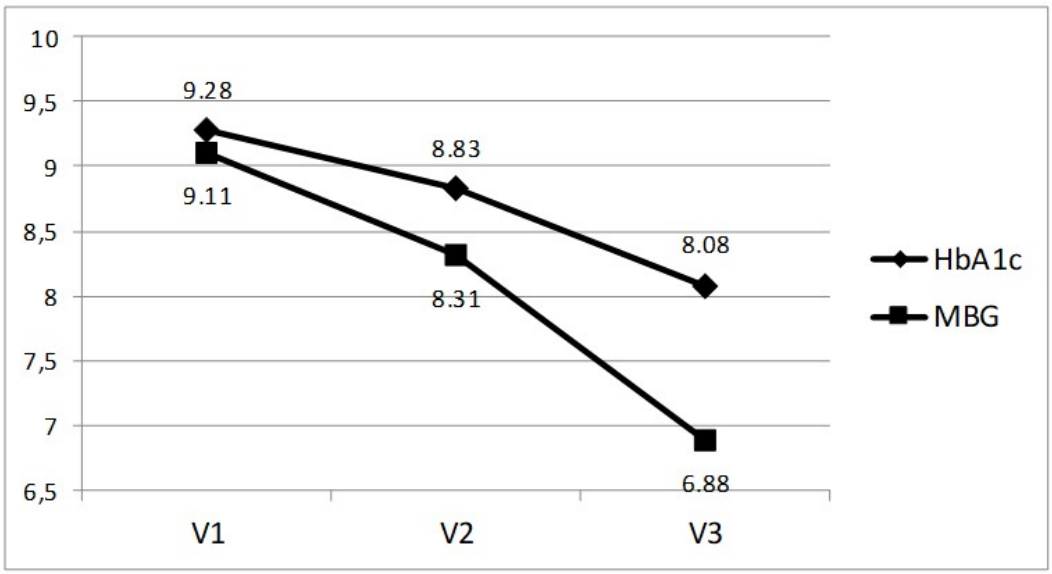

Fig. 1 – The change in the level of glycated hemoglobin (HbA1c) (%) (p = ns, V1 vs. V2; $p < 0.01$, V2 vs. V3; $p < 0.001$, V1 vs. V3) and mean blood glucose – MBG (mmol/L) ($p < 0.05$, V1 vs. V2; $p < 0.001$, V2 vs. V3; $p < 0.001$, V1 vs. V3) during therapy. ns – non significant. For other abbreviations, see Table 1.

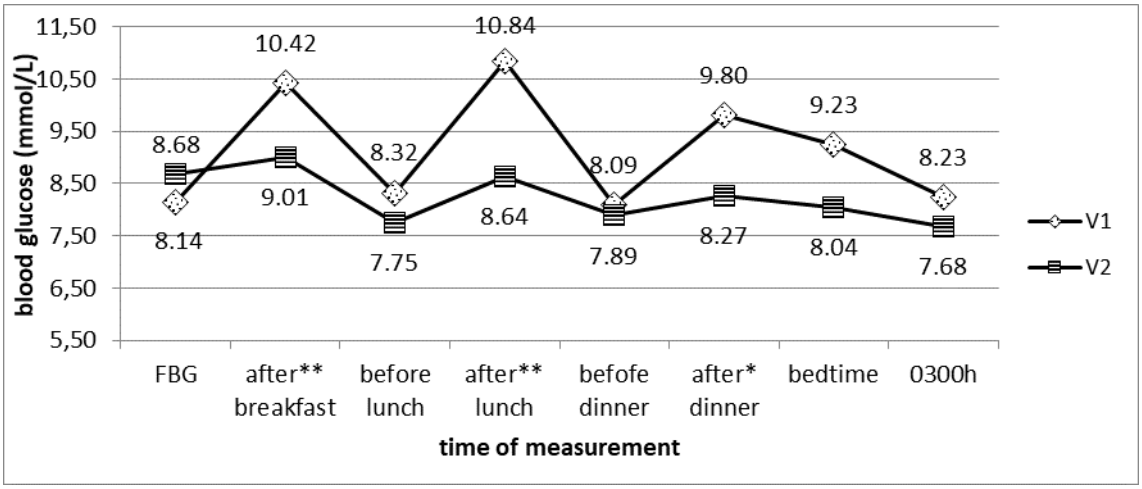

Fig. 2 – The mean of two eight-point blood glucose (BG) profiles at the beginning of therapy (V1) and after insulin aspart therapy, i.e., after the first three months (V2).
FBG – fasting BG; * $p < 0.05$; ** $p < 0.01$.

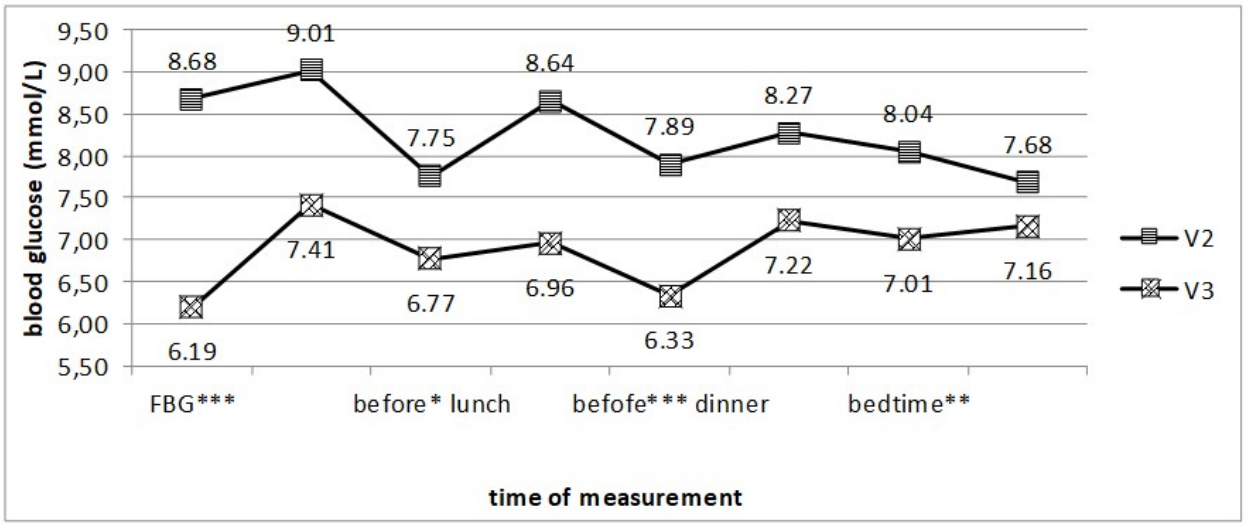

Fig. 3 – The mean of two eight-point blood glucose (BG) profiles after therapy with insulin aspart, i.e., after the first three months (V2) and after therapy with insulin aspart/glargine, i.e., after six months (V3).
FBG – fasting BG; * p < 0.05; ** p < 0.01; *** p < 0.001.

The levels of BG after breakfast, before and after lunch, before and after dinner, and at bedtime were almost all statistically significantly higher at the beginning of therapy compared to values after the third month of therapy as well as after the sixth month of therapy.

The level of BG during the night showed a slight decrease during the study and was statistically significantly lower after the sixth month of therapy than at the beginning ($p < 0.05$) (Wilcoxon test).

The values of the SD of BG in the profile were statistically significantly higher at V1 than at V2 ($p < 0.05$) (Student's *t*-test) and also at V3 ($p < 0.001$) (Wilcoxon test). The values were higher at V2 than at V3 ($p < 0.01$) (Wilcoxon test). The value of CV of BG in the profile was higher at the beginning of therapy than after the sixth month of therapy ($p < 0.05$) (Wilcoxon test).

In order to evaluate the effect of therapy on reducing the prandial increase (PI) in BG after all three meals individually and the total average PI in BG during the day, the mean PI in BG (BG after a meal – BG before a meal) was determined for all three main meals and the mean PI during the day. The results are shown in Table 2 and Figure 4.

The value of the PI in BG for dinner was higher at the beginning of therapy than after the third month ($p < 0.05$) (Student's *t*-test). The value of the PI in BG was at the same level of statistical significance for lunch, while higher levels of statistical significance were present for breakfast ($p < 0.01$) and the mean PI in BG ($p < 0.001$) (Wilcoxon test).

The value of the PI in BG for breakfast at the beginning of therapy was higher than after six months of therapy ($p < 0.05$) (Wilcoxon test); the values of the PI in BG for lunch and the mean PI in BG were statistically significantly higher at the beginning of therapy than after six months of therapy ($p < 0.001$) (Student's *t*-test).

The number of hypoglycemic episodes (number of hypoglycemia *per* patient *per* month) decreased during the

Table 2

Values of prandial increase in blood glucose (mmol/L) before and after insulin analog therapy

Prandial increase	V1		V2		V3	
	mean ± SD	median	mean ± SD	median	mean ± SD	median
Breakfast	2.28 ± 2.36	a**g* 2.40	0.33 ± 2.86	0.90	1.22 ± 2.07	1.30
Lunch	2.52 ± 3.44	a* g*** 3.10	0.89 ± 2.52	0.80	0.20 ± 1.94	0.60
Dinner	1.72 ± 3.24	a* 2.00	0.38 ± 2.42	0.60	0.89 ± 1.79	0.90
Mean	2.17 ± 1.74	ag*** 2.30	0.54 ± 1.67	0.73	0.77 ± 1.19	0.83

V1 – visit 1; V2 – visit 2; V3 – visit 3; SD – standard deviation.
Statistical significance: a – vs. V2; g – vs. V3; * $p < 0.05$ (Student's *t*-test); ** $p < 0.01$; *** $p < 0.001$ (Wilcoxon test).

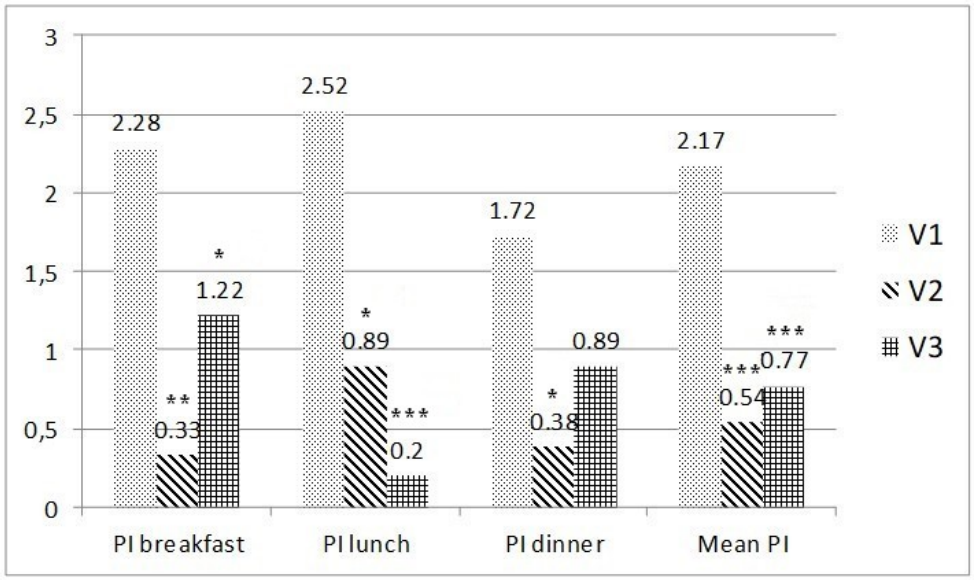

Fig. 4 – The change in the level of prandial increase (PI) in blood glucose (mmol/L) after all three meals individually and the total mean PI in blood glucose during the day (*$p < 0.05$ vs. V1; **$p < 0.01$ vs. V1; ***$p < 0.001$ vs. V1).
V2, V2, and V3 – visits, respectively. For an explanation, see Figures 2 and 3.

Table 3

Hypoglycemic episodes *per* patient *per* month before and after insulin analog therapy

Hypoglycemic episodes	V1		V2		V3	
	mean ± SD	median	mean ± SD	median	mean ± SD	median
Symptomatic	8.59 ± 6.32	a*g*** 10	6.10 ± 4.66	g*** 6	4.53 ± 3.60	4
Asymptomatic	5.96 ± 5.09	g** 6	4.96 ± 4.28	g*** 5	2.76 ± 3.25	1
Severe	0.08 ± 0.28	0	0.04 ± 0.20	0	0.02 ± 0.14	0
All	14.63 ± 9.77	g*** 16	11.10 ± 7.53	g*** 12	7.31 ± 5.09	8
Nocturnal	3.98 ± 3.91	g*** 4	3.14 ± 2.90	g*** 3	1.76 ± 1.75	2

V1 – visit 1; V2 – visit 2; V3 – visit 3; SD – standard deviation.
Statistical significance: a – vs. V2; g – vs. V3; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (Wilcoxon test).

course of therapy. Episodes of serious hypoglycemia whose number was too small for statistical analysis were excluded from the analysis. During one month of therapy with only human insulin, there were four severe hypoglycemias. During the first three months of therapy, there was a total of six severe hypoglycemias, i.e., two *per* month. During the last three months of therapy, a total of three hypoglycemias occurred, i.e., only one *per* month. Their number also evidently decreased during the study.

The total number of hypoglycemic episodes *per* patient *per* month was statistically significantly lower at V3 compared to the number of these episodes at V2, as well as compared to V1 ($p < 0.001$) (Wilcoxon test). The number of asymptomatic hypoglycemic episodes *per* patient *per* month was lower when comparing the V1 and V3 ($p < 0.01$) (Table 3). The lower number of symptomatic hypoglycemic episodes *per* patient *per* month was during the first three months of therapy compared to the period of human insulin therapy ($p < 0.05$). The number of nocturnal hypoglycemias did not statistically change after the first three months, but at V3, it was statistically significantly lower ($p < 0.001$) compared to V2 and V1.

Differences in insulin dose level and BW, i.e., BMI, are shown in Table 4 (compared by Student's *t*-test).

The total daily dose of insulin at the beginning of therapy was statistically significantly higher than after the sixth month of therapy ($p < 0.001$). Furthermore, the total daily dose of insulin at V2 was statistically significantly higher than at V3 ($p < 0.01$).

The total bolus dose at the beginning of therapy (dose of human regular insulin) was statistically significantly higher than the dose of insulin aspart at V2 and V3 ($p < 0.001$); also, the dose of insulin aspart at V2 was higher than at V3 ($p < 0.001$).

The total dose of basal insulin at the end of the third month of therapy and after the sixth month of therapy is statistically significantly higher compared to the dose of basal insulin at the beginning of therapy ($p < 0.001$).

The total daily dose of insulin expressed in unit (U) *per* kg of BW was the highest at the beginning of therapy, higher than at V3 ($p < 0.01$). The total daily dose of insulin at V2 was higher than at V3 ($p < 0.05$).

The values of BW and BMI were higher at the beginning of therapy ($p < 0.05$) and also after the third month of therapy ($p < 0.01$) compared to the values after the sixth month of therapy.

The values of all tested hematological parameters (complete blood count) did not differ statistically significantly during the test period (Table 5). There was no statistically significant difference between ESR values before and after insulin analog therapy in the study period. There was a statistically significant drop in the level of CRP, i.e., the level of CRP at the beginning of therapy was statistically significantly higher than after the third or sixth month of therapy at the level of maximum statistical significance of $p < 0.001$, while the level of fibrinogen was statistically significantly higher after the sixth month of therapy compared to the beginning of therapy ($p < 0.01$) (Wilcoxon test).

Discussion

T1DM is treated from the very beginning with insulin therapy. Over the years, insulin therapy has been improved by the discovery of new techniques for obtaining more and more purified preparations, as well as ways to change the pharmacokinetics of certain insulin preparations to provide daily therapy that would correspond as closely as possible to the physiological profile of insulin secreted by a healthy pancreas [13].

The leading type of modern insulin therapy for T1DM, and proposed by practically all recommendations, is the so-called IIT, or basal-bolus therapy, or multiple daily insulin injections. It involves providing basal insulinization with one or two injections of intermediate-acting human insulin (Neutral Protamine Hagedorn – NPH insulin) with the administration of three or more boluses of human soluble insulin (regular insulin) before the main meals [14–16]. The profile of the action of the human insulin preparation could not fully provide an insulin profile identical to the physiological one. In short, rapid- and short-acting human insulin has an effect that is neither fast enough nor short enough. Intermediate-acting insulin preparations (NPH or lente insulins) do not have an effect that is long enough and, more importantly, that is sufficiently uniform over 24 hrs [17–19].

Table 4

The levels of daily dose of insulin, body weight and body mass index before and after insulin analog therapy

Parameter	V1		V2		V3	
	mean ± SD	median	mean ± SD	median	mean ± SD	median
Bolus dose	34.29 ± 8.45	^{ag***} 36.00	31.55 ± 7.86	^{g***} 30.00	29.22 ± 7.77	30.00
Basal dose	25.59 ± 5.35	^{ag***} 26.00	27.18 ± 5.98	26.00	27.63 ± 6.28	28.00
TDD (U)	59.88 ± 12.79	^{g***}60.00	58.73 ± 13.02	^{g**} 60.00	56.86 ± 13.43	58.00
TDD (U/kg BW)	0.86 ± 0.18	^{g**} 0.88	0.84 ± 0.19	^{g*} 0.83	0.82 ± 0.19	0.80
BW (kg)	70.43 ± 10.84	^{g*} 70.00	70.49 ± 11.01	^{g**} 70.00	69.96 ± 11.20	70.00
BMI (kg/m²)	23.59 ± 2.81	^{g*} 23.95	23.61 ± 2.85	^{g**} 24.06	23.42 ± 2.88	23.71

TDD – total daily dose of insulin; BW – body weight; BMI – body mass index.
Statistical significance: a – vs. 2; g – vs. 3; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (Student's *t*-test).

Table 5

Values of parameters of inflammation and complete blood count before and after insulin analog therapy

Parameter	V1		V2		V3	
	mean ± SD	median	mean ± SD	median	mean ± SD	median
CRP (mg/L)	3.43 ± 2.03	^{ag***} 3.20	2.25 ± 1.39	2.10	2.07 ± 1.62	1.40
Fibrinogen (g/L)	3.33 ± 0.87	^{g**} 3.20	3.60 ± 1.04	3.50	3.87 ± 1.18	3.80
ESR (mm/h)	14.69 ± 13.04	12.00	13.47 ± 11.77	10.00	16.29 ± 14.55	14.00
Le ($\times 10^9$/L)	7.42 ± 1.81	7.30	6.98 ± 1.84	7.00	6.89 ± 1.93	6.70
Er ($\times 10^{12}$/L)	4.82 ± 0.42	4.84	4.84 ± 0.48	4.88	4.82 ± 0.45	4.85
Hb (g/L)	152.06 ± 11.20	152.00	150.63 ± 12.87	151.00	150.49 ± 12.03	148.00
Tr ($\times 10^9$/L)	256.84 ± 60.43	257.00	246.86 ± 65.66	242.00	264.00 ± 60.81	256.00

V1 – visit 1; V2 – visit 2; V3 – visit 3; SD – standard deviation; CRP – C-reactive protein; ESR – erythrocyte sedimentation rate; Le – leucocytes; Er – erythrocytes; Hb – hemoglobin; Tr – thrombocytes.
Statistical significance: a – vs. V2; g – vs. V3; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ (Wilcoxon test).

Modified insulins (insulin analogs), with their superior pharmacokinetic profile, can provide easier overcoming of the problem of strict metabolic control, i.e., provide better control of glycemia both during and after meals [20, 21].

The introduction of basal insulin analogs into therapy has resulted in a series of clinical studies that provide us with data on the most effective way to use these insulins in the treatment of T1DM and type 2 DM (T2DM) [22].

The effect of insulin analogs on the level of HbA1c, both fast-acting and long-acting, was reviewed through two significant analyses (Cochrane Reviews) [23, 24]. In an analysis that included 49 randomized clinical trials with rapid-acting insulin analogs compared to human insulins, a mean difference in HbA1c level of -0.1% was calculated in favor of analogs in T1DM. However, it is important to emphasize that the studies with basal insulins are designed in principle so that the dose is titrated according to the algorithm, with the aim of reaching the target level of HbA1c in all patients, hence a similar level of HbA1c achieved is not entirely unexpected [25].

Three clinical studies have compared an insulin regimen with insulin analogs (both rapid-acting and basal) with a regimen with human insulin alone in patients with T1DM [26–28]. In the largest of these three studies, a basal-bolus regimen with insulin aspart and insulin detemir was compared with a regimen with regular and NPH human insulin in 595 patients with T1DM for 18 weeks [26]. At the end of the study, the mean HbA1c level was lower in the aspart/detemir group than in the regular/NPH insulin group (7.88% vs. 8.11%; $p < 0.001$).

In a smaller but longer study with 56 patients with T1DM, a treatment regimen with insulin lispro and insulin glargine achieved a mean HbA1c level of 7.5% after 32 weeks compared to a mean HbA1c level of 8% achieved in patients treated human regular and NPH insulin [27]. A third study with a cross-over design, conducted in 28 adolescents with T1DM, showed no statistically significant differences in the achieved level of HbA1c among subjects treated with the combination of lispro/glargine compared with regular/NPH human insulin for 16 weeks (8.7% vs. 9.1%; $p = 0.13$) [28].

Our results showed a decrease in HbA1c levels after the introduction of insulin analog therapy. After three months of insulin aspart therapy, there was a non-significant decrease in the level of HbA1c. After the introduction of insulin glargine and three-month therapy with a combination of insulin analogs (aspart/glargine), there was a further significant decrease in the level of HbA1c.

After the first three months of therapy, a significant reduction in PPG was noted after all three main meals. There were no significant differences in FBG before meals, before bed, and at night, as well as in MBG values in the profile. The variability of daily BG was significantly lower. The mean PI in BG after a meal (the mean value of the difference between postprandial and preprandial BG) significantly decreased after the introduction of insulin aspart as prandial insulin. There are significant differences after all three meals individually and in the total PI during the day. In the second three-month period with aspart/glargine therapy, there are no further statistically significant differences in the level of BG increase after a meal.

After the introduction of both insulin glargine and three-month therapy with a combination of insulin analogs (aspart and glargine), there was a further significant reduction of most glycemic values in the daily profiles, especially FBG, MBG in the profiles, as well as the variability of daily glycemic values.

While improvements in HbA1c levels in the studies have been clearly linked to a lower incidence of the classic microvascular complications of diabetes [1], there is increasing evidence that a reduction in the incidence of macrovascular complications (ischemic heart disease, cerebrovascular disease, and peripheral vascular disease) may be more strongly related to the level of PPG, especially in T2DM. Isolated postprandial hyperglycemia (PPG > 7.8 mmol/L) in patients with normal FBG and optimal HbA1c (< 6.1%) doubles the risk of mortality from cardiovascular diseases [29]. Moreover, in large studies, in populations consisting of both DM and non-diabetics, the level of PPG was associated with total and cardiovascular mortality [30, 31].

In a review of published studies comparing rapid-acting insulin analogs with human insulin, in basal-bolus therapy, rapid-acting insulin analogs had a greater effect on PPG in each of the studies, with a lower PPG level compared to human insulin between 0.6 and 2.0 mmol/L [25]. In one of the longest studies (32 weeks) comparing a regimen with insulin analogs (lispro/glargine) to human insulins (regular/NPH human insulin), a significantly lower PPG area under the curve (AUC) was noted with the analog regimen (75 vs. 88 mmol/L/h; $p = 0.002$) [27].

Our results are in accordance with most of the results from the literature, i.e., the introduction of fast-acting insulin analogs into therapy significantly improves prandial glycemic regulation. In our results, this is reflected in a significant reduction in the level of PPG after all three main daily meals in the daily glycemic profiles, as well as in the decrease in the PI in BG after all three meals individually and in the total average PI in BG during the day.

A review analysis of studies (Cochrane Review) found a lower median incidence of severe hypoglycemic episodes with rapid-acting insulin analogs *per* 100 patients *per* year (21.8; range 0–247.4) compared with human insulin (46.1; range 0–544) [24]. Likewise, analysis of studies with long-acting insulin analogs found a significantly lower risk of nocturnal hypoglycemia in patients treated with insulin glargine ($p = 0.00003$) and insulin detemir ($p < 0.00001$) compared to NPH insulin. The risk of symptomatic hypoglycemia was also lower for insulin glargine compared to NPH insulin ($p = 0.005$) and for insulin detemir ($p = 0.00003$) [23]. The rate of severe hypoglycemia was lower with both basal insulin analogs compared with NPH insulin, although the threshold for statistical significance was not reached ($p = 0.2$) [32].

Our results showed a significant reduction in the number of hypoglycemias after the introduction of insulin analogs into therapy. The total number of hypoglycemias is lower after three months and the introduction of insulin

aspart into therapy (a finding slightly above the significance limit, $p = 0.06$), and it decreases especially after the introduction of insulin glargine into therapy, i.e., the total number of hypoglycemias expressed as the number of hypoglycemias *per* patient *per* month is lower at the end of the study and compared to the first three months of therapy, and especially compared to the period before the introduction of analogs in therapy ($p < 0.001$). There are significant differences, first of all, in the number of symptomatic hypoglycemia, which decreases after three months of therapy and at the end of the sixth month. The number of asymptomatic hypoglycemias was lower at the end of the study compared to the first three months of therapy and compared to the beginning of the study, while there was no difference in the number of asymptomatic hypoglycemias after three months compared to the beginning. This led to an increase in satisfaction with therapy and a reduction of the perception of both hyperglycemia and hypoglycemia, as we previously reported [33].

These data can be seen as part of the overall reduction in the number of hypoglycemias, both symptomatic and asymptomatic. They can be interpreted by better recognition of hypoglycemia described in patients in whom good control and avoidance of hypoglycemia improves the autonomic response to hypoglycemia and its recognition [34–37]. This effect takes some time to manifest itself; therefore, it was possibly more significant in the second half of our study. The number of nocturnal hypoglycemias also showed no changes after the first three months of therapy. Still, after the introduction of insulin glargine therapy, the number of nocturnal hypoglycemias was significantly lower compared to the moment before its inclusion and to the beginning of the study, i.e., to the period of therapy with human insulin alone ($p < 0.001$). This prominent and very significant advantage of insulin glargine compared to human basal insulins agrees with earlier reports, both worldwide and in our setting [21, 38]. In this context, the fact that at the end of the study, the level of almost all glycemia in the daily profiles was lower compared to the period after three months, except for night glycemia, should be seen as an advantage of insulin glargine in terms of its better safety, because the night period in the context of the phenomenon hypoglycemia is certainly the most sensitive and dangerous for patients. Reduction of the number of hypoglycemias is also connected with the variability of daily glycemia, which was significantly lower after the insulin analogs therapy, especially after the period of the introduction of glargine in therapy [39].

Our results showed some positive changes in the BW of our patients, which was lowest after the sixth month, and a difference at the end compared to the BW before the introduction of insulin glargine is statistically significant ($p < 0.01$), and also compared to the initial BW ($p < 0.05$). The same applies to the difference in BMI levels. The fact that the improvement of glycoregulation in the first three months was not accompanied by a significant change, i.e., a significant increase in BW, is positive for insulin aspart. An additional and even more significant improvement in glycoregulation after the introduction of insulin glargine, which was accompanied by a significant drop in BW, justifies the claims that basal insulin analogs, compared to NPH insulin, have a significantly more favorable effect on BW patients. The neutrality with regard to BW of new insulin preparations, which is also noted in our patients, is one of the significant advantages of insulin analogs compared to human insulins [40].

The fact that we recorded a discrete but significant drop in BW in our patients with a clear and very significant improvement in glycoregulation can also be interpreted through the analysis of the total daily dose of insulin. Namely, our results show a decrease in the total daily dose of insulin after three months of therapy (p = ns) and at the end of the study ($p < 0.01$ compared to three months and $p < 0.001$ compared to the beginning of the study). Achieving the same or even better degree of glycoregulation (as in our study) with lower doses of insulin is another advantage of insulin analogs. The dose reduction in our study is primarily due to the reduction of bolus and rapid insulin. The bolus dose at the beginning of the study fell after three months and even more at the end of the study (all these differences are highly statistically significant, $p < 0.001$). In contrast, the dose of basal insulin increased from the initial after three months ($p < 0.001$) and then further (p = ns compared to the value after three months). It can be concluded that the effect of fast, bolus insulin is potentially that which we associate with the increase in appetite, the increase in food intake, and the consequent increase in BW. The fact that this effect is manifested above all after the change of basal insulin and the introduction of insulin glargine in therapy confirms that good basal insulinization is necessary, which will enable good initial FBG and even insulinization between meals. In this way, bolus doses can be adjusted to the planned caloric intake, i.e., the size of the meal. That way there is no delay in the effect of bolus insulin after a meal and the need for additional food intake, which can potentially lead to an increase in BW. This is supported by a significant reduction in the variability of daily glycemia, which was recorded after three months of therapy and at the end of the study. Improvement of BG control with IIT with insulin analogs was accomplished without weight gain, like in some previous reports [41].

It has been pointed out that the state of low-grade chronic inflammation, followed by an increase in the circulatory level of inflammatory markers, primarily CRP, represents a risk for the development of various chronic complications. The US Centers for Disease Control and Prevention and the American Heart Association indicate that people with CRP values in the upper tertile for the adult population (> 3.0 mg/L) have double the cardiovascular risk compared to people whose CRP value is less than 1.0 mg/L [42].

Our results show a statistically significant decrease in CRP levels in the first three months, which is maintained until the end of the study and in the following three months. This decrease in CRP is primarily associated with the reduction of prandial stress, i.e., the value of PPG and the PI of BG. Studies support evidence that acute

hyperglycemia during a hyperglycemic clamp [43] or in the postprandial state [44] can increase the production of inflammatory cytokines, such as interleukin (IL)-6, tumor necrosis factor (TNF)-α and IL-18, and the concept that atherosclerosis is an inflammatory disease in diabetes has been confirmed [45].

The noted decrease in CRP levels can also be related to the finding of a discrete reduction in BW and a decrease in the total daily dose of insulin, which corresponds to previous report [46] in which intensive insulin therapy is also correlated with complex changes in inflammatory markers, which are potentially dependent on the degree of BW gain, and may represent a risk for the development of atherosclerosis. In this subgroup analysis from the DCCT study, among those on IIT, high sensitivity CRP (hsCRP) levels increased in those with the highest BW gain, while it decreased among those in the lowest third of BW gain. It is stated that IIT in patients with T1DM decreased the level of soluble intercellular adhesion molecule-1 and increased the levels of soluble TNF receptor 1 and hsCRP among those who increased BW. These data show that the effect of IIT on inflammation is complex and, since hsCRP is an inflammatory risk factor, it is suggested that the risk of atherosclerosis among diabetic patients may be influenced by the degree of gain in BW when on IIT [46].

Conclusion

Treating patients with T1DM with insulin analogs (insulin aspart and insulin glargine) in intensified insulin therapy leads to improvement of blood glucose control with a decrease in hypoglycemia, body weight, a dose of insulin, and C-reactive protein level.

Conflict of interest

The authors declare no conflict of interest.

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Analysis of fetal renal cortex development: cortical maturation index as a new potential guide in fetal renal cortex assessment

Analiza razvoja bubrežne kore fetusa: indeks maturacije korteksa kao novi potencijalni vodič u proceni razvoja fetalne kore bubrega

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Abstract

Background/Aim. To date, most of the scientific attention has been aimed at the morphometric analysis of the nephrogenic zone (NZ) of the fetal renal cortex, while the quantification and analysis of the maturation zone (MZ) and other indicators of renal maturity were missing. The aim of the study was to examine the characteristics of fetal kidney cortex maturation, as well as to propose the development of a new cortical maturity index (CMI). **Methods.** The study included 42 paraffin molds of the fetal kidney, divided into three groups according to gestational age (GA). After hematoxylin and eosin staining, tissue sections were analyzed through the following parameters: the thickness of the NZ and MZ, the renal corpuscles area (RCa) and the glomerular capillary tuft area (GCTa), and the maturation stages of the glomeruli. In addition, a new parameter, CMI, was formed as a ratio of NZ and MZ thickness. The collected data were statistically processed. **Results.** Changes in NZ and MZ thickness were statistically significant, and they correlated with GA. A value of CMI higher than 0.2 was recorded in the kidney samples of fetuses younger than the 20th gestational week (GW), while a value lower than 0.1 was recorded in the samples older than the 30th GW. With an increase in GA in all zones of the renal cortex, RCa and GCTa decreased. A statistically significant reduction of GCTa was observed in the oldest group in the juxtamedullary and intermediate zones of the cortex ($p < 0.01$). Glomeruli located in the deeper parts of the cortex were more mature than the superficial ones. **Conclusion.** The measured parameters can serve as a starting point for future studies that would analyze the histomorphological characteristics of the fetal kidney cortex. In the absence of clinical data, a newly formed parameter CMI can represent assistance with the determination of GA, as it significantly correlates with GA ($p < 0.01$).

Key words:
fetal development; fetus; histological techniques; kidney cortex.

Apstrakt

Uvod/Cilj. Do sada, pažnja naučne javnosti bila je većinski usmerena ka morfometrijskoj analizi nefrogene zone (NZ) bubrežne kore fetusa, dok su izostali kvantifikacija i analiza maturacione zone (MZ) i drugih indikatora zrelosti bubrega. Cilj rada bio je da se ispitaju karakteristike sazrevanja korteksa bubrega fetusa, kao i da se predloži formiranje novog indeksa maturacije korteksa (IMK). **Metode.** U studiju su bila uključena 42 parafinska kalupa fetalnih bubrega, podeljeni prema gestacijskoj starosti (GS) u tri grupe. Posle bojenja uzoraka hematoksilinom i eozinom, analizirani su sledeći parametri: debljina NZ i MZ, površina bubrežnog telašca (BTp) i glomerularnog klupčeta (GKp), kao i maturacioni stadijumi glomerula. Dodatno, formiran je novi parametar, IMK, kao odnos između debljine NZ i MZ. Sakupljeni podaci su statistički obrađeni. **Rezultati.** Promene u debljini NZ i MZ bile su statistički značajne i u korelaciji sa GS. Vrednost IMK viša od 0,2 zabeležena je u uzorcima bubrega fetusa mlađih od 20. gestacijske nedelje (GN), dok je vrednost niža od 0,1 zabeležena u uzorcima bubrega fetusa starijih od 30. GN. Sa porastom GS u svim zonama bubrežnog korteksa

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smanjile su se vrednosti BTp i GKp. Statistički značajna redukcija GKp primećena je u najstarijoj grupi u jukstamedularnoj i intermedijarnoj zoni kore ($p < 0,01$). Glomeruli locirani u dubljim delovima kore pokazivali su veći stepen zrelosti od onih koji su bili locirani površnije. **Zaključak.** Izmereni parametri mogu poslužiti kao početna tačka za buduće studije koje bi analizirale histomorfološke karakteristike korteksa bubrega fetusa. U nedostatku kliničkih podataka, novoformirani parametar IMK može pomoći pri određivanju GS, s obzirom na to da značajno koreliše sa GS ($p < 0,01$).

Ključne reči:
fetus, razvoj; fetus; histološke tehnike; bubreg, kora.

Introduction

The majority of people are aware of the kidneys' primary excretory function – to eliminate harmful substances from the human body. Furthermore, kidneys have many other homeostatic functions, such as water and electrolyte regulation, osmoregulation, arterial blood pressure regulation, acid-base regulation, hormone secretion, and the ability to perform gluconeogenesis [1]. As part of the urogenital system, kidneys are developed by a process of differentiation of the intermediate mesoderm, which forms urogenital folds and later a nephrogenic cord [2], so that three developmental forms can be obtained. The first one is the pronephros, which is rudimentary and nonfunctional in humans, the second one is the mesonephros, which functions for a short period, and the third is metanephros [3], referred to as the primordium of the definitive kidney [4–6]. During the development of the kidney cortex, two zones are observed. In the superficial parts of the cortex, beneath the capsule, the nephrogenic zone (NZ) is described as a zone of undifferentiated mesenchyme with developmental forms of glomeruli [7]. The subjacent, inner zone of the cortex is described as a maturation zone (MZ), spreading to the kidney's medulla. MZ is a place of differentiation and maturation of glomeruli and specific nephron segments. As a result, it contains various stages of maturing glomeruli, which are more or less immature in terms of histological appearance as well as function [8].

Active glomerulogenesis in NZ is a result of a complex interplay between ureteric bud branches and metanephric mesenchyme (blastema). Cells of the metanephric blastema condense under the influence of the ureteric bud epithelium, forming the following developmental forms of glomeruli: cap, renal vesicle, comma-shaped form, and S-shaped form [3]. Bowman's capsule (BC) is developed from the inferior part of the S-shaped form. Renal corpuscles (RC) are formed by the ingrowth of capillaries of adjacent mesenchyme into BC [2]. Those capillaries inside the RC are called a glomerulus, also known as the glomerular capillary tuft (GCT) [9]. Glomerulogenesis is a complex process during which cell differentiation occurs as a consequence of numerous intercellular and cell-matrix interactions [10]. Defects in this intricate process are known as impaired glomerulogenesis, providing a fundamental basis for understanding disorders such as glomerulocystic disease and for linking adult-onset conditions like chronic kidney disease (CKD) and hypertension to inadequate fetal development [11, 12]. Prematurity is one of the most common risk factors associated with impaired glomerulogenesis and is closely linked with oligonephropathy [13].

Analyzing the importance of the kidneys through their numerous functions, particularly in light of the rising prevalence of premature births [14], it becomes clear why their prenatal development and maturation should be studied further in the future. So far, most of the scientific attention has been aimed at the morphometric analysis of the NZ. In contrast, extensive and detailed morphometric studies of the MZ, as well as defining indicators of cortical maturity, are quite rare. Therefore, we aimed our research in that direction, believing that not only can this type of data be implemented into clinical and experimental research, but it can also provide important information about kidney maturity that neonatologists can expect in premature fetuses of the appropriate gestational age (GA).

Methods

This retrospective study was approved by the Ethics Committee of the University Clinical Center of Vojvodina, Novi Sad, Serbia (No. 00-1160, from December 16, 2019) since we used paraffin molds of autopsied fetal kidney tissue obtained from the archive of the Center for Pathology and Histology of the aforementioned Clinical Center.

The study included 42 specimens over a two-year period (January 1, 2019, until October 1, 2020), selected based on the inclusion criteria.

The inclusion criteria were the following: absence of malformations of the urinary system noted during the autopsy; clinical evidence indicating the absence of chromosomal abnormalities; absence of maceration and autolysis of the kidney tissue; presence of the capsule on the kidney surface. The chosen specimens were then classified into three groups, based on their fetal GA as determined by clinicians: G1 – kidney tissue samples from fetuses younger than the 19th gestational week (GW) (n = 18), G2 – kidney tissue samples from fetuses between the 20th and 24th GW (n = 10), and G3 – kidney tissue samples from fetuses older than the 25th GW (n = 14).

Histological staining

Sectioning and staining with hematoxylin and eosin was conducted at the Department of Histology and Embryology, Faculty of Medicine of the University of Novi Sad, during a noted period.

Morphometric analysis

Stained tissue slides were analyzed by a digital microscope, VisionTekTMSakura (Japan), under a magnification of x20. Measurement was done by the software VisionTek

Live 2.6 (Sakura, Japan). The kidney cortex was measured at five points in every specimen (option Ruler). The NZ thickness was measured from the capsule to the distal end of the S-shaped form, following the methodology of the previous study [15]. MZ thickness was measured from the inner border of NZ (distal end of the S-shaped form) to the medulla. The results were presented in micrometers (μm). Based on these values, we established a new parameter called the cortical maturity index (CMI), defined as the NZ/MZ ratio (presented as an absolute value).

At five randomly selected visual fields, an area of 2 mm^2 of the renal cortex was analyzed. The visualized glomeruli were classified into three groups, based on their localization within the cortex: (I) juxtamedullary (JM) glomeruli included a single row of glomeruli located closest to the medulla, (II) superficial glomeruli including a single row of glomeruli that were closest to the NZ, and (III) intermediate group which consisted of all glomeruli between the two previously described groups. At each visual field, five representative glomeruli were chosen in each cortical zone (superficial, intermediate, and JM), and the area of RC and glomeruli was measured (option free form area). The results were presented in μm^2.

Glomerular maturity assessment

The maturation stages of glomeruli were examined on all the glomeruli captured on the slides. The assessment was done by a modified classification proposed by Macdonald and Emery [16], which is given in the following text. First stage – glomerular bud is recognizable. It is fungi-shaped, and the real vascular pole cannot be obtained. The parietal layer of BC is made of cubic epithelium (Figure 1A). Second stage – the vascular pole of the glomerular bud can be obtained. The glomerulus is not lobulated, and it is covered by an uninterrupted visceral layer of BC. The parietal layer of BC is still built of the cubic epithelium (Figure 1B). Third stage – glomerular bud is distended and lobulated, but the uninterrupted visceral layer of BC is still present. BC epithelium of the parietal layer becomes thinner (Figure 1C). Fourth stage – glomerular buds continue to grow. The visceral layer of BC becomes fragmented, while the parietal layer is built of simple squamous epithelium. Capillary spaces are visible (Figure 1D).

Statistical analysis

For statistical analysis, we used Excel for Microsoft 365. A Kruskal-Wallis test was used to determine whether there was statistical evidence that the analyzed groups' means were significantly different. To analyze the correlation, we used Spearman's rank correlation test. Significant values were obtained at $p < 0.05$ and $p < 0.01$.

Results

NZ thickness decreased with increasing GA, with the highest values in the G1 (Figure 2). Statistical significance both in the reduction of the NZ thickness ($p < 0.05$) and the NZ thickness correlation with GA was noted ($p < 0.01$), and it showed a negative trend in the older specimens. On the other hand, MZ thickness increased with GA (Figure 3) and showed a positive trend of correlation with GA. Statistical significance was noted in both analyses ($p < 0.01$).

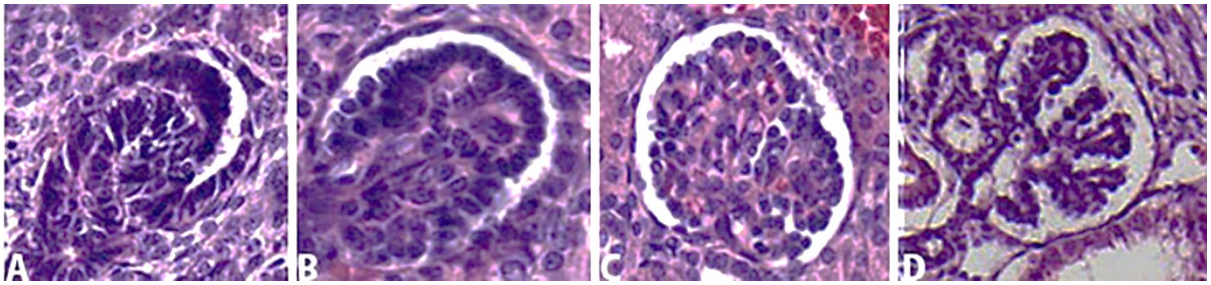

Fig. 1 – Micrographs showing maturation stages of glomeruli (hematoxylin and eosin staining × 630); A) 1st stage; B) 2nd stage; C) 3rd stage; D) 4th stage.

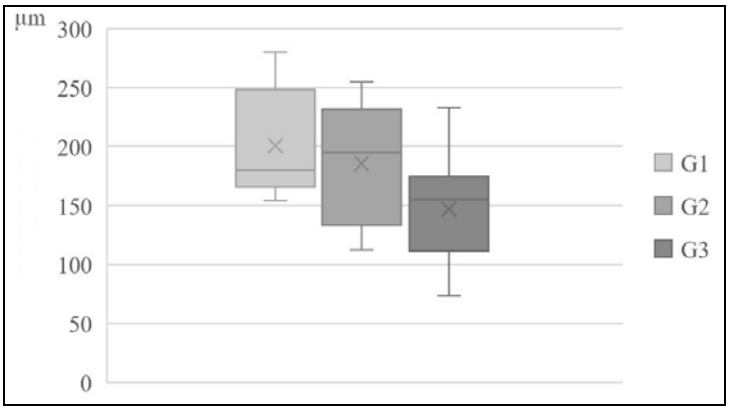

Fig. 2 – Distribution of the nephrogenic zone thickness. For abbreviations, see Table 1.

CMI decreased with the increase of GA within the analyzed groups, with statistical significance ($p < 0.01$) (Figure 4). A value of CMI that was higher than 0.2 was noted in the kidneys of fetuses younger than the 20th GW and a value lower than 0.1 in those older than the 30th GW. Correlation analysis showed a negative relationship between analyzed parameters, with statistical significance ($p < 0.01$).

RC area decreased in all three zones of the renal cortex with an increase in GA. GCT area also decreased in analyzed zones. Statistical significance in the reduction of GCT area was noticed in the third group ($p < 0.01$) in both JM and intermediate zones of the cortex (Table 1).

In the superficial zone of the renal cortex, in younger fetuses (G1 and G2), the first stage of glomerular maturation predominates. In fetuses older than 25 weeks, the second stage of glomerular maturation prevails. The third stage is slightly more frequent in older fetuses, and the fourth developmental stage was not present in the superficial zone of the cortex, regardless of GA. The intermediate cortical zone was predominantly populated by glomeruli in the third maturation

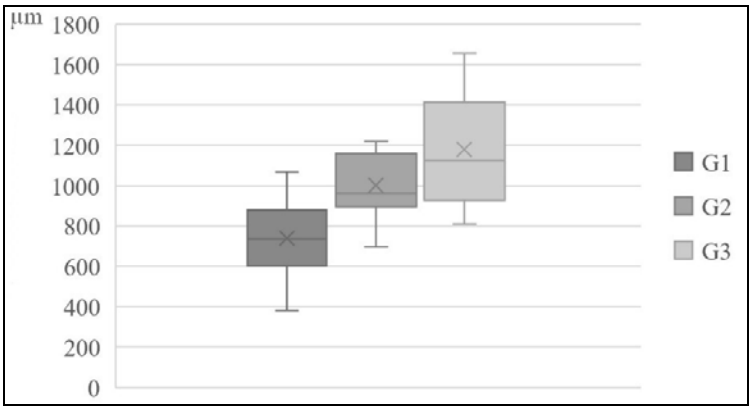

Fig. 3 – Distribution of the maturation zone thickness. For abbreviations, see Table 1.

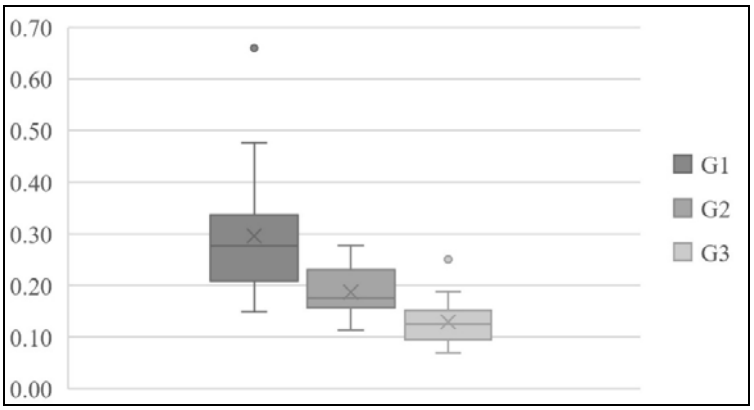

Fig. 4 – Distribution of the cortical maturity index. For abbreviations, see Table 1.

Table 1

Renal corpuscle area (RCa) and glomerular capillary tuft area (GCTa) in all three zones according to gestational ages

Zone	G1	G2	G3
Superficial			
RCa	5,350 ± 1,372	5,637 ± 855	5,101 ± 1,067
GCTa	2,719 ± 884	3,083 ± 805	3,014 ± 818
Intermediate			
RCa	9,494 ± 1,385	9,096 ± 1,360	6,507 ± 1,511
GCTa	5,443 ± 1,575	5,601 ± 1,624	3,744 ± 932*
Juxtamedullary			
RCa	16,669 ± 2,235	15,176 ± 2,597	11,916 ± 2,105
GCTa	9,266 ± 2,817	9,081 ± 2,070	6,428 ± 1,830*

Kidney tissue samples from fetuses: G1 – younger than 19th gestational week (GW) (n = 18); G2 – between the 20th and 24th GW (n = 10); G3 – older than the 25th GW (n = 14).

All values are given as mean ± standard deviation; all units of measurements are given as µm^2. *Statistical significance ($p < 0.01$).

Table 2

Distribution of the glomerular maturation stages

Group	Maturation stages of glomeruli (%)											
	superficial zone				intermediate zone				juxtamedullary zone			
	1st	2nd	3rd	4th	1st	2nd	3rd	4th	1st	2nd	3rd	4th
G1	48.6	40	11.4	0	0	3.2	95.2	1.6	0	0	83.3	16.7
G2	55.2	37.9	6.9	0	0	6.4	92.7	0.9	0	0	80	20
G3	32.1	49.1	18.8	0	0	10.1	76.9	13	0	0	34.1*	65.9*

For abbreviations, see Table 1; 1st, 2nd, 3rd, and 4th are different glomerular stages – see Figure 1.
***Statistical significance $p < 0.01$.**

stage, while the first stage was not recorded in any GA. In addition, statistical significance was not observed (Table 2).

In the JM cortical zone, the predominant form in G1 and G2 was the third maturation stage, but in G3, the oldest group, the fourth maturation stage was predominant. A statistically significant reduction of the third and increase of the fourth maturation stages was observed in fetuses with the highest GA ($p < 0.01$) (Table 2).

Discussion

The tendency to realize and understand complex processes, such as organ development, is getting more attention in modern science. About 20 years ago, David Barker [17] set up the fetal origin hypothesis and correlated low birth weight with a higher risk for the later development of numerous chronic diseases. Furthermore, Maringhini et al. [18] found that low birth weight is a significant risk factor for developing CKD. Knowing that congenital malformations are one of the most common causes of acute kidney injury and CKD [19, 20], the need to explore kidney development comes by itself.

The findings of our study showed that the thickness of NZ decreased with the increasing GA of the fetus and that it strongly correlated with GA, which is concordant with the results of previous studies that have analyzed kidney embryological development [7, 15, 21, 22]. A statistically significant decrease in NZ thickness was observed in the oldest group, similar to the results described by Ryan et al. [15]. By morphometric analysis of the kidney cortex, they recorded that the thickness of the NZ in the kidneys of fetuses younger than 20 GWs was around 200 μm, and while decreasing, NZ was still present in some of the specimens of the oldest group (37th GW). On the contrary, Tank et al. [23] stated in their study, conducted on 20 fetal kidney tissue specimens, that NZ could not be observed in the tissue material of fetuses older than 36th GW, and this variability about NZ still represents a kind of enigma. The oldest specimen in our study was 36 weeks old, and NZ was still present, indicating: a) variability in the disappearance of NZ and b) the possibility of postnatal glomerulogenesis. The presence of the NZ in the late periods of pregnancy points out that prematurely born neonates are exposed to a higher risk for two reasons. The first one is the fact that a higher percentage of glomeruli in less mature stages in younger fetuses indicates more immature kidneys with lower functional performances. Second, premature birth would stop, delay, or alter the development of new glomeruli in NZ, which implies that premature children would be born with fewer glomeruli compared to term children. Nephrogenesis and the morphogenetic activity in the renal cortex are down-regulated by unknown signals with the start of the perinatal period [8], and data suggest that the number of nephrons at birth is permanent during life [24]. Although some data suggest that nephrogenesis in the kidneys of preterm-born neonates continues and leads to a significant increase in the number of glomeruli and nephrons within the kidney after birth [25], this point of view is debatable, knowing that preterm and low-birth-weight neonates frequently suffer from oligonephropathy with lifelong disease risk. This is the reason some researchers are analyzing mechanisms by which nephrogenesis could be prolonged even after birth [26]. Similar to the disappearance of the NZ and the termination of glomerulogenesis, postnatal glomerular development is also not precisely determined and brings up disagreement and uncertainty.

While NZ represents a real area of interest for researchers, data about MZ is severely lacking, and it is important to understand the significance of this developmental zone. In our study, MZ increased within older specimens, and the exact values were collected. In this zone, maturating glomeruli, tubules, and blood vessels, such as cortical radiate arteries, can be obtained [27]. While the nephrons are maturing in the MZ, they are being formed in the NZ, and their following apposition to the MZ results in a radial extension of the renal parenchyma. During their development, RC located in NZ did not have established vessels or perfusion, and it is believed that these events happened later, after their relocation to the MZ. Vascular supply, including perfusion with erythrocytes, is in part developed in the MZ, so the initial perfusion of the GCT occurs here and not in the NZ, where vasculature is incomplete. It was believed that impaired nephrogenesis is caused by noxae altering the MZ and maturating glomeruli. However, actual data points out the significance of the primary and secondary steps of the nephrogenesis that are happening in the NZ [8]. Consequently, the last generation of maturating nephrons that are located in the MZ, as well as developing forms located in the NZ, are the targets of described noxae [28]. The functions of the MZ, other than maturation and vascularization of renal corpuscles, are not certain, and they represent a real field of possibilities for future research.

CMI, the ratio between NZ and MZ, decreased with the higher GA, which correlates with the decrease in the NZ thickness and the increase in the thickness of MZ. This index

showed a high statistically significant correlation ($p < 0.01$) with the decrease in fetal GA, which suggests that it could be used as a precise marker of this change. It can provide an estimate of the fetal GA and kidney maturity if other information is not available. In our research, a value of CMI higher than 0.2 was noted in the kidneys of fetuses younger than the 20th GW, while a value lower than 0.1 corresponded with the kidney tissue material of fetuses older than the 30th GW. Although this parameter showed a strong correlation with GA, these new findings need to be verified through future studies by expanding the number of specimens and their clinical implementation.

RC area analysis has shown that the value of this parameter decreased in all three cortical zones with the increase in GA. However, statistical significance was not noticed. Similarly, GCT area also decreased, and statistical significance in the reduction was noticed in the G3 ($p < 0.01$) in both JM and intermediate zones of the cortex. The presence of "abortive forms" of the glomeruli in the deeper parts of the renal cortex, which are thought to be direct arterio-venous anastomoses [7], could explain these findings. Crobe et al. [29] pointed out that with the increased GA, podocyte number decreases. This fact could correlate with the decrease of the GCT area, knowing that podocytes form the visceral layer of the BC and that their presence leads to glomerular hypercellularity in the early developmental stages of the GCT. Glomeruli of the superficial cortical zone contains fewer podocytes, and their GCT area are consequently lower compared to the GCT area of some more deeply placed glomeruli. In addition, Schell et al. [27] described changes in the shape of podocytes that occur during glomerular maturation. In the beginning, podocytes can be described as classic columnar epithelium, and later, they get thinner, which can additionally influence the GCT area values.

By analyzing the maturation stages of glomeruli, a common pattern of renal cortex maturation could have been observed, meaning that in each of the observed gestational periods, glomeruli located in the deeper parts of the cortex were more mature. The superficial zone of the renal cortex contained less mature glomeruli compared to the intermediate and, even deeper, JM zone. Furthermore, while the superficial zone did not contain glomeruli of the fourth stage of maturity, the JM zone did not contain glomeruli of the first and second stages of development. This finding is concordant with the previous studies, and it is a significant sign of cortical maturation [16].

Conclusion

This study has quantified and more thoroughly analyzed the developing renal cortex. The results have shown and numerically expressed the parameters of renal maturation, such as the thickness of the nephrogenic zone and maturation zone, as well as the glomerular maturation stages. In addition, a newly formed parameter, the cortical maturity index, can help with the estimation of gestational age as it significantly correlates with gestational age.

Obtained data are the basis for future research in the domain of histomorphology and ontogenesis of the kidney, which, we hope, will lead to an enhancement in prenatal, perinatal, and postnatal care.

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The significance of determining biomarkers of inflammation in chronic kidney failure

Značaj određivanja biomarkera zapaljenja u hroničnoj bubrežnoj slabosti

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Abstract

Background/Aim. Inflammation is the main cause of the onset, progression, and outcome of chronic kidney disease (CKD). The aim of the study was to examine the predictive value of inflammatory biomarkers in patients with CKD stages I–V and their association with parameters characteristic of CKD. **Methods.** A cross-sectional study analyzed 117 adult patients with CKD who were divided into two groups according to the glomerular filtration rate (GFR): Group 1, with normal to mild impairment of renal function (GFR ≥ 60 mL/min/1.73 m^2), stages I and II, and Group 2 with moderate and severe impairment of renal function (GFR < 60 mL/min/1.73 m^2), stages III, IV, and V, who have not started dialysis treatment. In addition to standard laboratory analyses, we determined derived parameters in patients, neutrophil-lymphocyte ratio (NLR), platelet- lymphocyte ratio (PLR), systemic immune-inflammation index (SII), and system inflammation response index (SIRI), as markers of inflammation. **Results.** A statistically significant difference between Groups 1 and 2 was observed for body mass index ($p < 0.003$), for platelets, hemoglobin, creatinine, urea, acidum uricum, iron, phosphorus, parathyroid hormone, and proteinuria 24 hrs ($p < 0.001$), for calcium ($p < 0.031$) and leukocytes ($p < 0.030$). By analyzing the values of NLR, PLR, SII, and SIRI in patients with CKD, a statistically significant difference ($p < 0.001$) was observed between the groups; the values were elevated in Group 2. NLR, PLR, and SII showed statistical significance for essential parameters in CKD (C-reactive protein, creatinine, GFR, hemoglobin, calcium, phosphorus, parathyroid hormone) and SIRI showed statistical significance for phosphorus in Group 2. The most sensitive was NLR at 87.7%, and PLR had the highest specificity, at 81.7%, with cut-off values for PLR – 151.75, NLR – 2.06, SII – 493.57, and SIRI – 0.739. **Conclusion.** Our results indicate that the detection of biomarkers NLR, PLR, SII, and SIRI could have a significant role in predicting inflammation in patients with CKD and would contribute to the timely recognition of patients at risk of developing complications.

Key words:
biomarkers; glomerular filtration rate; inflammation; renal insufficiency, chronic.

Apstrakt

Uvod/Cilj. Zapaljenje ima ključnu ulogu u razvoju, progresiji i ishodu hronične bolesti bubrega (HBB). Cilj rada bio je da se ispita prediktivna vrednost biomarkera zapaljenja kod bolesnika sa HBB stadijuma I–V i njihova povezanost sa parametrima karakterističnim za HBB. **Metode**. Studijom preseka analizirano je 117 odraslih bolesnika sa HBB koji su na osnovu brzine glomerulske filtracije (*glomerular filtration rate* – GFR) podeljeni u dve grupe: Grupu 1, sa normalnom do slabo redukovanom bubrežnom funkcijom (GFR ≥ 60 mL/min/1,73 m^2) stadijum I i II i Grupu 2, sa umerenim i teškim smanjenjem bubrežne funkcije (GFR < 60 mL/min/1,73 m^2), stadijum III, IV i V koji nisu započeli lečenje dijalizom. Pored standardnih laboratorijskih analiza, kao markeri zapaljenja, određeni su izvedeni parametri: odnos neutrofila prema limfocitima (*neutrophil-lymphocyte ratio* – NLR), odnos trombocita prema limfocitima (*platelet-lymphocyte ratio* – PLR) i indeksi zapaljenja *systemic immune-inflammation index* – SII i *system inflammation response index* – SIRI. **Rezultati**. Utvrđena je statistički značajna razlika između Grupa 1 i 2 za indeks telesne mase ($p < 0,003$), za trombocite, hemoglobin, kreatinin, ureu, mokraćnu kiselinu, gvožđe, fosfor, paratiroidni hormon, kao i za 24-satnu proteinuriju ($p < 0,001$), zatim za kalcijum ($p < 0,031$) i leukocite ($p < 0,030$). Analiziranjem NLR, PLR, SII i SIRI kod bolesnika sa HBB uočena je statistički značajna razlika ($p < 0,001$) između grupa; povišene vrednosti ovih markera pokazane su kod bolesnika u Grupi 2. NLR, PLR

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i SII su pokazali statističku značajnost za važne parametre u HBB (C-reaktivni protein, kreatinin, GFR, hemoglobin, kalcijum, fosfor, paratiroidni hormon), a SIRI je pokazao statističku značajnost za fosfor u Grupi 2. Najsenzitivniji je bio NLR sa 87,7%, a najveću specifičnost imao je PLR – 81,7%, uz *cut-off* vrednosti za PLR – 151,75, NLR – 2,06, SII – 493,57 i SIRI – 0,739. **Zaključak**. Naši rezultati ukazuju da bi detekcija biomarkera NLR, PLR, SII i SIRI mogla imati značajnu ulogu u predviđanju zapaljenja u obolelih od HBB i doprineti blagovremenom prepoznavanju bolesnika sa rizikom od nastanka komplikacija.

Ključne reči:
biomarkeri; glomerulska filtracija, brzina; zapaljenje; bubreg, hronična insuficijencija.

Introduction

Chronic kidney disease (CKD) occurs in all age groups, with a prevalence of 9.1%, which tends to increase, and data indicate that it will become the fifth leading cause of death by 2040 [1, 2]. CKD represents damage to the structure and/or function of the kidneys that lasts at least three months, with a glomerular filtration rate (GFR) < 60 mL/min/1.73 m^2, where microalbuminuria, proteinuria, and pathological urine sediment indicate significant kidney damage [3]. According to the GFR, there are five stages of CKD.

In patients with CKD, inflammatory processes, which are significant elements of increased morbidity and mortality, are manifested very early. Inflammatory tissue remodeling precedes and characterizes the progression of CKD, leading to fibrous changes, loss of kidney function, and numerous complications such as atherosclerosis and cardiovascular diseases (CVDs) [2, 4]. Determination of biomarkers of inflammation (BI) in patients with CKD is of great importance, bearing in mind that CVDs take a significant percentage (45%) of the cause of lethal outcomes already in the early stages of CKD [4–9]. In patients with CKD, cardiovascular system complications are the most common cause of morbidity and mortality. Acute myocardial infarction is observed in 30%–40% of patients with CKD [10]. Early detection of inflammatory processes is important in patients with CKD. Recently, four new BI potentially crucial in daily practice have been described: neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR), systemic immune-inflammation index (SII), and system inflammation response index (SIRI) [5–9, 11–14]. Elevated values of NLR and PLR are associated with the progression of CKD towards the end stage and with a high mortality rate [7–9, 11–13], and SII and SIRI have a higher predictive value than other BI and indices in predicting cardiovascular events [14, 15].

The aim of the study was to examine the potential predictive role of BI in patients with CKD stage I–V and their association with parameters characteristic of CKD.

Methods

The cross-sectional study included 117 adult patients with CKD, stages I–V, who did not start the dialysis procedure. Their average age was 56.97 ± 10.16 years; 63 (53.85%) were male and 54 (46.15%) female. The study was conducted according to the provisions of the Declaration of Helsinki, approved by the Ethics Committee of the Military Medical Academy, Belgrade, Serbia (No. 3000-1, from March 13, 2014). GFR was determined according to the CKD estimate GFR (eGFR) formula based on serum creatinine values [16]. We divided patients into two groups according to GFR. In Group 1, there were 60 patients with normal to mild impairment of renal function (GFR ≥ 60 mL/min/1.73 m^2), stages I and II, with the existence of one or more parameters of kidney damage (microalbuminuria, proteinuria, pathological urine sediment, or disorder of renal structures revealed by the visualization method). In Group 2, there were 57 patients with moderate and severe impairment of renal function (GFR < 60 mL/min/1.73 m^2), stages III, IV, and V, who had not started the dialysis treatment.

Patients excluded from the study were those with the following: acute myocardial infarction, cerebrovascular insult, with a diagnosis of inflammatory disease (pneumonia, bronchitis, rhinitis, angiitis, pancreatitis, cholangitis, cholecystitis, allergic dermatitis, urinary infection), with immunosuppressive therapy of malignant disease, and with data about a primary surgical intervention in the last six months.

Parameters determined for all respondents were body mass index (BMI) (normal 18.5–24.9 kg/m^2, overweight 25–29.9 kg/m^2, obese ≥ 30 kg/m^2) and smoking status. Blood samples for laboratory analysis were taken in the morning, after 12 hrs of fasting. The following were determined: blood count (BC) and differential BC, C-reactive protein (CRP), glucose, urea, creatinine, uric acid, total proteins, albumins, cholesterol, triglycerides, calcium (Ca), phosphorus (P), hemoglobin (Hb), iron (Fe), vitamin D3, parathyroid hormone (PTH); 24-hr proteinuria and urine culture were determined in the urine. Complete BC was performed on an ADVIA® 120 device using flow cytometry, and biochemical analyses were performed on an ADVIA® 1800 device using spectrophotometry. We determined BI (NLR, PLR, SII, and SIRI) in all patients from the BC. NLR was calculated by dividing the absolute number of neutrophils by the absolute number of lymphocytes. PLR was calculated from the ratio of platelets to lymphocytes. SII was obtained based on the form SII = platelet count (10^9/L) × neutrophil count (10^9/L) / lymphocyte count (10^9/L), and SIRI = neutrophil count (10^9/L) × monocyte count (10^9/L) / lymphocyte count (10^9/L).

Statistical analysis

The data were analyzed using the Statistical Package for the Social Sciences IBM-SPSS, version 26.0. Categorical variables were presented as frequency and were analyzed using the Chi-square test. All continuous variables are presented as mean ± standard deviation. The Kolmogo-

rov-Smirnov test was used to test the normality of data distribution. For intergroup comparisons, the Independent samples *t*-test for parametric variables was used. For testing the relationship between variables, Pearson's correlation was used.

Optimal thresholds (cut-off values) of biomarker values (NLR, PLR, SII, SIRI) were determined using the receiver operating characteristic (ROC) curve analysis. The sensitivity, specificity, and cut-off values for NLR, PLR, SII, and SIRI in patients with CKD were obtained. The ROC curve comparisons were performed to verify variations in sensitivity and false positive fractions (1 – specificity) of BI using overall cut-offs. Statistical significance was defined as $p < 0.05$ for all comparisons.

Results

In our patients, the underlying kidney diseases were arterial hypertension – 44 (37.6%), chronic glomerulonephritis – 27 (23.0%), diabetes mellitus – 16 (13.7%), polycystic kidney disease – 14 (12.0%), renal calculus – 12 (10.3%), and tubulointerstitial nephritis (TIN) – 4 (3.4%).

Demographic and laboratory data of patients with CKD are shown in Table 1, which shows individual parameters concerning GFR (Group 1: GFR > 60 mL/min/1.73 m^2; Group 2: GFR ≤ 60 mL/min/1.73 m^2) and summary parameters of patients.

Comparing age, gender, BMI, and smoking status, a statistically significant difference was observed only for BMI ($p < 0.003$). Observing the laboratory analyses, statistical significance ($p < 0.001$) was observed between the groups for platelets, Hb, creatinine, urea, acidum uricum, Fe, P, PTH, and proteinuria 24 hr, $p < 0.031$ for Ca and $p < 0.030$ for white blood cells (Table 1).

An analysis of BI values – NLR, PLR, SII, and SIRI – in patients with CKD showed a statistically significant difference ($p < 0.001$) between the groups. Elevated values of these markers were found in patients in Group 2 with a more severe degree of renal function impairment (Table 2).

Correlation of NLR, PLR, SII, and SIRI and significant parameters in Group 2 are presented in Table 3. A statistically significant correlation was obtained for NLR, PLR, and SII according to CRP, renal function parameters (creatinine and GFR), as well as according to Hb, Ca, and P. NLR and PLR statistically significantly correlated with PTH, and NLR and SII significantly correlated with Fe. SIRI correlated statistically significantly only with P, while the other parameters had no statistical significance.

Table 1

Demographic and laboratory parameters of patients with chronic kidney disease

Parameters	Reference range	All patients (n = 117)	Group 1 (n = 60)	Group 2 (n = 57)	*p*-value
Age (years)		56.97 ± 10.16	56.22 ± 12.08	57 ± 12	0.770
Men/women		63 (53.84)/54 (46.15)	32 (53.33)/28 (46.66)	31 (54.38)/26 (45.61)	1.000
BMI (kg/m^2)		26.26 ± 3.29	25.33 ± 3.16	27.19 ± 3.43	**0.003**
Smokers		44 (37.6)	18 (30)	26 (45.61)	0.081
CRP (g/L)	0.00–4.00	2.78 ± 2.01	2.50 ± 1.54	3.07 ± 2.39	0.172
RBC (×10^{12} /L)	4.50–6.50	4.88 ± 3.64	4.85 ± 0.38	4.91 ± 5.23	0.934
Hb (g/L)	1.30–1.80	130.09 ± 18.82	139 ± 10.15	120.44 ± 20.97	**< 0.001**
WBC (×10^9/L)	4–11	6.62 ± 1.95	6.24 ± 1.64	7.01 ± 2.17	**0.030**
PLT (×10^3/μL)	160–370	240.44 ± 79.44	215.98 ± 51.21	266.18 ± 94.84	**< 0.001**
Urea (mmol/L)	2.50–7.50	14.80 ± 13.64	5.63 ± 1.42	16.08 ± 8.00	**< 0.001**
Creatinine (μmol/L)	62–115	184.15 ± 164.00	76.73 ± 13.58	296.95 ± 171.27	**< 0.001**
Acidum uricum (mmol/L)	220–547	397.04 ± 104.08	357.93 ± 96.95	438 ± 96	**< 0.001**
Cholesterol (mmol/L)					
preferably	< 5.2				
borderline risk	5.2–6.2	5.14 ± 1.11	5.31 ± 1.09	4.91 ± 1.14	0.084
risk	> 6.2				
Triglycerides (mmol/L)					
preferably	< 1.7				
borderline risk	1.7–2.3	1.82 ± 0.98	1.86 ± 1.14	1.76 ± 0.77	0.971
risk	> 2.3				
Iron (μmol/L)	11–31	14.33 ± 6.18	16.27 ± 6.10	12.30 ± 5.63	**< 0.001**
Calcium (mmol/L)	2.15–2.60	2.37 ± 0.15	2.40 ± 0.10	2.34 ± 0.18	**< 0.031**
Phosphorus (mmol/L)	0.78–1.65	1.16 ± 0.29	1.02 ± 0.148	1.29 ± 0.32	**< 0.001**
PTH (pmol/L)	1.30–9.30	16.03 ± 21.54	5.66 ± 2.33	26.15 ± 26.40	**< 0.001**
Vitamin D (nmol/L)					
severe deficiency	< 25				
mild deficiency	25–50	65.99 ± 27.64	69.00 ± 25.15	62.49 ± 29.64	0.202
insufficiency	50–75				
recommended	> 75				
Proteinuria (g/24 hr)	0.00–0.150	0.73 ± 0.97	0.32 ± 0.47	1.15 ± 1.13	**< 0.001**

BMI – body mass index; CRP – C-reactive protein; RBC – red blood cells; Hb – hemoglobin; WBC – white blood cells; PLT – platelets; PTH – parathyroid hormone. Values are given as mean ± standard deviation or numbers (percentages).

Table 2

Parameters of inflammation in patients with chronic kidney diseases

Parameter	*Group 1 (n = 60)	**Group 2 (n = 57)	*p*-value
Neutrophils (10^9/L)	3.66 ± 1.31	4.57 ± 1.74	**0.002**
Lymphocytes (10^9/L)	1.83 ± 0.55	1.63 ± 0.7	0.063
Monocytes (10^9/L)	0.40 ± 0.12	0.57 ± 0.72	0.078
NLR	2.13 ± 0.98	2.98 ± 1.03	< **0.001**
PLR	125.61 ± 36.30	175.43 ± 66.69	< **0.001**
SII	451.82 ± 204.43	802.50 ± 447.36	< **0.001**
SIRI	0.88 ± 0.65	1.68 ± 1.99	< **0.001**

NLR – neutrophil-to-lymphocyte ratio; PLR – platelet-to-lymphocyte ratio; SII – systemic immune-inflammation index; SIRI – system inflammation response index.
Note: *glomerular filtration rate (GFR) ≥ 60 mL/min/1.73 m^2; **GFR < 60 mL/min/1.73 m^2.

Table 3

Correlation of NLR, PLR, SII, and SIRI and significant parameters in patients with GFR < 60 mL/min/1.73 m^2

Parameter		BMI	CRP	CRE	GFR	Hb	Fe	Ca	P	PTH	Proteinuria
NLR	Pearson Correlation	-0.134	0.362	0.368	-0.271	-0.302	-0.269	-0.334	0.328	0.337	0.072
	Sig.	0.321	**0.006**	**0.005**	**0.041**	**0.022**	**0.043**	**0.011**	**0.013**	**0.010**	0.595
PLR	Pearson Correlation	-0.111	0.375	0.467	-0.346	-0.375	-0.221	-0.446	0.329	0.400	0.131
	Sig.	0.411	**0.004**	**0.000**	**0.008**	**0.004**	0.098	**0.001**	**0.013**	**0.002**	0.331
SII	Pearson Correlation	-0.045	0.493	0.500	-0.333	-0.335	-0.260	-0.262	0.447	0.150	-0.070
	Sig.	0.740	**0.000**	**0.000**	**0.011**	**0.011**	**0.050**	**0.049**	**0.000**	0.265	0.604
SIRI	Pearson Correlation	0.014	0.011	0.234	-0.219	-0.074	-0.016	-0.085	0.274	0.074	0.224
	Sig.	0.917	0.935	0.080	0.101	0.583	0.907	0.530	**0.039**	0.582	0.094

CRE – creatinine; Fe – iron; Ca – calcium; P – phosphorus; PTH – parathyroid hormone. For other abbreviations, see Tables 1 and 2.

Table 4

Receiver operating characteristic curve analysis of NLR, PLR, SII, and SIRI

Variable	AUC	Asymptotic sig.	Asymptotic 95% CI		Sensitivity	Specificity	Cut-off value
			lower bound	upper bound			
NLR	0.766	0.000	0.680	0.853	87.7	58.3	**2.06**
PLR	0.758	0.000	0.671	0.845	66.7	81.7	**151.75**
SII	0.818	0.000	0.741	0.895	86.0	68.3	**493.57**
SIRI	0.725	0.000	0.634	0.816	86.0	51.7	**0.739**

AUC – area under the receiver operating characteristic curve; CI – confidence interval; sig. – significance. For other abbreviations, see Table 2.

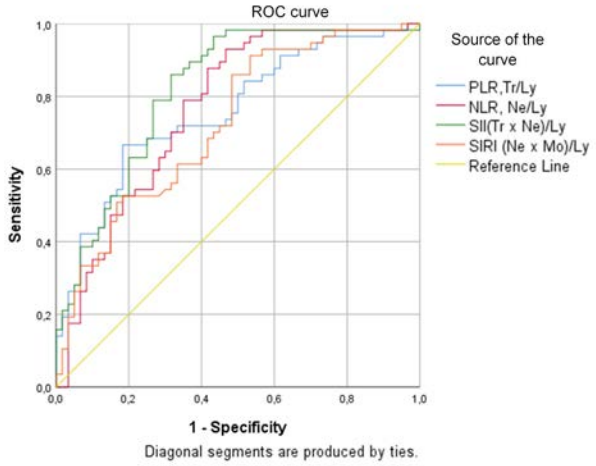

Fig. 1 – Receiver operating characteristic (ROC) curve of NLR, PLR, SIRI, SII for Group 2. For abbreviations, see Table 2.

The ROC analysis of the NLR, PLR, SIRI, and SII are shown in Table 4 and Figure 1. The area under the ROC curve (AUC) value of the NLR was 0.766, and the best cut-off value was 2.06 (p = 0.000). The AUC value of the PLR was 0.758, and the best cut-off value was 151.75 (p = 0.000). The AUC value of the SIRI was 0.725, and the best cut-off value was 0.739 (p = 0.000). The AUC value of the SII was 0.818, and the best cut-off value was 493.57 (p = 0.000).

Discussion

Inflammatory processes underlying the formation and rupture of atherosclerotic plaque, the formation of thrombus, and the subsequent development of cardiovascular complications have a proven influence on the development and progression of CKD [17–21]. Chronic inflammation causes in CKD are numerous (uremia, oxidative stress, infections, dyslipidemia, malnutrition, hypervolemia, dialysis) [22]. Ac-

cording to the results and investigations of previous studies, NLR, PLR, SII, and SIRI can be considered promising BI, progression, and predictors of mortality in patients with CKD [23]. Studies that included patients with different stages of CKD before the start of dialysis focused mainly on NLR and PLR markers. At the same time, the results for SII and SIRI were published somewhat less often and more often in patients on dialysis [23–26]. In our study comparing the group of patients with higher and lower GFR values, a statistically significant difference ($p < 0.001$) was obtained for the biomarkers NLR, PLR, SII, and SIRI, which had elevated values in the group with lower GFR .

Comparing NLR and PLR in patients with CKD, several authors found that PLR is a better BI than NLR and a superior predictor of mortality in patients with CKD [4, 24].

Our results indicated that both NLR and PLR statistically significantly correlated with parameters significant for CKD (CRP, creatinine, GFR, Hb, Ca, P, PTH), where the significance was more pronounced for PLR. However, unlike PLR, NLR statistically significantly correlated with Fe. Looking at SII in correlation with parameters significant in CKD, it is possible to see that it indicates statistical significance to CRP, creatinine, GFR, Hb, Ca, P, and Fe, indicating systemic inflammation in our patients. Similar results indicated that these biomarkers' association with inflammation was observed in studies focusing on patients with heart failure, autoimmune diseases, neurological disorders, etc. [10, 25, 27, 28].

In a study that included 85 patients with different stages of CKD (not on dialysis), Brito et al. [11] found increased values of NLR and PLR in patients with elevated high-sensitivity (hs)-CRP compared to the group of patients with hs-CRP within reference limits. In the same group of patients, a positive correlation between PLR and hs-CRP was observed. Similar results were observed by Li et al. [24], indicating a positive correlation of both NLR and PLR with hs-CRP in a group of 611 patients with CKD in the terminal stage of renal failure. The correlation between CRP and NLR, SII, and SIRI was observed in patients with acute lupus nephritis and heart failure [27, 28].

Analyzing the association of elevated values of BI with creatinine values, Toraman et al. [29] observed a positive correlation between NLR and creatinine in the studied group of 301 patients with CKD, indicating that an increase in inflammation leads to an increase in renal weakness and a negative correlation for NLR and PLR according to Hb and cholesterol.

We obtained similar results in our study. NLR, PLR, and SII correlated positively with creatinine and negatively with Hb. An increase in NLR, PLR, and SII is related to decreased Hb concentration and worsening of anemia in our patients. Moreover, these BI were related to an increase in creatinine and progression of renal failure.

Examining the association between SII and SIRI and mortality from all causes and cardiovascular mortality in 42,875 adult subjects, Xia et al. [30] observed that elevated values of SII and SIRI were significantly associated with lower levels of GFR.

In contrast to this study, in our patients, there was a correlation of NLR, PLR, and SII with elevated serum creatinine values and lower GFR values, but not for the SIRI.

The connection between inflammation and anemic syndrome in CKD has been confirmed in many studies, so the increased value of NLR and PLR in patients with CKD is described as having a negative correlation with the anemia parameters [31].

Considering that elevated NLR and PLR values were also registered in patients with resistance to erythropoietin, Valga et al. [32] indicate that NLR and PLR can be used as markers for monitoring resistance to erythropoietin. Our study also determined the association of NLR, PLR, SII, and anemia parameters.

Examining the relationship between markers of renal osteodystrophy and BI in patients with CKD, a positive correlation was observed in the relationship of PTH with NLR and PLR, which is independent of GFR and suggests that PTH could be a pro-inflammatory parameter independent of the degree of renal failure [29, 33, 34].

Furthermore, many authors have recently noticed a correlation between reduced vitamin D values and elevated BI values. One of the first studies related to the association between vitamin D concentration and the indices SII and SIRI, by Dziedzic et al. [35, 36] indicates a correlation between the concentration of vitamin D and the SII and SIRI as markers of inflammation significant in atherogenesis [35, 36].

The correlation of BI with parameters of renal osteodystrophy in our subjects with CKD verified the statistical significance of all four biomarkers with P (NLR, PLR, SII, and SIRI), three with Ca (NLR, PLR, and SII), and only two with PTH (NLR and PLR). None of the BI had statistical significance with vitamin D, probably because most subjects were on vitamin D therapy starting from stage III of CKD.

According to previous studies, there is no established standard threshold value of BI for an increased risk of an unfavorable outcome. Our results for NLR and PLR indicate that NLR sensitivity is 87.7% and specificity 58.3% and PLR sensitivity is 66.7% and specificity 81.7%. Our results are similar to those of Brito et al. [11], who, by examining BI in patients with CKD who are not on dialysis, indicated that the cut-off value for NLR (with 76.19% sensitivity and 48.44% specificity) was 1.98 and for PLR (with 85.71% sensitivity and 51.56% specificity) the cut-off value was 116.6. Similar results were also noted by Aneez et al. [13] in a study that included 85 subjects with proteinuria and CKD. The sensitivity of NLR according to the stages was as follows: for stage IIIa, it was 91.4%; for IIIb, it was 92.6%; for stage IV, it was 89.7%. The specificity of NLR was the following: for stage IIIa, 86.7%; for IIIb, 87.11%; for stage IV, 89.3%. The sensitivity of PLR was: for stage IIIa, 81.4%; for IIIb, 94.4%; for stage IV, 89.7%. The specificity of PLR was: for stage IIIa, 89.0%; for IIIb, 90.36%; for stage IV, 85.7%. The AUC for NLR was as follows: for IIIa, 0.976; for IIIb, 0.965; for stage IV, it was 0.962. The AUC for PLR was: for IIIa, 0.938; for IIIb, 0.981; for stage IV, 0.968.

The association of BI (NLR, PLR, SII, SIRI) and CKD is most often described in hemodialysis patients and less so in predialysis patients. 37. Tonyali et al. [37] were the first to publish a study on the predictive value of NLR on GFR in patients after partial or radical nephrectomy. NLR values were higher in patients with GFR < 60 mL/min/1.73 m^2 compared to the control group. The cut-off value for NLR was 3.18, with 39% sensitivity and 81% specificity.

By analyzing the statistical correlation in our group of patients, we observed that all four investigated BI (NLR, PLR, SII, and SIRI) in patients with CKD had statistical significance in Group 2 (p = 0.001); the most sensitive was NLR with 87.7%. The highest specificity was for PLR with 81.7%, with threshold values for PLR – 151.75, NLR – 2.06, SII – 493.57, and SIRI – 0.739. By analyzing SII and SIRI, we found that both markers had the same sensitivity of 86%. SII was more specific, with 68.3% vs. 51.7% for SIRI, with the cut-off values for SII being 493.57 and for SIRI 0.793.

In the available literature, several papers are on determining NLR and PLR BI in patients with CKD, while SII and SIRI are less common. According to recent studies, an essential place next to NLR and PLR is occupied by SII, which was more often examined in cardiac patients [25].

The first study related to the correlation of SII and CKD included 10,787 adult subjects from the United States of America when it was established that elevated SII values positively correlated with CKD and that the male population was more often affected [38]. In the future, the SIRI biomarker may be important in the assessment and prognosis of CKD patients, as it is associated with all-cause mortality and CVD mortality according to Wei et al. [39].

Additional tests and studies on a larger sample are needed to determine the exact role and importance of determining these BI further.

Conclusion

Our study's results are similar to those of other authors who indicated that biomarkers of inflammation (NLR, PLR, SII, and SIRI) were statistically significantly elevated in patients with moderate and severe impairment of renal function. In patients with moderate and severe impairment of renal function stages III, IV, and V who did not start dialysis treatment, statistically significant correlations were observed in relation to the NLR, PLR, and SII, for most of the examined parameters characteristic of chronic kidney disease, while SIRI had no statistical significance.

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Posterior single implants immediately loaded using one abutment at one time and temporary abutment in the posterior mandible without bone augmentation: a report on six-month outcomes data obtained from a prospective randomized controlled split-mouth clinical trial

Neposredno opterećenje posteriornih implantata u mandibuli primenom definitivnog i privremenog nosača: šestomesečni rezultati prospektivnog randomizovanog kontrolisanog *split-mouth* kliničkog istraživanja

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Abstract

Background/Aim. Given that frequent manipulation of the abutment during immediate loading can have a negative impact on the surrounding peri-implant hard and soft tissues, the concept "one abutment at one time" (OAO) has been introduced and documented in daily clinical practice. The aim of the study was to evaluate changes in peri-implant bone levels, clinical and radiographic parameters, and patient perspectives during the six-month follow-up period. **Methods.** The study was designed as a randomized controlled clinical trial. Patients with bilaterally healed sites in the posterior mandible received implants with a diameter of no less than 3.5 mm and a length of at least 8 mm. Based on randomization, patients were divided into a test group and a control group. Patients who were in the test group received implants that were immediately loaded with definitive abutments. In contrast, patients in the control group received implants where healing abutments were placed, followed by temporary abutments. Implants were immediately loaded with provisional restorations within the first seven days. They were delivered over the test group's definitive abutment and the control group's temporary abutment. Probing depth, bleeding on probing, clinical attachment level, plaque index, and keratinized tissue width were measured. Patient-Reported Outcome Measures and the Oral Health Impact Profile – 19 (OHIP-19) questionnaires were noted. **Results.** Out of 24 included patients, 22 completed the six-month follow-up. Peri-implant bone loss between study groups was comparable (mesial: t = -0.798, df = 21, p = 0.434; distal: t = 1.688, df = 21, p = 0.106), without statistical inter-group significance. OHIP-19 total scores significantly decreased after three months and remained similar six months after the implant placement in both groups without statistically relevant clinical inter-group changes. **Conclusion.** The OAO approach and provisional abutments showed comparable effectiveness regarding the immediate loading of posterior single implants.

Key words:
dental abutments; dental implants; mandible; methods; surveys and questionnaires.

Apstrakt

Uvod/Cilj. S obzirom na to da česta manipulacija nosačem (*abutment*-om) tokom neposrednog opterećenja može imati negativan uticaj na okolna tvrda i meka tkiva oko implantata, u svakodnevnu kliničku praksu uveden je i dokumentovan koncept „jedan po jedan nosač" (*one abutment at one time* – OAO). Cilj rada bio je da se tokom perioda praćenja od šest meseci procene promene u nivou periimplantne marginalne kosti, kliničkim i radiografskim parametrima i perspektivi pacijenata. **Metode**. Studija je osmišljena kao randomizovano kontrolisano kliničko ispitivanje. Pacijenti sa bilateralno zalečenim mestima u posteriornoj mandibuli dobili su implantate prečnika ne manjeg od 3,5 mm i dužine od najmanje 8 mm. Na osnovu randomizacije, pacijenti su podeljeni u testiranu grupu i kontrolnu grupu. Pacijenti u

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testiranoj grupi dobili su implantate neposredno opterećene odgovarajućim definitivnim nosačom, dok su pacijenti u kontrolnoj grupi dobili implantate na koje su postavljene kapice za zarastanje, a zatim privremeni nosači. Implantati su u prvih sedam dana neposredno opterećeni privremenim zubnim nadoknadama koje su postavljene preko definitivnih nosača u testiranoj grupi i preko privremenih nosača u kontrolnoj grupi. Praćeni klinički parametri bili su dubina sondiranja, krvarenje na provokaciju, nivo pripojnog epitela, plak indeks i širina keratinizovanog tkiva. Zabeleženi su rezultati upitnika mere ishoda i percepcije od strane pacijenata i upitnika o zadovoljstvu i uticaju na oralno zdravlje (*Oral Health Impact Profile 19* – OHIP-19). **Rezultati**. Od ukupno 24 uključena pacijenta, 22 su završila šestomesečno praćenje. Gubitak kosti oko implantata između ispitivanih grupa bio je uporediv (mezijalno: t = -0,798, df = 21, p = 0,434; distalno: t = 1,688, df = 21, p = 0,106), bez statističke međugrupne značajnosti. Ukupni rezultati OHIP-19 upitnika značajno su se smanjili posle tri meseca i ostali slični šest meseci nakon ugradnje implantata u obe grupe, bez statistički značajne kliničke promene među grupama. **Zaključak**. Pimena OAO koncepta opterećenja privremenim nosačom pokazala je uporedivu efikasnost u pogledu neposrednog opterećenja posteriornih pojedinačnih implantata.

Ključne reči:
zub, nosač proteze; zubi, implantati; mandibula; metode; ankete i upitnici.

Introduction

Over the past decade, implant dentistry has undergone a patient-centric transformation with the development of advanced techniques in implant placement and loading. The demand to accelerate the course of the treatment, in accordance with biological principles and patient's expectations and comfort, leads to the shift towards immediacy in implant dentistry. Evaluating outcomes in oral implantology by combining the placement and loading protocols is crucial. The literature data have shown that the combination of immediate loading of implants placed in healed posterior sites is associated with high survival and success rates [1].

Another important factor is the fact that the stability of both hard and soft tissues around the implant determines the lasting success of implant procedures. The integrity of the peri-implant supracrestal attachment depends on the good condition of the sulcular epithelium, the impermeability of the junctional epithelium, and the elasticity of the connective tissue [2]. The arrangement of peri-implant connective tissue fibers is parallel even without direct attachment to the implant/abutment and thus represents a vulnerable and weak point [3]. The repeated disruption of this implant-mucosal barrier due to repeated healing abutment/impression, copings/other prosthetic components manipulation, definitive abutment detachment, and reattachment could potentially lead to downward migration of the supracrestal attachment, thereby exacerbating marginal bone loss (BL) – MBL [4]. Furthermore, patients dislike frequent soft tissue manipulation due to the associated discomfort and prolonged chair time.

To reduce the oscillation of post-implantation changes, the existing literature presents several strategies aimed at consolidating peri-implant hard and soft tissues. There are various recommendations related to the characteristics of the abutment [5], morphology [6], and composition [7, 8], as well as measures to prevent microgap and micromovement [9, 10], refine implant-abutment platforms [11] and connections [12], and optimize the timing of definitive abutment activation [13, 14].

The one abutment at one time (OAO) approach, assisted by the implementation of the digital workflow, opposing conventional analog procedures, has exhibited comparable clinical outcomes in terms of MBL and soft tissue alterations [15]. Nevertheless, a scarcity of randomized, split-mouth clinical studies evaluating both clinical and radiological consequences, along with patient perspectives, interferes with a comprehensive assessment of these two methodologies within immediate loading protocols (ILPs).

Therefore, the aim of this study was to assess changes in peri-implant bone levels during the six-month follow-up period using the OAO concept, compared to provisional abutments of single immediately loaded posterior implant crowns, as well as to evaluate clinical parameters and changes in patient perspectives.

Methods

Study design

The study was designed as a split-mouth, double-blinded, randomized, patient-oriented, controlled clinical trial. All subjects were recruited at the Implant Center, Faculty of Dental Medicine, University of Belgrade, Serbia, from September 2021 to March 2022. The study was approved by the institutional Ethics Committee of the Faculty of Dental Medicine prior to study commencement (No. 36/16, from June 7, 2021), and each participant signed an informed consent. This research was conducted in full accordance with the Helsinki Declaration of 1975 and subsequent amendments. It was registered in ClinicalTrials.gov (NCT05668494) and carried out in accordance with the CONSORT statement.

Participants

From a pool of 38 adult patients evaluated for potential participation, 24 individuals met the inclusion criteria, which were as follows: patients showcasing bilaterally healed sites with at least one adjacent tooth, specifically in the posterior mandible premolar or molar region. Adequate restorative space was characterized by an interocclusal plane distance surpassing 20 mm in conjunction with a keratinized tissue band spanning a minimum of 2 mm (from crest to mucogingival junction). The osseous architecture was conducive to accommodating an implant with a diameter of no less than 3.5 mm and a length of at least 8 mm. Adult patients aged 20

and above, in sound physical and mental health conditions (classified as ASA I) were included. Patients were committed to adhering to the study protocol, as demonstrated by signing an informed consent.

Exclusion criteria involved patients with a history of head and neck radiotherapy or bisphosphonate medication, along with those presenting acute periodontitis, caries, or periapical radiolucency in the vicinity of adjacent teeth. Patients who had previous bone augmentation in the region of the posterior mandible were excluded from the study. Individuals with suboptimal oral hygiene, physical limitations impeding regular oral care, pregnant or breastfeeding females, smokers, and substance abusers were also excluded from the study.

Preoperative parameters

At the inclusion phase, a single calibrated examiner recorded clinical parameters, which were registered and stored in specially designed case record forms. Clinical periodontal parameters such as probing depth (PD), bleeding on probing (BOP), clinical attachment level (CAL), and plaque index (PI) were recorded for each patient around every tooth, and keratinized tissue width (KTW) was measured at the future implant site, as well as at sites mesially and distally to the planned implant position. Furthermore, Patient-Reported Outcome Measures (PROMs) and the Oral Health Impact Profile (OHIP-19) questionnaires were noted.

Prosthetic procedure

On the same day, a digital (3Shape, Trios system, Germany) abutment-level impression (GM™ Abutment Scanbody) was performed on the test group of implants; implant-level digital impression on the control group was also obtained (GM™ Universal Abutment Scanbody). Provisional restorations were made of polymethyl methacrylate (PMMA) (Telio® CAD, Ivoclar Vivadent, Schaan, Lichtenstein), following digital protocol. Within the first seven postoperative days, they were delivered over the definitive abutment for the test group and over the temporary abutment (GM™ temporary abutment) for the control group. The virtual design of provisionals was made in 3D design software (Exocad-Matera 2.3, Exocad, Darmstadt, Germany), and the milling of PMMA blocks was performed in a 5-axis milling machine (Zenotec Select, Wieland, Pforzheim, Germany). At the end of the twelve-week period, provisional restorations were removed. The control group received the selected GM™ abutment instead of the temporary abutment. Digital scanning was repeated in the same manner, using the abutment-level scanbodies, and the definitive restorations were delivered to the patients. Monolithic zirconia screw-retained single crowns (IPS emax ZirCad Prime Esthetic, Ivoclar, Liechtenstein) were placed over the GM™ abutments with an occlusal screw torque of 10 N-cm. The monolithic zirconia screw-retained restorations were fabricated following digital protocol.

Outcomes

The primary outcome variable was MBL changes, while the secondary outcome variables were clinical parameter changes, PROMs, and OHIP-19.

MBL was assessed as the difference between the marginal bone height and the implant shoulder. Intraoral radiographs using a customized radiographic holder and intraoral scans (3Shape, Trios system, Germany) were captured before the implant placement (T0), after temporary (T1) and definitive restoration delivery (T2), and on six-month follow-ups (T3). The mesial and distal vertical lines representing the distance between the marginal bone and implant shoulder were digitally measured and recalculated according to the radiographic distortion. Clinical parameters were assessed on T1 to T3.

OHIP-19 questionnaire was filled by the patients at T0 to T3. It consisted of 19 questions divided into seven domains: functional limitation, physical disability, physical pain, psychological disability, psychological discomfort, social disability, and handicap. Responses were delivered on a 5-point Likert-type scale ranging from 0 – "never" to 4 – "constantly". The score for each domain was calculated, and the sum of the seven domain scores represented the total OHIP-19 score. Additionally, PROMs were assessed using a questionnaire comprising five items: comfort, appearance, masticatory function, taste, and overall satisfaction. Patients were asked to answer these parameters by picking up one of the following answers: very unsatisfied, unsatisfied, fair, satisfied, and very satisfied [16].

Sample size

To detect an annual reduction of MBL, which is in accordance with the study of Canullo et al. [13], with a two-tailed 5% significance level and a power of 80%, a sample size of 24 patients was necessary, given an anticipated dropout rate of 10%.

Randomization was carried out using sealed envelopes with side allocation instructions, so both the surgeon and the patient were blinded for the group side during the implant placement. After implant placement, randomization envelope was opened, showing which side of the patient's mouth would be the test group and which the control group.

Statistical analysis

Descriptive statistics were calculated for patients' baseline and implant site characteristics, clinical parameters, patients' PROM levels of satisfaction, and OHIP-19 total scores. For the non-normality of the distribution of continuous variables, the Shapiro-Wilk normality test was used. The median of KTW at mesial and distal tooth, insertion torque, implant stability quotient (ISQ), clinical parameters [KTW, probing pocket depth (PPD), CAL, BOP, and PI] around the implant and for all teeth, BL and OHIP-19 total scores, together with 95% confidence interval (CI) for the median was calculated, based on exact Wilcoxon

sign rank test or sign test. Group differences were analyzed with the Wilcoxon signed rank test or Munzel-Brunner rank (MBR) test [17] for paired samples of numerical variables. An appropriate test was used after checking the symmetry of the distribution (for a single variable in CI of the procedure or the paired differences in the analysis of the group effect) with histogram and Miao-Gel-Gastwirth symmetry test [18]. Paired samples of ordinal variables (bone quality, PROMs domains' level of satisfaction) were compared with the MBR test.

Brunner-Langer repeated measures nonparametric analysis of variance (ANOVA) was used for testing the effects of group, time, and their interaction on KTW around the implant, on PROMs domains' level of satisfaction, and on OHIP for Edentulous Patients (OHIP-EDENT) total score. In the case of the significant effect, paired values were compared with the MBR test. Specifically, it examined the possibilities of improvement of PROMs domain level of satisfaction and decrease of OHIP-EDENT total scores during the time were tested for the control and test groups. In the case of the significant time effect on PROMs domains' level of satisfaction, without a significant effect of interaction, an increase in the level of satisfaction during time was further analyzed separately for the control group and the test group due to the ordinal nature of the satisfaction scale. In *post hoc* multiple comparisons, the false discovery rate was controlled using Benjamini-Hochberg's method [19].

The level of significance was set at 0.05. Statistical analysis was performed in statistical software R, version 4.3.0 (using R packages stats, lawstat [20], exactRankTests [21], DescTools [22], nparcomp [23], nparLD [24]).

Results

A total of 22 patients out of 24 included patients completed the six-month follow-up. Descriptive statistics of patients' characteristics are presented in Table 1.

Table 1

Descriptive statistics of patients' characteristics

Variable	Value
Patients age	40.45 ± 10.58
female	14 (63.6)
male	8 (36.4)
Smoker	
no	16 (72.7)
yes (light)	6 (27.3)
Systematic disease	
no	18 (81.8)
yes	4 (18.2)*
Disease	
no	17 (77.3)
yes, completed treatment	2 (9.1)
yes, ongoing treatment	3 (13.6)
Medication allergy	
no	19 (86.4)
yes	3 (13.6)
Parafunctional bruxism	
no	21 (95.5)
yes	1 (4.5)
Periodontal disease	
no	16 (72.7)
yes	6 (27.3)
Adjacent right mesial tooth	
natural dentition	20 (90.9)
crown or bridge	2 (9.1)
edentulous	0 (0)
Adjacent right distal tooth	
natural dentition	15 (68.2)
crown or bridge	2 (9.1)
edentulous	5 (22.7)
Adjacent left mesial tooth	
natural dentition	19 (86.4)
crown or bridge	3 (13.6)
edentulous	0 (0)
Adjacent left distal tooth	
natural dentition	12 (54.5)
crown or bridge	2 (9.1)
edentulous	8 (36.4)

Table 1 (continued)

Variable	Value
Right antagonist tooth	
natural dentition	12 (54.5)
crown or bridge	4 (18.2)
edentulous	6 (27.3)
Left antagonist tooth	
natural dentition	12 (54.5)
crown or bridge	2 (9.1)
edentulous	8 (36.4)
Time from right tooth extraction in years	13.77 ± 9.60
Time from left tooth extraction in years	12.91 ± 10.16
Reason for right tooth extraction	
endo complication	11 (50)
fracture	4 (18.2)
caries	3 (13.6)
anodontia	2 (9.1)
periodontitis	2 (9.1)
Reason for left tooth extraction	
endo complication	11 (50)
fracture	3 (13.6)
caries	4 (18.2)
anodontia	2 (9.1)
periodontitis	2 (9.1)

Data are described as numbers (percentages) for categorical variables and as mean ± standard deviation for continuous variables.
*** 3 patients with hypertension**

Table 2

Insertion torque (Ncm), implant stability quotient for implants in control temporary abutment and test (definitive) abutment groups

Variable	Mean ± SD	Median	Min–Max	95% CI for median
Insertion torque				
control group	45.23 ± 5.66	45	40–60	[42.5, 47.5]
test group	43.86 ± 2.64	45	40–50	[42.5, 45.0]
Mean ISQ *				
control group	78.52 ± 4.18	78.55	70–86	[76.5, 80.5]
test group	79.36 ± 5.42	80.65	61–86	[77.9, 81.8]

Ncm – Newton centimetre; SD – standard deviation; CI – confidence interval; *Mean of mesial, distal, vestibular, and oral implant stability quotient (ISQ).

The mean time elapsed from tooth extraction was 13.77 ± 9.60 months and 12.91 ± 10.16 months for the right and left jaw sides, respectively. In 50% of the cases, endodontic complication was the main reason for tooth loss. Descriptive statistics of insertion torque, mean ISQ, and 95% CI for median are presented in Table 2. There were no significant differences between the control and test groups concerning insertion torque values (V = 25.5, $p = 0.344$) and mean ISQ (V = 90, $p = 0.589$).

Changes in key clinical parameters in the control and test groups over time

Descriptive statistics of KTW, measured at mesial and distal tooth to the edentulous site at the beginning of the study, at future implant site, and at three months in control and test group, mean PPD, CAL, BOP, and PI, measured for all teeth at the beginning of the study and after six months, as well as mean PPD, CAL, BOP, and PI, measured at the implant site at six months in control and test group, together with 95% CI for median are presented in Table 3. In terms of the overall changes of clinical parameters over time (for all teeth), there were significant changes in values of PPD (V = 136.5, $p = 0.002$), CAL (V = 177.5, $p = 0.005$), and PI (V = 38, $p = 0.005$), but no significant changes of values of BOP ($t = -0.340$, df = 21, $p = 0.737$). Values of PPD and CAL were significantly lower at six months, while values of PI were slightly higher.

In terms of inter-group comparisons at six months, there were no significant differences in values of PPD (V = 129.5, $p = 0.369$), CAL (V = 138.5, $p = 0.436$), BOP (V = 50, $p = 0.773$), nor PI (V = 60.5, $p = 0.635$). Effect of time on KTW at implant site was statistically significant (F = 13.965, $df_1 = 1$, $df_2 = \infty$, $p < 0.001$), with no significant effect of group (F = 0,177, $df_1 = 1$, $df_2 = \infty$, $p < 0.674$), nor significant effect of interaction of time and group (F = 2.030, $df_1 = 1$, $df_2 = \infty$, $p < 0.154$). Values of KTW at the implant site, measured at three months, were significantly lower for both groups than values of KTW measured at the future implant site.

Table 3

Descriptive statistics of KTW around implant, PPD, CAL, BOP and PI by time and group

Variable	Group	Mean ± SD	Median	Min–Max	95% CI for median
KTW					
at mesial tooth					
beginning	control	3.82 ± 1.14	4	2–6	[3.0, 4.5]
	test	3.82 ± 1.10	3.5	2–6	[2.5, 4.5]
at distal tooth					
beginning	control	3.68 ± 1.43	3.5	1–7	[2.5, 4.5]
	test	3.64 ± 1.29	4	1–6	[2.5, 4.5]
at implant site					
beginning	control	3.77 ± 1.41	4	1–7	[3.0, 4.5]
3 months	control	3 ± 0.93	3	2–5	[2, 4]
beginning	test	3.68 ± 1.43	4	1–6	[2.5, 4.5]
3 months	test	3.18 ± 1.01	3	1–5	[2.5, 4]
Mean PPD					
all teeth					
beginning	both	2.49 ± 0.35	2.55	1.7–3.0	[2.35, 2.65]
6 months	both	2.35 ± 0.29	2.40	1.6–2.9	[2.2, 2.5]
around implant					
6 months	control	2.56 ± 0.46	2.70	1.5–3.3	[2.3, 2.8]
	test	2.50 ± 0.27	2.50	2.0–3.2	[2.35, 2.65]
Mean CAL					
all teeth					
beginning	both	2.59 ± 0.54	2.65	1.0–3.5	[2.40, 2.85]
6 months	both	2.45 ± 0.44	2.50	1.1–3.2	[2.35, 2.60]
around implant					
6 months	control	2.45 ± 0.77	2.50	0.8–3.8	[2.15, 2.80]
	test	2.41 ± 0.51	2.30	1.3–3.5	[2.25, 2.65]
BOP					
all teeth					
beginning	both	5.18 ± 3.91	4.0	2–18	[3, 5]
6 months	both	7.23 ± 9.70	3.5	1–45	[2,7]
around implant					
6 months	control	12 ± 18.48	1.0	0–71	[0, 16]
	test	11.36 ± 17.31	0	0–50	[0, 17]
PI					
all teeth					
beginning	both	7.95 ± 9.61	4	2–37	[3, 7]
6 months	both	17.77 ± 13.86	14	3–59	[7, 25]
around implant					
6 months	control	14.55 ± 21.01	8.5	0–83	[0, 18]
	test	14.45 ± 22.06	0	0–67	[0, 17]

KTW – keratinized tissue width; PPD – periodontal probing depth (in mm); CAL – clinical attachment level (in mm); BOP – bleeding on probing (in %); PI – plaque index (in %). For other abbreviations, see Table 2.

Table 4

Bone loss (BL) after six months of implant placement for control and test group

Group/Side	ΔBL			
	Mean ± SD	Median	Min–Max	95% CI for median
Control				
mesial	0.08 ± 0.15	0	0–0.57	[0, 0.10]
distal	0.10 ± 0.27	0	0–1.21	0
Test				
mesial	0.07 ± 0.14	0	0–0.48	[0, 0.10]
distal	0.12 ± 0.19	0	0–0.59	[0, 0.21]

ΔBL= BL at six months – BL at baseline. For other abbreviations, see Table 2.

Bone loss after six months

Descriptive statistics of BL six months after implant placement for the control and test group are presented in Table 4. BL was similar between the groups (mesial: t = -0.798, df = 21, p = 0.434; distal: t = 1.688, df = 21, p = 0.106).

Patients' self-reported measures (OHIP-19 and PROMs)

At the beginning of the study, patients of both groups were mostly very unsatisfied or unsatisfied concerning all of the PROMs domains, while they were mostly satisfied or

very satisfied at three months after the procedure and similarly at six months. These observations of the improvement in the level of satisfaction were confirmed with formal statistical testing (e.g., Brunner Munzel repeated measures ANOVA). Analyzing the comfort, there were significant effects of time (F = 123.813, df_1 = 1,67, $df_2 = \infty$, $p < 0.001$) and their interaction (F = 6.628, df_1 = 1,67, $df_2 = \infty$, $p < 0.003$). In multiple comparisons, there were significant improvements in PROMs comfort scores during the given time in the control group [before implantation (t_0) vs. three months after implantation (t_{3m}): t = 11, df = 21, $p < 0.001$, t_0 vs. six months after implantation (t_{6m}): t = 77.121, df = 21, $p < 0.001$] and also in the test group (t_0 vs. t_{3m} : t = 11, df = 21, $p < 0.001$, t_0 vs. t_{6m}: t = 11, df = 21, $p < 0.001$). In terms of the appearance of PROMs, there is a significant effect of time (F = 107.396, df_1 = 1,47, $df_2 = \infty$, $p < 0.001$). In multiple comparisons, there were significant improvements in PROMs appearance level of satisfaction during the given time in the control group (t_0 vs. t_{3m}: t = 9.562, df = 21, $p < 0.001$, t_0 vs. t_{6m}: t = 10.334, df = 21, $p < 0.001$) and also in the test group (t_0 vs. t_{3m}: t = 9.602, df = 21, $p < 0.001$, t_0 vs. t_{6m}: t = 9.949, df = 21, $p < 0.001$). Concerning the PROMs masticating function, there is a significant effect of time (F = 65.931, df_1 = 1,82, $df_2 = \infty$, $p < 0.001$). In multiple comparisons, there were significant improvements in PROMs masticating function level of satisfaction during the time in the control group (t_0 vs. t_{3m}: t = 11.599, df = 21, $p < 0.001$, t_0 vs. t_{6m}: t = 14.501, df = 21, $p < 0.001$) and also in the test group (t_0 vs. t_{3m}: t = 16.962, df = 21, $p < 0.001$, t_0 vs. t_{6m}: t = 18.110, df = 21, $p < 0.001$). In terms of PROMs taste, there is a significant effect of time (F = 65.909, df_1 = 1,71, $df_2 = \infty$, $p < 0.001$). In multiple comparisons, there were significant improvements in PROMs taste level of satisfaction during the given time in the control group (t_0 vs. t_{3m}: t = 20.029, df = 21, $p < 0.001$, t_0 vs. t_{6m}: t = 24.674, df = 21, $p < 0.001$) and also in the test group (t_0 vs. t_{3m}: t = 20.029, df = 21, $p < 0.001$, t_0 vs. t_{6m}: t = 35.611, df = 21, $p < 0.001$). Regarding the overall satisfaction of PROMs, there was a significant effect of time (F = 139.827, df_1 = 1,52, $df_2 = \infty$, $p < 0.001$). In multiple comparisons, there were significant improvements in PROMs overall satisfaction during the given time in the control group (t_0 vs. t_{3m}: t = 11, df = 21, $p < 0.001$, t_0 vs. t_{6m}: t = 11, df = 21, $p < 0.001$) and also in the test group (t_0 vs. t_{3m}: t = 11, df = 21, $p < 0.001$, t_0 vs. t_{6m}: t = 11, df = 21, $p < 0.001$). There was a significant improvement in the level of satisfaction after three months of the procedure in both groups, which was maintained after six months. Groups did not differ significantly in their level of satisfaction.

Descriptive statistics of the OHIP-EDENT total scores gathered at the beginning of the study, after three months, and after six months from the implant placement in each of the groups are presented in Table 5. At the beginning of the study, patients of both groups had much higher OHIP-EDENT total scores compared to three months after the implant placement and similarly at six months. These observations of a big decrease in OHIP-EDENT total scores are confirmed by formal statistical testing (e.g., Brunner Munzel repeated measures ANOVA). There is a significant effect of time (F = 179.050, df_1 = 1,80, $df_2 = \infty$, $p < 0.001$). In multiple comparisons, the time effect was further analyzed for both groups as a whole (taking the mean value of scores for each patient). There were significant decreases in OHIP-EDENT total scores after three months (t_0 vs. t_{3m}: t = -145.978, df = 21, $p < 0.001$) and after six months (t_0 vs. t_{6m}: t = -349.967, df = 21, $p < 0.001$), in comparison to the OHIP-19 total scores at the beginning of the study. OHIP-19 total scores significantly decreased after three months and remained similar six months after the implant placement in both groups.

Discussion

Implant dentistry has undergone a paradigm shift over the past decade, with a heightened focus on patient-centered care and innovative techniques for implant placement and loading. The demand for expedited treatment timelines that align with patient expectations and comfort has led to a reevaluation of adequate case selection in ensuring successful outcomes of an ILP. The present study tried to analyze the effects of the definitive abutment (OAO concept) within the ILPs of single posterior mandibular implants restored digitally, clinically, radiographically, and through patient-related outcomes. The results of this study revealed several key insights. The analysis of clinical parameters, such as KTW, PPD, CAL, BOP, and PI, indicated that both the control and the test group experienced significant changes over six months.

Table 5

Descriptive statistics of OHIP-EDENT total scores in control and test groups over time

OHIP-EDENT	Mean ± SD	Median	Min–Max	95% CI for median
Beginning				
control group	6.32 ± 5.37	4	2–26	[4, 8]
test group	5.77 ± 5.55	4	0–28	[4, 6]
3 months				
control group	0.18 ± 0.50	0	0–2	0
test group	0.18 ± 0.39	0	0–1	0
6 months				
control group	0.09 ± 0.29	0	0–1	0
test group	0.05 ± 0.21	0	0–1	0

OHIP-EDENT – Oral Health Impact Profile for Edentulous Patients; For other abbreviations, see Table 2.

Repetitive reconnection of the abutment in the post-implant placement period affects peri-implant soft and hard tissues [13, 25, 26, 27]. The OAO concept was introduced by Canullo et al. [13] to avoid and reduce aesthetical complications as a result of tissue changes. Numerous studies have demonstrated the applicability of this concept in clinical situations [13, 25, 26, 27], although strict patient selection seemed mandatory. The overall conclusion is that this concept is clinically relevant, with statistically slightly better results in terms of marginal bone levels, but without clinical relevance when compared to standardized prosthetic protocols [28]. The results of the present study are in line with the published data, emphasizing the fact that the application of one abutment one-time protocol can be introduced into daily clinical practice since it is very user-friendly equally from the patient's and clinician's point of view and is associated with the results comparable to conventional prosthetic procedures.

One of the primary outcome measures of the present study, MBL, was carefully evaluated in both groups. The analysis demonstrated no significant differences in BL between the control group (provisional abutments) and the test group (OAO approach) after six months. This suggests that both strategies can effectively manage bone stability in the short term. The results of this research are in line with the published data [28], with the additional comparative analysis of both ILPs within the same patient (split-mouth design). Moreover, careful case selection, adherence to proper surgical placement of implants, the use of Aqua® surface implants, and rigorous postoperative care protocols may contribute to the overall success of both approaches.

PROMs, as assessed through the OHIP-19, as well as the shortened version of the index for partially edentulous patients [16], revealed significant improvements in patients' levels of satisfaction, comfort, and overall well-being throughout the study. This suggests that not only do both implant strategies contribute to clinical success, but they also positively influence patients' quality of life and oral health-related quality of life. Slightly better indices have favored using a definitive abutment since this procedure is more comfortable for the patient.

The success rates of single monolithic zirconia screw-retained restorations achieved in this study are still the subject of the literature debate in terms of material selection and long-term follow-up but with promising results [29]. This study has verified that the implementation of digital prosthetic workflow and selection of ILP is a predictable, precise, effective, and dependable process [30]. Another study has demonstrated that patients reported greater satisfaction in terms of comfort when utilizing the intraoral scanner compared to the conventional polyether impression method [15].

It is important to acknowledge the limitations of this study. The six-month follow-up period offers insights into short-term outcomes, but a longer observation period is essential to ascertain the sustainability of these results over time. Additionally, the study focused on posterior single implants, and the applicability of these findings to different implant scenarios warrants further investigation.

Conclusion

The results of this study shed light on the comparable effectiveness of the OAO approach and provisional abutments in the context of immediate loading of posterior single implants. This study contributes to the existing body of knowledge in implant dentistry by offering insights into peri-implant tissue stability, patient satisfaction, and clinical outcomes within immediate loading protocols. Further research encompassing longer follow-up periods and diverse implant scenarios is necessary for further understanding of these implant strategies and their implications for long-term success.

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CASE REPORTS

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Masson's tumor of the thoracic spine: a rare cause of slowly progressive paraplegia

Masonov tumor torakalnog dela kičmenog stuba: redak uzrok sporoprogresivne paraplegije

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Abstract

Introduction. Intravascular papillary endothelial hyperplasia is an unusual reactive proliferation of endothelial cells around an organized thrombus, which occurs either in a dilated blood vessel, hematoma, or preexisting vascular lesion. These tumors rarely affect the central nervous system. Symptoms depend on the localization of the process itself. Localization in the central nervous system is limited to the intracranial space. Localization in the spinal canal is extremely rare, and only a few clinical cases have been described so far in the literature. **Case report.** A 67-year-old female patient was examined neurologically initially due to bilateral weakness of the lower extremities, accompanied by a feeling of pain and muscle tension, dominantly in the upper legs, more to the right. The complaints were present a year ago, and before that period, the patient was in a stable state of health. Due to a severe neurological deficit and the need for detailed exploration, the patient was hospitalized. A diagnosis was performed, which showed the localization of the pathological process in the thoracic 5–6 region of the spinal column. Decompression surgery was performed, and with the *ex tempore* findings metastasis was ruled out. Definitive pathohistological findings proved Masson's tumor. After the operation, the neurological weakness recovered. **Conclusion.** Masson's tumor, although rarely localized in spinal canal, is curable if it is correctly diagnosed and if an adequate therapeutic approach is applied. The initially presented symptoms may resemble numerous neurological or systemic diseases, which requires the clinician to be continuously aware of such rare pathological processes.

Key words:
diagnosis; differential diagnosis; histological techniques; magnetic resonance imaging; neoplasms, vascular tissue; neurosurgical procedures; spinal canal.

Apstrakt

Uvod. Intravaskularna papilarna endotelna hiperplazija je neobična reaktivna proliferacija endotelnih ćelija oko organizovanog tromba, koja se javlja ili u proširenom krvnom sudu, hematomu ili u postojećoj vaskularnoj leziji. Ovi tumori retko zahvataju centralni nervni sistem. Simptomi zavise od lokalizacije samog procesa. Lokalizacija u centralnom nervnom sistemu ograničena je na intrakranijalni prostor. Lokalizacija u kičmenom kanalu izuzetno je retka, a do sada je u literaturi opisano samo nekoliko kliničkih slučajeva. **Prikaz bolesnika.** Bolesnica stara 67 godina je prvobitno pregledana od strane neurologa zbog obostrane slabosti donjih ekstremiteta, praćene osećajem bola i napetosti mišića, dominantno u natkolenicama, više desno. Tegobe su se pojavile godinu dana ranije, pre toga je bila stabilnog zdravstvenog stanja. Zbog teškog neurološkog deficita i potrebe za detaljnim ispitivanjem, bolesnica je hospitalizovana. Urađena je dijagnostika kojom je utvrđen patološki proces u torakalnom 5–6 regionu kičmenog stuba. Urađena je operacija dekompresije, a *ex tempore* nalazom isključena je metastaza. Definitivnim patohistološkim nalazom utvrđen je Masonov tumor. Neurološka slabost se povukla posle operacije. **Zaključak.** Masonov tumor, iako je retko lokalizovan u kičmenom kanalu, izlečiv je ukoliko se pravilno dijagnostikuje i primeni odgovarajući terapijski pristup. Prvobitno ispoljeni simptomi mogu ličiti na mnogobrojne neurološke ili sistemske bolesti, zbog čega kliničari moraju neprekidno imati na umu tako retke patološke procese.

Ključne reči:
dijagnoza; dijagnoza, diferencijalna; histološke tehnike; magnetska rezonanca, snimanje; neoplazme, vaskularno tkivo; neurohirurške procedure; kičmeni kanal.

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Introduction

Intravascular papillary endothelial hyperplasia (Masson's vegetative hemangiothelioma) is a sporadic pathological condition affecting the nervous system [1]. It was discovered by Pierre Masson in 1923. However, a later neoplastic description by Henschen was criticized, who said that this type of lesion is reactive rather than neoplastic in nature. Although there are still controversies regarding the histological structure of Masson's tumor (MT), most authors believe it is a type of organized thrombus surrounded by a reactive proliferation of endothelial cells [2]. In 1975, Clearkin and Enzingen [3] described a lesion with unusual and marked thrombotic reorganization; MT was then renamed and became intravascular papillary endothelial hyperplasia (IPEH). Today, in the literature, these two names are equated [4]. MT can be classified into three types: the primary or pure, the secondary or mixed form, and the extravascular form. The primary/pure form, which is the most common form, typically occurs in a dilated vessel, most usually a vein rather than an artery, and arises in subcutaneous soft tissue. The secondary/mixed form presents in preexisting vascular abnormalities. Finally, the extravascular form, which is the least common form, occurs in hematomas. The symptoms given by this tumor depend on the localization, so they can often mislead clinicians, which speaks in favor of the importance of a multidisciplinary approach. Localization of Masson's hemangioma in the region of the central nervous system is rare and mostly limited to the intracranial space. Certainly, the localization of the process in the spinal canal (SC) is extremely unusual, so in the literature, there are only a few clinical cases of MT with this localization [5–7]. We presented the first diagnosed case of a patient with thoracic (TH) localization of IPEH, unique in Serbia.

Case report

A female patient, 67 years old, was initially examined for weakness of the lower extremities in September 2023. At that moment, the complaints lasted for about a year, and a month ago, she had severe pain and a feeling of tightness in the muscles of her legs, most intensely in her upper legs, which is why she used an orthopedic aid for movement (a walker). Due to the need for a detailed exploration, the patient was hospitalized in the Department of Neurology of the Clinical Hospital Center "Dr. Dragiša Mišović-Dedinje" in Belgrade, Serbia.

The patient's complaints started about a year before coming to the examination. Initially, the patient felt pain in the lower extremities, predominantly in the upper legs, which progressed over time in intensity and impact on gait. There was also a feeling of tightness in the leg muscles, more pronounced in the upper legs, more to the right. The weakness of the lower extremities progressed, so the patient started using a walker to move around. Two weeks before admission to the Hospital, she sprained her ankle. In her personal history, she is being treated for arterial hypertension, she had a tendon rupture of her right shoulder caused by trauma, and she is allergic to iodine.

The neurological examination was dominated by severe spastic paraparesis, more pronounced on the right. Her gait was spastic, paraparetic, possible only with the help of a lumbosacral (LS) spine walker. The sensation was reduced below the TH spine 6 level. The rest of the neurological exam was normal.

A magnetic resonance imaging (MRI) of the LS spine was performed on an outpatient basis (self-initiated): the hemangioma was central in the body of lumbar (L)5, occupying the entire height of the body. Smaller hemangiomas were found posteriorly in the body of L5 and TH10. Disc protrusions at the L3–L4 and L4–L5 levels were in close contact with the corresponding radix without direct radicular compromise. An electromioneurography examination of the lower extremities was performed: a moderately strong neurogenic lesion was found in all examined muscles except in the tibialis anterior bilaterally.

During the hospital treatment, the following diagnostics were performed: the MRI of the TH part of the spinal column and computed tomography (CT) of the chest. On the MRI (Siemens Aera 1.5 Tesla) of the TH part of the spinal column (Figures 1–3), paravertebral to the left, next to the bodies TH6 and TH5, a tumor mass that lied on the mentioned bodies could be seen with a wide base, with dimensions 18 × 38 mm, and cranio-caudal up to 50 mm.

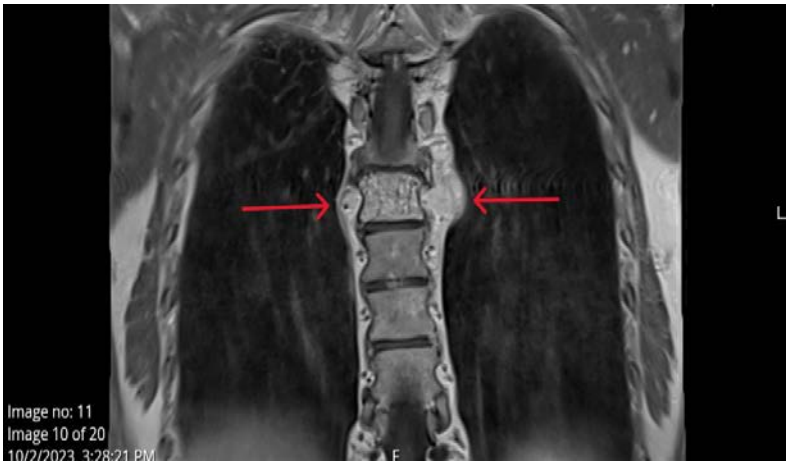

Fig. 1 – T2W coronal thoracic (TH): magnetic resonance imaging shows an affected TH 6 vertebra with a billateral tumor mass.

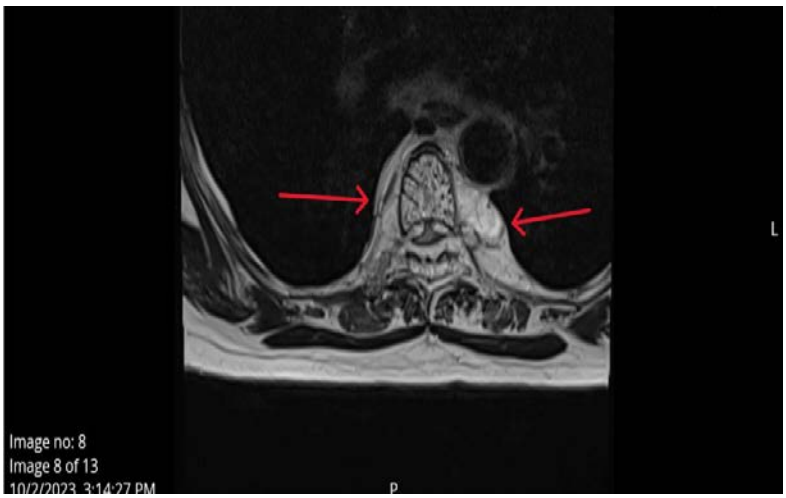

Fig. 2 – T2W axial: magnetic resonance imaging shows affected vertebra and paravertebral tumor mass more pronounced on the left.

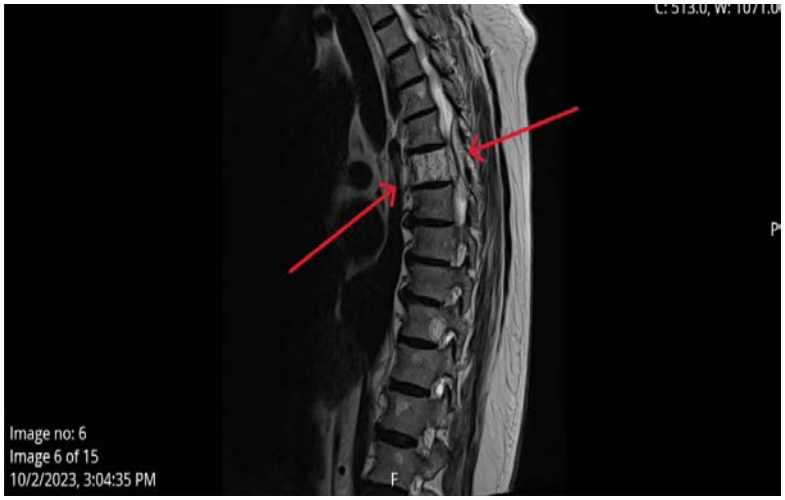

Fig. 3 – T2W sagittal: magnetic resonance imaging shows tumor mass in the spinal canal, complete obliteration.

There was an infiltration of the left underlying aspect of the body TH6, as well as the left pedicles, transversus, and lamina of this vertebra, without pathological fracture. There was an extension of the infiltrate into SC, which was critically stenosed at the level of the corpus TH6 with consequent compressive myelopathy in this segment. The differential diagnosis was thought to be in the direction of metastatic change of lung, breast, or pelvic region malignancy. The differential diagnosis, more precisely, was a suspected primary lung infiltrate with extension to underlying bodies or a package of pathological lymph nodes.

CT (Toshiba Aquilion One 320) finding of the chest was described as normal, without signs of consolidation and infiltration in the lung parenchyma. At the TH6 level of the vertebral body, the reduced bone structure could be seen with signs of destruction with edema and a soft tissue infiltrative component on both sides, more to the left. Differential diagnostic finding could have corresponded to a primary process on bone structures, but changes in the type of secondary deposits can not be ruled out either. Additional examination did not find any other pathological process.

After the orthopedist's examination, a decision was made on decompressive radical surgery. However, already during the extemporaneous biopsy, it was clear that it was not a metastatic tumor nor another malignant tumor, and tumor resection was performed while preserving the TH6 and TH5 vertebrae with stabilization on upper and lower levels (TH4 and TH7). MT was proven on the definitive pathohistological (PH) examination. The PH finding is given in the following lines. The microscopic analysis showed the following: several bone fragments with total dimensions of 3.5 × 3.5 × 1 cm; two soft tissue fragments with total dimensions of 2 × 1 × 0.5 cm. In the analyzed material, the following can be observed: fragments of trabecular bone, thin beds between which there is a vascular lesion represented by anastomosing blood vessels, thin wall, and dilated lumen with zones of papillary endothelial hyperplasia (Masson's tumor). The finding corresponds to an intraosseous hemangioma.

The postoperative course went smoothly. Within a month after the surgery, the neurological deficit gradually resolved.

Discussion

IPEH is a very rare benign pathological lesion that presents as a reactive vascular lesion with a tendency to expand and form a compressive mass. The mass was initially nominated as MT, but later, the name itself suffered numerous criticisms. Bearing in mind that the lesion itself is not malignant, considering the localization and the symptoms it causes, it still has the characteristics of a tumor. Therefore, the name was "softened" by introducing a term that actually corresponds to the PH structure of this pathological process. No preferential age of onset of IPEH has been shown, but data in the literature indicate there are differences in relation to gender. Namely, intracranial localization is significantly more common in women, with a ratio of 4:1 compared to men. Localization in SC, although extremely rare, affects men more often [4]. Usually, the articles published to date found IPEH in the skin and subcutaneous tissue of the head, neck, or extremities, and also in the oral mucosa, lips, sinus cavities, parotid gland, thyroid gland, lungs, superior *vena cava*, adrenal gland, renal vein, forearm, foot, intracranial. So far, only a few cases in SC have been published [8–12]. Our case report is the first to talk about the localization of the pathological process in the TH part of SC in Serbia and one of the few published worldwide. In our patient, it is most likely an MT due to a previous hemangioma of the vertebral body.

Considering the initially observed localization of the pathological process, as well as taking into account that the complaints occurred in a patient who was previously in a stable state of health, and bearing in mind the continuous progressive-deteriorative course of clinical symptoms, an expansive process with a possible primary cause was initially suspected with localization in the lung parenchyma or breast tissue, or in the pelvic organs. However, it turned out to be a mixed form of MT, formed on the site of a previous hemangioma in the body of the TH vertebra.

The pathogenesis of IPEH is still controversial. Different authors give different explanations for this lesion – from the fact that it is an overreaction to the normal process of thrombus reorganization to the fact that it is a benign proliferation of endothelial cells with secondary thrombosis and fibrin deposition [4]. Significantly, the diagnosis of IPEH itself is very demanding and challenging due to the presence of nonspecific MRI features. It is always necessary to consider cavernous/capillary hemangioma, Kaposi's sarcoma, endovascular papilloma and endothelioma, then schwannoma, neurofibromas, and arteriovenous malformations [12], and, in our case, secondary deposit as well. MRI and CT findings showed a pathologic substrate in our case, which was presented with tumor mass with infiltration of surrounded structures but without pathological fracture. In the essence of the pathological process, we found a hemangioma of the vertebral body, which transformed over time into a mixed form of MT.

The symptoms that appear depend on the localization of the pathological process. Namely, the localization of IEPH in SC can lead to pain in the chest and back, numbness of the lower extremities, paresis or paralysis, then bladder dysfunction as a result of compression of the spinal cord or cauda equina [1]. Treatment is considered in a situation where persistent pain and compressive symptoms occur. If diagnosed in time, complete surgical resection is possible, which is the most desirable treatment modality for the patient, with the most favorable outcome.

In our case, the patient underwent surgical treatment with complete resection, after which she recovered well, and further follow-up followed. Radiotherapy, as an adjuvant therapeutic modality, is considered when the lesion cannot be completely removed or when a recurrence occurs. To our knowledge, there is only one clinical case of IPEH localized in SC in which radiotherapy was applied after incomplete resection and with satisfactory success [13].

Conclusion

Although rarely localized in the spinal canal, MT is curable if diagnosed correctly and if an adequate therapeutic approach is applied. Better recognition of MT in the central nervous system can speed up and facilitate diagnosis. Certainly, magnetic resonance imaging is an irreplaceable method for preoperative orientation and operative plan. The significance lies in the domain of differential diagnostics because the initially presented symptoms may resemble numerous neurological or systemic diseases, which requires the clinician to be continuously aware of such rare pathological processes.

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CASE REPORT

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Thymic mucosa-associated lymphoid tissue lymphoma in a patient with Sjögren's syndrome with cutaneous vasculitis

Limfom ekstranodusnog limfnog tkiva u sastavu sluznica u timusu bolesnika sa Sjegrenovim sindromom i vaskulitisom kože

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Abstract

Introduction. The association between Sjögren's syndrome (SS) and the development of lymphoma is well known. The prevalence of lymphoma in patients with SS is 5%. Mucosa-associated lymphoid tissue (MALT) lymphoma is the most common lymphoma type in patients with SS. It is common for MALT lymphoma to develop in the stomach, while it is extremely rare in the thymus. **Case report.** We present a 61-year-old Caucasian male patient with primary SS, cutaneous vasculitis, and thymic MALT lymphoma. The patient had a two-year history of diffuse cutaneous palpable purpura on legs, intermittently enlarged left parotid gland, and dry mouth. The results of Schirmer's test were positive, labial salivary glands biopsy revealed a focus score ≥ 1, serology testing showed positive anti-Ro/SS-A and anti-La/SS-B antibodies, while skin biopsy findings revealed leukocytoclastic vasculitis. Diagnosis of primary SS with extraglandular cutaneous manifestations was confirmed. Cryoglobulinemia (Cg) and monoclonal gammopathy (MG) were detected, which increased the suspicion of hematological malignancy, and additional diagnostic procedures were performed. Computed tomography of the chest revealed an enlarged, multicystically altered anterior mediastinal mass. A thymectomy was performed through video-assisted thoracic surgery. Histological findings of the tissue confirmed the presence of tumor tissue consistent with MALT lymphoma in the thymus. Induction therapy with pulse doses of glucocorticoids was applied for three days, which was continued with medium doses of the drug. The doses were gradually reduced, and hydroxychloroquine was introduced. This has shown to be an effective therapy against features of SS. Postoperative local radiotherapy was performed. **Conclusion.** In SS patients with CV and in the presence of Cg and MG, attention should also be paid to the eventual development of MALT lymphoma, including the rare localization in the thymus.

Key words:
cryoglobulinemia; lymphoma, b-cell, marginal zone; sjogren's syndrome; thymus neoplasms; vasculitis, leucocytoclastic, cutaneous.

Apstrakt

Uvod. Veza između Sjegrenovog sindroma (SS) i nastanka limfoma je dobro poznata. Prevalencija limfoma kod bolesnika sa SS je 5%. Limfom ekstranodusnog limfnog tkiva u sastavu sluznica (*mucosa-associated lymphoid tissue* - MALT) je najčešći tip limfoma kod bolesnika sa SS. MALT limfom se često razvije u želucu dok je u timusu veoma redak. **Prikaz bolesnika.** Prikazujemo bolesnika starog 61 godinu, bele rase, sa primarnim SS i vaskulitisom kože (VK) i MALT limfomom u timusu. Bolesnik je imao difuznu palpabilnu purpuru na koži nogu prethodne dve godine, intermitentno uvećanje leve parotidne žlezde i osećaj suvih usta. Rezultati Širmerovog testa bili su pozitivni, histopatološki nalaz biopsije labijalnih pljuvačnih žlezda pokazao je fokus skor ≥ 1, serološkim analizama pokazana je pozitivnost na autoantitela anti-Ro/SS-A i anti-La/SS-B, a histopatološki nalaz biopsije kože potvdio je leukocitoklastični vaskulitis. Dijagnoza SS sa vanžlezdanom kutanom manifestacijom bila je potvrđena. Detektovane su krioglobulinemija (Kg) i monoklonska gamopatija (MG), što je povećalo sumnju na postojanje hematološke maligne bolesti i sprovedene su dodatne dijagnostičke procedure. Kompjuterizovanom tomografijom grudnog koša utvrđena je uvećana, multicistično izmenjena masa u prednjem medijastinumu. Urađena je timektomija primenom video-asistirane torakalne hiruške intervencije. Histopatološkim nalazom tkiva potvrđeno je prisustvo tumorskog tkiva koje je po karakteristikama odgovaralo MALT limfomu u timusu.

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Primenjena je indukciona terapija pulsnim dozama glukokortikoida tokom tri dana koja je nastavljena srednjim dozama leka. Doze su postepeno smanjivane i uveden je hidroksihlorokin. Ova terapija se pokazala kao delotvorna protiv manifestacija SS. Postoperativno je sprovedena lokalna radioterapija. **Zaključak.** Kod bolesnika sa SS i VK, i sa prisustvom Kg i MG, neophodno je obratiti pažnju na eventualni razvoj MALT limfoma, uključujući i veoma retku lokalizaciju u timusu.

Ključne reči:
krioglobulinemija; limfom, b-ćelijski, marginalna zona; sjegrenov sindrom; timus, neoplazme; koža, vaskulitis, leukocitoklastični.

Introduction

Mucosa-associated lymphoid tissue (MALT) lymphoma is an extranodal variant of marginal zone B-cell lymphoma, a distinct subtype of non-Hodgkin lymphoma (NHL). It is characterized by an indolent clinical course and typical histopathological features. It accounts for 7–8% of all B-cell lymphomas and usually occurs in the gastrointestinal tract in up to 50% of all cases. Other sites include the lungs, salivary glands (SG), ocular adnexa, skin, thyroid gland, breasts, and liver, whereas MALT in the thymus is very rare [1]. More than 40 years ago, Isaacson [2] defined the thymic MALT lymphoma (TML). Among patients with TML, approximately 80% of cases were Asian, the female-to-male ratio was 3 : 1, and the presence of cysts was often detected radiographically [3]. MALT lymphoma is frequently linked to chronic immune stimulation by bacterial or viral agents and autoimmune diseases, especially Sjögren's syndrome (SS), as well as with Hashimoto thyroiditis [4, 5]. We presented a rare case of a Caucasian male patient with primary SS, cutaneous vasculitis (CV), and TML.

Case report

A 61-year-old Caucasian man was admitted to our hospital for high suspicion of SS and CV. During the last two years, the patient suffered from recidivans diffuse purpura on the legs, myalgia of the legs, and painful knees. He had acute parotitis two times in the last six months. Furthermore, he had a dry mouth but not dry eyes. He complained of severe fatigue. Physical examination revealed an enlarged left parotid gland and cutaneous diffuse palpable purpura on the lower extremities. Blood tests showed: erythrocyte sedimentation rate 108 mm/h [reference range (RR) < 20 mm/h], C-reactive protein level 8.11 mg/L (RR 0–5 mg/L), leukocyte count 5.41 × 10^9/L (RR 4–11 × 10^9/L), lymphocyte count 1.01 × 10^9/L (RR 0.9–5.2 × 10^9/L), erythrocyte count 3.70 × 10^{12}/L (RR 3.8–5.8 × 10^{12}/L), hemoglobin level 110 g/L (RR 130–180 g/L), platelet count 259 × 10^9/L (RR 160–370 × 10^9/L). Results showed elevated serum IgA level [7.17 g/L, normal range (NR) < 3.5 g/L]. Serum monoclonal gammopathy (MG) was detected. Rheumatoid factor level was elevated (121 IU/mL, NR < 15 IU/mL), antinuclear antibodies were positive with a titer of 1/160, speckled pattern, anti-Ro/SS-A antibody was 220 IU/mL (NR 0–20 IU/mL) and anti- La/SS-B antibody was 60 IU/mL (NR 0–20 IU/mL), while the remaining antibodies to extractable nuclear antigens and anti-double stranded DNA were negative. Cryoglobulins were slightly positive. Extensive infectious disease workup, including hepatitis B, hepatitis C virus (HCV), Epstein-Barr virus, cytomegalovirus, parvovirus, herpes simplex, and human immunodeficiency virus, was negative. Labial salivary gland biopsy revealed lymphocytic infiltration focus (≥ 1) without neoplastic cells (Figure 1). The patients fulfilled the American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) classification criteria for SS [6]. Histological findings of skin biopsy revealed leukocytoclastic vasculitis. According to the above, the EULAR SS Disease Activity Index (ESSDAI) at SS diagnosis was 12. The patient was treated with pulse doses of methylprednisolone 500 mg daily for three days, followed by a dose of 0.5 mg/kg, with gradual dose tapering. Hydroxychloroquine was introduced at 400 mg daily. Smoking habit, the presence of cryoglobulinemia (Cg), MG, as well as CV increased suspicion of hematological malignancy and led to further diagnostic procedures – computed tomography (CT) of the whole body and bone marrow biopsy. CT of the chest revealed a retrosternal anterior mediastinal mass in the thymic region, dimensions 80 × 42 × 30 mm, with a multicystic appearance with part of fibrous changes (Figure 2). The video-assisted thoracic surgery (VATS) thymectomy was performed. Histological findings of the resected thymus tissue presented a lymphoma with heterogeneous diffuse infiltration of lymphocytes, centrocyte-like cells, monocyte-like cells, plasma, and blastoid cells (Figure 3A). Immunohistochemistry revealed positivity for CD20, CD79a, PAX5, and Bcl-2 in the neoplastic cells, while Bcl-6, CD10, cyclin D1, CD3, CD5, CD23, TdT, and CD1 were negative (Figure 3B). A low proliferative Ki-67 index was observed in up to 15% of neoplastic cells. Moreover, residual CK7 positive epithelioid thymic cells were detected in tumor tissue (Figure 3C).

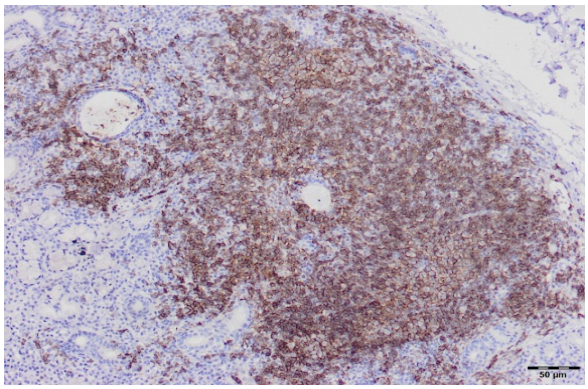


Fig. 1 – Histological findings of labial salivary glands biopsy. Immunohistochemical staining showing lobular infiltration with CD20 positive B-cells (HE, ×100).
HE – Hematoxylin and Eosin.

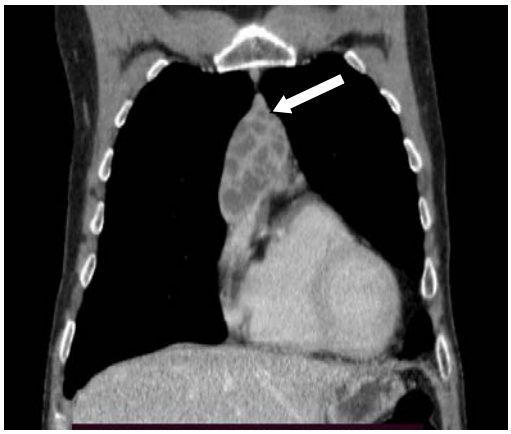
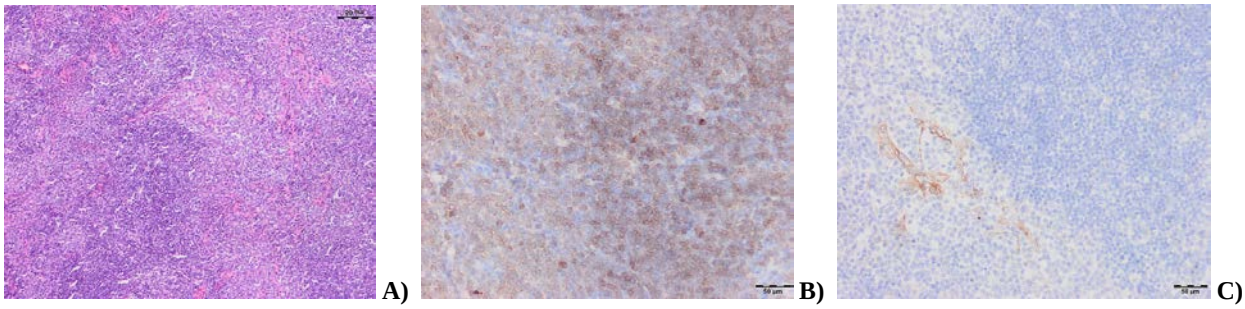


Fig. 2 – Computed tomography images reveal a large multicystic retrosternal mass in the anterior mediastinum (arrow).



A) B) C)

Fig. 3 – Histological findings of thymus tissue biopsy. Immunohistochemical staining showing: A) extranodal marginal zone B-cell lymphoma (HE, ×100); B) diffuse positivity for CD79a in neoplastic cells (HE, ×200); C) residual CK7 positive epithelioid cells (HE, ×200).
HE – Hematoxylin and Eosin.

Considering the results of all the tests that were done, a final diagnosis of extranodal non-Hodgkin lymphoma – MALT lymphoma in the thymus, was made. Bone marrow biopsy was without the presence of lymphoma. Post-tumor excision with the appliance of ^{18}F-fluoro-2-deoxy-D-glucose positron emission tomography/computed tomography (^{18}FDG-PET/CT) did not show probable postoperative residual lesions. Radiotherapy was performed with 24 Gy in eight courses. As an outpatient on follow-up after 20 months, the patient was without purpura, parotitis, or any systemic complaints. His therapy was prednisolone 5 mg every other day and 400 mg of hydroxychloroquine *per* day. Chest CT did not show any residue of tumor tissue.

Discussion

Primary SS is a systemic autoimmune disorder characterized by lymphocytic infiltration of exocrine glands, mainly the lacrimal and SG, leading to reduced secretory capacity. A subgroup of patients has experienced extraglandular manifestations that can involve any organ or system and worsen the disease outcome [7]. In addition to systemic manifestations, such as interstitial lung disease and cryoglobulinemic vasculitis, lymphoma is a systemic complication that leads to premature mortality in SS, adding an excess death rate of 9.4 cases *per* 1,000 patient at risk *per* year [8]. Among patients with autoimmune diseases, patients with SS are at a higher risk of developing lymphoma compared to patients with other autoimmune diseases (4-fold or 7-fold to patients with rheumatoid arthritis or systemic lupus erythematosus, respectively) and at an even higher risk compared to the general population (> 10-fold) [9]. The pathogenic role of B-cells in SS is a main feature of the disease, including the presence of circulating autoantibodies, alterations in peripheral B-cell subpopulations, B-cell predominance in advanced SG lesions, and increased risk of developing NHL B-cell MALT lymphoma in SS [10]. Lymphomagenesis in SS arises from persistent polyclonal B-cell activation due to chronic antigen stimulation in the SG, which can lead to oligoclonal/monoclonal B-cell expansion followed by the selection of premalignant B-cell clones and progress to lymphoma [11, 12]. Many predictors of lymphoma in SS have been investigated in the last several years. The most clinically relevant lymphoma predictor is persistent salivary gland enlargement (SGE), and then Cg and CV [13]. Although SGE is present in about one-third of SS patients, only a small number of them will develop lymphoma. An important study investigated and proposed a useful instrument for stratification of risk factors for lymphoproliferation in these groups of patients. Patients with at least two additional risk factors,

among four (Cg, low C4, leukopenia, anti-La positivity), are at high risk of lymphoma development [14]. Cg and CV occurred in 7–15% and 3–7% of patients with SS [14], respectively. According to the classification criteria for CV, a large majority of CV are HCV positive, while SS is the most common condition in HCV-unrelated CV [15]. A recent study of patients with SS and CV showed that first CV manifestations had begun in approximately 60% of patients within the first year from the SS onset, particularly with a high prevalence of CV, while other non-specific clinical manifestations such as arthralgia, myalgia, arthritis, or Raynaud's phenomena usually precede. One-third of associated NHL cases occurred during the first five years after CV onset, but diagnosis of NHL for the majority of patients was delayed, in some instances even for 20 years since CV diagnosis [16]. The presence of skin purpura has been reported as a key prognostic marker of lymphoma development in SS. Recent analysis confirmed a stronger association between patients with CV and lymphoma (hazard ratio = 7.47) than in those without CV (hazard ratio = 2.56) [17]. Approximately 10% of SS patients with MG had a hematologic malignancy, and MG increases the risk of developing either myeloma or, to a lesser extent, lymphoma [18]. There are attempts to determine and define subtype-specific predisposing factors for different subtypes of B-cell lymphoma in patients with SS. Several authors researched risk factors for developing MALT lymphoma in patients with SS. From a clinical point of view, the most important factors were Cg, higher focus score, and total ESSDAI at the time of SS diagnosis [19]. According to the World Health Organization classification, marginal zone B-cell lymphoma is classified into four subtypes: extranodal marginal zone lymphoma or MALT, primary cutaneous marginal zone lymphoma, nodal marginal zone lymphoma, and pediatric marginal zone lymphoma [20]. MALT lymphoma commonly arises from mucosal organs but rarely develops in tissue sites without mucosa: liver, thyroid, breast, skin, central nervous system. Therefore, marginal zone B-cell lymphoma in the thymus is MALT. TML is very rare, accounting for only 3% of anterior mediastinum mass [21]. Recent meta-analyses reported only up to 100 cases [22]. A recent article describing the association of SS and MALT lymphoma analyzed 142 patients in the last decade. All patients were diagnosed with MALT lymphoma simultaneously, as in our patient, or after SS. MALT lymphoma was found in the parotid glands (77.5%), lungs (14.8%), thymus in only eight patients (5.6%), submandibular glands (2.1 %), and other organs [23]. Currently, there are no standard treatment protocols or guidelines for the TML in SS. A therapeutic approach should be directed toward both diseases. Surgery, chemotherapy, radiotherapy, biological drugs – rituximab alone or in combination with chemotherapy has been commonly used [24, 25]. Some authors recommended active monitoring on the principle of "watch and wait" [26].

In our patient with clinical SGE and CV, laboratory findings of Cg and MG raised suspicion of lymphoma development. Hence, additional extensive diagnostic methods were performed, and TML was detected. Treatment with corticosteroids and hydroxychloroquine and maximal thymectomy surgery led to excellent results for both SS and TML disease treatments in our patient.

Conclusion

In patients with Sjögren's syndrome and cutaneous vasculitis, with the presence of monoclonal gammopathy and cryoglobulinemia in the early course of the disease, attention should also be paid to the rare localization of the MALT lymphoma in the thymus. According to available data, our case was only one in a few Caucasian patients, as well as the first Sjögren's syndrome-associated MALT lymphoma in the thymus in our country.

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Literatura

U radu literatura se citira kao superskript, a popisuje rednim brojevima pod kojima se citat pojavljuje u tekstu. Navode se svi autori, ali ako broj prelazi šest, navodi se prvih šest i *et al*. Svi podaci o citiranoj literaturi moraju biti tačni. Literatura se u celini citira na engleskom jeziku, a iza naslova se navodi jezik članka u zagradi. Ne prihvata se citiranje apstrakata, sekundarnih publikacija, usmenih saopštenja, neobjavljenih radova, službenih i poverljivih dokumenata. Radovi koji su prihvaćeni za štampu, ali još nisu objavljeni, navode se uz dodatak „u štampi“. Rukopisi koji su predati, ali još nisu prihvaćeni za štampu, u tekstu se citiraju kao „neobjavljeni podaci“ (u zagradi). Podaci sa interneta citiraju se uz navođenje datuma pristupa tim podacima.

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Tabele

Sve tabele pripremaju se sa proredom 1,5 na posebnom listu. Obeležavaju se arapskim brojevima, redosledom pojavljivanja, u levom uglu (**Tabela 1**), a svakoj se daje kratak naslov. Objašnjenja se daju u fus-noti, ne u zaglavlju. Svaka tabela mora da se pomene u tekstu. Ako se koriste tuđi podaci, obavezno ih navesti kao i svaki drugi podatak iz literature.

Ilustracije

Slikama se zovu svi oblici grafičkih priloga i predaju se kao dopunske datoteke u sistemu **aseestant**. Slova, brojevi i simboli treba da su jasni i ujednačeni, a dovoljne veličine da prilikom umanjivanja budu čitljivi. Slike treba da budu jasne i obeležene brojevima, onim redom kojim se navode u tekstu (**Sl. 1; Sl. 2** itd.). Ukoliko je slika već negde objavljena, obavezno citirati izvor.

Legende za ilustracije pisati na posebnom listu, koristeći arapske brojeve. Ukoliko se koriste simboli, strelice, brojevi ili slova za objašnjavanje pojedinog dela ilustracije, svaki pojedinačno treba objasniti u legendi. Za fotomikrografije navesti metod bojenja i podatak o uvećanju.

Skraćenice i akronimi

Skraćenice i akronimi u rukopisu treba da budu korišćeni na sledeći način: definisati skraćenice i akronime pri njihovom prvom pojavljivanju u tekstu i koristiti ih konzistentno kroz čitav tekst, tabele i slike; koristiti ih samo za termine koji se pominju više od tri puta u tekstu; da bi se olakšalo čitaocu, skraćenice i aktinome treba štedljivo koristiti.

Abecedni popis svih skraćenica i akronima sa objašnjenjima treba dostaviti pri predaji rukopisa.

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