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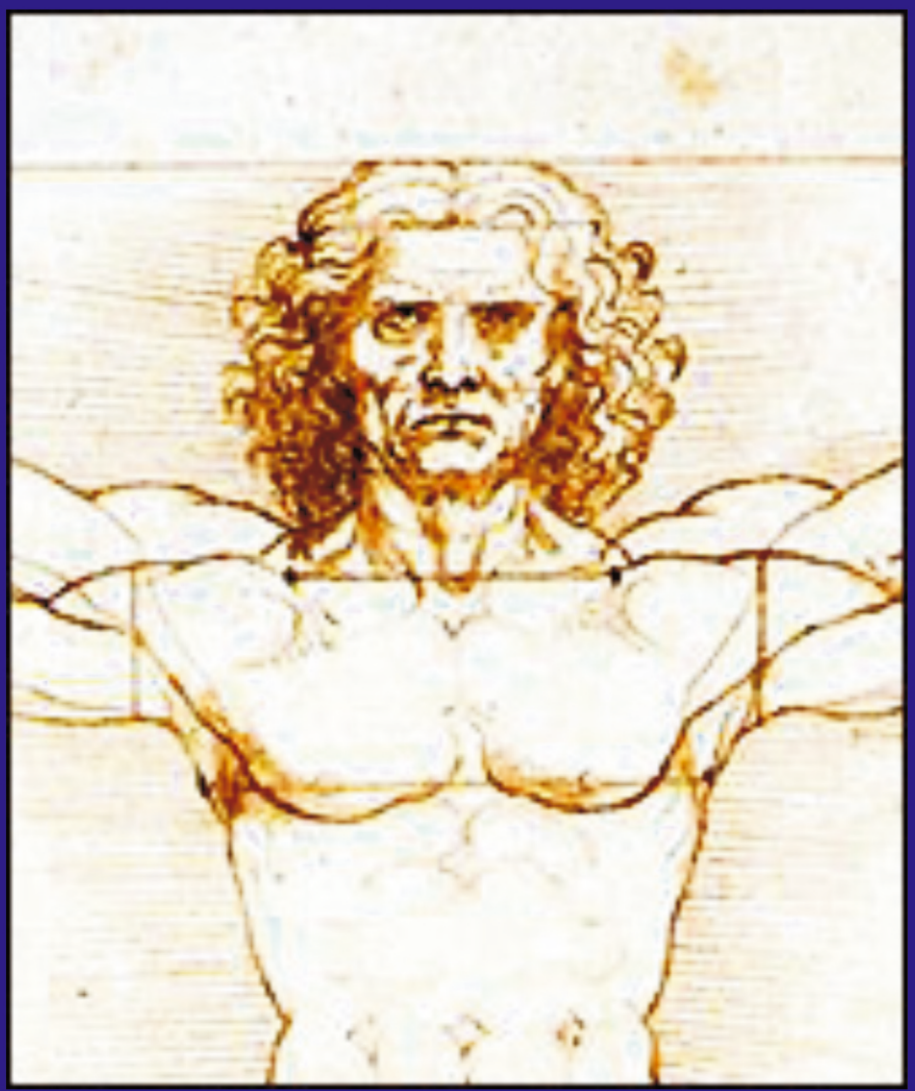

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ASSESSMENT OF THE RELATIONSHIP BETWEEN SERUM TWEAK LEVELS AND THE DEGREE OF VASCULAR INVOLVEMENT IN PATIENTS WITH STABLE ANGINA PECTORIS

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Abstract: **Introduction:** This study investigates the relationship between serum TWEAK levels and the degree of vascular involvement in patients with stable angina pectoris, offering insights beyond conventional cardiovascular risk factors.

Materials and Methods: This study involved 88 patients (33 women, 55 men) diagnosed with stable angina pectoris. Patients were classified based on angiographic findings. Comprehensive demographic and medical history data were collected, and morning blood samples were analyzed, focusing on TWEAK and IL-6 levels. To assess the severity of coronary artery lesions, a modified version of the Gensini scoring system was employed.

Results: Analyses revealed no significant correlation between TWEAK levels and the severity of coronary artery disease. Although some variations in biochemical markers were observed based on gender and diabetic status, these differences did not exhibit a statistically significant relationship with the degree of vascular involvement.

Conclusion: The findings indicate that serum TWEAK levels do not have a significant association with the severity of vascular involvement in patients with stable angina pectoris. These results highlight the limited efficacy of TWEAK as a sole biomarker in assessing the severity of coronary artery disease, emphasizing the complexity of its role.

Keywords: TWEAK, Angina pectoris, Gensini score.

INTRODUCTION

Cardiovascular diseases continue to rank among the leading causes of morbidity and mortality worldwide. Understanding the underlying causes of these diseases is of paramount importance for developing more effective treatment strategies and prevention. Beyond traditional risk factors such as metabolic syndrome, hypertension, and hyperlipidemia, recent years have witnessed significant findings pointing to the contributions of inflammation, particularly adipokines, to the pathogenesis of these diseases (1, 2). In this context, TWEAK (a member of the TNF superfamily) and its impact on cellular mechanisms have emerged as a novel research area in the understanding and management of cardiovascular diseases (3).

TWEAK, a member of the TNF superfamily and originally synthesized as a transmembrane protein with 249 amino acids, was first identified as a trigger of apoptosis (4). Subsequent studies have revealed that TWEAK is involved in various inflammatory and immune processes (5). Its action occurs through interaction with its only known receptor, fibroblast growth factor-inducible 14 (Fn14), which when activated can stimulate the release of cytokines such as TNF-α, IL-1, IL-6 and granulocyte colony-stimulating factor (G-CSF) and interferon-γ. In addition, TWEAK is involved in the secretion of other inflammatory mediators, including monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1 alpha (MIP-1α), intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) (6, 7). Macrophages and monocytes, particularly in inflamed tissues, have been identified as major producers of soluble TWEAK, highlighting the importance of the TWEAK/Fn14 signaling pathway in its contribution to the inflammatory environment and suggesting that its upregulation may influence the

pathogenesis of certain inflammatory and infectious diseases (8). The role of TWEAK is increasingly recognized in the progression of atherosclerosis, a disease characterized by inflammatory processes within the vessel wall (4). Studies of endothelial cells, macrophages, and other components of the vascular wall have increased our understanding of both chronic inflammation and acute responses to vascular injury. Furthermore, ongoing research on markers of oxidative stress, including IL-6, NO, and ROS, highlights their importance in the pathogenesis of cardiovascular disease and their potential as targets for therapeutic interventions (3, 4, 9). The association between TWEAK/Fn14 signaling and macrophage-mediated inflammation is a critical factor in the development of atherosclerotic plaques, with implications for the broader spectrum of cardiovascular disease.

This study aims to explore the relationship between TWEAK and cardiovascular diseases and potential correlations with oxidative stress markers like IL-6, NO, and ROS. By investigating the clinical and demographic relationships between TWEAK levels and these oxidative stress markers in patients with stable angina pectoris, this study seeks to contribute to the understanding of the use of biomarkers in the diagnosis, treatment, and management of coronary artery disease.

MATERIALS AND METHODS

Patients and Grouping

This study enrolled 88 patients (33 women, 55 men) diagnosed with stable angina pectoris who underwent coronary angiography. Patients were stratified into two groups based on the severity of observed coronary lesions: Group 1 included patients with non-significant lesions or a Gensini score below 50, while Group 2 consisted of patients with substantial vessel involvement or a Gensini score above 50. Detailed demographic profiles and comprehensive medical histories, encompassing gender, age, smoking status, diabetes, hypertension, dyslipidemia, coronary artery disease, family medical history, and current medication regimens, were meticulously documented through extensive survey forms. Height and weight data were also collected. Body Mass Index (BMI) was calculated using the formula: BMI = weight (kg) / height^2 (meters). The study was initiated following ethical approval from the local ethics committee (decision no: 2011-714190).

Inclusion and Exclusion Criteria

Inclusion Criteria: The inclusion criteria consisted of patients diagnosed with stable angina pectoris who had undergone coronary angiography.

Exclusion Criteria: The exclusion criteria encompassed patients with a history of myocardial infarction (MI) or prior coronary artery bypass surgery, individuals with significant valvular heart disease, active thyroid dysfunction, severe liver or kidney failure, systemic infectious or malignant diseases, and those who did not provide informed consent for participation in the study.

Laboratory Samples

Morning blood samples taken from the patients after 10-12 hours of fasting were used for biochemical analyses. Creatinine, glucose, lipid profile, hs-CRP, IL-6, ROS, and NO levels were measured. Serum Tweak and IL-6 levels were measured using East Biopharm brand ELISA kits with the sandwich ELISA method.

Angiography and Gensini Scoring

After comprehensive clinical and laboratory risk factor assessments, coronary angiography was performed on each patient by an experienced operator using the Judkins technique. Patients adhered to a twelve-hour fasting protocol for this procedure. Both the right and left coronary arteries were imaged in multiple angles in detail, to provide a comprehensive analysis. The assessment of angiographic images and scoring of lesions were independently carried out by two operators.

The coronary arteries were divided into 27 segments, and each segment was scored based on the degree of narrowing, ranging from 0.5 to 5.0. The Gensini score was calculated by multiplying these values, facilitating the classification of patients into two groups based on the prevalence of vascular lesions observed in angiography. In our Gensini scoring approach, the degree of angiographic stenosis was quantified as follows: 1 point for 0-25% narrowing, 2 points for 25-50% narrowing, 4 points for 50-75% narrowing, 8 points for 75-90% narrowing, 16 points for 90-99% narrowing, and 32 points for 100% occlusion. Specific coefficients were applied for each main coronary artery and the identified segments. These coefficients were as follows: 5 points for a left main coronary lesion, 2.5 points for proximal left anterior descending and left circumflex artery, 1.5 points for mid left anterior descending artery lesions, 1 point for the first diagonal branch, obtuse marginal branches, and the right coronary artery, and 0.5 points for the second diagonal and posterolateral branches of the left circumflex artery.

Statistical Analysis

In this study, statistical analyses were conducted using SPSS Version 20.0 (SPSS Inc., Chicago, IL,

USA) and R software (Version 4.1.3; R Foundation for Statistical Computing, Vienna, Austria). The Shapiro-Wilk test was employed to evaluate the normality of the distribution of continuous variables, which are presented as mean ± standard deviation (SD). Categorical variables are reported as frequencies and percentages (%). The Independent Sample T-test or Mann-Whitney U Test was utilized for comparisons of continuous variables between groups, while the Chi-Square test was applied for the comparison of categorical variables. To investigate the relationships between clinical and demographic characteristics, Pearson or Spearman correlation analyses were performed. Univariate logistic regression was carried out to identify potential predictors of outcomes, and Receiver Operating Characteristic (ROC) curve analysis was conducted to evaluate the diagnostic performance of the identified predictors. The level of statistical significance was established at $p < 0.05$.

RESULTS

In this comprehensive study, 88 participants were evaluated; of these, 34 were women (38.6%) and 54 were men (61.4%). The average age of the participants was 61.2 (age range 33-77). Half of the patients (50%) had a history of smoking. The most common comorbidities were hypertension (69.3%), Diabetes Mellitus (40.9%), and hyperlipidemia (34.1%). A family history of coronary artery disease was present in 38.6% of the participants.

In terms of vascular involvement, 21 patients (23.9%) had single-vessel involvement, 20 patients (22.7%) had two-vessel, 13 patients (14.8%) had three-vessel involvement, while 34 patients (38.6%) had no vessel involvement. The average Gensini score was calculated as 43.67 (range 0-268).

Patients were divided into two groups based on their Gensini score: Group 1 consisted of 50 patients (57%), and Group 2 consisted of 38 patients (43%). The demographic, comorbid disease, and laboratory findings of these groups are presented in Table 1.

In the analysis of IL-6 levels according to gender, the average was 119 ± 251 ng/L in women and 151±276 ng/L in men; this difference was not statistically significant ($p = 0.61$). Statistically significant differences were observed in ROS and NO levels when analyzed by gender (ROS $p = 0.054$, NO $p = 0.024$). When comparing Tweak values by gender, they were found to be 336.24 ± 137.09 ng/L in men and 323.42 ± 94.13 ng/L in women; this difference was also not statistically significant ($p = 0.988$). Comparisons related to gender are provided in Table 2.

Table 1. *Demographic and biochemical parameters of the patients*

	Total	Group 1 (n = 58)	Group 2 (n = 30)	P-value
Age (years)	61.2 ± 9.8	59.8 ± 10.1	63.7 ± 8.8	0.077
Sex (F/M)	34/54	30/28	4/26	< 0.001
Family History of CAD (%)	38.6	43.1	30.0	0.23
Smoking History (%)	50	55.2	40.2	< 0.001
DM (%)	40.9	43.1	36.7	0.56
HT (%)	69.3	70.7	66.7	0.70
Hyperlipidemia (%)	34.1	31.0	40.0	0.40
BMI (kg/m²)	29.19 ± 4.97	29.5 ± 5.6	28.6 ± 3.3	0.42
Glucose (mg/dl)	122 ± 47	123 ± 49	121 ± 45	0.865
HDL (mg/dl)	45 ± 12	45 ± 14	44 ± 10	0.645
hsCRP (mg/dl)	2.5 ± 1.9	2.43 ± 1.90	2.71 ± 2.1	0.52
Creatinine (mg/dl)	0.95 ± 0.15	0.95 ± 0.14	0.96 ± 0.19	0.88
LDL (mg/dl)	124 ± 37	121 ± 33	130 ± 43	0.31
Leukocytes (10^3/mm³)	6.9 ± 1.3	6.82 ± 1.26	7.30 ± 1.5	0.118
IL6 (ng/L)	139 ± 265	144 ± 282	129 ± 238	0.814
Tweak (ng/L)	354.27 ± 161.82	307,14 ± 95,50	354,27 ± 161,82	0.346
NO (ng/L)	3689 ± 1910	3861 ± 2043	3357 ± 1602	0.243
ROS (ng/L)	0.38 ± 0.2	0.37 ± 0.23	0.40 ± 0.3	0.654

Note: CAD - Coronary Artery Disease, DM - Diabetes Mellitus, HT - Hypertension, HDL - High-Density Lipoprotein, hsCRP - High-Sensitivity C-Reactive Protein, LDL - Low-Density Lipoprotein, IL-6 - Interleukin-6, NO - Nitric Oxide, ROS - Reactive Oxygen Species, BMI - Body Mass Index.

Table 2. *Comparison of Tweak, ROS, NO, and IL-6 levels by gender*

Indicator	Female	Male	P-value
IL-6 (ng/L)	119 ± 251	151 ± 276	0.61
ROS (ng/L)	0.4307 ± 0.026	0.3526 ± 0.24	0.054
NO (ng/L)	4220 ± 2038	3354 ± 1762	0.024
Tweak (ng/L)	323.42 ± 94.13	336.24 ± 137.09	0.988

Note: TWEAK - Tumor Necrosis Factor-like Weak Inducer of Apoptosis, ROS - Reactive Oxygen Species, NO - Nitric Oxide, IL-6 - Interleukin-6.

Table 3. *Comparison of Tweak, ROS, NO, and IL-6 levels by Diabetes Status*

Indicator	Non-Diabetic Mean ± SD	Diabetic Mean ± SD	P-value
Tweak (ng/L)	334.90 ± 120.11	329.27 ± 129.60	0.992
ROS (ng/L)	0.33 ± 0.20	0.45 ± 0.30	0.035
NO (ng/L)	3500 ± 600	3400 ± 650	0.43
IL-6 (ng/L)	150 ± 75	160 ± 80	0.52

Note: TWEAK - Tumor Necrosis Factor-like Weak Inducer of Apoptosis, ROS - Reactive Oxygen Species, NO - Nitric Oxide, IL-6 - Interleukin-6.

Table 4. *Univariate analyses of variables associated with significant vessel involvement or Gensini score exceeding 50*

Indicator	Odds Ratio	95% CI (Lower, Upper)	P-value
Age	0.991	0.946, 1.037	0.69
Sex	1.30	0.45, 3.79	0.63
Family History of CAD	0.42	0.16, 1.09	0.07
Smoking History	0.77	0.31, 1.87	0.56
DM	0.81	0.31, 2.11	0.67
HT	0.80	0.33, 1.96	0.62
Hyperlipidemia	0.62	0.25, 1.54	0.30
BMI	1.13	0.98, 1.30	0.08
Glucose	1.00	0.99, 1.01	0.85
HDL	1.03	0.98, 1.07	0.23
hsCRP	0.97	0.78, 1.20	0.76
Creatinine	0.41	0.05, 3.67	0.42
LDL	1.00	0.99, 1.01	0.73
Leukocytes	1.13	0.85, 1.52	0.40
IL6	1.00	0.998, 1.002	0.70
TWEAK	1.00	0.998, 1.004	0.45
NO	1.00	0.999, 1.000	0.33
ROS	1.45	0.28, 7.52	0.66

Note: CAD - Coronary Artery Disease, DM - Diabetes Mellitus, HT - Hypertension, HDL - High-Density Lipoprotein, hsCRP - High-Sensitivity C-Reactive Protein, LDL - Low-Density Lipoprotein, IL-6 - Interleukin-6, NO - Nitric Oxide, ROS - Reactive Oxygen Species, BMI - Body Mass Index.

When Tweak values were examined according to the presence of diabetes, they were 334.90 ± 120.11 ng/L in non-diabetic patients and 329.27 ± 129.60 ng/L in diabetic patients; this difference was not statistically significant (p = 0.992; Table 3). Finally, when evaluating the relationship between the number of affected vessels and TWEAK, ROS, NO, and IL-6 levels, no significant correlation was found among these parameters (p-values respectively 0.75, 0.27, 0.43, 0.52).

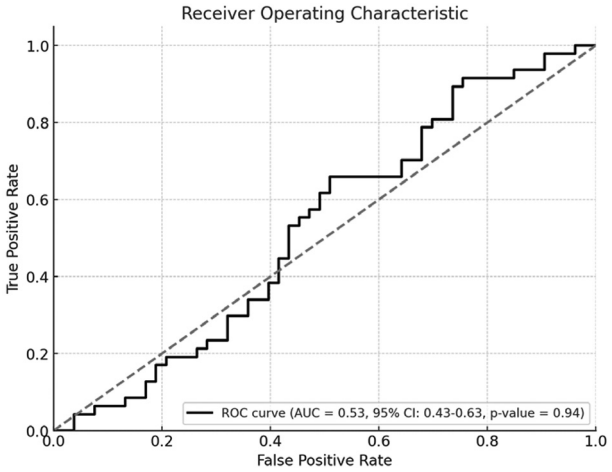

Figure 1. *ROC Curve Analysis of TWEAK for Predicting Significant Vessel Involvement or Gensini Score Above 50*

Univariate logistic regression analyses were conducted to evaluate potential predictors of inclusion in Group 2, considering factors such as age, gender, family history of coronary artery disease (CAD), smoking status, DM, HT, hyperlipidemia, BMI, glucose levels, HDL, hsCRP, creatinine, LDL, leukocytes, IL6, TWEAK, NO, ROS. The results showed that odds ratios and 95% confidence intervals for these variables were generally close to one, with all p-values being non-significant ($p > 0.05$), indicating that none of the variables studied were strong predictors of classification into Group 2, (Table 4). Additionally, the ROC curve analysis for TWEAK levels in Group 2 revealed an AUC of approximately 0.53(95%CI: 0.43-0.63). This underlines that TWEAK levels do not reliably discriminate between the presence and absence of the condition under investigation (Figure 1). These findings provide significant insights into the biological markers associated with coronary artery disease and establish a foundation for further research in this area.

DISCUSSION

Cardiovascular diseases hold a leading position among global causes of death, and numerous factors contributing to their etiology have been identified. Alongside common risk factors such as metabolic syndrome, the roles of inflammation and adipokines, particularly TWEAK, in the atherosclerotic process are drawing increasing interest (10). TWEAK, a member of the serine protease inhibitor family associated with adipose tissue dysfunction and insulin resistance, is currently under study for its connection to cardiovascular diseases (11). In this investigation, we examined the relationship between TWEAK levels and the degree of vascular involvement in patients with stable angina pectoris. Our findings indicate no significant correlation between the number or severity of vascular involvements and TWEAK levels. Furthermore, no significant differences were observed in the levels of inflammatory and oxidative stress indicators such as IL-6, NO, and ROS, between genders or in the presence of diabetes. These results suggest that TWEAK alone is not a sensitive marker for the presence or severity of coronary artery disease.

Recent studies have provided valuable insights into the relationship between TWEAK levels and cardiovascular diseases, aligning with our findings of no significant correlation between TWEAK levels and disease severity. In peripheral arterial disease, soluble TWEAK and the CD163/TWEAK ratio have been identified as predictors of long-term cardiovascular mortality (12). This highlights the prognostic importance of TWEAK in specific conditions rather than its direct correlation with disease progression. Similarly, while soluble TWEAK levels are linked to the presence of carotid atherosclerotic plaques in asymptomatic individuals, this does not necessarily indicate a progression towards severe cardiovascular conditions (13).

In patients with chronic kidney disease, soluble TWEAK levels are associated with major adverse cardiovascular events (14). This finding suggests a role for TWEAK in cardiovascular health that may not directly reflect disease severity. The observation of elevated soluble TWEAK levels in patients with ST-elevation myocardial infarction, which are related to adverse short-term outcomes, supports the idea of TWEAK being involved in specific cardiovascular events rather than indicating overall disease severity (15). Additionally, a study demonstrating that soluble TWEAK predicts hemodynamic impairment and functional capacity in pulmonary arterial hypertension underlines TWEAK's broader role in cardiovascular outcome prediction, independent of disease severity (16). These findings from diverse patient populations and conditions emphasize TWEAK's potential as a diagnostic or prognostic marker in cardiovascular diseases, rather than a direct indicator of disease severity. The evidence positions TWEAK as a key molecule in the complex interplay of inflammatory and cardiovascular processes, necessitating further investigation to fully understand its role in cardiovascular pathology.

Hypotheses regarding the mechanisms underlying the lack of association between TWEAK (tumor necrosis factor-like weak inducer of apoptosis) levels and the degree of vascular involvement suggest that this molecule's effects on the cardiovascular system may be modulated by various cofactors. Specifically, TWEAK's influence on the osteogenic transition and calcification of vascular smooth muscle cells (VSMCs) has been observed under both non-calcific and pro-calcific conditions. TWEAK increases the expression of markers

such as bmp2 mRNA (bone morphogenetic protein 2 messenger RNA), enhancing TNAP (tissue-nonspecific alkaline phosphatase) activity, thereby promoting calcification. However, it does not significantly alter MMP2 (matrix metalloproteinase 2) mRNA expression or activity, while it does increase both the expression and activity of MMP9 (matrix metalloproteinase 9). These findings indicate that TWEAK's effects on the vascular system are modulated by a range of factors including cell type, microenvironment, and disease stage, thus explaining the lack of a direct association between TWEAK levels and the degree of vascular involvement (6, 7, 17–19). Additionally, our demographic and biochemical analyses highlight that factors such as gender and smoking status are not associated with serum TWEAK levels. Research indicates that smoking affects the human serum metabolite profile, and these effects may be gender-specific. These studies show that the effects of smoking and cessation on serum metabolites are reversible, potentially reducing cardiovascular disease risks. However, these studies do not directly address serum TWEAK levels. Thus, specific relationships between serum TWEAK levels, gender, and smoking require further research for clarification (20).

There are several limitations to this study that warrant consideration. The sample size and demographic diversity of our study are limited, which may restrict the generalizability of the findings. Additionally, the design of the study precludes establishing causality, and the results are interpretative only in a correlative manner. The sensitivity and specificity of the methods used to measure TWEAK levels could also impact the outcomes. Moreover, the study did not evaluate other potential biomarkers and risk factors alongside TWEAK levels, limiting the scope of our findings.

CONCLUSION

This study marks a significant step in evaluating the potential roles of TWEAK and other biomarkers in the diagnosis and management of cardiovascular diseases. Our findings indicate that TWEAK levels do not show a significant relationship with the degree of vascular involvement in patients with stable angina pectoris nor are they associated with inflammatory and oxidative stress markers in the context of gender or the presence of diabetes. These results can guide further understanding of the potential use of TWEAK and other biomarkers in cardiovascular disease diagnosis and management. They also underscore the importance of tailoring the clinical use of biomarkers to specific patient populations and clinical scenarios. Comprehensive and multifaceted research in this field will advance the integration of these markers into clinical practice and refine strategies for the management of cardiovascular diseases.

Authorship: Conception and design of the study: Savaş Öztürk; Acquisition of data: Vehbi Şirikçi; Analysis and interpretation of data: Hüseyin Avni Fındıklı; Drafting the article: Vehbi Şirikçi; Critical revising: Vehbi Şirikçi; Final approval: Hüseyin Avni Fındıklı.

Ethical Standards:The study was conducted according to the Declaration of Helsinki and approved by the Research Ethics Committee at the Istanbul University Faculty of Medicine (decision no: 2011-714190).

Informed Consent: Written informed consent was obtained from all the participants prior to inclusion in the study online.

Conflict of Interests: The authors declare no conflicts of interest related to this article.

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Note: Artificial intelligence was not utilized as a tool in this study.

Sažetak

PROCENA ODNOSA IZMEĐU NIVOA TWEAK-a U SERUMU I STEPENA VASKULARNE UKLJUČENOSTI KOD PACIJENATA SA STABILNOM ANGINOM PEKTORIS

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Uvod: Ova studija istražuje odnos između serumskih nivoa TWEAK i stepena vaskularne uključenosti kod pacijenata sa stabilnom anginom pektorisom, pružajući uvide izvan konvencionalnih faktora rizika za kardiovaskularne bolesti.

Materijali i metode: U ovoj studiji učestvovalo je 88 pacijenata (33 žene, 55 muškaraca) dijagnostikovanih sa stabilnom anginom pektorisom. Pacijenti su klasifikovani na osnovu angiografskih nalaza. Prikupljeni su sveobuhvatni demografski i medicinski podaci, a

analizirani su uzorci jutarnje krvi, fokusirajući se na nivoe TWEAK i IL-6. Za procenu težine lezija koronarnih arterija korišćena je modifikovana verzija Gensini skale.

Rezultati: Analize nisu pokazale značajnu korelaciju između nivoa TWEAK i težine koronarne bolesti. Iako su primećene neke varijacije u biološkim markerima na osnovu pola i statusa dijabetesa, ove razlike nisu pokazale statistički značajnu povezanost sa stepenom vaskularne uključenosti.

Zaključak: Rezultati ukazuju da nivoi serumskog TWEAK nemaju značajnu povezanost sa težinom vaskularne uključenosti kod pacijenata sa stabilnom anginom pektorisom. Ovi rezultati ističu ograničenu efikasnost TWEAK kao jedinog biomarkera u proceni težine koronarne bolesti, ističući kompleksnost njegove uloge.

Ključne reči: TWEAK, Angina pektoris, Gensini skala.

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APPLICATION OF TRANSANAL TUBE AFTER ANTERIOR RECTAL RESECTION: IMPACT ON PREVENTION OF "ACHILLES HEEL" IN COLORECTAL SURGERY

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Abstract: Aim: The aim of our study was to investigate the potential reduction in the likelihood of anastomotic leakage occurrence in patients undergoing open anterior resection of the rectum without a protective stoma for stage III adenocarcinoma, by employing a transanal tube after performing stapled colorectal anastomosis.

Results: Considering the influence of all included risk factors, male gender (p = 0.032; OR = 2.873) and patients with type 2 diabetes mellitus (p = 0.033; OR = 2.873) demonstrated an increased likelihood of anastomotic leakage, while the presence of a transanal tube (p = 0.043; OR = 0.349; 95% CI: 0.126, 0.966) was associated with a statistically significant reduction in the likelihood of anastomotic leakage. T-test revealed that patients with anastomotic leakage without a placed tube had a statistically significantly longer mean postoperative hospitalization (20.94 days) compared to those with a transanal tube (19.43 days) (t = 2.375; p = 0.025). Kaplan-Meier analysis didn't show a statistically significant difference in the average estimated time to the occurrence of anastomotic leakage between patients without (3.86 days) and with a transanal tube (4.58 days) (p = 0.057).

Conclusion: Our study found that the placement of a transanal tube after colorectal anastomosis may be associated with a reduced likelihood of anastomotic leakage and shorter hospitalization in case leakage occurs. Additionally, although no statistically significant difference was found in the effect of the tube on the occurrence of anastomotic leakage depending on the presence of type 2 diabetes mellitus, the indication for its use in patients with type 2 diabetes mellitus may be of particular benefit.

Keywords: Equipment and supplies, postoperative complications, rectal neoplasms, surgical procedure.

INTRODUCTION

Colorectal cancer ranks as the third most common malignancy and poses a significant treatment challenge, particularly when diagnosed at an advanced stage (1, 2, 3). The preferred treatment approach for stage III colorectal cancer involves curative bowel resection followed by six months of adjuvant chemotherapy (4). Standard surgical procedures for stage III rectal cancer encompass anterior resection and abdominoperineal resection of the rectum with total mesorectal excision (5).

Anastomotic leakage (AL) is recognized as the "Achilles heel" of colorectal surgery, occurring in approximately 10.2% of patients following anterior resection for rectal carcinoma (6). AL involves the leakage of intestinal contents into the abdominal cavity, which can result in pelvic abscess, diffuse peritonitis, sepsis, metabolic disturbances, or multiple organ failure (7, 8). Managing this complication remains challenging, with a range of therapeutic options available including pharmacological, endoscopic, surgical therapies, or their combination, with treatment decisions heavily influenced by the surgeon's assessment and experience (9).

AL not only poses life-threatening risks and prolongs hospitalization but also increases treatment costs.

Moreover, it serves as an independent prognostic factor associated with reduced overall and cancer-specific survival (10).

Common preventive measures, such as controlling intraoperative risk factors (use of antibiotics, analgesia, surgical expertise, duration of surgery), are not always sufficient to prevent AL (11, 12). The investigation of the effect of placing a transanal tube (TnT) as an intervention to protect colorectal anastomosis after anterior rectal resection dates back several years (13). However, different opinions persist regarding its protective effect, considering both benign and malignant diseases, different heights and techniques for creating anastomosis, with recent studies also exploring various types, diameters, and materials of the tube (14, 15).

In our study, we focused on stage III adenocarcinoma of the upper and middle rectum due to its high prevalence in our elective surgical practice. The results of our research will provide valuable insights into the benefits of this widely available and simple intervention in preventing colorectal anastomotic dehiscence, with the potential to answer whether it should be a standard procedure in surgical practice for these patients.

Aim

The aim of our study was to investigate the potential reduction in the likelihood of anastomotic leakage (AL) in patients undergoing open anterior rectal resection for stage III adenocarcinoma, by employing a transanal tube (TnT) following stapled colorectal anastomosis.

PATIENTS AND METHODS

Patients and Study Design

Our retrospective cohort study included 102 patients classified as American Society of Anesthesiologists physical status I and II, who underwent elective radical (R0) anterior rectal resection with established mechanical colorectal anastomosis without protective ileo- or colostomy at the Department of General and Abdominal Surgery, Clinical Center of the University of Sarajevo, for rectal adenocarcinoma from May 2015 to November 2023 (16). Following surgery, patients underwent adjuvant chemotherapy (17). Only patients with type 2 diabetes mellitus (DM2) who had preoperative HbA1c values ranging from 6.5% to 8.8% and who had been diagnosed with DM2 for less than ten years, regardless of whether they were non-insulin or insulin controlled, were included in the study.

Exclusion Criteria

Patients with rectal adenocarcinoma TNM stage lower or higher than III, located outside the distance of 7-15 cm from the anal verge (upper and middle rectum), those who received neoadjuvant therapy, patients with tumors in other parts of the intestine besides the rectum, different histological tumor types, or metastatic tumors were excluded from the study (18). Additionally, patients undergoing emergency resectional procedures, those with Charlson Comorbidity Index scores equal to or less than 4, and patients with type 1 diabetes mellitus were excluded (19).

Methods

All patients included in the study underwent open anterior resection of the rectosigmoid colon after bowel preparation with oral laxatives the day before and receiving antibiotic prophylaxis within one hour before the incision (20-22). Two different approaches were used to access the vascular pedicle: high (ligation of the inferior mesenteric artery at its origin from the aorta above the origin of the left colic artery) and low (ligation of the inferior mesenteric artery below the origin of the left colic artery) (23). Macroscopic signs of intestinal viability (color of serosal surface, presence of bowel movement, pulsation, and bleeding from the resection margin) were used to assess the vitality of the colonic end of the anastomosis (24). A termino-terminal colorectal anastomosis was created using an appropriately sized mechanical stapler, followed by an air leak test to confirm integrity (24). A pelvic drain was placed in all patients through a separate incision on the skin.

Based on surgeon preference, after performing the anastomosis, a transanal tube (TnT) was placed in one group of patients (43 patients) while not in the other group (59 patients) (PVC, 10mm in width, 40cm in length), passing above the created anastomosis by approximately 5cm and additionally fixed to the skin of the gluteal region. The TnT was scheduled for removal 3 to 7 days after surgery, except in instances of anastomotic leakage (AL) when the removal of the TnT was delayed due to further assessment and additional treatment. The pelvic drain was removed following the absence of turbidity and a drainage quantity of $\leq$ 150 mL/day.

Patients were monitored during the postoperative hospital stay, with the key outcome parameter being the occurrence of anastomotic leakage (AL). Dehiscence was confirmed clinically, laboratory, and radiologically, and then surgically in patients indicated for revisional surgery (24). According to its occurrence, patients were divided into two main groups: without AL (72 patients) and with AL (30 patients). Pathohistological analysis was performed at the Clinic for Clinical Pathology, Cytology, and Human Genetics at the Clinical Center of the University of Sarajevo.

The research was conducted in accordance with the Helsinki Declaration of 1964 and its later amendments or comparable ethical standards, as well as institutional and national ethical standards.

RESULTS

Univariate regression analysis demonstrated a statistically significant higher likelihood of AL occurrence in males compared to females (p = 0.031; OR = 2.615; 95% CI: 1.093, 6.259), patients with DM2 compared to those without DM (p = 0.029; OR = 2.654; 95% CI: 1.107, 6.363), and in patients without a placed TnT compared to those with a placed TnT (p = 0.016; OR = 0.304; 95% CI: 0.116, 0.798). There was no statistically significant higher likelihood of AL occurrence in patients with high ligation of the inferior mesenteric artery compared to those with low ligation (p = 0.066; OR = 2.273; 95% CI: 0.948, 5.446) (Table 1).

In multivariate regression analysis, male gender (p = 0.032; OR = 2.873; 95% CI: 1.097, 7.519) and DM2 (p = 0.033; OR = 2.873; 95% CI: 1.091, 7.567) were statistically significant predictors of increased likelihood of AL occurrence, while the presence of TnT (p = 0.043; OR = 0.349; 95% CI: 0.126, 0.966) was associated with a statistically significant reduction in the likelihood of AL occurrence. High ligation of the inferior mesenteric artery didn't show a statistically significant increase in the likelihood of AL occurrence (p = 0.581; OR = 1.305; 95% CI: 0.507, 3.360) (Table 1).

The independent samples t-test revealed a statistically significant longer average duration of postoperative hospitalization in patients with AL and without a placed tube (20.94 days) compared to those with AL and with a placed TnT (19.43 days), (t = 2.375; p = 0.025). According to the results of linear regression, patients with a placed TnT had, on average, 1.51 days shorter hospitalization compared to patients with AL and without TnT (Figure 1).

In the group of patients without DM, univariate regression analysis revealed no statistically significant difference in the likelihood of AL occurrence between patients with and without a TnT (p = 0.668; OR = 0.727; 95% CI: 0.169; 3.121). However, among patients with DM2, a statistically significant lower likelihood of AL occurrence was observed in patients with a TnT compared to those without it (p = 0.032; OR = 0.250; 95% CI: 0.071; 0.886) (Table 2).

The analysis of the interaction effect didn't reveal a statistically significant difference in the effect

Table 1. *Predictors of anastomotic leakage with univariate and multivariate regression analysis*

Variable		N (%)	Anastomotic leakage		P*	95% CI*	P**	95%CI**
			Not observed 72 (70.6)	Observed 30 (29.4)				
			N (%)	N (%)				
Gender	Female	61 (59.8)	48 (78.7)	13 (21.3)	0.031	1.093;6.295	0.032	1.097;7.519
	Male	41 (40.2)	24 (58.5)	17 (41.5)				
Diabetes mellitus status	DM2	58 (56.9)	46 (79.3)	12 (20.7)	0.029	1.107;6.363	0.033	1.091;7.567
	Non-DM	44 (43.1)	26 (59.1)	18 (40.9)				
Ligation of the IMVS	Low	65 (63.7)	50 (76.9)	15 (23.1)	0.066	0.948;5.446	0.581	0.507;3.360
	High	37 (36.3)	22 (59.5)	15 (40.5)				
Transanal tube	Without tube	59 (57.8)	36 (61.0)	23 (39.0)	0.016	0.116;0.798	0.043	0.126;0.966
	With tube	43 (42.2)	36 (83.7)	7 (16.3)				

*Univariate regression analysis
**Multivariate regression analysis
CI, confidence interval; DM, diabetes mellitus; IMVS, inferior mesenteric vessels

Table 2. *Interaction effect between placement of transanal tube and diabetes on the probability of anastomotic dehiscence*

Groups	Subgroups	Anastomotic leakage					
		Not observed	Observed	P*	95%CI*	P**	95%CI**
		N (%)	N (%)				
No DM2	Without transanal tube	31 (79.5)	8 (20.5)	0.668	0.169;3.121	0.279	0.050;2.371
	With transanal tube	16 (84.2)	3 (15.8)				
DM2	Without transanal tube	6 (33.3)	12 (66.7)	0.032	0.071;0.886		
	With transanal tube	18 (66.7)	9 (33.3)				

*Univariate regression for anastomotic leakage in patients with and without transanal tubes, by diabetes mellitus status
**Interaction effect between transanal tube and diabetes mellitus
CI, confidence interval; DM, diabetes mellitus

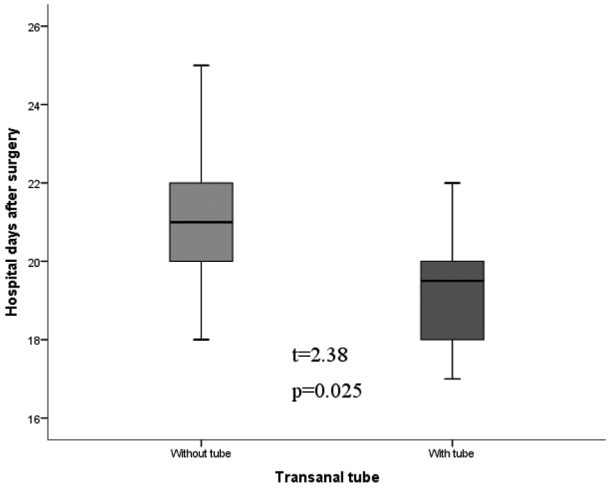

Figure 1. *Postoperative hospital stay (days) in patients with anastomotic leakage, due to transanal tube placement*

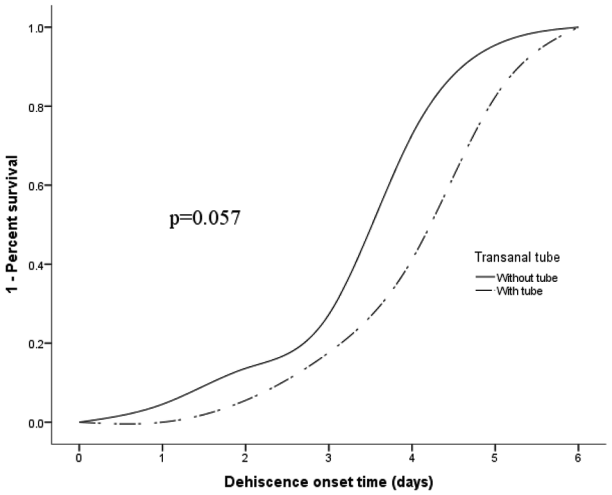

Figure 2. *Comparison of anastomotic leakage occurrence between patients with and without transanal tube*

of placing TnT on the likelihood of AL occurrence depending on the presence or absence of DM2 (p = 0.279; OR = 0.344; 95% CI = 0.050; 2.371) (Table 2).

There was no statistically significant difference in the average estimated time to AL occurrence between patients without (3.86 days) and with TnT (4.58 days) according to the results of the Log-rank test (p = 0.057) (Figure 2).

DISCUSSION

Our retrospective cohort study examined the significance of placing TnT after open anterior resection of the rectum due to stage III adenocarcinoma. This intervention has proven to be beneficial in reducing the likelihood of developing AL.

Anatomical differences between genders, such as the narrow and deep male pelvis, can complicate manipulation during the surgical procedure, which may increase the risk of AL in certain patients (25, 26).

In patients with type 2 diabetes mellitus (DM2), vascular damage, neuropathy, impaired collagen synthesis, and chronic inflammatory responses contribute to the increased risk of AL following surgical procedures (27, 28). This underscores the importance of interventions like TnT placement to mitigate AL risk in this patient population.

The results of previous research suggest that the level of ligation of the inferior mesenteric artery may not significantly affect the likelihood of AL because the blood supply to the anastomosis is complex and subject to the influence of multiple factors such as intraoperative technique, overall patient health status, tumor height, and other variables, which may partially explain the lack of correlation between the level of

ligation of the inferior mesenteric artery and the incidence of AL in our study (29, 30).

Previous studies suggest that TnT reduces endoluminal pressure in the intestinal anastomotic segment and allows drainage of exudate on the proximal side of the anastomosis, facilitating healing and reducing the risk of AL (31, 32). It also acts as a barrier against contamination of the anastomosis by fecal material from the intestinal lumen, reducing the risk of infection and aiding in maintaining its mechanical stability (33, 34).

In multivariate regression analysis, gender, DM2, and the method of ligating the inferior messenteric artery didn't disrupt the protective effect of TnT placement on the integrity of the anastomosis observed in univariate regression, indicating its consistent effect regardless of other risk factors. In a study conducted by Sueda et al. (35) on a sample of 392 patients using propensity score matching, the placement of TnT after mechanical colorectal anastomosis due to rectal carcinoma didn't show a significant protective effect against AL.

The results of our study demonstrated a significantly shorter duration of postoperative hospitalization among patients with AL who had TnT compared to those with AL but without TnT. Our findings are consistent with the research conducted by Brandl et al. (36), indicating a shorter postoperative hospital stay for the AL patient group with TnT compared to those without TnT (17.6 vs. 22.1 days; $p = 0.02$). The presence of TnT may contribute to better local infection control and reduce the risk of systemic complications, including sepsis (37). Additionally, we assume that patients with TnT had a smaller extent of dehiscence, leading to faster recovery and less need for revision surgery (38, 39).

Although a statistically significant interaction effect has not been proven, our analysis suggests that patients with DM2 may derive particular benefits from this intervention. A review of available medical literature didn't identify studies investigating the mentioned interaction, but based on the results of our study, it appears that placing TnT through colorectal anastomosis has the potential to compensate for what is compromised in patients with DM2, such as microcirculation and wound healing (27, 28, 32). The definitive cause of this phenomenon could be complex and requires further research to establish the exact relationship between the presence of DM2 and the benefits of TnT after surgery.

The absence of a significant difference in the time to AL occurrence between patients with and without TnT suggests that while TnT may reduce dehiscence incidence, other factors like overall health, surgical technique, and patient characteristics influence the timing of AL (40, 41).

Limitations of our study include its retrospective design, relatively small number of patients, and lack of investigation into interaction with other predictors of dehiscence included in the study. Additionally, the lack of identification of key confounding variables in the association between tube placement and AL, as well as imprecise specifications regarding the timing of tube removal, represent additional limitations. The need for reoperation and grading of dehiscence were not monitored, we didn't compare patients undergoing conventional and laparoscopic approaches, nor did we investigate the benefits of tube placement according to different tumor heights. Finally, we didn't identify any potential long-term benefits of this intervention.

CONCLUSION

Our study demonstrates that transanal tube (TnT) placement is associated with a reduced likelihood of anastomotic leakage (AL) and shorter hospitalization in case of dehiscence. Although we did not find a statistically significant difference in the effect of TnT on AL based on the presence of type 2 diabetes mellitus (DM2), the use of TnT in patients with DM2 may still be justified.

Our findings provide a basis for further research and consideration of introducing TnT placement as a routine protocol in patients undergoing anterior resection of the rectum for stage III adenocarcinoma, particularly in those concurrently with DM2.

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Abbreviations

DM2 - Diabetes Mellitus type 2
TnT - transanal tube
AL - anastomotic leakage

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Note: Artificial intelligence was not utilized as a tool in this study.

Sažetak

PRIMENA TRANSANALNOG DRENA NAKON PREDNJE RESEKCIJE REKTUMA: UTICAJ NA PREVENCIJU NASTANKA „AHILOVE TETIVE“ KOLOREKTALNE HIRURGIJE

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Cilj: Cilj naše studije bio je istražiti potencijalno smanjenje verovatnoće nastanka dehiscencije anastomoze primenom transanalnog drena nakon izvođenja staplerske kolorektalne anastomoze kod pacijenata podvrgnutih otvorenoj prednjoj resekciji rektuma bez protektivne stome zbog adenokarcinoma stadijuma III.

Rezultati: Uzimajući u obzir uticaj svih uključenih faktora rizika, muški pol (p=0.032; OR=2.873) i pacijenti sa diabetes melitusom tip 2 (p = 0.033; OR = 2.873) pokazali su povećanu verovatnoću za nastanak dehiscencije anastomoze, dok je prisutnost transanalnog drena (p = 0.043; OR = 0.349; 95% CI: 0.126, 0.966) bilo povezano sa statistički značajnim smanjenjem verovatnoće nastanka dehiscence anastomoze. T-testom utvrđeno je da pacijenti sa dehiscencijom anastomoze, a bez plasiranog drena, imaju statistički značajno dužu prosečnu postoperativnu hospitalizaciju (20.94 dana) u poređenju sa onima sa transanalnim drenom (19.43 dana), (t = 2.375; p = 0.025). Kaplan-Meier analizom nije uočena statistički značajna razlika u prosečnom procenjenom vremenu do pojave dehiscencije anastomoze između pacijenata bez (3.86 dana) i sa transanalnim drenom (4.58 dana) (p = 0.057).

Zaključak: Našom studijom utvrđeno je da plasiranje transanalnog drena nakon kolorektalne anastomoze može biti povezano sa smanjenom verovatnoćom nastanka dehiscencije anastomoze i kraćom hospitalizacijom u slučaju da je dehiscencija ipak nastala. Dodatno, iako nije pronađena statistički značajna razlika u efektu tubusa na nastanak dehiscencije anastomoze između pacijenata sa i bez diabetes melitusa tip 2, indikacija za njegovu primenu kod pacijenata sa diabetesom može biti od posebne koristi.

Ključne reči: Hirurške procedure, oprema i materijali, postoperativne komplikacije, rektalne neoplazme.

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CORRELATION BETWEEN TBARS VALUE IN SERUM AND TISSUE AS OXIDATIVE STRESS MARKERS IN PREMALIGNANT AND MALIGNANT CERVICAL LESIONS

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Abstract: **Introduction:** Numerous risk factors affect the development of cervical intraepithelial neoplasia (CIN) and cervical cancer (CC), with high-risk subtypes of the human papillomavirus (HPV) being the most significant. Oxidative stress (OS) plays an important role in the pathogenesis of CC and CIN as a risk factor. A commonly used marker of OS, which measures lipid peroxidation products in cells, tissues, and body fluids, is thiobarbituric acid reactive substances (TBARS). This study aimed to determine the correlation between TBARS levels in tissue and serum and evaluate their diagnostic significance in patients with cervical lesions.

Patients and methods: The research was conducted at the Clinical Center of the University of Sarajevo. The experimental group consisted of 200 female patients with biopsy-confirmed changes consistent with CIN, carcinoma in situ (CIS), and CC. The control group (N = 40) had biopsy-confirmed non-pathological findings. The concentration of TBARS was determined for all subjects from biopsy samples and serum according to standard laboratory practice.

Results: We found a significant difference in serum/tissue TBARS levels between study groups. Serum/tissue levels of TBARS in patients with CIS were significantly higher compared to the control group, patients with CIN 1, CIN 2, CIN 3, and patients with CC ($p < 0.05$ for all). There was a significant positive correlation between TBARS levels in serum (μM) and TBARS levels in tissue (μM) (Pearson›s $r = 0.494$, $p < 0.001$). Tissue and serum TBARS levels are major differentiation markers between CIS patients and the control group, as well as patients with CIN 1, CIN 2, CIN 3, and CC.

Conclusion: Patients with CIN and CC exhibit increased oxidative stress, indicated by higher levels of TBARS in their tissue and serum compared to healthy controls. TBARS levels in tissue are positively correlated with levels in serum. Tissue and serum TBARS levels are significant markers for differentiating the clinical stages of the disease.

Keywords: oxidative stress, cervical intraepithelial neoplasia, cervical cancer.

INTRODUCTION

Cervical cancer (CC) is one of the most common malignant diseases of the female reproductive system (1, 2). It represents a considerable health challenge worldwide, particularly in developing countries. CC develops through a series of pathological changes known as cervical intraepithelial neoplasia (CIN). Among the various risk factors for cervical cancer, the human papilloma virus (HPV), especially its high-risk subtypes, is the most significant (3). The therapeutic approach, prognosis, and survival depend on the clinical stage of the disease. Early diagnosis and treatment of CIN are crucial for the prevention of CC.

In the pathogenesis of CC and CIN, oxidative stress (OS) plays an important role as a risk factor (4-7). Oxidative stress and HPV infection primarily cause DNA damage. HPV and OS are closely associated because HPV proteins induce oxidative stress, which, in turn, promotes lipid peroxidation and cell damage (4).

Peroxidation of membrane lipids, as a result of oxidative stress, produces a broad range of oxidation products. The most frequent marker of this process is malondialdehyde (MDA), which binds to proteins and phospholipids of the membrane, exacerbating oxida-

tive cell damage (5). Another marker of oxidative stress that quickly and strongly binds to malondialdehyde is thiobarbituric acid reactive substances (TBARS). Lipid peroxidation products in cells, tissues, and body fluids are commonly evaluated by measuring TBARS.

AIM

The aim of this study was to determine the correlation between TBARS levels in tissue and serum and their diagnostic significance in patients with different severities of cervical lesions.

PATIENTS AND METHODS

The research was conducted at the Clinical Center of the University of Sarajevo. A total of 240 female respondents were included in the study, divided into two groups. The experimental group consisted of 200 patients, where the indication for biopsy was confirmed after a gynecological exam and Pap test. The biopsy indicated changes consistent with CIN, carcinoma in situ (CIS), and CC.

In the control group, which consisted of 40 subjects, the biopsy findings were confirmed to be non-pathological (excluding cervical carcinoma and any of the CIN stages).

The concentration of TBARS was determined for all subjects from simultaneously collected samples of biopsy material and serum using the spectrophotometric method according to standard laboratory practice.

The study was approved by the Ethics Committee of the Clinical Center of the University of Sarajevo (number: 0901-2-390/17). It was conducted in accordance with the ethical principles of the Declaration of Helsinki. The results were processed and analyzed using the Statistical Package for Social Sciences (SPSS), version 21.0. Results are expressed as mean (X) and standard deviation (SD). Differences in TBARS values between groups were tested using ANOVA with post-hoc analysis. Correlation between tissue and serum TBARS levels was analyzed by Pearson's correlation. A value of $p < 0.05$ was considered statistically significant.

RESULTS

The age statistics of the test subjects and the values of tissue and serum TBARS according to study groups are shown in Table 1.

ANOVA test (Table 2) showed a significant difference in TBARS levels in serum and tissue between the study groups. Post-hoc tests revealed that CIS patients had significantly higher levels of TBARS in both tissue and serum compared to the control group, patients with CIN 1, patients with CIN 2, patients with CIN 3, as well as patients with CC ($p < 0.05$ for all).

There is a significant positive correlation between levels of TBARS in serum (μM) and levels of TBARS in tissue (μM), Pearson's $r = 0.494$, $p < 0.001$ (Figure 1).

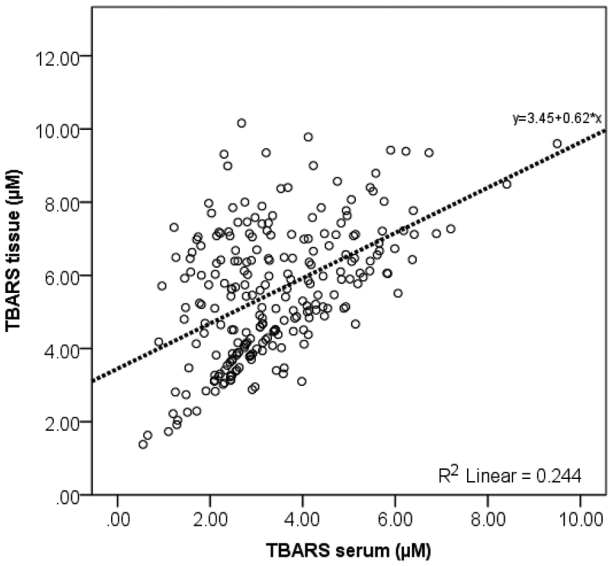

Figure 1. *Correlation between serum and tissue TBARS values (μM)*

Table 1. *Descriptive statistics for age and TBARS according to study groups*

Group	N	Age (years)	TBARS-tissue (μM)	TBARS-serum (μM)
Controls	40	50.28 ± 10.56	4.80 ± 0.22	2.81 ± 1.15
CIN 1	40	45.10 ± 11.32	4.78 ± 0.25	2.80 ± 1.18
CIN 2	40	45.53 ± 11.73	4.94 ± 0.24	3.23 ± 1.29
CIN 3	40	45.08 ± 11.63	5.94 ± 0.23	3.68 ± 1.27
CIS	40	49.78 ± 13.73	7.06 ± 0.31	4.59 ± 1.72
CC	40	54.08 ± 15.59	5.65 ± 0.24	3.07 ± 1.11

Table 2. *Difference in Age and TBARS Levels Between Study Groups*

Parameter	Sum of Squares	df	Mean Square	F	Sig.
TBARS serum (μM)	93.022	5	18.604	10.894	< 0.001
TBARS tissue (μM)	158.386	5	31.677	12.082	< 0.001

Table 3. *Sensitivity and specificity of TBARS in tissue as a marker of differentiation*

TBARS tissue	CIS/controls	CIS/CIN1	CIS/CIN2	CIS/CIN3	CIS/CC
Cut off	5.82	6.58	7.32	7.24	7.26
Sensitivity	78.95%	89.29%	95.24%	76.92%	76.92%
Specificity	76.19%	71.15%	66.10%	62.96%	62.96%
AUC	0.808	0,801	0.788	0.710	0.710
95%CI	0.712-0.903	0.705-0.897	0.688-0.887	0.561-0.799	0.595-0.824
p-value	< 0.001	< 0.001	< 0.001	0.006	0.001

Sensitivity and specificity of TBARS in tissue as a marker of differentiation between patients with CIS and: controls (CIS/controls), patients with CIN 1 (CIS/CIN1), patients with CIN 2 (CIS/CIN2), patients with CIN 3 (CIS/CIN3), patients with cervical carcinoma (CIS/CC). AUC - area under curve.

Table 4. *Sensitivity and specificity of TBARS in serum as a marker of differentiation*

TBARS serum	CIS/controls	CIS/CIN1	CIS/CIN2	CIS/CIN3	CIS/CC
Cut off	4.18	3.12	4.79	4.73	4.61
Sensitivity	88.0%	72.73%	76.72%	77.78%	84.0%
Specificity	67.27%	77.78%	62.96%	64.15%	65.45%
AUC	0.809	0.812	0.734	0.662	0.770
95%CI	0.713-0.904	0.705-0.897	0.622-0.847	0.542-0.783	0.667-0.873
p-value	< 0.001	< 0.001	< 0.001	0.013	0.001

Sensitivity and specificity of TBARS in serum as a marker of differentiation between patients with CIS and: controls (CIS/controls), patients with CIN 1 (CIS/CIN1), patients with CIN 2 (CIS/CIN2), patients with CIN 3 (CIS/CIN3), patients with cervical carcinoma (CIS/CC). AUC - area under curve.

Furthermore, we analyzed ROC curves to identify the sensitivity and specificity of the best cutoff points of TBARS in serum and tissue as a discriminator of the probability of significant cervical lesions (Tables 3 and 4).

DISCUSSION

In addition to the well-established association between the risk of developing CC and age (3, 6), as well as its correlation with HPV infection, other risk factors such as oxidative stress (OS) are also associated with CIN and uterine cancer (7, 8). As the organism ages, DNA damage accumulates, and during the division of such cells, this damage becomes permanent, leading to the development of mutations and malignant diseases (7, 8).

Oxidative stress (OS) holds an important place in the pathogenesis of a multitude of malignant diseases, including lung cancer, colorectal cancer, renal cancer, etc. (9).

The oxidized form of DNA, resulting from interactions with reactive oxygen species, leads to mutations and the development of carcinogenesis. This process is further enhanced by environmental factors such as radiation, pollution, and UV radiation (10). Cancer cells are often more exposed to OS compared to normal cells, although they can also develop resistance to OS through mechanisms that are not fully elucidated (11, 12). At the local level, OS within malignant tissue itself may have a beneficial effect on the apoptosis of carcinomatous cells and prevent their proliferation (12).

HPV infection is strongly associated with the initiation, promotion, and progression of cancer due to the expression of viral oncoproteins. While HPV oncoproteins are necessary for the progression of cervical cancer (CC), other conditions and factors are also required for the complete transformation of cells (13). OS, among commonly proposed factors, is often understudied but ultimately plays a role in CC. It acts synergistically or independently on HPV infection, contributing to the disease process.

In addition to DNA oxidation, lipid oxidation, or so-called lipid peroxidation, is a risk factor for carcinogenesis and its progression. Damaged lipid hydroperoxides yield a wide range of end products, including MDA. Lipid peroxidation plays a significant role in malignant transformation (14). Altered levels of lipid peroxidation have also been reported in many precancerous lesions (15). It has been confirmed that oxidative stress (OS) and its consequence, lipid peroxidation, occur in the early stages of carcinogenesis, including in relation to cervical intraepithelial neoplasia (CIN) (16).

Another cancer promoter arising as the end product of lipid peroxidation is highly cytotoxic MDA. Patients with CC have significantly increased levels of MDA,

which is also observed in patients with various cancer types compared to healthy controls (17, 18). Plasma MDA levels have been identified as an independent prognostic parameter of survival in these patients (19).

The pathogenesis of malignant diseases of the female reproductive system involves lipid peroxidation.

MDA is commonly used as a marker of oxidative stress, particularly for assessing lipid peroxidation. Acid thiobarbituric reactive substances (TBARS) are another marker of oxidative stress that quickly and strongly binds to malondialdehyde. Lipid peroxidation products in cells, tissues, and body fluids are commonly evaluated by measuring TBARS.

Our study results demonstrated that tissue levels of TBARS in CIS patients were significantly higher compared to other groups (controls, CIN 1, CIN 2, CIN 3, CC). Tissue TBARS levels reflected changes in cellular conditions, with significantly higher levels observed in the CIN 3 group compared to the control group and patients with CIN 1.

Tissue TBARS levels served as a significant differentiation marker between patients with CIS and various other groups, including CC.

Similarly, serum TBARS levels in our subjects showed significant differences between patients with CIS and the control group, as well as patients with different stages of CIN. Serum TBARS levels also differentiated between patients with CIS and CC.

These findings align with previous studies by Jelić et al. (20), which reported higher levels of lipid peroxidation in precancerous and uterine carcinoma tissues compared to controls. Elevated TBARS levels, indicative of oxidative stress, were observed in all examined groups and were significantly higher in women with advanced CC.

The production of oxygen radicals, which increase lipid peroxidation, is associated with disease progression, indicating greater cell membrane degeneration in advanced CC patients compared to those with lower stages.

Lipid peroxidation-induced tissue degeneration can spread through circulation, causing damage to other tissues (21).

Studies by Zahra et al. (17) and Carneiro et al. (21) further support the involvement of oxidative stress in cervical cancer pathogenesis, manifested by increased lipid peroxidation

Additionally, Visalli et al. found higher oxidative stress levels in patients with severe cervical intraepithelial lesions (SIL) compared to controls, even among patients with low-grade SIL (22).

Gonçalves et al. (23) demonstrated significantly higher TBARS levels in erythrocytes of women with malignant and premalignant lesions compared to controls. This study also identified a positive association between lipid peroxidation and lesion severity, suggesting that TBARS levels in erythrocytes can serve as early markers of oxidative stress, evident even in premalignant conditions.

Following the level of lipid peroxidation through TBARS concentration, Manju et al. (24) observed significantly higher levels in the plasma of patients with CC compared to healthy women. Furthermore, the researchers noted significantly lower levels of enzymatic antioxidants in CC patients compared to healthy subjects. They concluded that the increased consumption of enzymatic antioxidants was due to the removal of lipid peroxides and their sequestration by cancer cells.

Sahah et al. (25) demonstrated that the mean concentration of MDA in the serum of patients with CC was lower compared to the control group. Additionally, they found that the mean concentration of total antioxidant capacity (TAC) was significantly lower in the group of patients with CC compared to healthy subjects.

Naidu et al. (18) observed significantly higher levels of serum lipid peroxide in the form of MDA and NO in patients with CC compared to healthy controls. The maximum increase of MDA and NO was recorded in phase IV compared to healthy controls.

The association between oxidative stress (OS) and CC progression was described by Borges et al. (26), who detected a two to threefold increase in TBARS levels in the erythrocytes of patients with squamous intraepithelial lesions (SIL) or CC. Additionally, MDA levels in healthy women were almost three times lower than in women with SIL. Higher levels of MDA were recorded in women positive for HPV. These findings suggest that higher concentrations of MDA and TBARS reflect an increase in OS. Conflicting evidence indicates that malignant neoplasias are capable of releasing free radicals into the bloodstream, suggesting that the presence of cancer may cause increased OS, rather than being its consequence.

After forming at primary sites, lipid peroxidation or oxidative damage is transferred through the circulation. Therefore, we investigated whether there is a correlation between TBARS levels in tissue and serum (27).

Our research showed a significant positive correlation between TBARS levels in tissue and serum, with Pearson's $r = 0.494$, $p < 0.001$, indicating a relationship between tissue and serum oxidative stress markers.

We were unable to find existing literature on the correlation between tissue and blood levels of TBARS, not only in cervical cancer pathology but also in other cancers. Consequently, we are unable to offer a comprehensive explanation of our results. However, we speculate that the elevated TBARS serum levels may reflect heightened levels in tissue.

Biomarkers for lipid peroxidation hold promise as diagnostic tools in screening, predicting cancer recurrence, disease progression, or therapy effects in cancer patients. However, further comprehensive research is necessary to draw definitive conclusions.

Bearing in mind that the results of our research showed that both tissue and serum concentrations of TBARS were the highest in the group of subjects with CIS, and were significantly higher than the TBARS values in the control group, significantly higher than the TBARS values of patients with premalignant lesions, as well as significantly higher from the TBARS values of patients with uterine cancer, we are of the opinion that the increase in lipid peroxidation in this stage of the disease can serve as a potential biomarker for differentiating the transition of the disease from premalignant to malignant form. A possible increase in the level of TBARS in this stage may be a consequence of the progression of the disease, but the possibility that the organism at this stage responds to pronounced lipid peroxidation at the local and systemic level as a potential defense is not excluded, because oxide radicals can be harmful to cancer cells.

An increase in TBARS in the CIS compared to other stages may indicate the risk of disease progression. These results open up new perspectives in the diagnosis and therapy of the disease, in such a way that lipid peroxidation can serve as a possible biomarker of the stage of the disease, and as such can be a useful diagnostic tool.

CONCLUSION

Patients with CIN and CC have increased oxidative stress, indicated by higher levels of TBARS in their tissue and serum compared to healthy controls. The positive correlation between TBARS levels in tissue and serum underscores the significance of these markers in disease evaluation. Tissue and serum TBARS levels are a significant marker of differentiation of the clinical stage of the disease and can be a useful diagnostic tool influencing the selection of therapeutic procedures, but its application in screening is also possible.

Abbreviations

CC - Cervical Cancer
CIN - Cervical Intaepithelial Neoplasia
CIS - Carcinoma in Situ
DNA - Deoxyribonucleic acid
HPV - Human papillomavirus
MDA - Malondialdehyde
OS - Oxidative stress
TBARS - Acid tiobarbituric reactive substances

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Note: Artificial intelligence was not used as a tool in this study.

Sažetak

KORELACIJA IZMEĐU VREDNOSTI TBARS-a U SERUMU I TKIVU KAO MARKERA OKSIDATIVNOG STRESA U PREMALIGNIM I MALIGNIM LEZIJAMA GRLIĆA MATERICE

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Uvod: Brojni faktori rizika utiču na razvoj intraepitelnih neoplazija grlića materice (CIN) i cervikalnog karcinoma (CC), pri čemu su visoko rizični podtipovi humanog papiloma virusa (HPV) najznačajniji. Oksidativni stres (OS) igra važnu ulogu u patogenezi CC i CIN kao faktor rizika. Često korišćeni marker OS-a, koji meri produkte lipidne peroksidacije u ćelijama, tkivima i telesnim tečnostima, je reaktivna supstanca tiobarbiturne kiseline (TBARS). Ova studija ima za cilj da utvrdi korelaciju između nivoa TBARS-a u tkivu i serumu i proceni njihov dijagnostički značaj kod pacijenata sa lezijama grlića materice.

Pacijenti i metode: Istraživanje je sprovedeno u Kliničkom centru Univerziteta u Sarajevu. Ekspe-

rimentalnu grupu činilo je 200 pacijentkinja sa biopsijski potvrđenim promenama koje su konsistentne sa CIN, karcinomom in situ (CIS) i CC. Kontrolnu grupu (N = 40) činile su pacijentkinje sa biopsijski potvrđenim nepatološkim nalazima. Koncentracija TBARS-a određena je za sve subjekte iz uzoraka biopsije i seruma prema standardnoj laboratorijskoj praksi.

Rezultati: Utvrdili smo značajnu razliku u nivoima TBARS-a u serumu i tkivu između studijskih grupa. Nivoi TBARS-a u serumu i tkivu kod pacijenata sa CIS bili su značajno viši u poređenju sa kontrolnom grupom, pacijentima sa CIN 1, CIN 2, CIN 3 i pacijentima sa CC ($p < 0,05$ za sve). Postojala je značajna pozitivna korelacija između nivoa TBARS-a u serumu (μM) i nivoa TBARS-a u tkivu (μM) (Pearson-ov $r = 0,494$, $p < 0,001$). Nivoi TBARS-a u tkivu i serumu predstavljaju glavni marker diferencijacije između pacijenata sa CIS i kontrolnom grupom, kao i pacijenata sa CIN 1, CIN 2, CIN 3 i CC.

Zaključak: Pacijenti sa CIN-om i CC-om pokazuju povećani oksidativni stres, što ukazuju viši nivoi TBARS-a u njihovom tkivu i serumu u poređenju sa zdravim osobama. Nivoi TBARS-a u tkivu pozitivno su povezani sa nivoima u serumu. Nivoi TBARS-a u tkivu i serumu su značajni markeri za diferencijaciju kliničkih stadijuma bolesti.

Ključne reči: oksidativni stres, cervikalna intraepitelna neoplazija, karcinom grlića materice.

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Original article

PATIENT SATISFACTION WITH DENTAL HEALTH CARE DURING THE COVID-19 OUTBREAK IN SERBIA

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Abstract: **Introduction:** The COVID-19 virus belongs to the group of respiratory viruses. Due to the nature of their work, dentists are at greater risk of contracting the new coronavirus during dental interventions. Knowing the path of transmission of the virus is of great importance in preventing and suppressing the development of infection. Patients' fear of infection during the coronavirus pandemic was significant, and because of this, visits to the dentist for standard dental services were postponed, and patients came only for emergencies. Adherence to protective measures directly affected patients' satisfaction with dental services.

The aim of this work was to examine patient satisfaction with dental services during the coronavirus pandemic.

Materials and Methods: The study was designed as a cross-sectional study using an epidemiological questionnaire. A total of 120 patients were surveyed, 60 from private practice and 60 from the state sector. The questionnaire was anonymous and contained sociodemographic questions and questions related to knowledge of protocols and satisfaction with dental services. The data were processed in the SPSS 11.5 program.

Results: Out of 120 respondents with an average age of 38.92 ± 13.08 years, 98% were familiar with the protocol for protection against the coronavirus. There were no dissatisfied people in either private practice or the public sector; the only difference was in the degree of satisfaction. In private practice, more respondents were very satisfied as the highest level of satisfaction, while in the public sector, it was very satisfied.

Conclusion: A good knowledge of the protocol for protection and prevention of the spread of the virus raises awareness among dentists and patients. By following these measures, infection with the virus is reduced to a minimum, and therefore patient satisfaction with dental services is at a higher level.

Keywords: COVID-19, patient satisfaction, dentistry.

INTRODUCTION

Ensuring quality and safe health care during the COVID-19 pandemic was a challenge, especially in low and middle-income countries such as Serbia. In the Republic of Serbia, the first case of COVID-19 was reported on March 6, 2020, and more than 2.5 million cases have been detected so far, with the outbreak still ongoing (1).

The SARS-CoV-2 virus can spread through the inhalation of respiratory droplets from an infected individual (including aerosols generated through sneezing, coughing, speaking, singing, or breathing) or through direct contact with infected droplets through the eyes, mouth, or nose (2). Aerosol-generating procedures in dental health care can increase the risk of virus transmission, with dentists being on the frontline of infection (3). Due to the risk of infection for both dental healthcare workers and patients, additional COVID-19 infection control measures were strongly recommended for all patients when providing dental healthcare (4).

Patient satisfaction is an indicator of healthcare quality and effectiveness of healthcare services, and, as such, it is commonly used for measuring the quality of dental healthcare. The services provided by healthcare workers, good and efficient communication, and the willingness to provide medical measures play major roles in patient satisfaction (5). Measuring patients' satisfaction with dental interventions defines the quality of services provided, thus encouraging better work

in healthcare institutions. In this way, various deficiencies can be identified to improve the level of services in health institutions.

As a highly contagious disease, coronavirus required enhanced protection measures to prevent its spread (6, 7). The protection protocol was clearly defined and related to the wearing of protective equipment by dentists and patients, improved hygiene and disinfection, as well as adequate social distancing (8).

During the pandemic, the healthcare service system faced changes, causing different perceptions about the quality of interventions and, consequently, patient satisfaction (6). Therefore, it is necessary to immediately notice and remedy dissatisfaction factors to ensure the level of patient satisfaction remains high or even improves.

Apart from enhanced protection measures in dental offices, the fear of contracting the virus and the fear of dentists among patients greatly influenced the decision to seek dental services. In addition, the level of visiting patients' awareness of healthcare compliance to prevent the spread of the new coronavirus was important for the timely provision of dental services (9). In a clinical sense, it is essential to assess patient satisfaction with healthcare services during the COVID-19 pandemic, as the pandemic is expected to have reduced adherence to regular dental attendance and follow-up examinations and shifted focus to dental emergencies only (8, 9). Professional literature shows dental centers suffered damage during the pandemic due to neglected basic dental services.

In our country, several COVID-19-related studies were conducted about recommendations and the impact of the pandemic on the general population's health. Our study is the first to examine the satisfaction of patients in the public sector, which includes a wide population, as well as private practice.

This paper aims to examine patient satisfaction with dental services based on the protection protocol during the coronavirus pandemic.

MATERIAL AND METHODS

We performed a cross-sectional study of patients aged 18 years and older attending the Clinic of Dental Medicine and private healthcare facility, Implant Rendgen centar, in the city of Niš, situated in Southeastern Serbia. The study was approved by the Ethics Committee of the Clinic for Dental Medicine in Niš under number 14/14-2019 EO.

In 2022, Niš had a population of **249 816** inhabitants in an area of 596.71 km2. The study was carried out from February 1 to February 28, 2023. To adjust for the COVID-19 pandemic, we categorized visits after March 6, 2020, when the first case in Serbia was reported, as the COVID-19 period. The required information for the study was collected in face-to-face interviews with patients carried out by trained interviewers. The questionnaire contained 21 items and was divided into two parts: the respondents' sociodemographic characteristics and a patient satisfaction structured questionnaire adapted from previous publications and developed for this purpose (10, 11).

The patient satisfaction questionnaire consisted of 14 statements regarding various aspects of satisfaction. Of these statements, eight were close-ended questions, and six were to be scored on a five-point Likert scale to evaluate the patient satisfaction level from strongly disagree = 1 to strongly agree = 5. Closed-ended questions related to patient's familiarity with the protocol and protection against virus transmission, presence of fear of infection in dental centers, fear as a motivation for better oral hygiene, adequate workplace hygiene, whether dentists had prescribed protective equipment in place, whether adequate disinfection between patients was ensured, as well as whether the enhanced measures should continue to be applied (Table 1).

The study included 128 patients, of whom 66 were from the private sector and 62 were from public healthcare, and adequate responses were received from 120 individuals.

Patients were informed about the goals and procedures of the research and agreed to participate in the study. The authors followed the World Medical Association Declaration of Helsinki.

Collected data were statistically analyzed using SPSS 11.5 for Windows (SPSS Inc., Chicago, IL, USA). Descriptive statistics were computed for all variables. Mantel-Haenszel's chi-square test was used to compare frequencies of categorical variables between the two groups. Statistical significance was set at a level of 0.05.

RESULTS

The study included 128 patients, of whom 66 were from the private sector and 62 were from public healthcare, and adequate responses were received from 120 individuals.

The examined sample (n = 120) comprised 56 males (46.5%) and 64 females (53.5%). The average age of the respondents was 38.92 ± 13.08 years (range: 18-58). More than four-fifths of the respondents were from the city (83.3%), and a smaller number lived in rural areas (16.7%). The most significant number of respondents had university and college education (53.3%), followed by secondary education (44.2%), while as few as 2.5% had only primary education. In addition, the sample was dominated by employed people, namely 93 individuals or 77.5% (Table 2).

Table 1. *Questionnaire of patient satisfaction*

1.	Date of birth?_____
2.	Survey date?_____
3.	Gender? a) Male b) Female
4.	Education? a) Primary b) Secondary c) College and university
5.	Place of living? a) Rural area b) City
6.	Live in Serbia? a) Yes b) No
7.	Employment status? a) Employed b) Unemployed c) Retired
8.	Are you familiar with the protocol? a) Yes b) No
9.	Chosen physician a) Private sector b) Public sector
10.	How many times did you brush your teeth? a) None b) 1-2 times a day c) 3-4 times a day
11.	How many check-ups did you have during the pandemic? a) None b) 1-2 times c) 3-4 times d) Only in emergencies
12.	Was the mouth disinfected before the intervention? a) Yes b) No
13.	Was the fear of the virus a motivation for better hygiene behavior? a) Yes b) No
14.	Did the doctors have adequate protection in place? a) Yes b) No c) Partly
15.	Was the work area well disinfected? a) Yes b) No
16.	What was the patient appointment schedule like? a) Poor b) Good c) Excellent
17.	Did the patients use protection against the virus? a) Yes b) No
18.	Were you afraid of getting infected in the office? a) Yes b) No
19.	Did you need emergency dental intervention while you were infected with the virus? a) Yes b) No
20.	Should enhanced protection measures continue to be applied? a) Yes b) No c) Partly
21.	How satisfied are you with dental interventions during the pandemic?? a) Dissatisfied b) Satisfied c) Moderately satisfied d) Very satisfied

Table 2. *Demographic characteristics of respondents*

Variable		N	%	statistics
Gender	Males Females	56 64	46.5 53.5	$\chi^2 = 0.533$ df = 1 p = 0.465
Education	Primary Secondary College and university	3 53 64	2.5 44.2 53.3	$\chi^2 = 52.85$ df = 2 p = 0.000
Place of living	Rural area City	20 100	16.7 83.3	$\chi^2 = 53.333$ df = 1 p = 0.000
Employment status	Employed Unemployed Retired	93 22 5	77.5 18.3 4.2	108.95 df = 2 p = 0.000
Age	18-24 25-34 35-44 45+	17 31 34 38	14.2 25.8 28.3 31.7	$\chi^2 = 8.333$ df = 3 p = 0.0396

Table 3*. Satisfaction with services by sociodemographic characteristics*

Variable	Category	Satisfied (n, %)	Moderately satisfied (n, %)	Very satisfied (n, %)	Statistics
Gender	Males Females	23 (41.1) 23 (35.9)	20 (35.7) 29 (45.3)	13 (23.2) 12 (18.8)	$\chi^2(1) = 1.165$ p = 0.559
Education*	Primary Secondary College and university	2 (66.7) 20 (37.7) 24 (37.5)	1 (33.3) 20 (37.7) 28 (43.8)	0 13 (24.5) 12 (18.8)	$\chi^2(1) = 0.596$ p = 0.7422
Place of living	Rural area City	13 (65.0) 33 (33.0)	3 (15.0) 46 (46.0)	4 (20.0) 21 (21.0)	$\chi^2(1) = 8.383$ p = 0.0151
Employment status	Employed Unemployed Retired	36 (38.7) 8 (36.4) 2 (40.0)	40 (43.0) 8 (36.4) 1 (20.0)	17 (18.3) 6 (27.3) 2 (40.0)	$\chi^2(2) = 2.3871$ p = 0.665
Age	15-24 25-34 35-44 45+	9 (52.9) 8 (25.8) 14 (41.2) 15 (39.5)	6 (35.3) 13 (41.9) 15 (44.1) 15 (39.5)	2 (11.8) 10 (32.3) 5 (14.7) 8 (21.1)	$\chi^2(3) = 5.771$ P = 0.449

The results referring to the respondents' answers and the differences in distribution among them are given in Table 3.

Gender, age, education, and employment status did not affect respondents' satisfaction with the dental services provided. The only sociodemographic characteristic by which the respondents differed statistically significantly in terms of satisfaction with the services provided is the place of living. Respondents living in rural areas were more often "satisfied" (the lowest registered degree of satisfaction) - 65%. At the same time, they were less often "moderately satisfied," 15%, compared to 46% of respondents living in the city. In the "very satisfied" category (the highest degree of satisfaction), the responses of respondents from rural areas did not differ from those of respondents from the city.

Patient responses to questionnaire questions affecting satisfaction are shown in Table 4

With regard to satisfaction with the dental services provided in the private and public sectors, there are no dissatisfied patients. Satisfied patients are almost equal in number, with the only difference being that there are more very satisfied patients in the private sector and more moderately satisfied patients in the public sector (Figure 1).

Table 4. *Patient satisfaction structured questionnaire*

Question number	Questions and answers	Number of answers: (%)
1.	Are you familiar with the protocol?	
	Yes	118 (98)
	No	2 (2)
2.	Was the fear of the virus a motivation for better hygiene behavior?	
	Yes	33 (27.5)
	No	87 (72.5)
3.	Did the doctors have adequate protection in place?	
	Yes	118 (98.3)
	Partly	2 (1.7)
4.	Was the work area well disinfected?	
	Yes	119 (99.2)
	No	1 (0.8)
5.	Did the patients use protection against the virus?	
	Yes	111 (92.5)
	No	9 (7.5)
6.	Was the mouth disinfected before the intervention?	
	Yes	78 (65)
	No	42 (35)
7.	Were you afraid of getting infected in the office?	
	Yes	29 (24.2)
	No	91 (75.8)
8.	What was the patient appointment schedule like?	
	Poor	2 (1.7)
	Good	73 (60.8)
	Excellent	45 (37.5)
9.	How many check-ups did you have during the pandemic?	
	None	25 (20.8)
	1-2 times	60 (50)
	3-4 times	17 (14.2)
	Only in emergencies	18 (15)
10.	Should enhanced protection measures continue to be applied?	
	Yes	44 (36.7)
	No	28 (23.3)
	Partly	48 (40)
11.	Chosen physician	
	Private sector	60 (50)
	Public sector	60 (50)
12.	How many times did you brush your teeth?	
	b) 1-2 times a day	116 (96)
	c) 3-4 times a day	4 (4)
13.	Did you need emergency dental intervention while you were infected with the virus?	
	a) yes	10 (10)
	b) no	110 (90)
14.	How satisfied are you with dental interventions during the pandemic??	
	b) satisfied	46 (38.3)
	c) moderately satisfied	49 (40.8)
	d) very satisfied	25 (20.8)

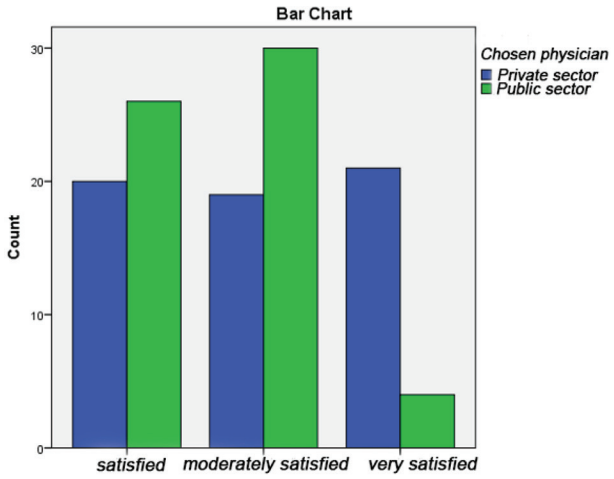

Figure 1. *Distribution of satisfaction in the private and public sectors*

DISCUSSION

The oral cavity has diverse microbiota harboring numerous species of bacteria participating in the protection of systemic health. It is also the initial part of two systems - digestive and respiratory - and thus the entrance for pathogenic microorganisms (11).

Complications of untreated tooth and periodontal diseases may disrupt the entire body's immune system and cause serious diseases (12). During the coronavirus pandemic, the fear of infection increased awareness of and concern about the health status of the entire population. The imperative was to improve the disinfection and hygiene of the workspace, ensure better protection of dentists, and reduce waiting room crowding, which minimized the possibility of virus spread (13).

A large number of studies have been carried out by surveying dentists around the world since all countries were affected by the pandemic (COVID-19 virus). In contrast, very few studies examined patients' satisfaction with services and knowledge of virus transmission and protection. Compliance with protocols by dentists results in higher patient satisfaction with services (14).

Factors affecting patient dissatisfaction include medical staff qualifications, work organization, and workplace cleanliness, especially during a pandemic. Patients' concerns about their health, especially among the elderly population, demand high-quality and impeccable dental services (15, 16).

In our country, communication with the population about virus transmission and protection protocols was mainly done through the public information system.

Nair et al. (16) report in their study that 98% of respondents had knowledge of the virus protection protocol. Their research result fully correlates with results in our research, where also 98% of respondents were familiar and only 2% were partially familiar with the protection protocols. The high level of knowledge shows that the pandemic-related advice and measures issued by the World Health Organization and conveyed through the media contributed to good knowledge of infection control and spread.

In his study, SAAD A. (5) reports that of the total respondents, 55.3% had higher education and also a high satisfaction level (73.4%). In terms of education, the highest percentage of respondents in our study (53.3%) had higher education, of which 43.7% were moderately satisfied, 37.5% were satisfied, and 18.8% were very satisfied with the services. Of the 44.2% with secondary education, the number of satisfied and moderately satisfied respondents was equal (37.7%), while 24.5% were very satisfied. Only 2.5% of respondents had primary education, of which 66.7% were satisfied, and 33.3% were moderately satisfied. These results indicate that higher education may influence a higher degree of patient satisfaction.

In the study by Paravie and Osmani conducted in Iran, 65% of respondents were familiar with the protocol, and 43.5% and 47.1% of respondents with primary education and from urban areas, respectively, were satisfied with the services. The overall frequency of satisfaction was 39.3% high level, 45.2% medium level, and 15.2% low level of satisfaction (9). Compared to this study, a smaller percentage of respondents were familiar with the protocol, hence the lower percentage of high-level satisfaction. Also, in our study, there were more respondents from the city (83.3%), 33% of whom were satisfied, 46% were moderately satisfied, and 21% were very satisfied with the services provided, while there were no dissatisfied respondents. Respondents from rural areas reported a lower degree of satisfaction than those from the city, which result is also a consequence of less frequent visits to the dentist. The general conclusion is that respondents from rural areas were less satisfied with the services provided than respondents from the city. They were not dissatisfied, but their level of satisfaction was somewhat lower than that of respondents from the city.

Wardani et al. (13) report in their study that 64% of patients visited a dentist during the COVID-19 pandemic 1-2 times a year. The results are similar to our study, where 60% of patients visited dental offices 1-2 times a year.

N. Gutierrez-Marin notes in her study that 74.7% of respondents were very satisfied and only 0.4% were dissatisfied, which is the result most similar to that of our study. The only difference is that in this study, there were no dissatisfied patients (17).

N. Sandy et al. report that 60.1% of patients had a high level of satisfaction, 20.08% had a moderate level of satisfaction, and 19.32% had a low level of satisfaction (18).

Most studies that have investigated patient satisfaction report that patients are generally satisfied with services. The first thing they notice during a visit is the appearance of offices, cleanliness, tidiness, and organization of patient appointments, giving the impression that the services will also be at a high level. The kindness and empathy of dentists and staff are essential, as in this way, patients' fear is overcome, and their trust is gained, contributing to a higher degree of satisfaction. A lower degree of satisfaction reported in studies can only further motivate dentists to improve their workspace and the quality of services and, therefore, achieve higher patient satisfaction.

CONCLUSION

Patients treated during the COVID-19 pandemic in the dental health care system in Niš, Serbia, were very satisfied with their treatments. A good knowledge of patient protection protocols for the COVID-19 virus raises awareness among dentists and patients to take better care of their own and others' health. The study revealed good knowledge of preventive measures; however, patient awareness of the risk of contracting other viral infections should be increased to help control transmission in dental institutions.

Abbreviations

COVID-19 - coronavirus disease of 2019

SARS-CoV-2 - Severe acute respiratory syndrome coronavirus 2

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Sažetak

ZADOVOLJSTVO PACIJENATA STOMATOLOŠKOM ZDRAVSTVENOM ZAŠTITOM TOKOM IZBIJANJA VIRUSA COVID-19 U SRBIJI

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Uvod: Virus Covid-19 spada u grupu respiratornih virusa. Stomatolozi zbog prirode posla su u većem riziku od zaražavanja novim korona virusom tokom stomatoloških intervencija. Poznavanje puta prenošenja virusa od velikog je značaja u prevenciji i suzbijanju razvoja infekcije. Strah pacijenata od zaražavanja tokom pandemije korona virusa bio je veliki i zbog toga su odlagane posete stomatologu zbog standardnih stomatoloških usluga već su se pacijenti javljali samo zbog hitnih stanja. Poštovanje mera zaštite direktno je uticalo na zadovoljstvo pacijenata stomatološkim uslugama.

Cilj ovog rada je bio ispitivanje zadovoljstva pacijenata stomatološkim uslugama tokom pandemije korona virusa.

Materijali i metode: Studija je dizajnirana kao studija preseka uz upotrebu epidemiološkog upitnika. Ukupno je anketirano 120 pacijenata, 60 iz privatne prakse i 60 iz državnog sektora. Upitnik je bio anonimnog karaktera i sadržao je pitanja sociodemografskog tipa i pitanja vezana za poznavanje protokola i ocenu zadovoljstva stomatološkim uslugama. Podaci su obrađeni u programu SPSS 11.5.

Rezulati: Od 120 ispitanika prosečne starosti 38,92 ± 13,08 godina, 98% je bilo upoznato sa protokolom zaštite od korona virusa. Nezadovoljnih nije bilo ni u privatnoj praksi ni u javnom sektoru, jedina razlika je u stepenu zadovoljstva. U privatnoj praksi je više bilo veoma zadovoljnih kao najviši nivo zadovoljstva, dok je u javnom sektoru bilo više veoma zadovoljnih.

Zaključak: Dobro poznavanje protokola zaštite i sprečavanja širenja virusa podiže svest kod stomatologa i pacijenata. Poštovanjem mera od strane stomatologa i pacijenata zaražavanje virusom svedeno je na minimum, a samim tim je zadovoljstvo pacijenata stomatološkim uslugama na višem nivou.

***Ključne reči**:* Covid-19, zadovoljstvo pacijenata, stomatologija.

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COULD WEARING FACE MASKS DURING THE PANDEMIC HAVE CREATED AN ENVIRONMENT FOR DEMODEX MITES?

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Abstract: **Background:** During the COVID-19 pandemic, wearing face masks became mandatory in our country to prevent the spread of the virus, as in many other countries.

Objective: The study aimed to examine whether wearing face masks during the pandemic created a microenvironment for *Demodex* mites.

Materials and Methods: The study included three groups: (i) those who wore N95/FFP2 masks, (ii) those who wore a 3-ply surgical mask, and (iii) a control group (who rarely wore masks). The age, gender, occupation, smoking status, alcohol consumption, past medical history, and face-washing habits were questioned, and dermatological examination was performed. The presence of Demodex mites was detected by the standard superficial skin biopsy (SSSB) from three regions of the face.

Results: Sociodemographic characteristics and the findings of dermatological examination of the three groups were similar ($p > 0.05$). There was no significant difference across the groups concerning the presence of Demodex mites in corresponding regions (the right cheek, nose, forehead) and corresponding numbers of Demodex mites (> 5 D/cm$^{2)}$; the total number of Demodex mites in each region; presence of Demodex mites in any area on the face, number of Demodex mites in any region > 5 D/cm^2 on the face, the total number of Demodex mites found on the face, and the presence of Demodex mites in the areas under the mask (the nose and the right cheek together) ($p > 0.05$).

Conclusion: We can conclude that wearing masks by healthy adults with no known skin diseases during the pandemic does not create a proper microenvironment for the lives of Demodex mites.

Keywords: COVID-19, *Demodex* mites, mask.

INTRODUCTION

COVID-19 has profoundly impacted the world, leading to millions of deaths and causing sequelae in various organs, particularly the lungs (1). To prevent the spread of the infection, which was declared a pandemic, wearing surgical masks was recommended and subsequently mandated in many countries (2). During the pandemic, N95/FFP2 and 3-ply surgical masks were among the most commonly worn types, with healthcare professionals, especially those treating COVID-19 patients, frequently using N95/FFP2 masks during work hours (3). These masks, especially N95/FFP2, provide a tighter fit to the face compared to three-ply surgical masks.

Various skin conditions like seborrheic dermatitis, acne vulgaris, acne rosacea, and contact dermatitis have been reported to worsen due to mask-wearing during the pandemic (3, 4). Demodex mites have also been implicated in exacerbating certain skin conditions post-mask-wearing (4, 5).

Demodex follicularum and Demodex brevis are the main species residing in seborrheic areas such as the chin, cheeks, nose root, and forehead, and they can be found in healthy individuals, contributing to some skin diseases (5). These mites feed on sebum and epithelial contents, with Demodex folliculorum located in the follicular infundibulum and Demodex brevis residing in the sebaceous duct and meibomian glands (5, 6). Factors like humidity, temperature, and acidic pH are favorable environments for these mites, and their presence increases significantly with age (7-10).

The presence of a single mite is considered Demodex positive, and having more than five parasites in an area of 1 cm^2 is considered sufficient to cause disease, although not all individuals with this parasite count develop symptoms (5, 11). Demodex mites are associated with the etiology of common skin diseases such as rosacea and seborrheic dermatitis (5).

Considering reports of an increase in demodex-related dermatoses due to mask-wearing, this study aims to investigate whether wearing masks by healthy in-

dividuals during the COVID-19 pandemic created an environment conducive to Demodex mites, compared with a control group.

MATERIAL AND METHODS

The study was conducted on March 15, 2021, and data collection was completed within 6 months. Participants were randomized after stratification by age using a simple randomization table. Exclusion criteria included having dermatosis (such as acne, acne rosacea, seborrheic dermatitis) or other dermatological diseases associated with Demodex mites, receiving or having received treatment for Demodex-associated dermatosis (topical and/or systemic), using face wash gel or cologne that prevents the life of Demodex mites, using cosmetic cream with pore-clogging features, applying disinfectant, being obese (BMI > 30), regularly consuming alcohol, receiving oral therapy that suppresses the immune system, or using creams that may affect the immune system when applied topically on the face, as well as developing any skin complaints with wearing masks.

Participants were categorized into three groups: (ı) those who wore N95/FFP2 masks during all working hours except for less than 1 hour; (ıı) those who wore a 3-ply surgical mask during all working hours except for less than 1 hour; and (ııı) people who wore a 3-ply surgical mask for less than 3 days a week, less than 2 hours a day, spending most of the time at home during the day, or those who worked alone all day in their rooms and wore a 3-ply surgical mask for less than 1 hour during the day as required. The third group represented the control group, consisting of individuals who presented to the hospital during the study period (as there were no individuals without masks during the day).

The sample size was calculated to be at least 48 people for each group, with a test power of approximately 80,151%. The study received approval from the Clinical Research Ethics Committee of Katip Çelebi University (approval number: 027, March 11, 2021) and was conducted following the principles of the Declaration of Helsinki. Participants provided informed consent before inclusion.

Sociodemographic characteristics including age, gender, and occupation, tobacco and alcohol use, past medical history, and face-washing habits were evaluated. Dermatological examination results, including skin type (oily or dry-normal) and Fitzpatrick skin typing, were also analyzed.

In order to detect the presence of Demodex mites, the standard superficial skin biopsy (SSSB) was performed onthe face. Dry sterile gauze was used to wipe the face after dropping cyanoacrylate, marking a 1 cm² area on the slide wiped with dry sterile gauze from three areas of the face: the right cheek, nose, and mid-forehead. After leaving the slide for 60 seconds and slowly removing it from the face, immersion oil was dripped onto the slide

The slide was covered with a coverslip, and Demodex mites were counted first under the light microscope at x10 and then x20 magnification (Demodex folliculorum and brevis were not differentiated). This process was repeated for each region. The presence of Demodex mites in each region and their number, if any, were recorded on the inquiry form.

SPSS 17.0 program was used for data analysis. Frequency and percentage values were determined for discontinuous variables, while mean and standard deviation values were calculated for continuous variables. Statistical analysis included the following tests: One-sample Kolmogorov-Smirnov test, Chi-Square test, Fisher's Exact Test and Kruskal-Wallis Test.

RESULTS

The study included a total of 144 people who met the inclusion criteria, with 48 people in each group. Table 1 shows the distribution of the sociodemographic characteristics of the participants and the comparison of these characteristics, including smoking status, alcohol use, past medical history, facial washing habits (only water, water and soap, non-specific face wash), findings of dermatological examination, skin type (oily or dry-normal skin), and data on the distribution of Fitzpatrick skin type, along with comparisons of these data.

Table 2 presents data and comparisons categorized according to the presence and number of Demodex mites obtained from the right cheek, nose, and forehead regions of the participants using the SSSD technique. It also includes the results of SSSD regarding whether the number of Demodex mites was 5 > D/cm².

DISCUSSION

During the pandemic, wearing masks became mandatory in many countries, including our own, from August 9, 2020, to April 22, 2022, with recommendations for their use in closed working areas, public transportation, and entertainment venues (1, 2). Healthcare workers in COVID-19 departments, those in close contact with suspected cases, mostly wore FFP2/N95 masks for better protection, while others, including patients, primarily used single 3-ply surgical masks (4, 12). Studies indicate that mask use may lead to the occurrence or worsening of various facial conditions (12-16), including acne vulgaris, perioral der-

Table 1. *Distribution of the sociodemographic characteristics of the participants*

	N95\FFP2 mask N (%)	**A 3-ply surgical mask N (%)**	**Controls N (%)**	**P**
Mean age (SD)*	36.50 (8.91)	38.65 (7.81)	35.04 (9.59)	0.19
Gender				
Female	33 (68.8)	33 (68.8)	33 (68.8)	1.00
Male	15 (31.2)	15 (31.2)	15 (31.2)	
Total	48 (100)	48 (100)	48 (100)	
Occupational group†				
Health worker (doctor, nurse, staff)	48(100)	48(100)	15 (31.5)	0,00
Housewife	0 (0)	0 (0)	24 (50)	
Other	0 (0	0 (0)	9 (18.75)	
Total	48(100)	48(100)	48(100)	
Smoking				
Yes	33 (61.8)	30 (62.5)	36 (75.0)	0.51
No	15 (31.3)	18 (37.5)	12 (25.0)	
Total	48 (100)	48 (100)	48 (100)	
Alcohol consumption				
Yes (social drinker)	29 (60.4)	37 (77.1)	36 (75.0)	0.14
No	19 (39.6)	11 (22.9)	12 (25.0)	
Total	48 (100)	48 (100)	48 (100)	
Face washing habit				
Water only	17 (35.4)	19 (36.6)	15 (31.3)	0.90
Water and soap	24 (50)	24 (50.0)	27 (56.3)	
Non-specific				
Face-wash product	7(14.6)	5 (10.4)	6 (12.5)	
Total	48 (100)	48 (100)	48 (100)	
The number of face washes per day				
Once	30 (62.8)	25 (52.1)	24 (50)	0.66
Twice	13 (27.1)	14 (29.2)	15 (31.3)	
Three times and more	5 (10.4)	9 (18.8)	9 (18.8)	
Total	48 (100)	48 (100)	48 (100)	
Skin characteristic				
Normal-Dry	17 35.4)	26 (54.2)	25 (52.1)	0.20
Oily	31 (64.6)	22 (45.8)	23 (47.9)	
Total	48 (100)	48 (100)	48 (100)	
Fitzpatrick Skin Type				
Type 2	16 (33.3)	24 50.0)	23 (47.9)	0.15
Type 3	32 (66.7)	24 (50.0)	25 (52.1)	
Total	48 (100)	48 (100)	48 (100)	

Participants had no past medical history.

* Age was evaluated with the one-sample Kolmogorov-Smirnov test ($p < 0.05$); since it was not consistent with the normal distribution, the Kruskal-Wallis test was used for analysis.

† Fisher's Exact Test used.

The Chi-square test was used for other analyses in the table.

Table 2. *Datas and analysis related to demodex mites by groups*

	N95\FFP2 mask N (%)	**A 3-ply surgical mask N (%)**	**Controls N (%)**	**P**
1	2	3	4	5
The presence of Demodex mites				
The right cheek				
No	30 (62.5)	25 (52.1)	29 (60,4)	0.54
Yes	18 (37.5)	23 (47.9)	19 (39.6)	
The nose				
No	40 (83.3)	34 (70.8)	38 (79.2)	0.32
Yes	8 (16.7)	14 (29.2)	10 (20.8)	
The forehead				
No	38 (79.2)	35 (72.9)	33 (68.8)	0.50
Yes	10 (20.8)	13 (27.1)	15 (31.3)	
The number of Demodex mites5>D/cm²				
The right cheek				
No	39 (81.3)	36 (75.0)	41 (85.4)	0.09
Yes	9 (18.8)	12 (25.0)	7 (%4.6)	
The Nose*				
No	47 (97.9)	45 (93,8)	47 (97,9)	0.67
Yes	1 (2.1)	3 (6.3)	1 (2.2)	
The Forehead*				
No	46 (95.8)	45 (93.8)	45 (93.8)	0.93
Yes		3 (6.3)	3 (6.3)	
The number of Demodex mites Mean (SD)†				
The right cheek	2.33 (4.06)	3.53 (5.06)	1.92 (3.56)	0.29
The Nose	0,52 (1,36)	0,98 (2,46)	0,56 (1,64)	0.34
The Forehead	0.85 (2.34)	1.23 (3.52)	1.08 (3.52)	0.57
The presence of *Demodex* mites on any part of the face (right cheek, nose, forehead)				
No	29 (60.4)	24 (50.0)	25 (52.1)	0.55
Yes	19 (39.6)	24 (50.0)	23 (47.9)	
The presence of *Demodex* mites on any part of the face (the right cheek, nose, forehead) **5 > D/cm²**				
No	37 (77.1)	31 (64.6)	40 (83.3)	0.90
Yes	11 (22.9)	17 (35.5)	8 (16.7)	
The total number of Demodex mites on areas of the face (the right cheek, nose, forehead)				
Mean (SD)†	3.71 (5.76)	5.71 (8.64)	3.54 (6.87)	0.48
The presence of *Demodex* mites under the mask on any part of the face (the right cheek, nose)				
No	29 (60.4)	25 (52.1)	28 (58.3)	0.69
Yes	19 (9.6)	23 (47.9)	20 (41.7)	

1	2	3	4	5
The presence of *Demodex* mites under the mask on any part of the face (the right cheek, nose) **5 > D/cm²**				
No	38 (79.2)	32 (66.7)	40 (83.3)	0.13
Yes	10 (20.8)	16 (33.3)	8 (16.7)	
The number of Demodex mites on areas under the mask (the right cheek, nose) **mean (SD)†**	2.85 (4.66)	4.47 (6.2)	2.45 (4.73)	0.46

*Fisher's Exact Test used

† The number of *Demodex* mites in each region of the face, and the total number of Demodex mites on the face, and the number of Demodex mites in the areas under the mask (right cheek and nose) were not consistent with the normal distribution when evaluated with the One-Sample Kolmogorov-Smirnov test ($p < 0.05$). The Kruskal-Wallis test was used to analyze these data. The chi-square test was used for other analyses in the Table.

matitis, and acne rosacea, which are associated with Demodex mites (5, 15, 16). Some dermatoses exacerbated by mask use have been linked to Demodex mites (17, 18).

Demodex mites preferentially lay their eggs in the deep parts of hair follicles or sebaceous glands and are more commonly found in oily skin, feeding on sebum. They thrive in moist, warm, and acidic environments (5-8). Implicated in various skin conditions such as rosacea, non-specific facial dermatitis, androgenetic alopecia, Demodex mites are associated with factors like age, genetics, immunosuppression, increased sebum production, UV exposure, and hygiene habits (5, 7, 9). Although they can live on human skin without symptoms, an increase in their numbers may lead to inflammation, although density alone may not be the sole factor (5, 11).

The standard superficial skin biopsy (SSSB) is a common diagnostic method used to detect Demodex mites in clinical settings (5, 10, 11). In our study, SSSB was used to detect Demodex mites. No significant differences were found among the three groups regarding sociodemographic characteristics, skin type, gender, smoking, alcohol use, face washing habits, or skin condition (Table 1), indicating similarity between the groups.

During the pandemic, various skin conditions associated with mask use have been reported, including perioral dermatitis, acne vulgaris, rosacea, eczema, contact dermatitis, and seborrheic dermatitis (13-16). The term "maskne" has even been coined to describe these conditions related to mask-wearing during Covid-19. Factors such as changes in skin microbiota, duration of mask-wearing, textile properties, friction, and occlusion have been implicated (19). Textile dyes and formaldehyde found in masks may also contribute to dermatoses (4, 19, 20).

Some studies have suggested an increase in Demodex mites associated with mask use (9, 20, 21). For instance, patients with acne have reported worsening of lesions with mask use, possibly due to an increase in Demodex mites along with other microorganisms (15, 16, 19). A prospective study investigating mask use and seasonal changes in individuals with rosacea and similar diseases found an association between these conditions, Demodex mites, and seasonal temperature (17).

Because Demodex mites thrive in a humid, warm, and acidic environment with increased sebum production, and wearing masks creates such an environment, our study aimed to investigate whether mask use contributes to the presence of Demodex mites (22). To our knowledge, no study has compared mask use and the presence of Demodex mites in healthy individuals without skin diseases with a control group.

We found no statistical difference across the three groups regarding the presence of Demodex mites on the right cheek, nose, and forehead; the number of Demodex mites exceeding 5 per square centimeter in these regions; the total number of Demodex mites on the face; or the presence of Demodex mites under the mask (Table 2). These findings suggest that mask use by healthy individuals without skin diseases does not create a favorable environment for Demodex mites.

In the context of our study, mask-wearing habits throughout the day, regardless of duration, might have influenced the presence of Demodex mites, potentially leading to no significant difference across the groups. Our control group consisted of individuals who predominantly stayed at home, wearing a surgical mask for less than 3 days a week, less than 2 hours a day or those who spent their entire day alone in their rooms. While we didn't find any statistically significant difference across the groups, we observed that individuals wearing a single 3-ply surgical mask had proportionally more Demodex mites in almost all analyses compared to those wearing N95/FFP2 masks and the control group (Table 2).

This discrepancy might be attributed to unfavorableconditions created by N95/FFP2 masks for Demodex mites. The tight grip of N95/FFP2 masks creates a CO2-rich environment, potentially affecting the respiration of Demodex mites (21). Additionally, chemicals like formaldehyde and textile dyes left on N95/FFP2 masks were considered harmful to Demodex mites (4, 19). Makeup materials were also reported to block pores and prevent Demodex mites from feeding (9). The tight fit of N95/FFP2 masks might have led to pore-clogging, further affecting Demodex mites. Moreover, individuals wearing N95/FFP2 masks were often healthcare workers in high-risk environments, using more disinfectants, which could have affected the life of Demodex mites due to chemical exposure through evaporation or residual contact.

While a relationship has been established between the number of Demodex mites and the occurrence of dermatosis, recent studies have emphasized the association between HLA groups and the role of individual immunological mechanisms in triggering diseases caused by Demodex mites. Furthermore, it is well-documented that the parasite reproduces more easily under conditions of local or systemic immunosuppression. The exacerbation of Demodex-related dermatoses following mask usage may be attributed to mechanisms influenced by mask-wearing, particularly in individuals sensitive to Demodex mites. It's worth noting that our study was conducted with healthy volunteers devoid of any skin disease. However, the exclusion of individuals with skin complaints during the period of mask-wearing could have influenced our study results.

Limitations

The control group consisted of individuals who presented to the hospital during the study period, as wearing a mask was mandatory in our country at that time.

CONCLUSION

In conclusion, wearing masks by healthy adults without known skin diseases during the pandemic did not create an environment conducive to Demodex mites.

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Note: Artificial intelligence was not utilized as a tool in this study.

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Sažetak

DA LI JE NOŠENJE ZAŠTITNIH MASKI TOKOM PANDEMIJE MOGLO STVORITI OKRUŽENJE POGODNO ZA DEMODEX GRINJA?

Doner Aktas Nurhan

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Uvod: Tokom pandemije COVID-19 nošenje zaštitnih maski postalo je obavezno u našoj zemlji radi sprečavanja širenja virusa, kao i u mnogim drugim zemljama.

Cilj: Cilj studije bio je ispitati da li je nošenje zaštitnih maski tokom pandemije stvorilo mikrookruženje pogodno za Demodex grinje.

Materijal i Metode: Studija je uključivala tri grupe: (i) osobe koje su nosile N95/FFP2 maske, (ii) osobe koje su nosile troslojnu hiruršku masku i (iii) kontrolnu grupu (osobe koje retko nose masku). Ispitivani su se godine starosti, pol, zanimanje, pušački status, konzumiranje alkohola, medicinska istorija kao i navike pranja lica, a obavljena je i dermatološka analiza. Prisustvo Demodex grinja otkriveno je standardnom površinskom biopsijom kože (SSSB) sa tri regije lica.

Rezultati: Sociodemografske karakteristike i nalazi dermatološkog pregleda tri grupe bili su slični ($p > 0,05$). Nije bilo značajne razlike između grupa u vezi sa prisustvom Demodex grinja na odgovarajućim regijama (desni obraz, nos, čelo) i odgovarajućim brojevima Demodex grinja (> 5 D/cm2); ukupan broj Demodex grinja u svakoj regiji; prisustvo Demodex grinja na bilo kojoj regiji lica, broj Demodex grinja u bilo kojoj regiji > 5 D/cm2 na licu, ukupan broj Demodex grinja prona-

đenih na licu i prisustvo Demodex grinja na područjima ispod maske (nos i desna obraz zajedno) (p > 0,05).

Zaključak: Možemo zaključiti da nošenje maski od strane zdravih odraslih osoba bez poznatih kožnih bolesti tokom pandemije ne stvara odgovarajuće mikrookruženje za Demodex grinje.

Ključne reči: COVID-19, Demodex grinje, maska.

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ITEM ANALYSIS OF MULTIPLE-CHOICE QUESTIONS IN AN UNDERGRADUATE SURGERY COURSE-AN ASSESSMENT OF AN ASSESSMENT TOOL

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Abstract: **Introduction:** In the field of medical education, multiple-choice questions (MCQs) represent the most commonly utilized method of assessment. It is necessary to analyze the assessment results through item analysis to ensure the quality is appropriate. This study evaluated the quality of the MCQs utilized for summative evaluation of the students in the General Surgery Course conducted in the year 2023-24, at the College of Medicine (Unaizah), Qassim University, Saudi Arabia.

Methods: Using a number of established parameters for item analysis, the study evaluated the multiple-choice questions for difficulty, discrimination power, and quality of distractors.

Results: The quality of the questions varied. The means of the facility index, discrimination index, discriminative efficiency, and distractor efficiency were, in order, 76.31%, 0.28, -0.7743, and 32%.

Conclusion: Item analysis is a crucial technique for evaluating the quality of MCQs. There were multiple defects in the MCQs used in summative assessments, revealing the scope for further improvement in future courses. It is important to plan faculty development events often to impart knowledge and skills related to creating MCQs that are valid, reliable, and of high quality.

Keywords: Assessment**,** Item analysis, Multiple-choice questions, Facility index, Discriminative index, Distractor efficiency, Non-functional distractor, Distractor analysis.

INTRODUCTION

Assessment is an essential process used in medical education to evaluate a student's clinical competency and capacity to meet predetermined learning objectives (1). The assessment tools are devised to enhance students' comprehension and learning while enabling them to identify their areas of weakness (2). From the teachers' perspective, the assessment outcomes provide evidence to uphold or alter the educational objectives and pedagogical approaches (3). To satisfy various evaluation demands, a wide variety of assessment methods are available, including multiple-choice questions (MCQs), the Objective Structured Clinical Exam (OSCE), short answer questions (SAQs), modified essay questions (MEQs), extended matching questions (EMQs), mini-clinical evaluation exercises (Mini-CEX), direct observation of procedural skills (DOPS), and viva-voce.

Whether an assessment is being used for formative (diagnosis, feedback, and improvement) or summative (promotion and certification) reasons will determine which method is best. There are several recognized attributes of assessment tools, including affordability, practicality, validity, reliability, and educational impact. Regardless of the reason, a single assessment method cannot evaluate every competency domain, and hence, a range of assessment methods is needed, allowing the benefits of one to offset the drawbacks of another (4).

MCQs are the most widely used assessment tool in medical education worldwide, and they come in many types. Type-A MCQ comprises two parts: a stem that states the question or lead-in; and a set of alternatives, or potential answers, which include a key, which is the best response to the question, and several distractors (three to four), which are reasonable but incorrect responses. MCQs are supposed to assess all levels of teaching objectives within Bloom's taxonomy of learning, including knowledge recall, comprehension, application, analysis, synthesis, and evalua-

tion. Making an excellent multiple-choice test (MCQ) can be difficult and time-consuming. Nevertheless, because answers to MCQs can be evaluated quickly and precisely, this approach is usually chosen over other evaluation tools due to its objectivity and reduction of human bias (5,6,7). A good-quality MCQ has key answers distributed throughout the test, thereby minimizing placement bias, and the key and distractors must be similar in length, style, and grammatical form. To audit the quality of the assessment, the MCQs are analyzed by a process termed item analysis (8).

The aim of this study is to evaluate the quality of the multiple-choice questions (MCQs) utilized in the surgery block during the academic year 2023-24 for the Year-4 undergraduate medical students at the College of Medicine, Qassim University, Saudi Arabia, as part of their summative assessment.

MATERIAL AND METHODS

A cross-sectional study was conducted at the Department of Surgery, College of Medicine (Unaizah branch), Qassim University, Saudi Arabia, during the academic session 2023-24. The college was established in 2012 and has adopted a team-based learning (TBL) curriculum for undergraduate medical training, leading to the award of an MD degree upon successful completion. The assessment is based on the curricular contents related to the intended learning objectives, and multiple-choice questions (MCQs) are one of the tools utilized for assessment in the surgery course.

The study analyzed the results of the MCQs used in the summative assessment of 38 male students in Year 4 of the medical undergraduate course, conducted at the end of 8 weeks of teaching. The MCQs were analyzed for:

i. Their level of difficulty, measured by the facility index (P),

ii. Power of discrimination, measured by the discrimination index (DI), and

iii. Distractor analysis, measured by distractor efficiency (DE).

There were 50 items, and the time allotted for each item was 2 minutes (total time: 100 minutes). Each item was of the one-best type, having a single stem and five answer options, one of them being correct and the other four being 'distractors.' The test was conducted paperless on a computer, and students were required to log in through their university-allotted usernames and passwords. Every correct response was awarded 1 mark, and there was neither a mark nor a negative mark for any blank or incorrect response. Thus, the maximum and minimum attainable scores for the test were 50 and 0, respectively. The test was criterion-referenced, and passing standards were expressed in absolute terms, with a passing score of 60%. For the test, 70% of MCQs were newly constructed, and 30% were taken after modification from the question bank created from the tests conducted over the previous five years.

The scores of the students were arranged in descending order, and then the DI was determined using Kelley's technique, which takes into account the difference between the scores achieved by 27% of students on the higher side (high achievers) and those of the 27% on the lower side (low achievers). Calculations of the values of P and DI were undertaken by the application of the formulae depicted in Table 1.

Table 1. *Formulae for calculation of facility index (P) & discrimination index (DI) of MCQs*

Facility Index & Discrimination Index Calculations	
Facility Index (P) = [(H+L) / N] x 100	**Inference** *: Less than 30%, the MCQ is very difficult.* *Greater than 70%, the MCQ is easy.* *30% to 70%, the MCQ lies within an acceptable range.*
Discrimination Index (DI) = 2 x [(H-L) / N]	**Inference** : The DI measures the differences obtained in correct responses between the higher achievers and the lower achievers. The calculated value ranges between 0 and 1. The higher the DI, the more the test item can discriminate better between students with higher and lower test scores. Accordingly, if the value is: ***1.*** *0.19 or less, the MCQ has poor discrimination.* ***2.*** *Between 0.2 and 0.29, the MCQ has acceptable discrimination.* ***3.*** *Between 0.3 and 0.4, the MCQ has good discrimination.* ***4.*** *Greater than or equal to 0.4, the MCQ has excellent discrimination.*
• *N is the sum total of the students in both high and low groups.* • *H and L respectively stand for the number of correct responses in the high and low groups.* *MCQs that attained a P between 30 - 70 and DI > 0.24 were termed* ***'ideal'****.*	

The distractors in the MCQ are measures of its functioning. When a distractor is chosen by more than 5% of the examinees, it is considered a functioning distractor (FD), and if chosen by less than 5%, it is termed a non-functioning distractor (NFD). On the basis of the number of NFDs in an MCQ, distractor efficiency (DE) ranged from 0 to 100%, as shown in Table 2.

Table 2. *Relationship of Distractor Efficiency (DE) with the Number of Non-Functional Distractors (NFDs)*

Non-Functional Distractors (NFDs)	Distractor Efficiency (DE)
4	0
3	25
2	50
1	75
0	100

RESULTS

The item analysis of the surgery course multiple-choice question (MCQ) exam revealed important findings regarding the quality of the assessment tool. There were 50 five-option MCQs, with a single key and four distractors in each. Thirty-two students appeared in the examination and attained grades ranging from 16.3 to 50 out of the maximum of 50, with a mean score of 40.47 ± 8.08, as shown in Table 3. There were only 12 MCQs (24%) with the facility index (FI) lying in the desired range of 30–70%. Thirty-five MCQs had a facility index above 70%, hence qualifying as easy to solve. The examination demonstrated an adequate level of discrimination overall between high- and low-performing students, as 40 (80%) MCQs had a positive discrimination index (DI). However, since only 21 (42%) MCQs had good or excellent discrimination, there is significant scope for improvement.

Tables 4 and 5 show the item analysis of distractors for MCQs 1-25 and MCQs 26-50 respectively, while Table 6 displays their overall psychometric analysis. This analysis pointed towards a deficiency in the design of the distractors, as depicted in the following data:

i. Seventeen (34%) MCQs had 4 non-functioning distractors (NFDs) and hence, a distractor efficiency (DE) percentage of 0%.

ii. Twelve (24%) MCQs had 3 NFDs and hence, a DE percentage of 25%.

iii. Twelve (24%) MCQs had 2 NFDs and hence, a DE percentage of 50%.

iv. Eight (16%) MCQs had 1 NFD and hence, a DE percentage of 75%.

v. One (2%) MCQ had no NFD and hence, a DE percentage of 100 %.

Table 3. *Item analysis report*

Serial Number	Parameter	Results
1.	Exam Median score	42
2.	Exam Average Score (Mean)	40.47
3.	Standard Error of Measurement	1.4
4.	Standard Deviation (SD)	8.08
5.	Minimum Score	16.3
6.	Maximum Score	50
7.	Total Difficulty	76.31%
8.	MCQs with Facility Index < 30%	3
9.	MCQs with Facility Index > 70%	35
10.	MCQs with –ve discrimination	5
11.	MCQs with zero discrimination	5
12.	MCQs with +ve discrimination Index < 0.19	13
13.	MCQs with +ve discrimination Index = 0.2-0.29	6
14.	MCQs with +ve discrimination Index = 0.3-0.39	3
15.	MCQs with +ve discrimination Index >=0.4	18

Table 4. *Distractor Analysis (MCQs 1-25)*

Serial	Key Answer	Number and percentage of students selecting the options					NFDs
		A	B	C	D	E	
1.	*C*	1 (3.13%)	21 (65.63%)	5 (15.63%)	3 (9.38%)	2 (6.25%)	1
2.	*D*	0 (0.00%)	4 (12.50%)	0 (0.00%)	23 (71.88%)	5 (15.63%)	2
3.	*B*	1 (3.13%)	16 (50.00%)	10 (31.25%)	2 (6.25%)	3 (9.38%)	1
4.	*B*	0 (0.00%)	32 (100.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	4
5.	*B*	0 (0.00%)	23 (71.88%)	5 (15.63%)	2 (6.25%)	2 (6.25%)	1
6.	*C*	1 (3.13%)	0 (0.00%)	30 (93.75%)	1 (3.13%)	0 (0.00%)	4
7.	*C*	7 (21.88%)	15 (46.88%)	10 (31.25%)	0 (0.00%)	0 (0.00%)	2
8.	*A*	31 (96.88%)	1 (3.13%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	4
9.	*A*	29 (90.63%)	1 (3.13%)	1 (3.13%)	1 (3.13%)	0 (0.00%)	4
10.	*E*	2 (6.25%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	30 (93.75%)	3
11.	*C*	3 (9.38%)	2 (6.25%)	24 (75.00%)	3 (9.38%)	0 (0.00%)	1
12.	*B*	0 (0.00%)	32 (100.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	4
13.	*B*	0 (0.00%)	30 (93.75%)	0 (0.00%)	1 (3.13%)	1 (3.13%)	4
14.	*D*	2 (6.25%)	0 (0.00%)	1 (3.13%)	29 (90.63%)	0 (0.00%)	3
15.	*B*	0 (0.00%)	30 (93.75%)	0 (0.00%)	0 (0.00%)	2 (6.25%)	3
16.	*A*	0 (0.00%)	0 (0.00%)	1 (3.13%)	0 (0.00%)	31 (96.88%)	4
17.	*B*	0 (0.00%)	29 (90.63%)	3 (9.38%)	0 (0.00%)	0 (0.00%)	3
18.	*E*	10 (31.25%)	0 (0.00%)	2 (6.25%)	5 (15.63%)	15 (46.88%)	1
19.	*D*	0 (0.00%)	0 (0.00%)	0 (0.00%)	31 (96.88%)	1 (3.13%)	4
20.	*E*	2 (6.25%)	3 (9.38%)	0 (0.00%)	0 (0.00%)	27 (84.38%)	2
21.	*A*	25 (78.13%)	2 (6.25%)	1 (3.13%)	4 (12.50%)	0 (0.00%)	2
22.	*A*	31 (96.88%)	0 (0.00%)	1 (3.13%)	0 (0.00%)	0 (0.00%)	4
23.	*D*	5 (15.63%)	0 (0.00%)	0 (0.00%)	27 (84.38%)	0 (0.00%)	3
24.	*D*	0 (0.00%)	1 (3.13%)	1 (3.13%)	22 (68.75%)	8 (25.00%)	3
25.	*C*	8 (25.00%)	0 (0.00%)	22 (68.75%)	0 (0.00%)	2 (6.25%)	2

Table 5. *Distractors analysis (MCQs 26-50)*

Serial	Key Answer	Number and percentage of students selecting the options					NFDs
		A	B	C	D	E	
26.	*E*	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	32 (100.00%)	4
27.	*B*	2 (6.25%)	1 (3.13%)	20 (62.50%)	8 (25.00%)	1 (3.13%)	2
28.	*B*	30 (93.75%)	0 (0.00%)	0 (0.00%)	2 (6.25%)	0 (0.00%)	3
29.	*D*	0 (0.00%)	13 (40.63%)	0 (0.00%)	17 (53.13%)	2 (6.25%)	2
30.	*B*	1 (3.13%)	30 (93.75%)	0 (0.00%)	0 (0.00%)	1 (3.13%)	4
31.	*B*	5 (15.63%)	25 (78.13%)	2 (6.25%)	0 (0.00%)	0 (0.00%)	2
32.	*B*	2 (6.25%)	22 (68.75%)	2 (6.25%)	5 (15.63%)	1 (3.13%)	1
33.	*D*	11 (34.38%)	1 (3.13%)	1 (3.13%)	19 (59.38%)	0 (0.00%)	3
34.	*B*	0 (0.00%)	31 (96.88%)	1 (3.13%)	0 (0.00%)	0 (0.00%)	4
35.	*E*	0 (0.00%)	2 (6.25%)	1 (3.13%)	1 (3.13%)	28 (87.50%)	3
36.	*B*	14 (43.75%)	10 (31.25%)	3 (9.38%)	3 (9.38%)	2 (6.25%)	0
37.	*A*	29 (90.63%)	0 (0.00%)	1 (3.13%)	2 (6.25%)	0 (0.00%)	3

38.	*D*	22 (68.75%)	2 (6.25%)	0 (0.00%)	8 (25.00%)	0 (0.00%)	2
39.	*C*	1 (3.13%)	0 (0.00%)	31 (96.88%)	0 (0.00%)	0 (0.00%)	4
40.	*A*	13 (40.63%)	0 (0.00%)	0 (0.00%)	17 (53.13%)	2 (6.25%)	2
41.	*C*	0 (0.00%)	1 (3.13%)	31 (96.88%)	0 (0.00%)	0 (0.00%)	4
42.	*C*	0 (0.00%)	0 (0.00%)	28 (87.50%)	2 (6.25%)	2 (6.25%)	2
43.	*D*	2 (6.25%)	3 (9.38%)	4 (12.50%)	23 (71.88%)	0 (0.00%)	1
44.	*C*	0 (0.00%)	1 (3.13%)	23 (71.88%)	5 (15.63%)	3 (9.38%)	2
45.	*B*	0 (0.00%)	30 (93.75%)	0 (0.00%)	1 (3.13%)	1 (3.13%)	4
46.	*C*	1 (3.13%)	5 (15.63%)	26 (81.25%)	0 (0.00%)	0 (0.00%)	3
47.	*C*	2 (6.25%)	2 (6.25%)	24 (75.00%)	3 (9.38%)	1 (3.13%)	1
48.	*D*	1 (3.13%)	3 (9.38%)	0 (0.00%)	28 (87.50%)	0 (0.00%)	3
49.	*D*	0 (0.00%)	1 (3.13%)	0 (0.00%)	30 (93.75%)	1 (3.13%)	4
50.	*D*	0 (0.00%)	0 (0.00%)	0 (0.00%)	32 (100.00%)	0 (0.00%)	4

Table 6. *Psychometric Analysis of Multiple-Choice Questions*

Serial Number	***Facility Index***	***Discrimination Index***	***Discriminative Efficiency***	***Distractor Efficiency***	***Serial Number***	***Facility Index***	***Discrimination Index***	***Discriminative Efficiency***	***Distractor Efficiency***
1	15.63%	0.17	33.33%	75%	***26***	100.00%	0%	0%	0%
2	71.88%	0.39	48.39%	50%	***27***	62.50%	0.19	22.94%	50%
3	*50.00%*	*0.17*	*2.16%*	*75%*	***28***	*0.00%*	*0%*	*0%*	*25%*
4	*100.00%*	*0%*	*0%*	*0%*	***29***	*53.13%*	*0.54*	*68.83%*	*50%*
5	*71.88%*	0.67	83.85%	75%	***30***	93.75%	0.22	37.10%	0%
6	93.75%	0.78	13.51%	0%	***31***	78.13%	0.48	61.15%	50%
7	31.25%	-0.04	-5.68%	50%	***32***	68.75%	0.23	28.02%	75%
8	96.88%	-0.09	-17.37%	0%	***33***	59.38%	0.56	69.26%	25%
9	90.63%	0.52	79.26%	0%	***34***	96.88%	0.04	7.34%	50%
10	93.75%	-0.01	-2.21%	25%	***35***	87.50%	0.34	48.94%	25%
11	75.00%	0.28	34.14%	75%	***36***	31.25%	0.15	21.09%	50%
12	100.00%	0%	0%	0%	***37***	90.63%	0.19	28.06%	25%
13	93.75%	0.55	95.91%	0%	***38***	68.75%	0.20	25.40%	50%
14	90.63%	0.52	79.26%	25%	***39***	96.88%	-0.06	-11.20%	0%
15	93.75%	0.55	95.91%	25%	***40***	53.13%	0.59	75.89%	50%
16	96.88%	-0.06	-11.20%	0%	***41***	96.88%	0.04	7.34%	0%
17	90.63%	0.52	81.68%	25%	***42***	87.50%	0.57	84.66%	50%
18	46.88%	0.15	20.22%	75%	***43***	71.88%	0.11	13.76%	75%
19	96.88%	0.04	7.34%	0%	***44***	71.88%	0.45	56.41%	50%
20	84.38%	0.45	61.34%	50%	***45***	93.75%	0.48	83.63%	0%
21	78.13%	0.47	59.38%	50%	***46***	81.25%	0.30	38.96%	25%
22	96.88%	0.49	100.00%	0%	***47***	9.38%	0.19	44.99%	75%
23	84.38%	0.26	34.82%	25%	***48***	87.50%	0.63	92.33%	25%
24	68.75%	0.13	15.79%	25%	***49***	93.75%	0.29	50.90%	0%
25	68.75%	0.19	23.18%	50%	***50***	100.00%	0%	0%	0%

Mean Values: Facility Index 76.31%; Discrimination Index 0.28; Discriminative Efficiency -0.774; Distractor Efficiency 32%.

DISCUSSION

Assessment is an important component of medical education that, when executed appropriately, indicates whether or not students have achieved the intended learning goals (9). Instructional and curriculum modifications are also influenced by the assessment results (10). A variety of methods are available to assess knowledge, but multiple-choice questions (MCQs) are currently one of the most popular options because of their ease of use, impartiality, consistency, simplicity in administration, and capacity to cover a larger range of subject matter. MCQs can reveal information on students' comprehension, knowledge, and analytical abilities, allowing for the identification of both the strengths and flaws in their grasp of a subject. It has been shown that, when properly constructed, MCQs can assess higher-order cognitive domains such as synthesis and application, in addition to discriminating between students' individual abilities (11). On the other hand, if a test has a greater rate of errors, it tends to be less reliable and penalizes participants (12). As a result, it's critical to have robust distractors, rationally sound keys, and an effective stem that integrates the various learning levels and the directive verbs that go along with them within each learning domain (8).

Even though creating multiple-choice questions (MCQs) seems easy, it takes a lot of work and time to design them correctly, especially for faculty members who have never undergone dedicated training in assessment methodology (13). MCQs present a number of design challenges, such as confusing stems, multiple correct answers, contentious answers, give-away keys, poor distractors, and a high likelihood of predicting the right responses. Nonetheless, item analysis can offer the relevant data required to raise the caliber and efficacy of MCQs. As shown in Tables 3-5, our study identified a number of weaknesses regarding the degree of difficulty, the quality of distractions, and discrimination. The number of non-functional distractors (NFDs) in MCQs was of particular concern. The negative and zero discrimination index implied, contrary to expectations, that low-performing students accurately recognized the MCQ's key more often than or as frequently as good performers. This could be the result of an incorrect key, imprecise item phrasing, or possibly deficient student preparation (13).

To maintain fairness and the integrity of the test, various actions were initiated as per the academic regulations to neutralize the impact of defective items with a negative or zero discrimination index, including dropping out or modification of the questions. The shortcomings identified by item analysis have been discussed in many other published studies, and a high percentage of items with writing flaws (IWFs) and non-functional distractors (NFD) have been demonstrated (7, 14). Chauhan et al. (15) reported 1, 2, 3, and 4 NFDs with rates of 7.69%, 30.77%, 60.00%, and 1.54%. The percentage of 0, 1, 2, and 3 NFDs was 65.00%, 25.00%, 10.00%, and 0.00%, respectively, in Patel's (16) item analysis, where the MCQs included only three distractors. Similarly, Mahjabeen et al. (17) reported the figures at 25.00%, 46.00%, 25.00%, and 5.00%, respectively. The distractor effectiveness of the MCQs in this study is 32%, which is significantly lower than the values in the literature. Rao et al. (18), Gajjar et al. (19), and Patel (16) have achieved values as high as 90%, 89.6%, and 84.9%, respectively. In a study by Lama et al. (20), 43.3% of MCQs used in summative assessment of undergraduate dental students had a poor discrimination index (≤ 0.2), and distractor efficiency was 100 in only 6%. About 37% of the MCQs were either very difficult or very easy and hence inappropriate. The item analysis of MCQs used in the assessment of ophthalmology block in undergraduate medical courses found 50% to be defective, with bad stems or distractors being the prime culprits (21).

In a recent study by Baste (22), the correlation of the actual difficulty level of MCQs in the physiology block of undergraduate medical students as derived by item analysis was compared with the difficulty level as perceived by the faculty. It was found that the correlation between the actual and perceived difficulty level was poor, even though the enrolled faculty in the study were experienced. It was concluded that mere experience does not assure the accuracy of the perceived difficulty level and that item analysis needs to be properly conducted after every MCQ-based assessment. The study further revealed underutilization of item analysis of MCQs, and the reasons provided by the faculty included lack of motivation, involvement in difficult calculations, staff shortage, and lack of skills.

In a multidisciplinary and integrated curriculum, designing MCQs consumes a lot of time and is usually a difficult undertaking. Hence, there are recommendations about the disposal of MCQs found defective in item analysis. Bhat and Prasad (21) suggest such MCQs be analyzed to detect the item writing flaws and then optimized into a viable question. In their study, 16 out of 40 (40%) MCQs were either very easy or very difficult on the basis of item analysis, and after proper edits, 15 MCQs were salvaged for entry into the question bank.

The study's findings concur with the recommendation in the literature (21, 22) that faculty members should more frequently attend faculty development programs aimed at improving their ability to construct

excellent multiple-choice questions and create viable question banks. Numerous studies have demonstrated that carefully designed, longitudinal faculty development workshops improve the writing abilities of multiple-choice questions (MCQs) in terms of discriminating and difficulty indices, as demonstrated by the cognitive levels of Bloom's taxonomy, decreased item writing errors, and increased functioning distractors (13, 23, 24, 25).

CONCLUSION

Properly constructed multiple-choice questions (MCQs) are an objective and reliable tool to assess the learning performance of students. Item analysis is an important activity that must be properly conducted after MCQ tests in order to assess the level of difficulty and their capacity to distinguish between good and weak students. The results of this analysis have the potential to identify the sections of the course material that require revision or adjustment. Item analysis identified an array of weaknesses with our multiple-choice questions that required rectification as per the rules. Regular faculty development activities are required so that the MCQ constructors know how to correctly interpret the item-analysis data and, on that basis, undertake meaningful steps to improve the quality of questions, thereby achieving the objective of holding valid, effective, and fair tests. Only the question items with a good difficulty index, acceptable discrimination power, and zero non-functional distractors should be utilized for student promotion and retained in the question bank for possible reuse.

Abbreviations

MCQ - Multiple-choice question
NFD - Non-functioning distractor
DE - Distractor efficiency
DI - Discrimination index
P - Facility Index
IWF -Item writing flaw

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Sažetak

ANALIZA STAVKI PITANJA SA VIŠESTRUKIM IZBOROM NA DODIPLOMSKOM ISPITU IZ HIRURGIJE - PROCENA ALATA ZA OCENJIVANJE

Al Sulaim Sulaiman Lamees, Salati Ahmad Sajad

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Uvod: U oblasti medicinskog obrazovanja, pitanja sa višestrukim izborom (MCQs) predstavljaju najčešće korišćeni metod procene. Neophodno je analizirati rezultate putem analize stavki kako bi se osigurao odgovarajući kvalitet. Ova studija je procenila kvalitet MCQ-ova koji se koriste za sumativnu procenu studenata na ispitu iz opšte hirurgije sprovedenih tokom 2023-24. godine na Medicinskom fakultetu (Unaizah), Univerziteta Qassim u Saudijskoj Arabiji.

Metode: Koristeći nekoliko uspostavljenih parametara za analizu stavki, studija je procenila pitanja sa višestrukim izborom u pogledu težine, diskriminatorne moći i kvaliteta distraktora.

Rezultati: Kvalitet pitanja je varirao. Srednje vrednosti indeksa težine, indeksa diskriminacije, efikasnosti diskriminacije i efikasnosti distraktora bile su, redom, 76.31%, 0.28, -0.7743 i 32%.

Zaključak: Analiza stavki je ključna tehnika za procenu kvaliteta MCQ-ova. Postojale su višestruke greške u MCQ-ovima korišćenim u sumativnim procenama, što otkriva prostor za dalje poboljšanje u budućim kursevima. Važno je redovno organizovati edukacije nastavnog osoblja kako bi se prenela potrebna znanja i veštine za kreiranje MCQ-ova koji su validni, pouzdani i visokog kvaliteta.

Ključne reči: Procena, Analiza stavki, Pitanja sa višestrukim izborom, Indeks težine, Indeks diskriminacije, Efikasnost distraktora, Neefikasan distraktor, Analiza distraktora.

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Original article

HAEMATOLOGICAL VARIATIONS ASSOCIATED WITH ALCOHOL CONSUMPTION IN A NIGERIAN UNIVERSITY COMMUNITY

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Abstract: **Introduction:** Alcohol consumption is widespread, contributing to approximately 3 million deaths worldwide. This study aimed to assess haematological indices among alcohol consumers compared to non-consumers in the University of Calabar Community, Nigeria.

Materials and Methods: This case-control study enrolled 100 subjects (50 alcohol consumers and 50 non-consumers) from the University of Calabar community. Blood samples were collected aseptically for Full Blood Count and Thrombin Time analysis. Rigorous study design, meticulous data collection, and thorough analysis ensured the reliability of our findings.

Results: The mean corpuscular volume (MCV) (76.21 ± 7.35 fl), mean corpuscular haemoglobin (MCH) (25.31 ± 3.26 pg), Platelet (PLT) (154.38 ± 54.89 x 10^9) and Platelet Crit (PCT) (0.14 ± 0.04%) of alcohol consumers were significantly lower than that of the controls respectively (79.36 ± 5.76 fl, 26.47 ± 2.15 pg, 187.22 ± 58.34 x 10^9 and 0.17 ± 0.04%) ($p < 0.05$). At the same time, the red cells distribution width (RDWCV), Platelet distribution width (PDW), and thrombin time (TT) varied significantly among alcohol consumers (15.14 ± 1.05%, 14.91 ± 0.43fl and 15.22 ± 2.53s) respectively than in the controls (14.74 ± 0.95%, 14.76 ± 0.29 fl and 12.70 ± 1.52s). Other parameters were comparable ($p > 0.05$) between the two groups of subjects studied. A significant negative correlation ($r = 0.801$, $p = 0.001$) was observed between Thrombin time and Plateletcrit in the test subjects. In contrast, a significant positive correlation ($r = 0.698$, $p = 0.001$) was observed between Mean Corpuscular Haemoglobin Concentration and Haemoglobin in alcohol consumption. A significant negative correlation ($r = 0.973$, $p = 0.001$) was observed between lymphocytes and granulocytes in percentage in the test subjects. These significant findings provide valuable insights into the haematological variations associated with alcohol consumption.

Conclusion: These significant differences observed in this study underscore the impact of alcohol consumption on haematological indices. The study concluded that derangements in some haematology parameters occur in alcoholics, which can affect their quality of life.

Keywords: Haematology, Alcohol consumers, Platelet count.

INTRODUCTION

Alcohol is a chemical substance known for its ability to induce intoxication and alter mental and physical functions when consumed (1). The American Society of Addiction Medicine (ASAM) defines alcoholism as a primary chronic disease influenced by genetic, psychosocial, and environmental factors. This condition is often progressive and fatal, characterized by continuous or periodic impaired control over drinking despite adverse consequences and cognitive distortions, most notably denial (2). Similarly, Godwin defines alcoholism as a compulsion to drink, causing harm to oneself and others (3).

Alcohol is a psychoactive substance with dependence-producing properties and has been widely used across various cultures and ethnic groups for many years. The harmful use of alcohol in society today is significant, leading to a burden of diseases and illness-

es, as well as substantial social and economic consequences. High rates of problems are associated with family members, friends, and co-workers of individuals with alcohol use disorder (4, 5).

The consumption of alcohol predisposes individuals to various diseases, accidents, domestic violence, and other health-related conditions. These include liver cirrhosis, cardiovascular diseases, certain carcinomas, heart diseases, and irregular heartbeat. Alcohol also affects the pancreas, immune system, and can lead to mental and behavioral impairments (6, 7). Alcoholism often reduces a person's life expectancy by around ten years, with cardiovascular complications being the most common cause of death among alcoholics (8, 9).

It has been reported that there is a high rate of suicide among chronic alcohol consumers, which increases with the duration of alcohol exposure. Approximately 3–15% of alcoholics die by suicide, and research has found that over 50% of all suicides are associated with alcohol or drug dependence (10, 11).

This is believed to be due to alcohol causing physiological distortion of brain chemistry and social isolation. Suicide is also very common among adolescent alcohol abusers, with 25% of adolescent suicides being related to alcohol abuse (12).

Drinking during pregnancy can result in foetal alcohol spectrum disorders. Women are generally more sensitive than men to the harmful effects of alcohol, primarily due to their smaller body weight, lower capacity to metabolize alcohol, and a higher proportion of body fat (13). Alcohol consumption is widespread, with about 3 million deaths worldwide attributed to it. In 2016, a global burden of disease study estimated that Nigeria is one of the countries with the highest prevalence of current alcohol use among adults 15 years and older in Sub-Saharan Africa (5).

Haematological parameters, including red blood cells, white blood cells, and haemoglobin concentrations, are widely used as clinical indicators of health and disease (14, 15). This study aims to provide information useful to healthcare practitioners on the dangers of alcohol consumption and its management.

MATERIALS AND METHODS

Study Area

This study was conducted between July and December 2023 at the University of Calabar Community, Calabar, located in Cross River State in the South-South Geographical Zone of Nigeria.

Study Design

A case-control study design was adopted for this study. Demographic information was obtained and documented from the study subjects through interviews.

Sampling

A purposive sampling method was adopted for this study.

Selection of Subjects

A total of 100 subjects between the ages of 15 and 65 were enrolled for this study. Fifty (50) were alcohol consumers, and fifty (50) were non-alcohol consumers, including both males and females. Informed consent was duly obtained from all participants. For the minors who participated in the study, consent to obtain blood samples was obtained from their parents.

Inclusion Criteria

Both alcohol consumers and control subjects who gave their informed consent were recruited for this study.

Exclusion Criteria

Both alcohol consumers and control subjects who did not give their informed consent were excluded from this study. Subjects with a history of liver disease, viral hepatitis, renal disease, and haematology malignancies were excluded from participating in the study.

Ethical Consideration

Ethical clearance was sought and obtained from the Cross River State Ministry of Health's Ethical Committee with the number REC No: CRSMOH/HRP/REC/2023/416. A well-structured questionnaire was administered, and their consent was obtained to participate in the study.

Sample Collection

Five (5) milliliters of blood were collected from the antecubital vein of the subjects after properly disinfecting the venepuncture site with 70% ethanol. Three (3) milliliters were dispensed into an Ethylene Diamine Tetra Acetic Acid (EDTA) bottle with a final concentration of 2mg/ml for Full Blood Count (FBC) determination. Two (2) milliliters were dispensed into a tri-sodium citrate anticoagulated bottle with a concentration of 3.13% for thrombin time determination.

Sample Handling, Storage, and Processing

The samples were transported to the laboratory in an ice pack cooler. The samples were stored in a refrigerator at 4-8°C for full blood count, and those for thrombin time were spun at 3000 rpm. The plate-

let-poor plasma was separated from the whole blood and stored refrigerated.

The full blood count sample was processed using a haematology auto-analyser (BC—2800 Mindray). The thrombin time samples were analyzed using a manual one-stage quick method with the Helena thrombin Kit purchased in Lagos, Nigeria.

Assay of Parameters

Determination of Full Blood Count

Full Blood Count Samples were determined using the Automated Haematology analyzer Mindray BC-2800, UK.

Determination of Thrombin Time

Thrombin time samples were analyzed manually using a water bath at 37°C on the STA-Compact. The fibrinogen concentration in plasma was determined quantitatively by the Clauss clotting method, which involves measuring the rate of fibrinogen-to-fibrin conversion in diluted samples under the influence of excess thrombin.

Statistical Analysis

The Statistical Package for Social Science (SPSS) version 20.0 was used for the analysis. The data were summarized as percentages, charts, mean, standard deviation, and Student's t-test. The results were presented in tables and figures. Pearson's correlation was used to correlate the variables of alcohol consumers, with a significance level set at $P \leq 0.05$.

RESULTS

This research examined some haematological parameters of alcohol consumers and non-alcohol consumers who served as control groups in the University of Calabar Community. The parameters assayed were Full Blood Count and thrombin time. A total of 100 subjects, 64 males and 36 females, were enrolled for the study.

Table 1 shows the demographic data of alcohol consumers and non-alcohol consumers. The majority of the alcohol consumers were between ages 15-25 years, while the majority of the controls were between ages 26-35 years. There were more male alcohol consumers than females, with a frequency of 40 (80.0%) males and 10 (20.0%) females, respectively. In contrast, non-alcohol consumers were 24 (48.0%) males and 26 (52.0%) females. Single individuals participated more in both groups: 38 (76.0%) alcohol consumers and 44 (88.0%) non-alcohol consumers, compared to married individuals, who comprised 12 (24.0%) alcohol consumers and 5 (10.0%) non-alcohol consumers. Additionally, only 1 (2.0%) non-alcohol consumer was cohabiting.

Tertiary education had higher attendance in both groups: 34 (68.0%) alcohol consumers and 43 (86.0%) non-alcohol consumers, compared to secondary institutions: 16 (32.0%) alcohol consumers and 7 (14.0%) non-alcohol consumers.

Students participated more in both groups, with 28 (56.0%) alcohol consumers and 39 (78.0%) non-alcohol consumers, followed by business owners. The least participants were academic and non-academic staff alcohol consumers, 3 (6.0%) respectively.

Among alcohol consumers, those who consumed alcohol for 1 to 5 years were the majority, 27 (54.0%), followed by those who drank for 11 years and above, 16 (32.0%), and the least were those who drank for 6 to 10 years, 7 (14.0%). Consumers of spirits were 18 (36.0%), followed by other types of alcohol at 32.0%, and the least were red wine and palm wine consumers, 8 (16.0%) each. Those who changed their brands of alcohol were 39 (78.0%), more than those who did not, 11 (22.0%). Consumers of alcohol with 42% alcohol content were the most, 12 (24.0%), followed by those with 5% and 17.5% alcohol content, 10 (20.0%) each, and the least were those with 8% (8 (16.0%)), 65% (3 (6.0%)), and other percentages (3%, 25%, 35%, and 45% (1 (2.0%) each)).

Table 2 shows the comparison of full blood count and thrombin time between alcohol consumers and controls. The mean corpuscular volume (MCV) was significantly lower ($P = 0.01$) in alcohol consumers compared to controls, and the mean corpuscular haemoglobin (MCH) was significantly lower ($P = 0.03$) in alcohol consumers. The red cell distribution width (RDWCV) was significantly higher ($P = 0.04$) in alcohol consumers compared to controls. The platelet count (PLT) was significantly lower ($P = 0.01$) in alcohol consumers compared to the control group. The platelet distribution width (PDW) was significantly higher ($P = 0.05$) in alcohol consumers compared to the control group. Plateletcrit (PCT) was significantly lower ($P = 0.01$) in alcohol consumers compared to controls. Meanwhile, the thrombin time (TT) was significantly higher ($P = 0.01$) in alcohol consumers compared to the control group. All other haematological parameters were comparable between the two groups.

Figure 1 shows the distribution of alcohol and non-alcohol consumers based on ethnicity. Figure 2 shows a significant negative correlation ($r = 0.801$, $p = 0.001$) between thrombin time and plateletcrit. Figure 3 shows a significant positive correlation ($r = 0.698$, $p = 0.001$) between mean corpuscular haemoglobin concentration and haemoglobin. Figure 4 shows a sig-

Table 1. *Demographic data of alcoholics and apparently healthy non-alcoholics in the University of Calabar Community*

Demographics	Alcoholics	Non-alcoholics
Age range		
15-25	24 (48.0%)	22 (44.0%)
26-35	17 (34.0%)	25 (50.0%)
36-45	6 (12.0%)	2 (4.0%)
46-65	3 (6.0%)	1 (2.0%)
Gender		
Male	40 (80.0%)	24 (48.0%)
Female	10 (20.0%)	26 (52.0%)
Marital status		
Singles	38 (76.0%)	44 (88.0%)
Married	12 (24.0%)	5 (10.0%)
Cohabiting	0 (0.0%)	1 (2.0%)
Level of education		
Secondary	16 (32.0%)	7 (14.0%)
Tertiary	34 (68.0%)	43 (86.0%)
Occupation		
Students	28 (56.0%)	39 (78.0%)
Business owners	16 (32.0%)	10 (20.0%)
Academic staff	3 (6.0%)	0 (0.0%)
Non-academic	3 (6.0%)	1 (2.0%)
Duration of consumption (years)		
1-5	27 (54.0%)	0 (0.0%)
6-10	7 (14.0%)	0 (0.0%)
11 & above	16 (32.0%)	0 (0.0%)
Brand of alcohols		
Beer	16 (32.0%)	0 (0.0%)
Red wine	8 (16.0%)	0 (0.0%)
Spirit	18 (36.0%)	0 (0.0%)
Palm-wine	8 (16.0%)	0 (0.0%)
Ever changed brand of alcohol		
Yes	39 (78.0%)	0 (0.0%)
No	11 (22.0%)	0 (0.0%)
Percentage of alcohol in a brand		
3 (%)	1 (2.0%)	0 (0.0%)
5%	10 (20.0%)	0 (0.0%)
6%	2 (4.0%)	0 (0.0%)
8%	8 (16.0%)	0 (0.0%)
17.5%	10 (20.0%)	0 (0.0%)
25%	1 (2.0%)	0 (0.0%)
35%	1 (2.0%)	0 (0.0%)
42%	12 (24.0%)	0 (0.0%)
45%	1 (2.0%)	0 (0.0%)
65%	3 (6.0%)	0 (0.0%)

Table 2. *Mean values of haematological parameters of alcoholics and non-alcoholics*

Parameters	Alcoholics n = 50	Non-alcoholics n = 50	p-value < 0.05
Total WBC	5.71 ± 1.83	5.53 ± 1.41	0.57
Lymphocyte	2.70 ± 0.91	2.54 ± 0.74	0.35
Mid L/L	0.54 ± 0.19	0.55 ± 0.16	0.70
Gran L/L	2.47 ± 1.07	2.43 ± 0.91	0.85
Lymph %	47.23 ± 8.26	46.33 ± 8.13	0.58
Mid %	9.91 ± 1.99	10.36 ± 2.86	0.36
Gran %	42.84 ± 8.40	43.29 ± 8.99	0.79
Haemoglobin g/l	133.72 ± 27.83	135.60 ± 17.49	0.68
Red blood cells l/l	5.24 ± 0.88	5.11 ± 0.70	0.41
Haematocrit l/l	0.40 ± 0.07	0.40 ± 0.50	0.86
MCV fl	76.21 ± 7.35	79.36 ± 5.76	0.01*
MCH Pg	25.31 ± 3.26	26.47 ± 2.15	0.03*
MCHC g/dl	33.10 ± 1.72	33.39 ± 0.99	0.31
RDWCV %	15.14 ± 1.05	14.74 ± 0.95	0.04
RDWSD %	41.47 ± 3.64	42.54 ± 3.29	0.12
Platelet	154.38 ± 54.89	187.22 ± 58.34	0.00*
MPV	9.53 ± 0.04	9.30 ± 0.56	0.10
PDW	14.91 ± 0.43	14.76 ± 0.29	0.05*
PCT	0.14 ± 0.04	0.17 ± 0.04	0.00*
TT	15.22 ± 2.53	12.70 ± 1.52	0.00*

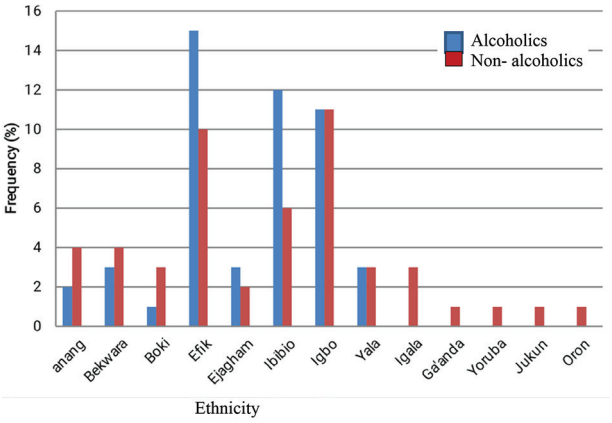

Figure 1. *Distribution of alcohol consumers and non-alcohol consumers based on Ethnicity*

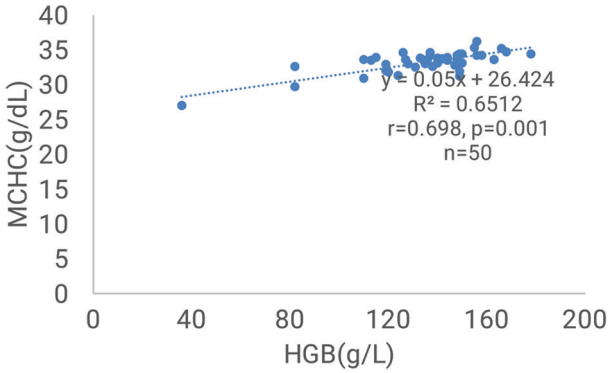

Figure 3. *Correlation of mean corpuscular haemoglobin concentrations (MCHC) and Haemoglobin (HGB) of Alcohol Consumers*

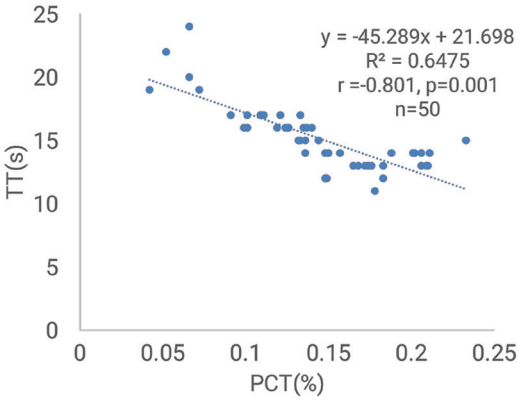

Figure 2. *Correlation of Thrombin Time and Plateletcrit of Alcohol Consumers*

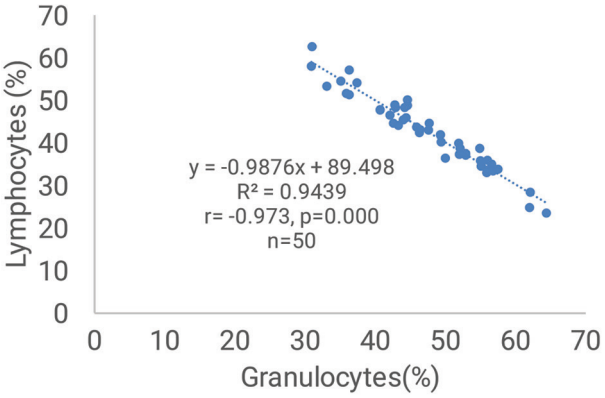

Figure 4. *Correlation of Lymphocytes (%) and Granulocytes (%) of alcohol consumers*

nificant negative correlation (r = 0.973, p = 0.001) between lymphocytes and granulocytes percentages.

DISCUSSION

The findings of this research have shown that alcohol consumption affects the Full Blood Count and Thrombin time of alcohol consumers. Recent studies have reported that no amount of alcohol use is beneficial to the body and also the associated health risks. Its use among university students is a severe cause of concern. In this study, more males were found to consume alcohol than females; this could be a result of peer pressure, cultural upbringing, or environmental influences. This aligns with a study by Ajayi et al. (4). Students who were between 15 and 25 years old had a higher prevalence of alcohol use. This may be because this age group signifies a period of adulthood; it is a time when many young adults experiment and try new things, including alcohol consumption. Also, this age group is subjected to so many societal pressures and the zeal to attain success, which may lead them to embrace the use of alcohol. Many of the students in this study were, in one way or another, dependent on their parents for their livelihood and mostly had small sources of income.

There is a higher prevalence of alcohol use among consumers of cultural origin who are Efiks, followed by Igbos and Ibibios. This could be due to their frequent engagement in cultural and religious activities. Similar findings were observed in a study by Olashore et al. (16) in Nigeria.

The distribution of marital status among alcohol consumers in this study was majorly singles; this may be attributed to the young age of the alcohol consumers since the majority were between the ages of 15-25 years. Additionally, the study reveals a higher representation of tertiary education among alcohol consumers, and a similar case was observed between the controls. This study showed that the majority of the consumers had taken alcohol for 1-5 years, and mostly, about 18(36.0%) of the consumers prefer spirit and 16 (32.0%) beer. About 39 (78.0%) of alcohol consumers in this study had changed from one brand of alcohol to another and preferred brands with alcoholic content of about 42.0% being about 12 (24.0%). Data obtained from this study has shown that the duration of alcohol consumption has a statistically significant difference when compared to alcohol consumers.

The complete blood count and Thrombin Time of alcohol and non-alcohol consumers were compared. A comparison of the haematological parameters of alcohol consumers with non-alcohol consumers reveals a statistically significantly lower mean corpuscular volume (MCV). However, this observation disagrees with the findings of a study conducted by Ballard (17), who reported a significant increase in the MCV of alcohol consumers but agreed with the work of Okafor et al. (18). A substantial decrease in the mean cell haemoglobin (MCH) was observed in this study. This does not align with a similar study by Jain et al. (19) in India. Also, in this study, there was a statistically significant decrease in some platelet parameters of alcohol consumers. This observed decrease may be a result of the effect of the long duration of consumption of alcohol since most of the consumers have been exposed for about 1-5 years. Similar findings were observed in previous studies carried out by Quraish et al. (20) and Etura et al. (21), who reported that the effect of prolonged alcohol exposure can stimulate some level of inflammatory response in the test subjects. Also, there was a significant decrease in the red cell distribution width (RDWCV). A significant decrease was observed in the Plateletcrit (PCT) of alcohol consumers and a substantial increase in the thrombin time (TT) of alcohol consumers, which could be due to the effect of alcohol on the clotting factors.

Similarly, there was a statistically significant strong negative correlation between thrombin time and Platelet Crit. The reason for this is not clearly understood, but it implies that long-term alcohol consumption may alter Thrombin time and Plateletcrit.

CONCLUSION

The findings of this study reveal a significant decrease in Mean Corpuscular Volume and Mean Corpuscular Haemoglobin, as well as in some platelet parameters (platelet count, platelet distribution width, and plateletcrit) of alcohol consumers. Additionally, the Red Cell Distribution Width Coefficient of Variation and Thrombin time of alcohol consumers were significantly increased. These significant differences underscore the impact of alcohol consumption on haematological indices. The study concludes that derangements in some haematological parameters occur in alcoholics, which can affect their quality of life.

Abbreviations

ASM - American Society of Addiction Medicine
FBC - Full Blood Count
HCT - Haematocrit
HGB - Haemoglobin
MCH - Mean Corpuscular Haemoglobin
MCHC - Mean Corpuscular Haemoglobin Concentration
MCV - Mean Corpuscular Volume
MPV - Mean Platelet Volume

PCT - Platelet Crit

PDGF - Platelet-Derived Growth Factor

PDW - Platelet Distribution Width

PLT - Platelet

RBC - Red Blood Cells

RDWCV - Red Cells Distribution Width Coefficient of Variation

RDWSD - Red Cells Distribution Width Standard Deviation

TT - Thrombin Time

WBC - White Blood Cells

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Note: Artificial intelligence was not utilized as a tool in conducting this study.

Sažetak

HEMATOLOŠKE VARIJACIJE POVEZANE SA KONZUMIRANJEM ALKOHOLA U UNIVERZITETSKOJ ZAJEDNICI U NIGERIJI

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Uvod: Konzumiranje alkohola je široko rasprostranjena i dovodi do otprilike 3 miliona smrtnih slučajeva širom sveta. Ova studija je imala za cilj da proceni hematološke pokazatelje kod onih koji konzumiraju alkohol u poređenju sa onima koji ne konzumiraju alkohol u zajednici Univerziteta u Kalabaru, Nigerija.

Materijali i metode: Ova case-control studija uključila je 100 ispitanika (50 potrošača alkohola i 50 nekonzumenata) iz zajednice Univerziteta u Kalabaru. Krvni uzorci su aseptično prikupljeni radi analize kompletne krvne slike i trombinskog vremena. Rigorozan dizajn studije, pedantno prikupljanje podataka i temeljna analiza osigurali su pouzdanost naših rezultata.

Rezultati: Prosečni volumen eritrocita (MCV) (76.21 ± 7.35 fl), prosečna količina hemoglobina u eritrocitima (MCH) (25.31 ± 3.26 pg), trombociti (PLT) (154.38 ± 54.89 x 109) i trombokrit (PCT) (0.14 ± 0.04%) kod konzumenata alkohola bili su značajno niži u odnosu na kontrolnu grupu, redom (79.36 ± 5.76 fl, 26.47 ± 2.15 pg, 187.22 ± 58.34 x 109 i 0.17 ± 0.04%) ($p < 0.05$). Istovremeno, koeficijent varijacije širine distribucije eritrocita (RDWCV), širina distribucije volumena trombocita (PDW) i trombinsko vreme (TT) značajno su varirali kod konzumenata alkohola (15.14 ± 1.05%, 14.91 ± 0.43fl i 15.22 ± 2.53s) u poređenju sa kontrolama (14.74 ± 0.95%, 14.76 ± 0.29fl i 12.70 ± 1.52s). Ostali parametri su bili slični ($p > 0.05$) između dve grupe ispitanika. Primećena je značajna negativna korelacija ($r = 0.801$, $p = 0.001$) između trombinskog vremena i trombokrita kod ispitanika. Nasuprot tome, primećena je značajna pozitivna korelacija ($r = 0.698$, $p = 0.001$) između srednje koncentracije hemoglobina u eritrocitu i hemoglobina kod konzumenata alkohola. Takođe je primećena značajna negativna korelacija ($r = 0.973$, $p = 0.001$) između limfocita i granulocita izražene procentima kod ispitanika. Ovi značajni nalazi pružaju dragocene uvide u hematološke varijacije povezane sa konzumacijom alkohola.

Zaključak: Ove značajne razlike uočene u ovom istraživanju naglašavaju uticaj konzumacije alkohola na hematološke pokazatelje. Studija zaključuje da dolazi do poremećaja u nekim hematološkim parametrima kod alkoholičara, što može uticati na njihov kvalitet života.

Ključne reči: hematologija, konzumenti alkohola, broj trombocita.

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Original article

THE PROGNOSTIC AND PREDICTIVE VALUE OF KI-67 PROLIFERATION INDEX AND uPA/PAI-1 COMPLEX IN SERUM FOR PATIENTS WITH EARLY INVASIVE BREAST CANCER

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Abstract: **Introduction:** Breast cancer, the most common malignancy in women, represents a significant health issue, and biomarkers such as the Ki-67 index and uPA/PAI-1 complex can provide insight into treatment outcomes and therapeutic response.

Objective: The primary outcome of the study was the assessment of 5-year disease-free survival (DFS), defined as the postoperative period until the occurrence of loco-regional or distant metastases and death from any cause.

Patients and Methods: A retrospective cohort study included 166 patients with early invasive breast cancer, in whom the prognostic and predictive significance of the uPA/PAI-1 complex and Ki-67 biomarkers in surgically treated patients at the Clinic for General and Abdominal Surgery of the University Clinical Center in Sarajevo was evaluated during the period from September 2015 to February 2017.

Results: Univariate regression analysis identified an increased probability of DFS shorter than five years in patients with negative hormone receptors, positive HER-2 receptor, ≥ 8 positively mph nodes, and a Ki-67 index ≥ 14% ($p < 0.05$). Multivariate regression analysis revealed that T2 stage, tumor size of 20-50 mm, and a Ki-67 index ≥ 14% were associated with a higher probability of DFS shorter than five years ($p < 0.05$). The five-year DFS rate was higher in patients with a Ki-67 index < 14% compared to those with ≥ 14% ($p = 0.011$), while there was no difference in five-year DFS among patients with different levels of the uPA/PAI-1 complex ($p = 0.636$).

Conclusion:Our study highlights the importance of the Ki-67 proliferative index as a strong prognostic and predictive factor for DFS in patients operated on for early invasive breast cancer. Additional monitoring and tailored therapeutic strategies may be beneficial in patients with elevated Ki-67 index values, T2 stage, and tumor size of 20-50 mm.

Keywords: biomarkers, general surgery, treatment outcome, women's health.

INTRODUCTION

Breast cancer is the most common cancer in women worldwide and represents a significant public health issue, being the fifth leading cause of cancer death in the developed world (1, 2).

Early invasive breast cancer, which includes stages T1, T2, N0, N1, and T3N0, can be genetically analyzed to classify into four main molecular subtypes: Luminal A, Luminal B, HER2-positive, and "basal-like" or "triple-negative" (3, 4). The Ki-67 antigen proliferative index, a marker of cell proliferation in breast cancer, shows positive protein expression in all phases of the cell cycle (except the G0 phase), with its elevated expression being associated with an increased risk of disease recurrence and a reduced response to systemic therapy (5).

However, due to the lack of standardized laboratory analysis methodology and clear cut-off values for the application of systemic therapy, the Ki-67 proliferative index has not yet been accepted as a universal biomarker for breast cancer prognosis (6).

The occurrence of metastasesis the main cause of mortality in breast cancer, with extracellular matrix degradation playing a key role in this process, facilitated

by the urokinase plasminogen activator (uPA) and plasminogen activator inhibitor-1 (PAI-1) complex (7, 8).

Due to its clinical relevance, the uPA/PAI-1 complex determined in tumor tissue or cytosol has been recognized as a prognostic and predictive biomarker for breast cancer, as confirmed by the recommendation of the American Society of Clinical Oncology (9).

In today's medical practice, increasing emphasis is placed on the prognostic-predictive value of genetic panels that play a key role in the individualization of treatment and improvement of treatment outcomes in various diseases, including breast cancer (10). However, in transitional countries, these genetic markers are not yet widely accepted and available in practice (11, 12, 13).

Existing short comings and controversies underscore the need to investigate the role of the Ki-67 proliferative index and uPA/PAI-1 complex in serum and their integration into the existing concept of prognosis and prediction in patients with early invasive breast cancer.

Aim

The aim of our studywas to investigate the prognostic and predictive significance of Ki-67 proliferative index values and preoperative levels of the uPA/PAI-1 complex in serum in patients operated on for early invasive breast cancer, to contribute to the improvement of their treatment efficacy.

PATIENTS AND METHODS

Our prospective-retrospective cohort study included 166 patients older than 18 years with pathologically verified early invasive breast cancer, surgically treated at the Department of General and Abdominal Surgery, Clinical Center of the University of Sarajevo (CCUS), from September 2015 to February 2017. This study was approved by the Ethical Committee of CCUS. All procedures were in accordance with the institutional and national research committee's ethical standards and with the 1964 Helsinki Declaration and its later amendments.

Patients without cutaneous manifestations of the disease and those without previous premalignant or malignant breast diseases were included. Additionally, patients with a negative history of immune, chemo, radio, and hormonal therapies, as well as those without previous breast or axillary lymph node surgeries, were included.

Patients with advanced forms of cancer, including infiltration and inflammation of the breast skin region, and those with multiple breast cancers were excluded from the study. Patients with systemic liver, kidney, or cardiovascular diseases, as well as those who didn't provide informed consent to participate in the study, were also excluded.

Surgical treatment involved radical modified mastectomies or breast-conserving surgeries. Furthermore, complete dissection of the first and second layers of ipsilateral axillary lymph nodes or sentinel lymph node biopsy was performed (14).

Laboratory tests were conducted at the Clinical Biochemistry with Immunology Department of CCUS. The preoperative concentration of the uPA/PAI-1 complex in serum ranged from 0.1 to 100 ng/ml according to the manufacturer's instructions (15).

Pathohistological analysis was performed at the Clinical Pathology, Cytology, and Human Genetics Department of CCUS. The thres hold value to distinguish "high" and "low" Ki-67 proliferation index was set at 14% (16).

Disease-free survival (DFS) was defined as the postoperative time until the occurrence of locoregional or distant metastases and death from any cause, expressed in months.

Patient follow-up included five-year monitoring through annual mammographic and clinical examinations, following the standard protocol for early invasive breast cancer (17).

IBM SPSS Statistics version 22.0 for Windows was used for statistical analysis. The Chi-square (X^2) test was used to examine the association between variables. Univariate and multivariate regression analyses were applied to assess the independent and adjusted effects of the predictors of DFS, respectively. Receiver operating characteristic (ROC) analysis was used to evaluate the discriminative power of uPA/PAI-1 markers in predicting DFS. Kaplan-Meier analysis was used to evaluate the assessment of five-year DFS according to Ki-67 index and uPA/PAI-1 complex values. The most important results were presented in the form of tables and figures.

RESULTS

Univariate regression analysis revealed that patients with negative estrogen receptors (OR = 2.89; p = 0.040; 95% CI: 1.050, 7.975), negative progesterone receptors (OR = 2.91; p = 0.022; 95% CI: 1.170, 7.261), positive human epidermal growth factor receptor 2 (HER-2) (OR = 0.349; p = 0.029; 95% CI: 0.136, 0.897), eight or more positive lymph nodes (OR = 0.148; p = 0.004; 95% CI: 0.041, 0.537), those without Luminal A tumors (OR = 3.67; p = 0.008; 95% CI: 1.410, 9.599), and a Ki-67 index ≥ 14% (OR = 3.301; p = 0.014; 95% CI: 1.117, 0.787) had a higher likelihood of DFS shorter than five years. No statistically significant association was found between other predictors and five-year DFS, as shown in Table 1.

Table 1. *Representation of predictors of five-year disease-free survival and the results of univariate regression analysis*

Variables		N (%)	5Y-DFS		P*	95% CI*
			Achieved 144 (86.7)	Not Achieved 22 (13.3)		
			N (%)	N (%)		
Age	< 45 years	19 (11.4)	16 (84.2)	3 (15.8)	0.729	(0.336; 4.747)
	45 years and above	147 (88.6)	128 (87.1)	19 (12.9)		
Menstrual status	Premenopause	25 (15.2)	22 (88.0)	3 (12.0)	0.832	(0.237; 3.185)
	Postmenopause	140 (84.8)	121 (86.4)	19 (13.6)		
Tumor stage	T1 stage	74 (44.6)	65 (87.8)	9 (12.2)	0.710	(0.338; 2.209)
	T2 stage	92 (55.4)	79 (85.9)	13 (14.1)		
Tumor size (mm)	0.1-19.9 mm	78 (47.0)	71 (91.0)	7 (9.0)	0.132	(0.185; 1.247)
	20-50 mm	88 (53.0)	73 (83.0)	15 (17.0)		
Estrogen receptor	Negative	27 (16-3)	20 (74.1)	7 (25.9)	0.040	(1.050; 7.975)
	Positive	139 (83.7)	124 (89.2)	15 (10.8)		
Progesteron receptor	Negative	54 (32.5)	42 (77.8)	12 (22.2)	0.022	(1.170; 7.261)
	Positive	112 (65.7)	102 (91.1)	10 (8.9)		
HER-2 receptor	Negative	129 (77.7)	116 (89.9)	13 (10.1)	0.129	(0.136; 0.897)
	Positive	37 (22.3)	28 (75.7)	9 (24.3)		
Lymph nodes	Negative	83 (50.0)	76 (91.6)	7 (8.4)	0.073	(0.161; 1.085)
	1-3 positive lymph nodes	51 (30.7)	43 (84.3)	8 (15.7)	0.539	(0.291; 1.906)
	4-7 positive lymph nodes	21 (12.7)	19 (90.5)	2 (9.5)	0.592	(0.329; 7.031)
	8 or more positive lymph nodes	11 (6.6)	6 (54.5)	5 (45.5)	0.004	(0.441; 0.537)
Molecular subtypes	Luminal A	98 (59.0)	91 (92.9)	7 (7.1)	0.008	(1.410; 9.599)
	Luminal B, HER-2 positive	16 (9.6)	14 (87.5)	2 (12.5)	0.926	(0.228; 5.097)
	Luminal B, HER-2 negative	25 (15.1)	19 (76.0)	6 (24.0)	0.093	(0.141; 1.164)
	HER-2 positive	9 (5.4)	6 (66.7)	3 (33.3)	0.085	(0.064; 1.193)
	Triple negative	18 (10.8)	14 (77.8)	4 (22.2)	0.243	(0.485; 1.634)
uPA/PAI-1 complex levels	0-0.99 ng/ml	35 (21.1)	29 (82.9)	6 (17.1)	0.203	(0.653; 1.258)
	1-1.99 ng/ml	93 (56.0)	80 (86.0)	13 (14.0)		
	2-2.99 ng/ml	33 (19.9)	30 (90.9)	3 (9.1)		
	3 ng/ml or above	5 (3.0)	5 (100)	0 (0.0)		
Ki-67 index	< 14%	144 (86.7)	119 (90.2)	13 (9.8)	0.014	(0.117; 0.787)
	14 % or above	34 (13.3)	25 (73.5)	9 (26.5)		

*Univariate regression analysis for 5Y-DFS

5Y-DFS, Five-year disease-free survival; CI, Confidence interval; HER-2, Human epidermal growth factor receptor 2; Ki-67, Antigen Kiel 67; uPA/PAI-1, Urokinase plasminogen activator/plasminogen activator inhibitor-1

Table 2. *Multivariate regression analysis of predictors of five-year disease-free survival*

		5Y-DFS*	
		P	95% CI
Age	< 45 years	0.564	(0.220; 6.053)
	45 years and above		
Menstrual status	Premenopause	0.585	(0.081; 4.128)
	Postmenopause		
Tumor stage	T1 stage	0.009	(1.991; 2.622)
	T2 stage		
Tumor size (mm)	0.1-19.9 mm	0.005	(0.007; 0.413)
	20-50 mm		
Estrogen receptor	Negative	0.994	(0.080; 2.773)
	Positive		
Progesteron receptor	Negative	0.961	(0.229; 4.057)
	Positive		
HER-2 receptor	Negative	0.508	(0.195; 2.244)
	Positive		
Lymph nodes	Negative	0.070	(0.957; 3.044)
	1-3 positive lymph nodes		
	4-7 positive lymph nodes		
	8 or more positive lymph nodes		
Molecular subtypes	Luminal A	0.366	(0.638; 3.387)
	Luminal B, HER-2 positive		
	Luminal B, HER-2 negative		
	HER-2 positive		
	Triple negative		
uPA-PAI-1 complex levels (ng/ml)	0-0.99 ng/ml	0.178	(0.239; 1.303)
	1-1.99 ng/ml		
	2-2.99 ng/ml		
	3 ng/ml or above		
Ki-67 index (%)	< 14%	0.031	(0.097; 1.152)
	14 % or above		

*Multivariate regression analysis for 5Y-DFS

5Y-DFS, Five-year disease-free survival; CI, Confidence interval; HER-2 receptor, Human epidermal growth factor receptor 2; Ki-67, Antigen Kiel 67; uPA/PAI-1, Urokinase plasminogen activator/plasminogen activator inhibitor-1

In multivariateregression analysis, patients with T2 stage tumors (OR = 0.302; p = 0.009; 95% CI: 1.991, 2.622), tumor size of 20-50 mm (OR = 0.304; p = 0.005; 95% CI: 0.007, 0.413), or Ki-67 index ≥ 14% (HR = 0.292; p = 0.031; 95% CI: 0.097, 1.152) had a significantly higher likelihood of DFS shorter than five years. Multivariate regression analysis did not demonstrate statistically significant predictive roles of other variables for five-year DFS, as shown in Table 2.

ROC analysis revealed a low discriminatory power of uPA/PAI-1 markers for predicting five-year DFS (AUC = 0.472; p = 0.675; 95% CI: 0.340, 0.605), as depicted in Figure 1.

For patients with a Ki-67 index < 14%, the estimated DFS was 48.08 months, while for those with a Ki-67 index ≥ 14%, it was 44.03 months, with a statistically significant difference demonstrated by the Log-rank test (X^2 = 7.08; p = 0.008). The five-year DFS rate for patients with a Ki-67 index < 14% was 90.2%, while for those with a Ki-67 index ≥ 14%, it was 73.5%, with a statistically significant difference (p = 0.011), as shown in Figure 2A.

No statistically significant difference in estimated DFS was found among patients with different levels of uPA/PAI-1 markers by the Log-rank test (X^2 = 1.706; p = 0.636). The five-year DFS rates for different marker

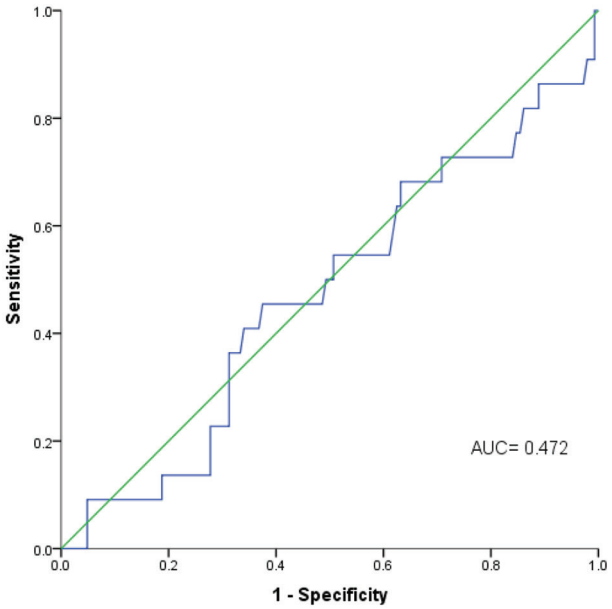

Figure 1. *ROC Curve for uPA-PAI-1 complex in predicting five-year disease-free survival*

ROC, Receiver operating characteristic; uPA/PAI-1, Urokinase plasminogen activator/plasminogen activator inhibitor-1

levels were as follows: 0-0.99 ng/ml (82.9%), 1-1.99 ng/ml (86.0%), 2-2.99 ng/ml (90.9%), and 3 ng/ml and above (100.0%), with no statistically significant difference (p = 0.623), as shown in Figure 2B.

DISCUSSION

Our study analyzed the prognostic and predictive significance of the Ki-67 proliferation index and preoperative values of the uPA/PAI-1 complex in serum in patients with early invasive breast cancer. Unlike the uPA/PAI-1 complex in serum, the Ki-67 proliferation index proved to be a significant prognostic-predictive factor for DFS in these patients.

Patients with negative estrogen receptors have a statistically significantly higher risk for a shorter DFS, likely due to biologically more aggressive tumors and reduced benefit from hormonal therapy (18, 19, 20). Negative progesterone receptors are also associated with an increased risk of shorter DFS, emphasizing the importance of hormonal signaling (21). The HER-2 hormonal expression system plays a crucial role in therapy selection and response intensity but is associated with a higher risk of unfavorable outcomes (22, 23). Axillary lymph node analysis is crucial for accurately determining disease stage and adjusting therapy, especially in patients with multiple positive lymph nodes (24-27). The Luminal A tumor subtype of breast cancer typically responds positively to hormonal therapy, which may contribute to longer DFS, particular-

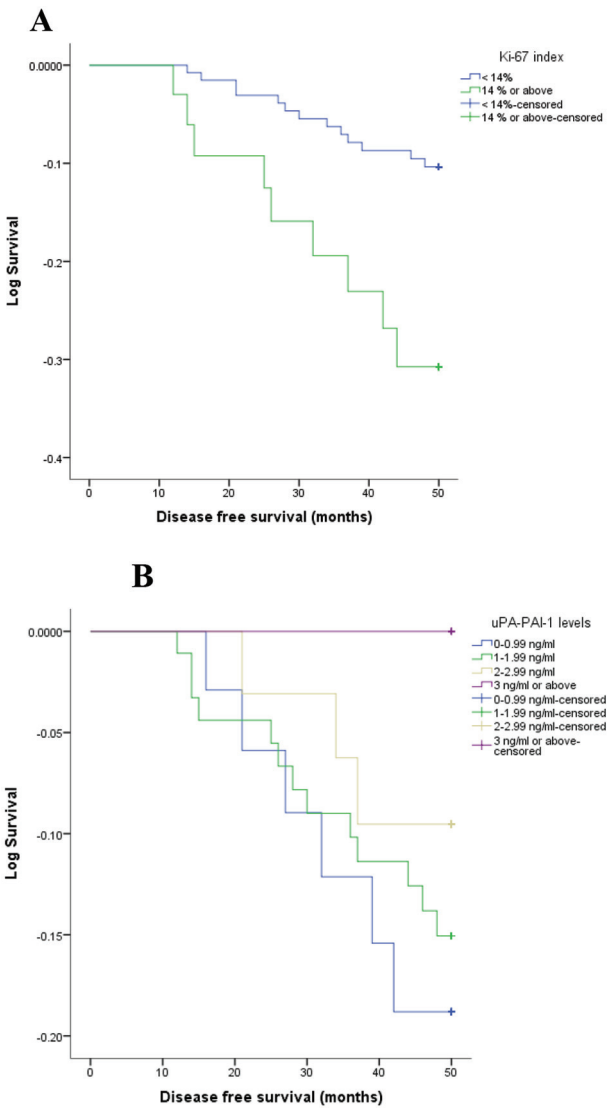

Figure 2. *Five-year disease-free survival based on Ki-67 index (A) and uPA-PAI-1 (B) marker levels*

Ki-67, Antigen Kiel 67; uPA/PAI-1, Urokinase plasminogen activator/plasminogen activator inhibitor-1

ly in the first five years (28, 29). Various studies have confirmed that patients with high Ki-67 indices have a greater risk of shorter DFS (30, 31). The Ki-67 index, which measures the rate of tumor cell proliferation, is associated with accelerated cell division and faster tumor growth (32). Reduced sensitivity of these tumors to certain therapeutic protocols can also contribute to an increased likelihood of shorter DFS, as noted in our study (33).

Multivariate regression analysis has demonstrated the association of tumor stage, tumor size, and Ki-67 index with DFS, supporting previous findings regarding their prognostic-predictive significance (34, 35). These factors together reflect the complexity of the disease and its potential impact on outcomes. The Ki-

67 index, as a proliferation marker, further contributes to understanding the disease dynamics (32, 36-39).

The study conducted by Mahmood et al. (40) investigated serum uPA/PAI-1 in the contex to fearly invasive breast cancer, highlighting the need to consider systemic factors in interpreting serum biomarkers and emphasizing the importance of considering potential influences of cytokines and other tumor markers on uPA/PAI-1 complex expression. Additionally, the values of this complex measured in serum do not represent a reliable prognostic and predictive parameter (41), unlike its values in the cytosol or tumor tissue (42, 43, 44).

Limitations of our study include the lack of analysis of the interaction between the Ki-67 index and the uPA/PAI-1 complex, both mutually and with other standard clinicopathological characteristics. Such analysis would enable better identification of patient subsets that could benefit from a combined analysis of these markers. Further more, other genetic or molecular characteristics that could affect the prognostic and predictive significance of these biomarkers were not included (11, 12, 13). The lack of long-term follow-up, as the follow-up only covered the first five postoperative years, is also considered a limitation of the study (45).

Our results indicate a statistically significant effect of elevated Ki-67 values on shortening DFS, with this effect consistent regardless of the presence of uPA/PAI-1 and other previously documented prognostic factors considered in our study.

CONCLUSION

Our study emphasizes the strong and consistent prognostic and predictive ability of the Ki-67 index in assessing DFS in patients who have undergone surgery for early invasive breast cancer. In contrast, preoperative values of the uPA/PAI-1 complex in serum, whether alone or in combination with other predictors, did not show significant prognostic and predictive potential for assessing DFS in these patients. Additional monitoring and tailored therapeutic strategies may be beneficial for patients with elevated Ki-67 values, T2 stage tumors, and tumor sizes of 20-50 mm.

Abbreviations

TNM (Tumor, Node, Metastasis) – tumor classification based on tumor size, lymph node involvement, and the presence of distant metastases

ER – estrogen receptor

HER-2 – human epidermal growth factor receptor 2

Ki-67 – marker of proliferation Ki-67

PR – progesterone receptor

uPA – urokinase plasminogen activator

PAI-1 – plasminogen activator inhibitor 1

DFS – disease-free survival

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Sažetak

PROGNOSTIČKO-PREDIKTIVNI ZNAČAJ Ki-67 PROLIFERATIVNOG INDEKSA I PREOPERATIVNIH VREDNOSTI uPA/PAI-1 KOMPLEKSA U SERUMU KOD PACIJENTKINJA SA RANIM INVAZIVNIM KARCINOMOM DOJKE

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Uvod: Karcinom dojke, najčešći malignitet kod žena, predstavlja značajan zdravstveni problem, a biomarkeri poput Ki-67 indeksa i uPA/PAI kompleksa mogu pružiti uvid u ishode lečenja i terapijski odgovor.

Cilj: Glavni ishod studije bila je procena petogodišnjeg preživljavanja bez bolesti (DFS), definisanog kao postoperativno razdoblje do pojave loko-regionalnih ili udaljenih metastaza i smrti od bilo kojeg uzroka.

Materijal i metode: Retrospektivna kohortna studija uključivala je 166 pacijentkinja sa ranim invazivnim karcinomom dojke, kod kojih se procenjivao prognostički i prediktivni značaj uPA/PAI-1 komplek-

sa i Ki-67 biomarkera kod hirurški tretiranih pacijentkinja na Klinici za opštu i abdominalnu hirurgiju Kliničkog centra Univerziteta u Sarajevu, u periodu od septembra 2015 do februara 2017.

Rezultati: Univarijantnom regresionom analizom utvrđena je povećana verovatnoća za DFS kraći od pet godina kod pacijentkinja sa negativnim hormonskim receptorima, pozitivnim HER-2 receptorom, sa ≥ 8 pozitivnih limfnih čvorova i Ki-67 indeksom ≥ 14% ($p < 0.05$). Multivarijantnom regresionom analizom utvrđeno je da su T2 stadijum, veličina tumora od 20-50 mm i Ki-67 indeks ≥ 14% povezani sa većom verovatnoćom za DFS kraći od pet godina ($p < 0.05$). Petogodišnja stopa DFS-a bila je veća kod pacijenata sa Ki-67 indeksom < 14% u odnosu na one sa ≥ 14% ($p = 0.011$), dok nije bilo razlike u petogodišnjem DFS-u među pacijenticama sa različitim nivoima uPA/PAI-1 kompleksa ($p = 0.636$).

Zaključak: Naša studija ističe važnost Ki-67 proliferativnog indeksa kao snažnog prognostičko prediktivnog faktora za DFS kod pacijentkinja operisanih zbog ranog invazivnog karcinoma dojke. Dodatni nadzor i prilagođene terapijske strategije mogu biti korisni kod pacijentkinja sa povišenim vrednostima Ki-67 indeksa, T2 stadijumom i veličinom tumora od 20-50 mm.

Ključne reči: biomarkeri, opšta hirurgija, ishodi lečenja, zdravlje žena.

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Original article

DIFFERENTIAL DIAGNOSIS OF IRON DEFICIENCY ANEMIA AND BETA THALASSEMIA IN PORT HARCOURT PREGNANT WOMEN USING THE MENTZER INDEX

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Abstract: **Introduction:** The Mentzer index is a cost-effective and accurate method for differentiating between iron deficiency anemia (IDA) and beta-thalassemia. Anemia in pregnancy is a global health concern, especially in developing countries like Nigeria, where it is primarily linked to iron deficiency and may also include other underlying hemoglobin disorders, with beta-thalassemia (βT) being the most common. This cross-sectional study aimed to differentiate between iron deficiency anemia and beta-thalassemia in pregnant women attending tertiary hospitals in Port Harcourt, Nigeria, based on the Mentzer Index.

Materials and Methods: A total of 120 apparently healthy pregnant women aged between 20 and 50 years were recruited for the study. Five milliliters (5 ml) of venous blood were collected from each participant using a disposable syringe and placed into an ethylene diamine tetraacetic acid (EDTA) anticoagulated bottle for full blood count determination using a Mindray automated analyzer. The Mentzer Index was calculated from the mean cell volume (MCV) and red blood cell (RBC) count values. The data obtained were analyzed statistically using Statistical Package for Social Sciences (SPSS) Version 23.

Results: Mean age ± SD of the study population (28.68 ± 5.6years), Mean + SD of Hb (11.38 + 2.08g/dl), PCV (31.72 + 4.59%), RBC count (4.65 ± 1.86 x 10^{12}/L), MCV (79.92 ± 5.91 fl) and Platelets count (272.9 ± 42.10 x 10^{9}/L) respectively. Out of 120 participants, 48 (40%) had Hb and PCV values above 11.0 g/dl and 33% respectively. A total of 5 (4.17%) had a Mentzer index < 13 and confirmed beta-thalassaemia, while 67 (55.83%) had a Mentzer index > 13 and confirmed iron-deficiency anaemia indicating a 60% prevalance of anaemia in study population with iron-deficiency anaemia been more common amongst the study population than beta-thalassaemia.

Conclusion: Based on the Mentzer Index calculation, this study revealed a high prevalence of iron deficiency anemia (IDA) and a lower prevalence of beta-thalassemia among pregnant women attending tertiary hospitals in Port Harcourt, Nigeria. Healthcare practitioners should consider incorporating the Mentzer Index as a cost-effective means of differentiating iron deficiency anemia from beta-thalassemia in pregnant women, particularly in rural areas. Additionally, increased awareness and educational programs focusing on proper nutrition and iron supplementation during pregnancy should be advocated.

Keywords*:* Mentzer Index, Iron deficiency anemia, Beta-Thalassemia, Pregnant Women.

INTRODUCTION

The Mentzer index is a cost-effective and accurate method for differentiating iron deficiency anemia (IDA) from beta-thalassemia, though it remains underutilized in clinical settings. Anemia during pregnancy is a significant global health concern, particularly in developing countries like Nigeria. It is primarily associated with iron deficiency but may also involve other underlying hemoglobin disorders, with beta-thalassemia (βT) being the most prevalent.

Anemia during pregnancy is characterized by low circulating hemoglobin (Hb), the oxygen-carrying pigment in blood. This condition is defined as a drop in hemoglobin concentration below the normal threshold, which is two standard deviations below the median value for a population of the same sex, age, and stage of pregnancy. This results in decreased oxygen-carrying capacity in pregnant women (1, 2, 3).

Iron is crucial for effective hematopoiesis (4), and research by Ugwu and Uneke (2) highlights multiparity, starting from the seventh month of pregnancy (third trimester), and low socioeconomic status as risk factors for iron deficiency anemia in pregnant women. Poor screening for IDA exacerbates the challenge. Globally, iron deficiency anemia remains one of the most common causes of anemia, and oral iron supplementation during pregnancy is widely advocated, although dietary interventions are less emphasized (5). Hemoglobin concentration (Hb) and packed cell volume (PCV) are standard components of a complete blood count and are essential for diagnosing and monitoring anemia in pregnant women (6, 7, 8).

According to the Nigeria Demographic and Health Survey (NDHS) (9), the prevalence rate of anemia among women of reproductive age (15-49 years) is 58%, particularly challenging in rural communities where awareness is limited. Thalassemia is characterized by quantitative defects in hemoglobin synthesis, resulting in hypochromic microcytic anemia due to reduced or absent synthesis of the globin chain of hemoglobin (10, 11, 12).

Distinguishing between beta-thalassemia, a hereditary disorder (10), and iron deficiency anemia is crucial, as they have distinct etiologies and require different management approaches. While iron deficiency anemia primarily requires oral iron supplementation or controlled dietary intake, beta-thalassemia may necessitate interventions such as blood transfusions. Accurate diagnosis ensures that pregnant women receive appropriate treatment, optimizing maternal and fetal health. Misdiagnosis of beta-thalassemia as iron deficiency anemia could lead to inadequate management and complications such as fetal growth restriction or preterm birth (13, 14). Proper diagnosis and differentiation are essential to prevent adverse outcomes, making the Mentzer index a relevant and cost-effective tool.

Research by Tabassum *et al*. (15) indicates that while various measures have been used to differentiate between beta-thalassemia and iron deficiency anemia, none are 100% sensitive or specific except for the Mentzer index. Bose and Maimoon (16) found that the Mentzer index has high sensitivity and specificity among available measures.

The Mentzer index is calculated by dividing the mean cell volume (MCV) in femtoliters (fl) by the total red blood cell (RBC) count in millions per microliter. An index greater than thirteen (> 13) suggests iron deficiency anemia, while an index less than thirteen (< 13) indicates a likelihood of beta-thalassemia. Individuals with a Mentzer index < 13 may undergo HbA2 estimation through hemoglobin electrophoresis, a cost-effective method for confirming beta-thalassemia in high-risk patients (15, 16).

Diagnosing IDA and beta-thalassemia, particularly in developing countries, poses challenges due to high costs, which affect affordability for pregnant women. Additionally, there is limited information on the diagnosis of iron deficiency anemia and beta-thalassemia in pregnant women in Port Harcourt, Nigeria.

This study aims to determine the prevalence of iron deficiency anemia and beta-thalassemia among pregnant women attending tertiary hospitals in Port Harcourt. It will help allocate resources more efficiently by targeting specific laboratory tests and potentially advance medical knowledge in clinical practice through the reliable differentiation of anemia in pregnant women based on the Mentzer index.

MATERIALS AND METHODS

Study Design

This cross-sectional observational study utilized randomized sampling to differentiate between iron deficiency anemia (IDA) and beta-thalassemia in pregnant women attending a tertiary hospital in Port Harcourt, Nigeria, based on the Mentzer Index. The study was conducted from August 2023 to December 2023.

Study Population

The study population consisted of 120 apparently healthy pregnant women aged 20 to 50, randomly recruited from a tertiary hospital in Port Harcourt, Nigeria.

Eligibility Criteria

Pregnant women attending the tertiary hospital in Port Harcourt, Nigeria, who were willing to provide oral consent, were included in the study. Non-pregnant individuals and those unwilling to provide informed consent or participate in the research were excluded.

Ethical Considerations

Ethical approval for this study was obtained from Rivers State University Teaching Hospital (RSUTH), Port Harcourt.

Blood Collection and Sample Preparation

Five milliliters (5 ml) of venous blood were collected from each participant using a disposable syringe and transferred into ethylene diamine tetraacetic acid (EDTA) anticoagulated bottles. Full blood count was determined using a Mindray automated analyzer, and the Mentzer index was calculated from the mean cell volume (MCV) and red blood cell (RBC) count values.

Calculation of Mentzer Index

The Mentzer Index was calculated using the formula:

$$\text{Mentzer Index} = \frac{MCV\,(ft)}{RBC\ (million\ per\ microlitre)}$$

Differentiation between Iron Deficiency Anemia and Beta-Thalassemia

Differentiation between IDA and beta-thalassemia was based on the research by Tabassum *et al*. (15), which states that a Mentzer Index greater than 13 is indicative of IDA, while an index less than 13 suggests beta-thalassemia.

Data Analysis

The data obtained were analyzed using Statistical Package for Social Sciences (SPSS) Version 23.

RESULTS

Socio-Demographic Characteristics of Study Participants

Table 1 presents the socio-demographic characteristics of the study population. The mean age ± SD of the participants was 28.68 ± 5.6 years. The majority, 89 participants (74.2%), were aged 20-39 years, 30 participants (25.0%) were aged 40-59 years, and 1 participant (0.8%) was aged 60-79 years. Regarding gestational age, 39 participants (32.5%) were within 1-19 weeks, 68 participants (56.7%) were within 20-39 weeks, and 13 participants (10.8%) were 40 weeks and above. Based on parity, 94 participants (78.3%) had 1-3 pregnancies, while 26 participants (21.7%) had 4 or more pregnancies.

Hematological Parameters of Study Population

Table 2 summarizes the hematological parameters of the study population. The mean values were: Hemoglobin: 11.38 ± 2.08 g/dL, Packed Cell Volume: 31.71 ± 4.59%, White Blood Cell Count: 4.65 ± 1.86 × 10^12/L, Red Blood Cell Count: 5.93 ± 1.75 × 10^9/L, Mean Cell Hemoglobin: 26.97 ± 2.54 pg, Mean Cell Volume: 79.92 ± 5.91 fL, Mean Cell Hemoglobin Concentration: 34.58 ± 1.87 g/dL, Platelet Count: 272.9 ± 42.10 × 10^9/L.

Table 1. *Socio-demographic characteristics of study participants*

Characteristics of the Population	Number Of Subjects	Percentage (%)
Age Range (years)		
10-39	89	74.2
40-59	30	25.0
60-79	1	0.8
Mean Age ± SD (years)	28.68 ± 5.6	
Gestational age (weeks)		
1-19	39	32.5
20-39	68	56.7
40 and above	13	10.8
Parity		
1-3	94	78.3
4-6	26	21.7

Table 2. *Haematological parameters of study population*

Haematological Parameter	Mean ± SD
Haemoglobin concentration	11.38 ± 2.08 g/dl
Packed Cell Volume	31.71 ± 4.59 %
Red Blood Cell Count	4.65 ± 1.86 x 10^{12}/L
White Blood Cell Count	5.93 ± 1.75 x 10^{9}/L
Mean Cell Volume	79.92 ± 5.91 fl
Mean Cell Hemoglobin	26.97 ± 2.54 pg
Mean Cell Hemoglobin Concentration	34.58 ± 1.87 g/dl
Platelet Count	272.9 ± 42.10^{9}/L

Differentiation of Iron Deficiency Anemia (IDA) and Beta-Thalassemia in Pregnant Women

Table 3 illustrates the differentiation between iron deficiency anemia and beta-thalassemia among the pregnant women attending a tertiary Hospital in Port Harcourt Nigeria. Out of the 120 participants, 48 (40%) had Hb and PCV values above 11.0 g/dl and 33% respectively and classified as non anaemic pregnant individuals, 67 (55.83%) were diagnosed with iron deficiency anemia based on the Mentzer Index, while 5 participants (4.17%) were diagnosed with beta-thalassemia.

Table 3. *Differentiation of iron deficiency anaemia (IDA) and B-thalassaemia in pregnant women based om Mentzer Index*

Mentzer Index	Disorder/Condition	Number of Subjects	Percentage (%)
	Non anaemic individuals	48	40.00
Less than 13 (< 13)	ß-Thalassaemia	5	4.17
Greater than 13 (> 13)	Iron Deficiency Anaemia	67	55.83

DISCUSSION

This study utilized the Mentzer Index to differentiate between iron deficiency anemia (IDA) and beta-thalassemia in pregnant women attending a tertiary hospital in Port Harcourt.

The hematological parameters observed in the study population revealed that while values for hemoglobin (Hb) concentration, red blood cell (RBC) count, mean cell hemoglobin concentration (MCHC), white blood cell (WBC) count, and platelet count were within normal reference ranges, packed cell volume (PCV), mean cell volume (MCV), and mean cell hemoglobin (MCH) were slightly below the lower limits of normal/reference ranges. These variations are typical in pregnancy due to physiological changes, including dilutional anemia and increased physiological stress, which can alter hematological parameters. These findings are consistent with the research by Dapper *et al*. (17) and Mba *et al.* (18), who observed similar variations in hematological parameters in pregnant women in Port Harcourt. Additionally, Amah-Tariah *et al*. (19) reported non-significant variations in platelet counts across different trimesters of pregnancy.

The differentiation between iron deficiency anemia and beta-thalassemia based on the Mentzer Index showed that 5 participants (4.17%) had a Mentzer Index of less than 13, indicative of beta-thalassemia, while 67 participants (55.83%) had a Mentzer Index greater than 13, indicative of iron deficiency anemia. This distribution indicate a prevalence of 60% anemia with high prevalence of iron deficiency anemia compared to beta-thalassemia among the study participants.

The predominance of iron deficiency anemia in this study may reflect the participants' nutritional status, particularly concerning dietary iron intake during pregnancy. The increased demand for iron during pregnancy can exacerbate iron deficiency, especially in women who enter pregnancy with insufficient iron stores. Multi-parity further contributes to the depletion of iron stores, as observed in this study's predominantly multiparous participants. This high prevalence of iron deficiency anemia although lower in terms of percantage aligns with findings from Ndukwu and Dienye (20), who reported a 62.6% prevalence rate for anemia among pregnant women in Rivers State. Ugwu and Uneke (2) highlighted that iron deficiency accounts for 75% of all types of anemia in pregnancy globally, with a reported prevalence of 25% in Nigeria. Azinge *et al*. (21) also reported a prevalence of 57.5% for anemia in pregnant women in Nigeria, which is lower than the 60% prevalence observed in this study. Onimawo and Onuoha (22) reported a lower prevalence of 14% for iron-deficiency anemia in pregnant women in Abia State.

The low prevalence of beta-thalassemia in this study can be attributed to its hereditary nature, resulting from mutations or deletions in the beta-globin gene (HbB) on chromosome 11. Since beta-thalassemia prevalence is not influenced by pregnancy, this finding is consistent with the observations of Needs *et al.* (10). The lower prevalence of beta-thalassemia compared to iron deficiency anemia highlights the susceptibility of pregnant women to iron deficiency and reinforces the need for effective screening and nutritional interventions.

CONCLUSION

This study highlights a high prevalence of iron deficiency anemia (IDA) and a lower prevalence of beta-thalassemia among pregnant women attending tertiary hospitals in Port Harcourt, Nigeria. The Mentzer Index proved to be an effective tool for distinguishing between these conditions, even as variations in hematological parameters were observed.

Recommendation

Healthcare practitioners are encouraged to integrate the Mentzer Index as a cost-effective method for differentiating between iron deficiency anemia and beta-thalassemia in pregnant women, particularly in rural areas where resources may be limited. Additionally, there should be a concerted effort to enhance awareness and education on proper nutrition and iron supplementation during pregnancy to address and prevent iron deficiency anemia.

Abbreviations

IDA - Iron Deficiency Anemia
P**CV** - Packed Cell Volume

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Conflict of Interest: The authors declare no conflicts of interest regarding the writing and publication of this article.

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Authors' Contributions: RJ contributed to the conceptualization, detailed review, and statistical analysis. PA conducted literature reviews and laboratory analyses. EE and ZJ participated in the review and editing of the manuscript. All authors have reviewed and approved the final manuscript.

Note: Artificial intelligence was not utilized as a tool in this study.

Sažetak

DIFERENCIJALNA DIJAGNOZA ANEMIJE USLED NEDOSTATKA GVOŽĐA I BETA TALASEMJE KOD TRUDNICA U PORT HARCOURTU KORIŠĆENJEM MENTZER INDEKSA

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Uvod: Mentzer indeks je isplativ i tačan metod za razlikovanje anemije usled nedostatka gvožđa (IDA) i beta-talasemije. Anemija u trudnoći je globalni zdravstveni problem, posebno u zemljama u razvoju kao što je Nigerija, gde je prvenstveno povezana sa nedostatkom gvožđa i može uključivati i druge osnovne hemoglobinopatije, pri čemu je beta-talasemija (βT) najčešća. Ova studija preseka imala je za cilj da diferencira anemiju usled nedostatka gvožđa i beta-talasemiju kod trudnica koje posećuju tercijarne bolnice u Port Harcourtu, Nigerija, na osnovu Mentzer indeksa.

Materijali i Metode: U studiju je uključeno ukupno 120 naizgled zdravih trudnica uzrasta između 20 i 50 godina. Pet mililitara (5 ml) venske krvi je uzeto od svake učesnice studije koristeći jednokratni špric i stavljeno u bočicu sa antikoagulansom etilen-diamin-tetra-sirćetnom kiselinom (EDTA) za određivanje kompletne krvne slike koristeći Mindray automatski analizator. Mentzer indeks je izračunat na osnovu prosečnog volumena eritrocita (MCV) i broja eritrocita (RBC). Dobijeni podaci su statistički analizirani korišćenjem Statističkog paketa za društvene nauke (SPSS) verzija 23.

Rezultati: Prosečna starost ± SD populacije studije bila je 28,68 ± 5,6 godina. Srednje vrednosti ± SD su bile sledeće: Hb 11,38 ± 2,08 g/dl, HCT 31,72 ± 4,59%, broj eritrocita 4,65 ± 1,86 x 10^{12}/L, i MCV 79,92 ± 5,91 fl. Od ukupno 120 učesnica, 5 (4,17%) je imalo Mentzer indeks < 13 i kod njih je potvrđena beta-talasemija, dok je 115 (95,83%) imalo Mentzer indeks > 13 i kod njih je potvrđena anemija usled nedostatka gvožđa. Ovo ukazuje da je anemija usled nedostatka gvožđa češća među populacijom u studiji nego beta-talasemija.

Zaključak: Na osnovu izračunatog Mentzer indeksa, ova studija je otkrila visoku prevalenciju anemije usled nedostatka gvožđa (IDA) i nižu prevalenciju beta-talasemije među trudnicama koje posećuju tercijarne bolnice u Port Harcourtu, Nigerija. Zdravstveni radnici bi trebali razmotriti upotrebu Mentzer indeksa kao isplativog načina za razlikovanje anemije usled nedostatka gvožđa od beta-talasemije kod trudnica, posebno u ruralnim područjima. Pored toga, potrebno je povećati svest i organizovati edukativne programe koji se fokusiraju na pravilnu ishranu i suplementaciju gvožđem tokom trudnoće.

Ključne reči: Mentzer Indeks, Anemija ssled nedostatka gvožđa, Beta-Talasemija, Trudnice.

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Short Communication

HOW TO PREPARE FOR AN AVIAN INFLUENZA H5N1 PANDEMIC OUTBREAK: LESSONS LEARNED FROM THE INFLUENZA H1N1 PANDEMIC OF 1918

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Abstract: In this paper, we highlight the significant challenges encountered during the influenza H1N1 pandemic of 1918. These lessons are being utilized to prepare for potential avian influenza H5N1 pandemic outbreaks, as this virus will impact both humans and food-serving animals. Preparation for this threat is crucial because H5N1 infections are already affecting the United States and many other countries.

Keywords: Avian influenza H5N1, Bird flu, Pandemic outbreak, Influenza A, Food security.

INTRODUCTION

The recent SARS-CoV-2 pandemic outbreak has ended, but the virus continues to circulate within the human population, infecting numerous people. The avian influenza virus H5N1 is currently attempting to mutate to infect humans and mammalian populations, including cows, pets, foxes, and whales. In the United States, the presence of this virus has been confirmed in pasteurized milk, and 71 people are currently being monitored for the virus. As of May 13, 2024, one person has been infected, despite May typically not being a peak month for influenza infections (1).

The author has analyzed literature on the influenza virus H1N1 1918 outbreak and cases of influenza H5N1. In this and future work, we aim to develop blueprints to tackle potential H5N1 outbreaks, as this virus poses a threat to human life and the food industry, including poultry, milk, and beef.

PANDEMIC INFLUENZA VIRUS H1N1 1918

The influenza virus H1N1 1918 outbreak revealed several essential traits, such as the virus's rapid spread throughout the human population in the United States and the United Kingdom. This is evidenced by literature available from scanned newspaper content online from the University of Utah Marriott Library and the BBC's audio documentaries. The infection's symptoms included fever, headache, body pains, and coughing with colored sputum, which led to pneumonia in fatal cases. Rarely, there was also diarrhea and brain inflammation.

In the United States, this virus came in three waves: July, September to November 1918, and February to March 1919. It caused a large number of fatalities among the afflicted population in a short period of time, with the fatality rate reaching 10% to 15% in certain situations. Many notable people became infected, including the King of Spain and prominent leaders from the United Kingdom and the United States. Measures used to prevent this virus included masking the mouth and nose, gargling, and oral disinfection. Public institutions and schools were closed. Pneumonia was often caused by subsequent bacterial infections. Most fatalities occurred in young people aged 26 to 40. Symptoms appeared several hours to a day after infection and lasted three days, which is why this illness is also known as three-day fever. In certain cases, symptoms could last up to six days. Infected persons were asked to stay at home and rest until symptoms subsided. The virus was brought to the United States and the United Kingdom by armed forces fighting in Europe during World War I, which was nearing its end. Vitamin D was recommended and used. Social distancing was employed to prevent infection from coughing and sneezing people (2-9).

In the USA, vaccination was used against secondary bacterial infections. These vaccines were made by growing bacteria and inactivating them through a heating process. This bacterial vaccination led to a

reduction in pneumonia cases, saving many lives, as no antibiotics were available at that time. The vaccine was administered both as a curative measure for early-stage infections and as a prophylactic measure for non-infected persons (10, 11, 12).

AVIAN INFLUENZA VIRUS H5N1

To date, the avian influenza virus H5N1 has caused around 700 infections and approximately 300 fatalities. From the literature, it is evident that this virus causes flu-like symptoms similar to those of influenza H1N1 1918. However, many reports indicate brain involvement in several cases. Publications have emphasized learning lessons from the 1918 pandemic outbreak (13, 14, 15). Today, better therapies are available for treating secondary bacterial infections, and various monitoring and diagnostic tools can quickly identify outbreaks of this virus. A narrow choice of antiviral therapies, such as neuraminidase and matrix inhibitors, is also available.

This virus spreads through migratory birds in wildlife, transmitting the virus to livestock, which serve as food and are in close contact with humans. Therefore, there are two important aspects of this virus: its impact on the human population and animals (Figure 1).

Transmission to pets, particularly cats, is another urgent area of attention. In the USA, several cats have suffered fatal infections with Clade 2.3.4.4b from infected cows. Similar feline infections have been reported in other countries like Poland and Korea. This raises concerns about the possibility of transmission from infected cats to their owners (16).

Another alarming issue is that house mice in the USA have been infected with H5N1 strains, potentially playing a significant role in spreading the virus to other species. Many wild species and pets, such as cats, feed on mice as a source of nutrition. Controlling and monitoring the mouse population is challenging,

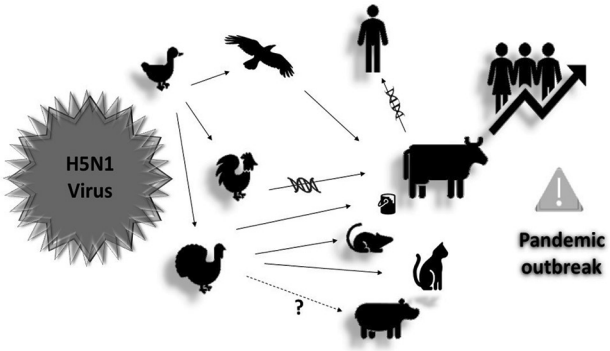

Figure 1. *Possible transmission routes of avian influenza virus H5N1 in USA*

and total elimination is not feasible, as it could lead to an imbalance in the ecosystem (17).

Several publications indicate that individuals infected with H5N1 strains are often under 40 years of age, another similarity with the H1N1 pandemic outbreak (18, 19, 20). It is crucial to educate young people to break the infection cycle.

In this research work, we present new ideas to combat future avian influenza pandemic outbreaks. Our opinion is that this virus spreads very rapidly, as evidenced by its impact on poultry, leading to limited preventive options, particularly culling infected birds. The virus has now been found in many cows in the USA, and pasteurized milk has been found to be contaminated with it.

FOOD SECURITY

An important question arises regarding food safety: should contaminated milk, even if pasteurized, be consumed? The answer is no. If the milk is heavily contaminated, it should not be used for human or animal consumption, as pasteurization may not inactivate all pathogens. Additionally, killed viruses may still contain virulent parts that can cause complications. This example highlights the issue of food security.

We suggest that countries worldwide store milk and beef from infection-free cows in various forms, such as powder and frozen meat. Similarly, poultry products like eggs and meat from infection-free birds should be stored as frozen meat and egg powder to be used during a pandemic outbreak.

There is a need to define standards for contamination levels that can be treated with disinfectants like chlorine and hydrogen peroxide for meat. For milk and eggs, standards for pasteurization must be defined while ensuring that workers handling these products remain infection-free. Today, we have molecular tools to establish these standards. For instance, real-time Polymerase Chain Reaction (PCR) can be used to determine the Cycle Threshold (Ct) value of infected samples (21). If the Ct value exceeds 30, the food can be disinfected or pasteurized and deemed safe for human and animal consumption.

Similarly, antigen-detecting immunological tests and advanced nanotechnological tests can be utilized. Users must be instructed to cook food thoroughly before consumption. There is an urgent need for experimental research in this area.

Reducing the lifespan of poultry birds could also minimize infection risk, while their meat can be frozen for human consumption. Governments must invest heavily in creating new storage capacities, similar to those for oil and food grains.

PREVENTION

Developing avian influenza-resistant poultry birds and cows is crucial. Inexpensive therapeutic molecules should be developed for use in animal populations to reduce or eliminate these infections economically. Plant-derived molecules with antiviral properties show promise and can be synthesized to enhance efficacy and lower production costs. Research in this field must be accelerated. Boosting the immune system of animals is another important strategy. These immune-boosting molecules could also serve as preventive measures for human populations in endemic areas of influenza virus H5N1, as well as for travelers to these regions.

Genekam Biotechnology AG has developed an inexpensive molecule called Genekampowermolecule, which has shown effectiveness against SARS-CoV-2. Testing its efficacy against H5N1 in humans could provide a preventive and therapeutic option.

While vaccination development is crucial for controlling influenza viruses, our current understanding of bird and mammal physiology is insufficient for developing perfect vaccines. More research is needed in this area to avoid developing vaccines with questionable efficacy, as seen in the case of SARS-CoV-2 (22).

Improving short- and long-term preventive measures is urgent, as the 1918 pandemic showed that this virus spreads rapidly. It's crucial to determine whether H5N1 will affect young populations as it did in 1918. Additionally, understanding the role of SARS-CoV-2 in an H5N1 pandemic outbreak is essential for preparing effective prevention strategies against mixed infections, which can be highly fatal.

The roles of other avian influenza virus strains, such as H9N2 and H7N9, during human infections should also be investigated.

Each country should prepare with molecular testing kits stored in advance. My laboratory has developed a double-check real-time PCR test that provides broader coverage of circulating strains and is easy to use. Quality assurance of such tests is essential to avoid issues seen with questionable tests during the SARS-CoV-2 pandemic. Training for healthcare professionals, including medical, dental, and veterinary students, is crucial for identifying different pathological conditions during potential pandemic outbreaks and understanding the various blood profiles associated with these conditions, as observed during the SARS-CoV-2 outbreak (23).

This work serves as a global call to prepare for potential avian influenza pandemic outbreaks.

CONCLUSION

Here we have shown the lessons learned from pandemic outbreak H1N1 1918 and the steps needed to improve for potential H5N1 pandemic outbreak. We are working to write the full versions of this publication about the preparation of the influenza virus H5N1 pandemic outbreak.

Abbreviations

BBC - British Broadcasting Corporation
Ct - Cycle Threshold
PCR - Polymerase Chain Reaction
SARS-CoV-2 - Severe Acute Respiratory Syndrome Coronavirus 2
USA - United States of America

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Note: Artificial intelligence was not utilized as a tool in this study.

Sažetak

KAKO SE PRIPREMITI ZA PANDEMIJU PTIČJEG GRIPA H5N1: POUKE IZ PANDEMIJE INFLUENCE H1N1 IZ 1918

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U ovom radu ističemo značajne izazove s kojima smo se susreli tokom pandemije influence H1N1 1918. godine. Ove lekcije koristimo kako bismo se pripremili za potencijalna izbijanja pandemije ptičjeg gripa H5N1, s obzirom na to da ovaj virus može uticati kako na ljude tako i na životinje koje služe kao hrana. Priprema za ovu pretnju je od ključnog značaja jer su H5N1 infekcije već prisutne u Sjedinjenim Američkim Državama i mnogim drugim zemljama.

Ključne reči: Ptičja influenca H5N1, ptičji grip, izbijanje pandemije, influenca A, sigurnost hrane.

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Case report

SOFT LINING OF IMMEDIATE COMPLETE DENTURES: CASE REPORT

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Abstract: **Introduction:** With the placement of dental implants in edentulous patients, creating and adapting immediate dentures is crucial to allow patients to perform basic physiological functions during the osseointegration period without affecting implant healing. This report presents a case of direct soft lining of immediate complete dentures following implant placement.

Case Report: Six dental implants were inserted in the upper jaw, while the patient retained natural teeth in the lower jaw. An immediate complete denture was fabricated and adjusted for the upper jaw. The prosthesis base was prepared and processed to accommodate soft lining material. Adhesive was applied to the prepared base, followed by silicone application for soft fitting onto the prosthesis base, which was then inserted into the patient's mouth. After three minutes, the denture was removed, and soft lining treatment of the immediate complete denture was completed.

Conclusion: Direct soft lining facilitated patient adaptation to the immediate prosthesis and supported safer healing and osseointegration until definitive work could be completed. The addition of a soft silicone layer reduced pressure on the residual alveolar ridge and improved adhesion of the immediate prosthesis.

Keywords: soft lining of dentures, direct denture lining, immediate dentures.

INTRODUCTION

Soft lining of acrylic prostheses is a routine procedure in everyday dental practice. Complete acrylic prostheses, composed of PMMA (Poly(methyl methacrylate)), are hard acrylic resins designed to possess robust mechanical and physical properties capable of withstanding the pressures and forces generated during chewing (1).

Within the oral cavity, the residual alveolar ridge areas are unevenly covered by oral mucosa, varying in type and thickness. Consequently, certain regions of the ridge may experience greater pressure from the prosthesis base. To compensate for differences in mucosal thickness and elasticity, the inner part of the prosthesis base can be lined with a soft material when appropriately indicated (2).

Soft lining materials for prostheses create a thin, absorptive layer that reduces occlusal pressure on the bone and promotes more even force distribution (3). These materials are particularly useful for patients with alveolar ridge resorption, bruxism, and xerostomia (4).

It's noteworthy that these materials are biocompatible, tasteless, odorless, and commonly used as bases for immediate prostheses.

Soft lining materials can be classified based on composition into vinyl polysiloxane, silicone rubber, acrylate resin materials, and others. They can also be categorized as short-term or long-term lining materials. Curing methods include cold-curing, heat-curing, microwave-curing, and others. Currently, vinyl polysiloxane and silicone materials are most widely used for long-term soft lining in dental practice (5).

Over time, soft lining materials naturally become softer. Compared to acrylate, silicone lining materials excel in elasticity and long-term tissue pressure relief due to their lack of plasticizers (6).

CASE REPORT

This paper presents a case of a patient who underwent direct soft lining of an upper total prosthesis following upper jaw implant placement. Due to the 4-6 month osseointegration period required for the implants, an immediate upper total prosthesis was fabricated. An anatomical impression for the prosthesis was taken on the same day as implant placement, and the production of the immediate prosthesis followed standard procedures including anatomic and functional impressions, inter-jaw relationship determination, tooth alignment testing, and prosthesis delivery.

Direct soft lining of the prosthesis was performed for two primary reasons. First, impressions for the prosthesis were taken immediately after installing six endosteal implants, and the fabrication process itself spanned seven days. The appearance of the mucosa of the residual alveolar ridge changed before and after implant placement, affecting fit. Soft direct application of the prosthesis allowed for better adaptation. Secondly, total dentures rest on the mucous membrane of the residual alveolar ridge, transferring all loads to the tissues. Implant placement may exert unwanted pressure on surrounding bone. Applying a thin layer of silicone relieves tissue pressure.

In this case report, the A-silicone basis relining material (*Mollosil standard-Dentax dental*) was used for soft denture lining. This material, designed for long-term soft lining, belongs to the group of cold-polymerizing materials used for direct intraoral application. It is packaged in two tubes—a base and a catalyst—and was applied according to the manufacturer's instructions.

Before applying the soft lining material, a part of the acrylate was first removed from the underside of the prosthesis base using a hand piece and drill. This process involved removing 0.5-1 mm of acrylate from the denture base where it rests on the mucous membrane to create space for the new material. After removing the acrylate, adhesive was applied evenly using the brush provided with the adhesive bottle. The adhesive was left for 40 seconds without subsequent drying or rinsing, allowing it to prepare the surface for the soft lining material application (Figure 1).

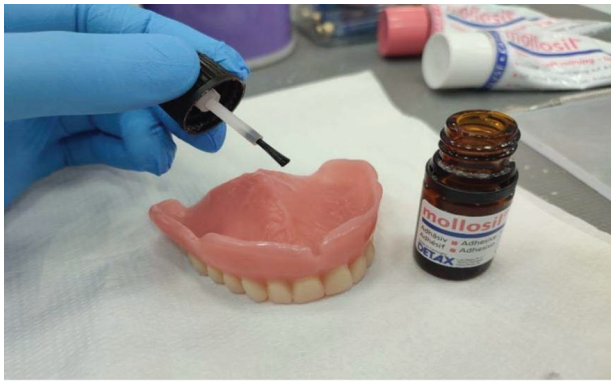

Figure 1. *Applying adhesive to the denture base*

After applying the adhesive, the prosthesis is ready for the application of the base material. Using a glass plate and a metal spatula, the soft lining material is mixed thoroughly. The mixed mass is then evenly distributed and applied onto the base of the prosthesis (Figure 2).

At the end of this procedure, the prosthesis was inserted into the patient's mouth. The patient, who retained

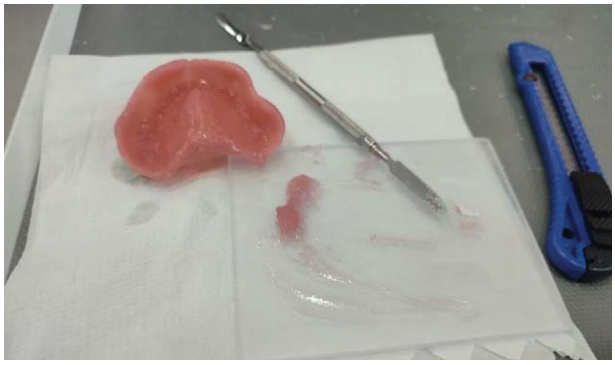

Figure 2. *Mixing mass for soft lining and placing it on the base of the prosthesis*

natural lower teeth, received an upper total denture with a soft lining material. After the patient bit down, the jaw was brought into the intercuspation position. The prosthesis remained in the mouth for 3 minutes without opening to allow the soft lining material to bond.

After the specified time, the prosthesis was removed from the mouth, and any excess soft lining material was carefully removed using a scalpel. Following this procedure, the prosthesis was handed over to the patient (Figure 3).

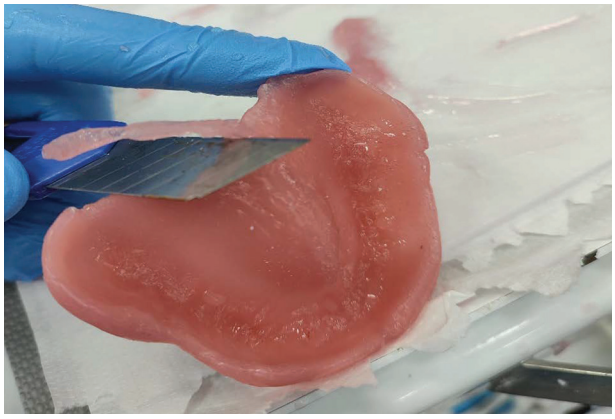

Figure 3. *Removal of excess mass from the prosthesis with a scalpel*

DISCUSSION

The lining of mobile dental prostheses is a common clinical procedure used to correct the gingival surface of prostheses. These procedures involve the use of materials with specific elastic characteristics, categorized into conditioners and liners based on their intended use and longevity.

Conditioners, such as soft acrylates, are temporary lining materials composed of polymer methacrylates modified with plasticizers. On the other hand, liners are permanent lining materials that typically consist of two-component formulations that polymerize at elevated temperatures. One drawback of liners is the potential for plasticizer dissolution over time.

Soft silicone liners, which polymerize at room temperature, are another category of lining materials. They are supplied in the form of two different colored pastes containing base polymer (dihydro-poly-dimethyl-siloxane), silicone fillers, pigments, crosslinkers (methyl-orthosilicate or ethyl-orthosilicate, tetraethoxy-silane), polymerization activators (benzoyl peroxide or dibutyl zinc dilaurate), and pigments (7).

These components work together to create a durable, elastic lining suitable for prosthetic applications.

The research conducted by Petković et al. evaluated four soft lining materials, comprising two based on soft acrylates and two silicone elastomers. Their findings revealed a greater increase in bond strength with silicone elastomers compared to soft acrylates. Importantly, they observed that there were no changes in the fracture type of the samples before and after exposure to water (8).

Ariyani et al. conducted a study demonstrating that treating PMMA (Poly(methyl methacrylate)) with an adhesive primer before applying soft backing material resulted in the highest bonding intensity (9). This highlights the importance of surface preparation for optimal bonding in prosthetic applications.

In recent research by Chladek et al., the positive effects of silicone for soft lining were investigated in the context of exposure to *Candida albicans* suspension. Their in vitro study showed that exposure to fungi did not adversely affect the mechanical properties of the silicone lining material. Despite *Candida albicans* colonization on the material's surface after 60 days, there was no penetration of fungi into the soft silicone lining material (10). These findings suggest that silicone liners may offer effective resistance against fungal colonization without compromising material integrity in prosthetic applications.

CONCLUSION

In modern dentistry, due to the development of technologies, materials, and implant systems, new methods and improved procedures are explored every day. It is important to correctly define indications and select appropriate procedures and therapies tailored to each patient's unique needs. Based on the findings discussed, the application of silicone-based preparations for direct soft lining in immediate prostheses following implant installation should be considered a standard practice and method of choice for such cases.

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Author contribution: All authors have contributed equally.

Note: Artificial intelligence was not utilized as a tool in this study.

Sažetak

MEKO PODLAGANJE IMEDIJATNE TOTALNE PROTEZE:PRIKAZ SLUČAJA

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Uvod: Kod ugradnje zubnih implantata kod bezubih pacijenata, izuzetno je važno izraditi i prilagoditi imedijatne proteze, kako bi pacijenti mogli da obavljaju osnovne fiziološke funkcije tokom perioda oseointegracije implantata bez uticaja na period zarastanja implantata. Prikazan je postupak direktnog mekogpodlaganja imedijatne proteze nakon ugradnje implantata.

Prikaz slučaja: U gornjoj vilici postavljeno je šest zubnih implantata, dok je pacijent imao sopstvene zube u donjoj vilici. Imedijatna totalna proteza je izrađena i prilagođena za gornju vilicu. Baza proteze je pripremljena i obrađena, stvarajući prostor za masu za meko podlaganje. Potom je adheziv postavljen na pripremljenu bazu proteze, nakon čega je usledilo nanošenje silikona za meko podlaganje na bazu proteze i postavljanja proteze u usta pacijenata. Posle tri minuta proteza je izvađena iz usta i urađena je obrada meke mase za podlaganje kompletnih proteza.

Zaključak: Direktno meko podlaganje olakšalo je adaptaciju pacijenta na imedijatnu protezu i omogućilo sigurniji i bolji period zarastanja i oseointegracije implantata do konačnog rada. Postavljanjem mekog silikonskog sloja smanjeno je opterećenje koje proteza vrši na rezidualni alveolarni greben i omogućeno bolje prianjanje imedijatne proteze.

Ključne reči: meko podlaganje proteze, direktno podlaganje proteze, imedijatne proteze.

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Case report

TREATMENT OF OPEN EXTRUDED FRACTURE NECK OF THE TALUS USING THE COMBINED METHOD OF EXTERNAL FIXATION MODIFIED FOR DYNAMIC ANKLE JOINT FIXATION AND KIRSCHNER WIRES

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Abstract: **Introduction:** Open extruded fractures of the talus occur in 2% of talar fractures. These fractures are challenging for surgeons due to complications such as infection, nonunion, and arthritis. The most common treatment method is talus reimplantation and osteosynthesis.

Case report: A 19-year-old presented with an open extruded fracture of the talus, classified as Hawkins II, following a fall from a height. Emergency surgery involved wound debridement, talus reimplantation, osteosynthesis with modified external fixation for dynamic ankle joint fixation (Mitkovic-type), and two Kirschner wires. Fragments were repositioned under C-Arm fluoroscopy. The patient received a 14-day antibiotic regimen (*Cephalosporins, Aminoglycoside, Metronidazole*) and thromboembolic prophylaxis for 35 days. After 6 weeks we allowed movements in the ankle joint with physical therapy. Gradual weight-bearing was allowed after 8 weeks. The osteosynthetic material was removed after 18 weeks, with full weight-bearing achieved after 6 months. Radiographic follow-up was conducted up to 24 months postoperatively, showing excellent healing with minimal dorsiflexion restriction.

Conclusions: Treating this injury is a significant challenge. External fixation can be a viable method for managing open luxation fractures of the talus.

Keywords: accidental falls, ankle joint, Kirschner wires, external fixators, talus.

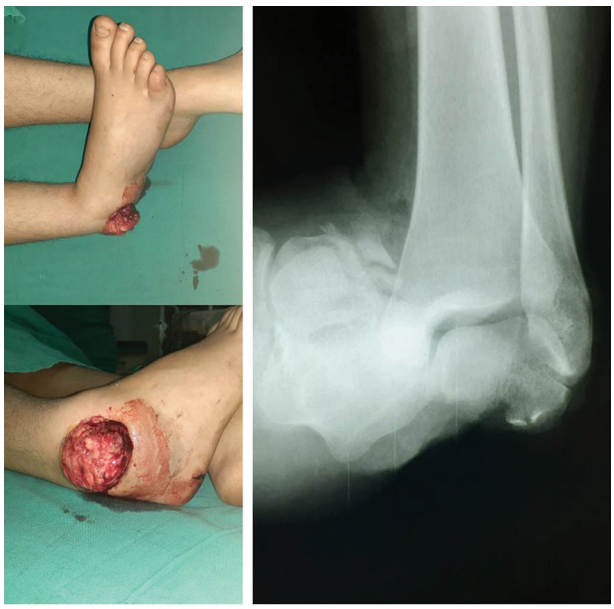
INTRODUCTION

An open fracture and extrusion of the talus is a rare injury, accounting for 2% of all talar fractures. It typically results from high-energy impacts involving significant tibiotalar dorsiflexion or plantar flexion with subtalar pronation or supination (1). All soft tissue connections of the talus are severed and the anatomical relationship with the tibia, calcaneus, and navicular bone is disrupted (2). The main complications include infection, arthritis, and avascular necrosis of the talus. The common treatment methods are reimplantation and osteosynthesis, with talectomy and tibiocalcaneal arthrodesis used less frequently (3–5).

CASE REPORT

A 19-year-old female presented with an open extruded fracture of the right talus following a fall from a height of approximately 1 meter. An ambulance transported the patient to our hospital 1 hour after the incident.We established with the first clinical examination a laceration on the lateral side of the ankle joint measuring about 10 cm with a fracture of the neck of the talus and extrusion of the posterior part of the talus, lateral malleolus and part of tibial pilon through the wound. The radiographs show a fracture of the neck of the talus and luxation (extrusion) of the posterior part of the talus (position in ankle joint) from the talocalcaneal without tarsus-metatarsus dislocation (Figure 1). We classified it as Hawkins II type talar fracture according to the modified classification of these injuries according to Canale and Kelly (6).

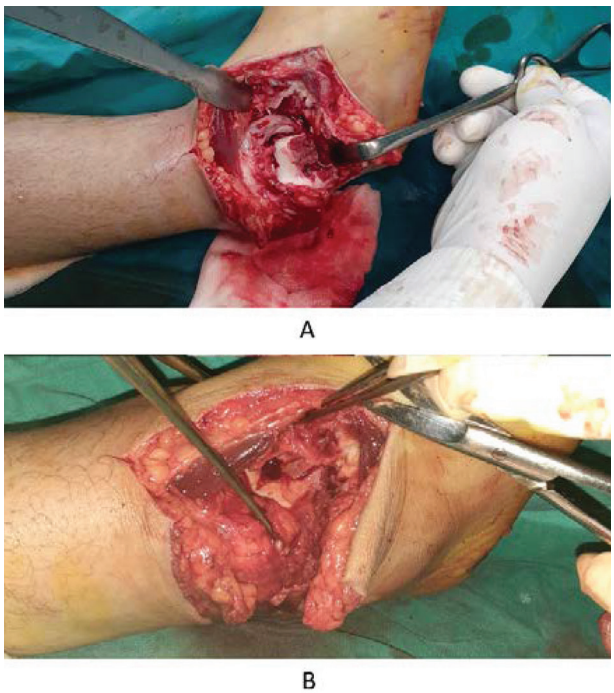
After a short preoperative preparation and radiological diagnosis of the injury, the operative procedure was started within four hours of the patient's admis-



A B

Figure 1. *A - clinical and B - radiography images show approximately 10 cm laceration on the lateral aspect of the right ankle with a posterior talus, part of the tibial pilon, and lateral malleolus completely extruded through the skin*

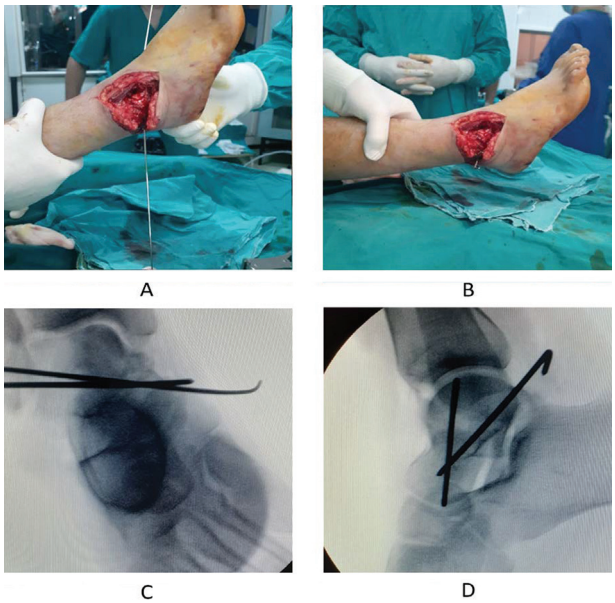
sion. Before the actual operation, two hours after admission to the hospital, the patient received antibiotic therapy (second-generation *Cephalosporins* and *Aminoglycosides*) as well as anti-tetanus protection. Under general anesthesia, we performed wound irrigation, debridement, and intraoperative swab collection. The extruded talus was repositioned under C-Arm fluoroscopy (Figure 2) and fixed with two Kirschner wires (Figure 3).



A

B

Figure 2. *Excision of necrotic soft tissue (**A**) and reimplantation (**B**) of the talus*



A B C D

Figure 3. *Minimally percutaneous osteosynthesis of the talus with two Kirschner wires. A and B - Intraoperative look (shew), C - Anterior Posterior (AP) dorsoplantar radiograph view and D - lateral radiograph view*

After radiographic confirmation that the fragments of the talus were successfully brought to the anatomical position in both the ankle and talocalcaneal joints, we tested the movement and stability of the ankle joint fragments using C-Arm fluoroscopy. Following this assessment, we proceeded with the external fixation of the ankle joint in a neutral dorsiflexion position. This was achieved using the Mitkovic type M20 external fixator, which has been modified for rigid and dynamic external fixation of the ankle joint (Figure 4). The procedure concluded with wound drainage and closure.

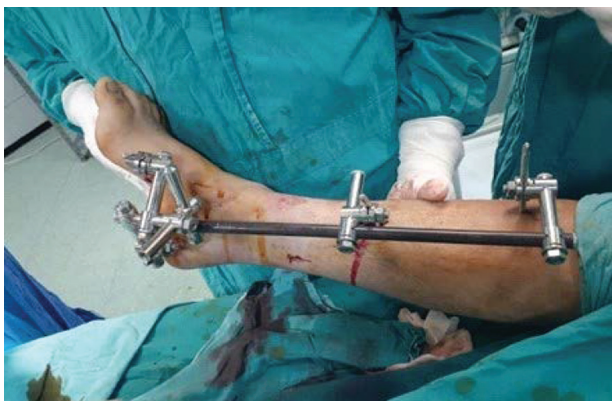


Figure 4. *Placement of Mitkovic-type external fixator M20 modified for rigid and dynamic ankle joint fixation*

The patient was verticalized on the 1st postoperative day and trained to walk using crutches. We prescribed a regimen of second-generation cephalosporin antibiotic therapy (*Cefuroxime* 4.5 g/day i.v), aminoglycosides (*Amikacin* 4g/day i.v) and *Metronidazole* (1,5 g/day i.v) for 7 days. Thromboembolic prophylaxis was administered for 35 days, alongside analgesics for pain management. The patient remained hospitalized for 14 days until the sutures were removed, during which the wound healed without infection.

Pin site care was performed twice a week while in the hospital, and subsequently at another medical institution. A wound swab revealed the presence of two bacteria: *Staphylococcus aureus* and *Pseudomonas aeruginosa.* Despite this finding, we did not change the antibiotic therapy. Laboratory findings for inflammation remained within normal limits during the hospital stay. We also closely monitored clinical signs of wound healing and, based on this assessment, determined the appropriate duration of antibiotic therapy.

The external fixator was unlocked to allow movement in the ankle joint after 6 weeks, and the patient was referred to physical therapy, which included kinesiotherapy, electrotherapy, and pulsed magnetic field therapy. Partial weight-bearing of 10-20% was allowed after 8 weeks. At the 10-week mark post-trauma, ankle radiographs were taken to examine the Hawkins sign. After 12 weeks, the patient was permitted to bear 50% of their weight.

The Kirschner wires were removed after 12 weeks, and the fixator was removed under intravenous anesthesia after 18 weeks. Following this, the patient was referred for further physical therapy with a plan to gradually transition off crutches within two weeks and achieve full weight-bearing on the injured leg. There were no signs of deep infection around the pins.

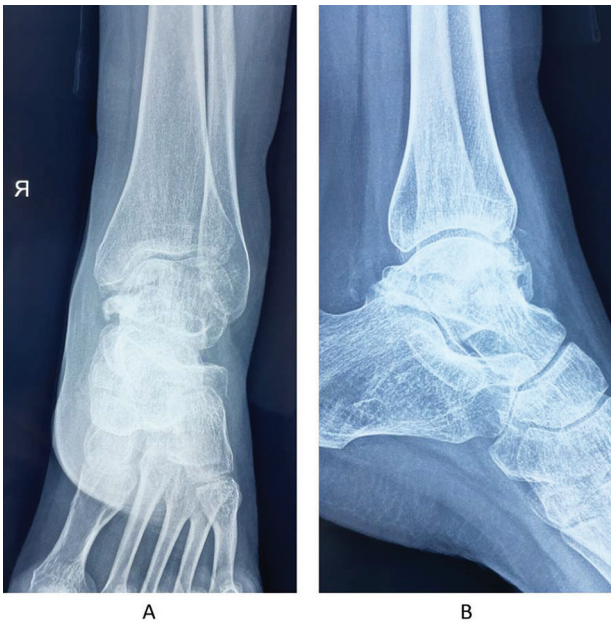


Figure 5. *Radiography 24 months after operation shows well-maintained density of the talus; A - Anterior Posterior (AP) dorsoplantar radiograph view and B - lateral radiograph view*

The patient attended check-ups every two weeks initially, and after the removal of the fixator, every two months. Radiographic follow-ups were conducted up to 24 months postoperatively (Figure 5).

On the last radiographs, there were no signs of arthritis in the ankle joint, and the talus healed in an excellent position. A radiographic examination showed no sign of avascular necrosis of the talus. The clinical findings of the ankle joint at the end of the treatment were excellent with optimal plantar flexion of 50° and with a restriction of ankle motions of 10° in dorsiflexion that which did not significantly impair walking or normal daily activities (Figure 6).

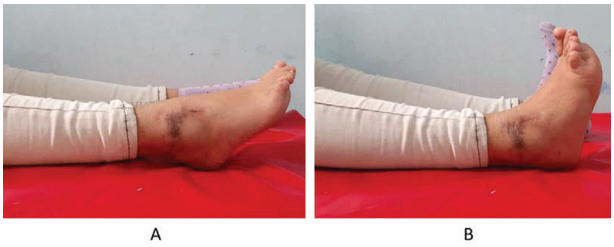


Figure 6. *Functional recordings 24 months after operation; A - maximal plantar flextion of ancle joint (50°); B - Dorsiflection of ancle joint (-10°)*

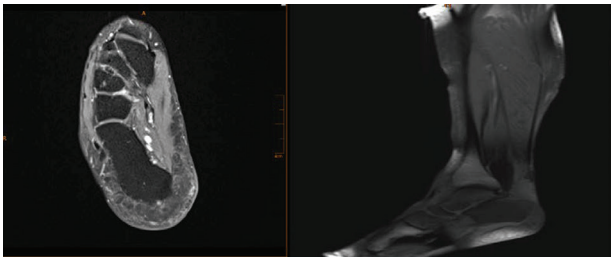


Figure 7. *MRI of the right ankle and foot after 24 months of follow-up. A and B views show complete bony union with no signs of instability, avascular necrosis, or talar collapse*

DISCUSSION

Traumatic extrusion of the talus is a rare injury that occurs after high trauma energy. Exaggerated ankle plantar flexion with extreme subtalar supination causes dislocation and disruption of ligaments. Many treatment options are described in the literature, but none of them guarantee a successful result. Of course, there is no consensus about appropriate treatment (7).

Not so long ago, talectomy with primary tibiocalcaneal arthrodesis was preferred over reimplantation

(8). In his study, Issaoui suggested that completely extruded or grossly contaminated thallus should be replaced (9). Some authors, to reduce the risk of infection, delay reimplantation of the talus and use antibiotic cement in the form of talus while awaiting results. Immediate talus reimplantation and osteosynthesis are most often performed. After reimplantation osteosynthesis of the talus can be performed by ORIF (open reduction and internal fixation), percutaneous Kirschner wires, or external fixation (10). We decided to use the Mitkovic-type external fixator M20, modified for dynamic external fixation of the ankle joint (11, 12). In our case, respecting all operational postulates treatment of an open fracture, using antibiotics and anti-tetanus protection, surgical debridement, and using external fixation very carefully, we reduced the risk of infection and avascular necrosis.

Otherwise, external fixation can be a definitive method of treatment, although often in practice it is temporary (13). In our case, we used the method of external fixation as a definitive method of treatment, with additional stabilization by percutaneous Kirschner wires.

The use of antibiotics in the case of an open fracture is long-term and lasts at least 2 weeks. The combination advised initially is similar to that for other open fractures (14). We used triple antibiotic therapy for two weeks. We had no wound infection.

The pin site care that we implemented was by the standard recommendations for pins site care (15).

The mean healing time of a talus neck fracture in this type of fracture is 10-12 weeks on average (16). In our case, talus union was visible on radiographs at about 14 weeks, but we kept the external fixator for another 4 weeks as a precaution.

Of course, the percentage of occurrence of avascular necrosis of the talus is of great concern to the surgeon. Unfortunately, that is hard to predict today. The most important and only indicator of revascularization of the talus which is observed on conventional radiographs, 6 to 12 weeks after the injury is the Hawkins sign. We had Hawkins sign on the control radiographs in that period. Most authors agree that the follow-up time for patients with a talus fracture is about 2 years (17). In our case, no avascular necrosis occurred 24 months after the operation.

The functional results of the treatment of open and extruded fractures of the talus neck in our case correspond to the results of the treatment described in the literature (18, 19). In the literature, there are no clearly defined treatment guidelines for this injury. The first choice of treatment which showed good and promising functional results is the reimplantation of fractured talus and osteosynthesis. Recent studies recommend that immediate reimplantation of extruded talus in open fracture is the safest procedure with a very favorable treatment effect (18, 19).

CONCLUSIONS

In conclusion, in our case, by observing all the principles of treating an open fracture, using antibiotics, anti-tetanus protection, irrigation, debridement of the wound, repositioning of the talus, and fixation with the method of external fixation and additional Kirschner wires, we obtained a good anatomical and functional result treatment. Certainly, this type of treatment does not exclude arthrodesis or arthroplasty of the ankle joint. The success of such treatment supports our therapeutic choice. We had no side effects such as avascular necrosis, infection, or talar collapse, achieving a good functional outcome with a relatively less invasive procedure contributing to a better quality of life for the patient.

Abbreviations

AP - Anterior Posterior

ORIF - Open reduction and internal fixation

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki. Ethical approval was not required, as this was a case report.

Informed Consent Statement: Written informed consent was obtained from the patient for the publication of this report.

Data Availability Statement: All data generated or analyzed for this report are included in the published article.

NOTE: Artificial intelligence was not used as a tool in this study.

Sažetak

LEČENJE OTVORENOG EKSTRUDIRANOG PRELOMA VRATA TALUSA KOMBINOVANOM METODOM SPOLJAŠNJE FIKSACIJE MODIFIKOVANE ZA DINAMIČKU FIKSACIJU SKOČNOG ZGLOBA I KIRSCHNEROVIM IGLAMA

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Uvod: Otvoreni ekstrudirani prelom talusa predstavlja 2% od svih preloma talusa. Zbog brojnih komplikacija (infekcija, nesrastanje, artritis) predstavlja veliki izazov za hirurga. Najčešće korišćeni metod lečenja je reimplantacija talusa i osteosinteza.

Prikaz slučaja: 19-godišnji pacijent zadobio je otvoreni ekstrudirani prelom talusa tipa Hawkins II nakon pada sa visine. Hitni operativni zahvat koji smo uradili bio je debridman rane, reimplantacija talusa, osteosinteza sa spoljnom fiksacijom modifikovanom za dinamičku spoljašnju fiksaciju skočnog zgloba (Mitković-tip) i dve Kirschnerove igle. Repoziciju fragmenata kontrolisali smo C-Arm fluoroskopijom. Pacijentu je ordinirana 14-dnevna antibiotska terapija (cefalosporin, aminoglikozid, metronidazol) i tromboembolijska profilaksa u trajanju od 35 dana. Posle 6 nedelja dozvolili smo pokrete u skočnom zglobu uz fizikalnu terapiju. Postepeno povećanje oslonca na povređenoj nozi dozvoljeno je nakon 8 nedelja. Osteosintetski materijal je uklonjen nakon 18 nedelja, a tretman je nastavljen fizikalnom terapijom sa punim opterećenjem na nozi nakon 6 meseci. Rendgensko postoperativno praćenje talusa rađeno je do 24. meseca. Odličan rezultat dobijen je sa zalečenim talusom i minimalnim ograničenjem dorzalne fleksije stopala.

Zaključak: Lečenje ove povrede predstavlja veliki izazov za hirurga. Spoljna fiksacija može biti izabrana kao metoda lečenja otvorenog luksacionog preloma talusa.

Ključne reči: slučajni padovi, skočni zglob, Kirschnerove igle, spoljni fiksatori, talus.

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Case report

INTRACYSTIC HEMORRHAGE IN THE LUMBAR SPINE AS A CAUSE OF SUDDEN LEG WEAKNESS – A CASE REPORT

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Abstract: **Introduction**: Synovial cysts of the facet joint are rare benign changes occurring in the spinal synovial joints, predominantly in the lumbar region. Although these cysts are generally benign, intracystic hemorrhages are extremely rare and can lead to severe complications.

Case Presentation: This case report details a patient who developed cauda equina syndrome due to hemorrhage within a synovial cyst in the lumbar spine. The patient's symptoms included acute radicular pain and neurological deterioration. Diagnostic imaging revealed a large synovial cyst with evidence of intracystic bleeding.

Conclusion: Acute radicular pain and rapid neurological deterioration can result from a synovial cyst in the lumbar spine, particularly when complicated by intracystic bleeding. Urgent decompressive surgery and removal of the cyst resulted in complete recovery for the patient.

Keywords: synovial cysts, bleeding, leg weakness.

INTRODUCTION

Synovial cysts, which arise as a consequence of facet osteoarthritis, can cause pain syndromes and radicular complaints due to the direct compression of the dural sac and nerve roots in the lateral recesses (1). Their etiology is likely related to increased mobility of the facet joints and herniation of the synovium through a ruptured capsule. Although these cysts are most commonly located epidurally and posterolaterally outside the vertebral canal, anterior cysts that project towards the canal can also occur. Occasionally, these cysts may enlarge and exacerbate symptoms, with hemorrhage potentially causing rapid and significant cyst expansion (2). Conversely, spontaneous resolution of symptoms can occur following decompression of the facet joint cyst. Synovial cysts located in the upper lumbar spine are relatively rare, and only a few cases involving acute intracystic bleeding have been reported (3). Intracystic bleeding can lead to the development of acute cauda equina syndrome, a serious neurological condition that requires immediate intervention (3).

In this report, we present the clinical course, diagnostic approach, and treatment of a patient with this rare but potentially serious condition.

CASE REPORT

Patient SM, a 54-year-old man, presented to a neurologist at the Clinical Hospital Center in Gračanica, Serbia, with complaints of lumbar spine pain, leg weakness, and urination difficulties, which had started a week prior. On his own initiative, he had been using non-steroidal anti-inflammatory drugs (NSAIDs) to manage the pain. The day before hospitalization, while driving, he experienced tightness in his right calf. When attempting to stand, he noticed weakness in his legs and difficulty moving. Over the following two days, his mobility was severely restricted, and he could only walk and stand with assistance.

Neurological examination upon admission revealed polyradicular motor and sensory deficits at the L5 and S1 levels. The most significant weakness was in the feet, with the manual muscle test (MMT) scoring 1/5 for the right peroneal and tibial muscles, and 2/5 for the left foot. Motor and sensory deficits followed a radicular distribution pattern.

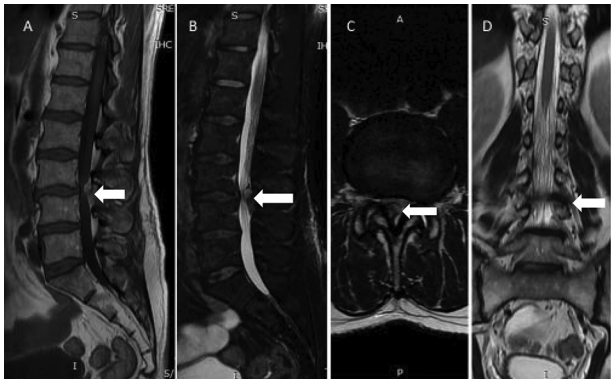

Figure 1. *Lumbosacral spine MR: sagittal TW1 (A), sagittal TW2 (B), axial TW2 (C), and coronal TW2 (D) show a synovial cyst of the right L3-4 facet joint with signs of hemorrhage and critical spinal canal stenosis. (Changes are indicated by arrows.)*

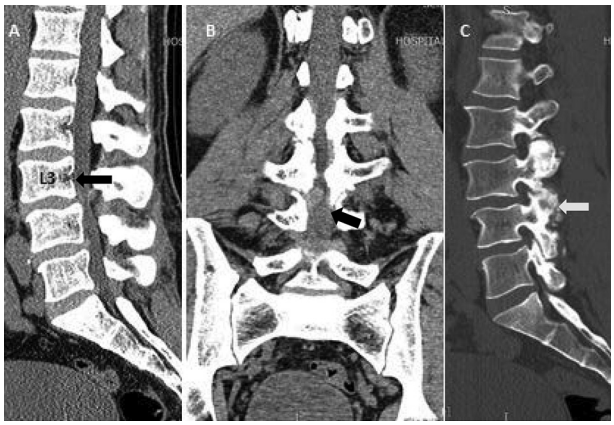

Figure 2. *CT scan of the lumbosacral spine: (A) spondylolisthesis L3 corresponds to Taillard grade I (< 10%), (B) synovial cyst in the posterolateral part of the L3-4 right facet joint, (C) hypertrophic facet arthropathy at the level of the facet joint L3-4. (Changes are indicated by arrows.)*

Given the clinical presentation and neurological findings, neuroradiological diagnostics were promptly performed, confirming the presence of a synovial cyst in the right L3-4 facet joint, measuring 2 x 1.5 cm, with intraspinal extension and critical stenosis of the spinal canal (Figures 1 and 2). The patient underwent urgent surgical intervention, during which the tumor-like mass in the spinal canal was removed. Intraoperative findings revealed a degeneratively altered synovial cyst with areas of fresh bleeding.

Postoperatively, the patient experienced gradual neurological recovery. One month after surgery, he was able to move independently. Follow-up magnetic resonance imaging showed satisfactory postoperative results, with no recurrence of the original pathology (Figure 3). The patient adhered to a rehabilitation program and regular check-ups. One year after the surgery, he resumed his daily activities without issues.

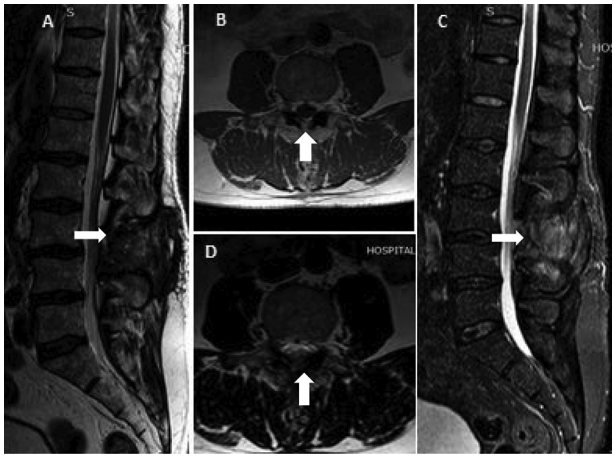

Figure 3. *MR of the lumbosacral spine: (A-D) sagittal and axial tomograms - state after L3 decompressive laminectomy and medial facetectomy. (Changes are indicated by arrows.)*

Informed consent for publication was obtained from the patient.

DISCUSSION

Synovial cysts are rare but significant complications of facet osteoarthritis that can lead to severe neurological symptoms. Facet syndrome typically presents with pain that worsens with prolonged sitting, standing, backward bending, lateral bending, and twisting motions (4). The term “juxtafacet cyst” is commonly used in the literature to describe synovial and ganglion cysts located in the extradural region of the spine. Studies indicate that most non-hemorrhagic lumbar synovial cysts are found at the L4-L5 level (5). In contrast, the cyst in our patient was located in the upper lumbar region, underscoring the rarity and severity of the condition.

The patient’s symptoms included lumbar pain, leg weakness, and urination problems, which are characteristic of cauda equina syndrome. Clinical findings on admission revealed polyradicular motor and sensory deficits at the L5 and S1 levels, consistent with the neurological examination. The manual muscle test (MMT) demonstrated significant weakness in the feet, highlighting the severity of the condition. Cannarsa et al. (6) reported that bleeding within synovial cysts occurs more frequently in men and at a younger age, suggesting a link to microtrauma based on occupation and lifestyle. Although NSAIDs were used by the patient to manage pain, they are not a primary cause of bleeding. However, their use might exacerbate bleeding risks due to reduced blood clotting, particularly in predisposed individuals (7).

Intracystic hemorrhage can rapidly enlarge the cyst and lead to acute compression of nerve roots, as

observed in our case. While clinical and neurological evaluations are essential for assessing spinal synovial cysts, radiological diagnostics are crucial for accurate identification of these lesions. In our patient, magnetic resonance imaging (MRI) confirmed a synovial cyst in the right L3-4 facet joint, with intraspinal extension and spinal canal stenosis (Figures 1 and 2). Computed tomography (CT) revealed L3 spondylolisthesis and hypertrophic facet arthropathy at the same level. The combined use of MRI and CT provided a comprehensive view of the pathological changes, facilitating precise diagnostic and therapeutic planning. MRI is particularly useful in differentiating synovial cysts from peripheral nerve tumors, such as neurofibromas, especially in younger patients (8).

Conservative management is typically the first-line treatment for patients without neurological deficits, as synovial cysts can sometimes regress spontaneously (9). For our patient, surgical intervention, including decompressive laminectomy and cyst excision, led to a gradual recovery of neurological function and significant improvement in clinical condition.

CONCLUSION

Acute radicular pain can result from a synovial cyst in the upper lumbar spine, with or without associated intracystic hemorrhage. In our patient, acute intracystic bleeding led to rapid and severe neurological deterioration. Urgent diagnostic evaluation was crucial for determining prognosis and selecting an appropriate therapeutic strategy. Decompressive surgery and removal of the synovial cyst resulted in complete resolution of acute symptoms and significant improvement in neurological deficits.

Abbreviations

NSAIDs - Non-steroidal anti-inflammatory drugs
MMT - Manual Motor Test
CT - Computed Tomography
MR - Magnetic Resonance

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Sažetak

INTRACISTIČNO KRVARENJE U LUMBALNOJ KIČMI KAO UZROK IZNENADNE SLABOSTI NOGU – PRIKAZ SLUČAJA

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Uvod. Sinovijalne ciste fasetnog zgloba su retke benigne promene koje se javljaju u kičmenim sinovijalnim zglobovima, često u lumbalnoj regiji. Ove promene mogu izazvati različite simptome, a intracistične hemoragije su izuzetno retke, ali mogu biti ozbiljne komplikacije.

Prezentacija slučaja. U ovom prikazu slučaja opisujemo klinički tok, dijagnostički pristup i terapiju pacijenta koji je imao sindrom cauda ekuina izazvanog krvarenjem unutar sinovijalne ciste u lumbalnoj kičmi.

Zaključak. Akutni radikularni bol može biti uzrokovan sinovijalnom cistom u gornjoj lumbalnoj kičmi. Intracistična krvarenja, kod našeg pacijenta, uzrok su brzog i ozbiljnog neurološkog pogoršanja, pa hitna dekompresivna operacija i uklanjanje sinovijalne ciste dovode do potpunog oporavka.

Ključne reči: ciste, krvarenje, slabost nogu.

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IMPACT OF VITAMIN E ON DIFFERENT ORGAN SYSTEMS

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Abstract: Vitamin E, present in both plant and animal-based foods, is a lipophilic compound with multifaceted biochemical functions. Its deficiency can lead to severe health consequences, while excessive intake may result in hypervitaminosis. Among tocopherols, Alpha-tocopherol stands out for its pharmacokinetic properties and potent antioxidant effects. It exerts significant influence on the immune, nervous, and cardiovascular systems, as well as on skin health. Notably, it plays a crucial role in preventing carcinogenesis. While Alpha-tocopherol garners attention, other tocopherol group members should not be overlooked, as advancements in science uncover their diverse biological impacts. In recent years, tocotrienols have emerged with distinct biochemical properties that profoundly affect human health.

Keywords: vitamin E, alpha-tocopherol, immune system.

INTRODUCTION

Vitamin E was first found in green vegetables (1). Isolated in 1922, its numerous roles in biological functions have since become the focal point of study for many scientists. It serves as a powerful antioxidant and plays a crucial role in preventing lipid peroxidation and removing free radicals. The vitamin E family consists of two major groups: tocopherols and tocotrienols, both of which are fat-soluble. These groups can be further divided into four subgroups. All tocopherols share an aromatic chromanol head, leading to several isomers such as alpha, beta, gamma, and delta isomers. In contrast, tocotrienols, the other group, possess hydrocarbon chains of unsaturated fatty acids, differing from tocopherols, which have saturated fatty acids in their tail comprised of 16 carbons. Tocotrienols demonstrate higher solubility in lipid membranes compared to tocopherols (2).

Vitamin E, particularly α-tocopherol, is abundant in rice bran, palm oil, olives, soybeans, and various grains (1). Its antioxidant properties play a crucial role in neutralizing free radicals generated by lipid oxidation. Consequently, an inadequate diet leads to decreased vitamin E levels, increasing the risk of cardiovascular diseases. Certain nutrients exhibit the ability to reduce free radicals and offer protective effects against oxidative stress (3), with alpha-tocopherol being the most potent and prevalent vitamin in this group. Various studies provide adequate recommendations for this vitamin (4).

A year-long study conducted in India on two groups of subjects evaluated the influence of antioxidants, specifically vitamins A and E. Subjects with well-controlled diabetes exhibited significantly higher serum vitamin levels compared to those with uncontrolled disease (5). Tocotrienols possess a molecular structure distinct from tocopherols, featuring a farnesyl group (6). Tocopherol serves as a ubiquitous antioxidant in nature and plays a pivotal role in neutralizing free radicals. Consequently, it is predominantly found in mitochondria and the sarcoplasmic reticulum within cells (7).

Vitamin E encompasses eight different compounds. Like other fat-soluble vitamins, they are absorbed into the bloodstream via the small intestine. Metabolic processing in the liver primarily occurs for alpha-tocopherol, while other forms are excreted unchanged (8). Common causes of deficiency often stem from irregularities in dietary fat absorption or inadequate metabolism. The optimal serum tocopherol level for adults should not exceed 5 mcg/ml (8)

Impact on the immune system

Vitamin E exerts its effects on the immune system by influencing certain inflammatory mediators, including eicosanoids and cyclooxygenase-2 (1). Stud-

ies primarily focusing on alpha-tocopherol have elucidated its immunomodulatory effects through various mechanisms. Alpha-tocopherol directly affects T lymphocytes by stabilizing their membranes and also contributes to the generation of intercellular signals that influence inflammatory factors (9). Hypovitaminosis of vitamin E adversely affects both cellular and humoral immunity. Furthermore, vitamin E enhances the phagocytic capacity of macrophages (10) and increases the production of interleukin-2 (IL-2). Tocopherols have also been shown to protect respiratory tract cells during viral infections, including SARS-CoV-2 infection (11).

Supplementation, sources, and recommendations of Vitamin E

Some studies suggest that tocopherol supplementation reduces erythrocyte deformities, although the evidence remains inconclusive, warranting further research. A dosage of 1600 IU of vitamin E for several weeks has been shown to reduce oxidative stress, thereby decreasing the susceptibility to diseases associated with this issue (7). However, data from randomized trials indicate that routine supplementation does not reduce the risk of preeclampsia and poor fetal growth (12). For adolescents of both sexes, the recommended daily intake is 9 mg for ages 10 to 13 and 12 mg for ages 14 to 19 (4). The safe and optimal intake of tocopherol for adults is up to 15 mg per day (13).

Vitamin E is exclusively obtained through dietary sources and is not synthesized by intestinal flora bacteria or within the organism itself (14). Breastfeeding mothers may need to supplement their vitamin E intake to achieve the recommended daily dose of 19 mg. Daily vitamin E supplementation from prenatal multivitamins for the mother can be safe and modestly increase vitamin E levels in breast milk, thereby improving the baby's vitamin E status. Additionally, women with higher polyunsaturated fatty acid intake tend to have higher levels of alpha-tocopherol in breast milk (15). However, it's essential to prioritize a healthy diet over vitamin E supplementation, and any supplementation should be recommended by a healthcare professional (16).

In practice, vitamin E has not demonstrated toxicity within recommended doses for humans (17). Results from a randomized study conducted in Pakistan suggest that delta-tocotrienol supplementation, when added to other glycemic control agents, may prevent long-term diabetic complications in patients with type 2 diabetes mellitus (18).

Vitamin E hypervitaminosis

Hemorrhage can occur as a result of excessive doses of vitamin E. Toxic doses ranging from 100 mg to 1 gram can lead to cerebral hemorrhage (13). While vitamin E deficiency is uncommon, routine supplementation is not recommended. Instead, patients should be encouraged to maintain a balanced and varied diet to obtain adequate levels of alpha-tocopherols. If supplementation is deemed necessary, it's crucial to monitor for common adverse reactions, potential drug interactions, and the risk of bleeding, particularly in patients receiving anticoagulant therapy (16).

Vitamin E and carcinogenesis

Recent studies have examined the impact of tocopherols and its isomers on cancer prevention. Vitamin E has been found to have a preventive effect against certain types of cancer. For instance, a meta-analysis demonstrated an inverse relationship between increased vitamin E intake and the risk of uterine cancer (19). However, comparisons between tocopherol and tocotrienol have shown that while alpha-tocopherol exhibits higher systemic bioavailability, gamma and delta forms have a stronger protective effect against cancer (19). Additionally, research investigating the combined effect of vitamin E and doxorubicin on breast cancer cells in vitro revealed significant cytotoxicity with high doses of vitamin E (20). Certain members of the vitamin E group have also shown preventive effects against mechanisms leading to prostate cancer (21).

Genetic factors and the influence of Vitamin E on the nervous system

Vitamin E supplementation has been associated with reductions in certain neurological symptoms (22, 23). Moreover, vitamin E plays a role in gene expression regulation (24). Nutrigenomics, as a complex field, must consider the polymorphic properties of different genetic groups involved in vitamin E metabolism (25). Mutations in the alpha-tocopherol transfer protein gene can lead to familial vitamin E deficiency, oxidative stress, and progressive neurological diseases such as spinocerebellar ataxia (26). Current research indicates that vitamin E and its metabolites have neuroprotective effects (27).

Alzheimer's disease, mitochondrial dysfunction, and Vitamin E

Alzheimer's disease, characterized by memory loss and cognitive decline, is associated with mitochondrial dysfunction and increased oxidative stress. Vitamin E's potent antioxidant properties make it effective in combating free radicals, which play a role in neuronal damage. Walnuts, rich in vitamin E, are linked to Alzheimer's prevention (28, 29). Studies

have also shown that ascorbic acid and high doses of vitamin E can positively impact cognitive function (30). Furthermore, several meta-analyses have found correlations between serum antioxidant levels, particularly vitamin E, and depression (29).

Impact on the skin

The skin serves as a crucial barrier, safeguarding the body's integrity against various external factors. Research conducted by the World Health Organization reveals that nearly a third of the global population suffers from skin issues, attributable to deficiencies in specific vitamins and minerals (31). Among these, Vitamin E stands out for its profound impact on skin health. Its primary function is to protect the skin from oxidative damage induced by sunlight through neutralizing free radicals (32).

Studies indicate that pre-exposure application of alpha-tocopherol can shield the skin from various types of damage caused by ultraviolet radiation (33). For example, a regimen of 1000 IU of Vitamin E daily for six months has been effective in treating yellow nail syndrome. Additionally, it plays a role in managing other skin disorders. In cases of skin amyloidosis, Vitamin E, when combined with other active ingredients, significantly contributes to the disease's treatment (34, 35).

CONCLUSION

Vitamin E, found in both plant and animal foods, holds significant importance for overall health. While alpha tocopherol has traditionally received the most attention, recent research has highlighted the biological significance of other tocopherol forms and tocotrienols. The powerful antioxidant properties of these compounds play a crucial role in preventing various diseases discussed in this paper, emphasizing the importance of maintaining optimal levels of vitamin E in the body.

The impact of vitamin E on skin health is particularly notable, with its antioxidant properties offering protection against photooxidant damage. Studies on photoprotection demonstrate its effectiveness in reducing acute skin reactions, while its therapeutic potential is evident in dermatological disorders such as yellow nail syndrome and subcorneal pustular dermatoses.

Special attention should be given to pregnant and breastfeeding women, as well as individuals with diets deficient in vitamin E. Prevention remains key in avoiding pathology associated with vitamin E deficiency, underscoring the importance of ensuring adequate intake of this essential vitamin.

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Sažetak

UTICAJ VITAMINA E NA RAZLIČITE ORGANSKE SISTEME

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Vitamin E se nalazi u hrani biljnog i životinjskog porekla. Vitamin E je lipofilno jedinjenje koje ima brojne biohemijske funkcije. Nedostatak vitamina E može imati ozbiljne zdravstvene posledice kao i hipervitaminoza sa datim vitaminom. Među svim jedinjenjima koja pripadaju grupi tokoferola, alfa-tokoferol se posebno ističe po svojim farmakokinetičkim svojstvima i funkciji. Alfa tokoferol je veoma jak antioksidans. Njegovo dejstvo na ljudski organizam ogleda se u brojnim antioksidativnim reakcijama, posebno na imuni, nervni sistem i kardiovaskularni sistem, kao i na kožu. Od posebnog značaja u proučavanju ovog vitamina je prevencija kancerogeneze. Međutim, ne treba zanemariti ni druge predstavnike ove grupe, jer su njihovi biološki uticaji postali sve očigledniji sa napretkom nauke. Poslednjih decenija otkrivena su različita biohemijska svojstva tokotrienola koja značajno utiču na ljudski organizam.

Ključne reči: vitamin E, alfa tokoferol, imuni sistem.

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Review article

DIABETIC KETOACIDOSIS IN PREGNANCY

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Abstract: Diabetic ketoacidosis (DKA) is one of the most serious and life-threatening complications of diabetes mellitus (DM), especially when it occurs during pregnancy, with a prevalence ranging from 0.5% to 3%. Pregnancy is considered a susceptible environment for the development of this type of metabolic imbalance due to its inherent physiological changes. Unspecific symptoms (vomiting, diarrhea, abdominal pain, etc.), especially in pregnant women, and the fact that ketoacidosis can develop even with normal glucose values (defined as euglycemic ketoacidosis), often lead to a delayed diagnosis. Evidence suggests that timely diagnosis and appropriate management of ketoacidosis are crucial in preventing adverse outcomes for both the mother and the fetus. Fetal outcomes are often dichotomous, resulting in either fetal demise (miscarriage/stillbirth) with a prevalence of 10% to 35%, or the birth of a healthy baby, with possible complications primarily related to diabetes mellitus itself. Additionally, case reports of ketoacidosis developing even in non-diabetic women due to other diseases (such as acute pancreatitis, appendicitis), as well as in those with gestational diabetes mellitus (GDM), further emphasize the importance of considering this condition in everyday clinical practice. The aim of this paper is to further elucidate the causes and course of this complication, as well as the outcomes for both mother and fetus, to contribute to a better overall understanding.

Keywords*:* diabetes mellitus, diabetic ketoacidosis, pregnancy.

INTRODUCTION

Diabetic ketoacidosis (DKA) represents one of the most severe complications of diabetic pregnancies, posing a life-threatening risk for both mother and fetus. Initially mostly associated with type 1 diabetes mellitus (DM), it can also develop in type 2 DM and gestational diabetes mellitus (GDM). Its significance lies not only in the metabolic imbalance it causes but also in the potentially fatal consequences for both mother and fetus, necessitating immediate and proper management (1)**.** The prevalence of DKA in pregnancies complicated by either pregestational or gestational DM is estimated to be around 0.5-3% (2).

Physiological aspects of pregnancy make it a state susceptible to ketoacidosis. Pregnancy is recognized as a state of accelerated starvation, associated with an increase in insulin counter-regulatory hormones (human placental lactogen, progesterone, prolactin, tumor necrosis factor α), as well as impaired buffering capacity due to respiratory alkalosis (with compensatory renal loss of bicarbonates), and a notable rise in insulin demands, especially in the second trimester (2). Additionally, there is an increase in gluconeogenesis and glycogenolysis, a decrease in peripheral glucose uptake, followed by lipolysis and enhanced ketone production in the liver. All these factors, along with increased fetoplacental glucose demands, in the context of relative or absolute insulin deficiency, contribute to the development of DKA (3, 4, 5).

DKA can be provoked by vomiting, starvation, non-compliance with insulin therapy, poor glycemic control, infection, glucocorticoid therapy, β-mimetic therapy, stress, labor, etc. (2). The classic triad of hyperglycemia, ketonemia (beta-hydroxybutyrate, acetoacetate, and acetone), and a high anion gap clearly suggests the presence of DKA (1). Diagnostic criteria for DKA, proposed by The Joint British Diabetes Societies Inpatient Care Group, include: ketonemia > 3 mmol/L or ketonuria (> 2+ on urine strips), blood glucose > 11.0 mmol/L or known diabetes, bicarbonates < 15 mmol/L, and/or venous pH < 7.3 (3). DKA can

be further classified based on pH values as mild (7.25-7.30), moderate (7.0-7.24), or severe (< 7.0) (1).

Treatment focuses on excessive fluid resuscitation, continuous insulin therapy, potential need for electrolyte (potassium) or bicarbonate replacement, treatment of contributing factors (such as infection), and close monitoring of maternal and fetal responses. Maternal monitoring involves hourly measurements of blood glucose and ketones, with other parameters (blood urea nitrogen, creatinine, electrolytes, venous pH) measured every 2 hours, along with monitoring blood pressure and urine output (6). A potential improvement in maternal monitoring in diabetic pregnancies could be the implementation of continuous glucose monitoring devices, which, due to their precision and non-invasiveness, might lead to prompt detection of glycemic excess (7).

Fetal monitoring in diabetic pregnancies is crucial due to the risk of congenital malformations, macrosomia, polyhydramnios, etc. In the case of ketoacidosis, fetal monitoring involves confirming viability (with further ultrasound assessments as needed), cardiotocography (if suitable), and delivery if indicated. Recovery is defined by pH > 7.3, ketonemia < 0.6 mmol/L, anion gap < 12 mEq/L, and bicarbonates > 15 mmol/L (6, 8). Suggested glucose values post-DKA are 5.6-8.3 mmol/L (9).

CLINICAL POINT OF VIEW

Being extremely endangering but not very frequent, previous experiences in clinical practice are reflected through retrospective studies and case reports. This complication has been observed mostly in women with type 1 diabetes mellitus (DM) and in the third trimester of pregnancy, most frequently precipitated by infections, vomiting, steroid therapy, and medical malpractice. The main associated risk factors include lower socioeconomic status and at least one microvascular complication of DM before pregnancy (9).

A retrospective study conducted at Mayo Clinic showed that most women with DKA events had poor preconceptual glycemic control with high HbA1c values (9%), which improved during pregnancy but still remained high (7.5% and 7.6%). Cases of fetal loss (17%) mostly occurred either at the time of admission or within 1 week after discharge from the hospital after DKA treatment (miscarriage or stillbirth). More than half of the neonates required admission to the neonatal intensive care unit (NICU), 2/3 experienced neonatal hypoglycemia, 1/3 were large for gestational age (LGA), and every 10th had a congenital anomaly. Additionally, it was observed that administering antenatal corticosteroids might precipitate this condition, therefore, the need for an adjustment of insulin doses when administering these drugs was pointed out (4).

Another retrospective study conducted in South Africa showed similarly that DKA occurs predominantly in women with type 1 DM, in the early third trimester, in poorly controlled DM cases (with an average HbA1c of 9.2% at the time of diagnosis). The most often observed conjoined factors were infections (especially of the urinary tract) and discontinuation of insulin therapy. Overall, a higher rate of fetal losses occurred (28%), with a significant association between low potassium levels and fetal demise (1). No maternal deaths were noted in any of the previously mentioned studies (1, 4, 10).

Considering these facts, the developing fear of fetal loss seems justified. However, an approach which attempts to reverse maternal metabolic acidosis rather than undergoing immediate delivery seems to be preferable. Submitting a mother with unregulated ketoacidosis to an emergency cesarean section might lead to the worsening of her condition with not much benefit to the fetus itself. One of the many studies that supported this approach is a case report of a woman at 29 weeks gestation, with poorly regulated type 1 DM and diagnosed DKA. Despite reduced variability of the fetal heart rate pattern, and repeated unprovoked decelerations presented at admission, a decision was made to mainly handle maternal metabolic imbalance. After successful and prompt treatment, the patient was discharged and later regularly controlled by obstetricians and endocrinologists. She delivered vaginally at 34 weeks due to premature spontaneous rupture of the membranes, to a healthy baby (11). Although uncommon, this condition was also observed in gestational DM at 30 weeks gestation, unfortunately, with intrauterine fetal demise at the time of DKA presentation (12).

FETAL OUTCOMES

In addition to the well-known consequences DKA might have on maternal health (acute renal failure, respiratory distress syndrome, cerebral edema, and even death) with reported maternal mortality rates ranging from 5 to 15%, short- and long-term outcomes for children have also been of interest (6, 9).

Fetal outcomes can be severe, and fetal mortality rates vary from 10 to 35%, with developed countries such as the United Kingdom and the United States reporting rates around 16% (4, 10). To provide an updated assessment of fetal outcomes in DKA-complicated pregnancies, a retrospective cohort study covering a 20-year period in Boston was conducted. The authors reported that the most frequent outcomes were NICU admissions (59%), preterm birth (46.3%), and fetal de-

mise (15.6%), all of which were significantly higher than in the general population of women with diabetes mellitus. Contributing factors to fetal demise were mainly associated with the severity of DKA event (the need for maternal intensive care unit admission and higher serum osmolarity), while factors associated with preterm birth and NICU admissions were more related to maternal health (smoking, preeclampsia, higher HbA1c, etc.) (13).

The true pathophysiological mechanisms that lead to fetal complications are still to be fully understood, but several are presumed: maternal dehydration due to osmotic diuresis leading to fetal distress in the light of decreased uteroplacental perfusion; fetal acidosis induced by maternal acidosis; maternal hypophosphatemia leading to fetal hypoxia; fetal hyperinsulinemia followed by hypokalemia causing fetal arrhythmias, etc. (11, 14). Overall, complications such as miscarriage and stillbirth have mainly been related to the metabolic imbalance of DKA itself. Other outcomes, such as large for gestational age (LGA), NICU admissions, neonatal hypoglycemia, shoulder dystocia, etc., are more related to diabetes mellitus itself (3). Higher rates of fetal demise are more frequently seen in newly discovered type 1 diabetes mellitus, and better outcomes seem to be associated with lower glycemia values, lower insulin requirements, gestational age at DKA presentation, as well as a shorter duration of metabolic imbalance (14).

Concerns regarding long-term outcomes for children are predominantly based on the exposure of the fetal brain to increased maternal ketone and lactate concentrations, which are presumed to be associated with fetal brain injury (6). Contributing to these findings, an inverse connection between maternal ketonemia and mental development was found in 2-year-old children, suggesting impaired brain development in these children might be present (15). Furthermore, subsequent neurological and intellectual problems, including autism and seizures, have also been noted (16).

EUGLYCEMIC DIABETIC KETOACIDOSIS IN PREGNANCY

Euglycemic ketoacidosis also represents an acute, life-threatening condition, with the diagnosis being often unintentionally delayed due to unspecific symptoms and the absence of hyperglycemia, and the prevalence of up to 30% of all DKA cases in pregnancy (2).

It is defined as ketoacidosis with normal or marginally elevated levels of serum glucose, but is still presumed as a diagnosis of exclusion because other causes of high anion gap must first be excluded (alcohol intoxication, drug overdose, sepsis, renal failure, etc.) (2, 17).

Treatment resembles the one used for diabetic ketoacidosis, but it is to be noted that fluid replacement requires an additional 5% dextrose along with saline, to avoid hypoglycemia caused by insulin infusion (which remains necessary to prevent further ketone production) (6). Although some evidence-based clinical approaches had suggested that lowering insulin dose in half might be equally effective, Algaly et al. had a different experience. Their case included a 28-year-old woman with DM type 1 presenting with normal glucose levels and anion gap, low pH, and bicarbonate levels. Initial therapy, besides intensive rehydration with crystalloids and potassium supplementation, included insulin at a dose of 0.05 units/kg/h and resulted in further slow progression of metabolic acidosis. Only when the insulin dose was increased to the usual 0.1 units/kg/h, her condition began to improve. The authors concluded that starting insulin rate at a lower dose might not be as effective, and suggesteded that the insulin treatment should resemble the one given for non-euglycemic DKA (16). The significant role of placental counterregulatory hormones in the development of this complication was pointed out in a case report of a woman at 33 weeks gestation with DM type 1 and diagnosed with euglycemic DKA. Despite close monitoring and adequate therapy, her condition had not improved (and unfortunately resulted in fetal demise 8 hours after admission), until the delivery of the fetus and more importantly, the placenta. Soon after delivery, her insulin sensitivity and acidosis rapidly improved, pH continued to rise and the anion gap eventually closed. She was soon discharged without any further events (15). Nevertheless, two cases of euglycemic DKA in the same DM type 1 patient, with contrary outcomes (first pregnancy resulting in fetal demise and the second one with the late preterm birth of a healthy baby), demonstrated the impact of immediate and proper management of this condition, as well as the necessity for further education of pregnant women, obstetricians and diabetologists regarding this entity (2). Frise et al. pointed out the importance of having this condition in mind even in non-diabetic pregnant women because in the 3 case reports, acute pancreatitis was the cause of ketoacidosis (without detected hyperglycemia or previously diagnosed DM) (18). Also, Dikowita et al. reported a case of ketoacidosis in a non-diabetic woman due to fasting and vomiting because of appendicitis, therefore, the need to avoid and prevent long periods of starvation in pregnant women was emphasized (19).

CONCLUSION

Diabetic ketoacidosis in pregnancy represents one of the most serious and life-endangering complications

for both mother and fetus. Due to its nonspecific symptoms, potential to be the first presentation of diabetes mellitus, and the fact that it can occur even within normal glucose values, its diagnosis might not always be immediate. The evidence so far consistently emphasizes that diagnosing this complication promptly and subjecting these women to immediate adequate treatment remains crucial. Therefore, having this entity in mind and understanding its course might help prevent misdiagnosis and subsequent adverse pregnancy outcomes in everyday clinical practice.

Abbreviations

DKA - diabetic ketoacidosis
DM - diabetes mellitus
GDM - gestational diabetes mellitus
LGA - large for gestational age
NICU - neonatal intensive care unit

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Sažetak

DIJABETIČNA KETOACIDOZA U TRUDNOĆI

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Dijabetična ketoacidoza važi za jednu od najozbljnijih i životno ugrožavajućih komplikacija dijabetes melitusa, posebno kada se javi u trudnoći, sa prevalencom od 0,5 do 3%. Trudnoća se smatra stanjem koje je podložno razvoju ovog tipa metaboličkog disbalansa, zbog već prisutnih fizioloških promena. Nespecifični simptomi (povraćanje, dijareja, bol u abdomenu itd.) posebno kod trudnica, kao i činjenica da ketoacidoza može da se razvije čak i pri normalnim vrednostima glikemije (euglikemijska ketoacidoza), dovode do kasne pretpostavke o ovoj dijagnozi. Dosadašnji dokazi sugerišu da se jedino kod blagovremeno dijagnostikovane i adekvatno tretirane ketoacidoze mogu prevenirati loši ishodi za majku i fetus. Fetalni ishodi su uglavnom isključivi, ili je u pitanju fetalni gubitak (pobačaj ili intrauterina smrt ploda) sa prevalencom od 10 do 35%, ili rođenje zdravog deteta sa mogućim komplikacijama za koje se smatra da su najviše povezani sa dijabetesom kao takvim. Dalje, prikazi slučajeva ketoacidoze koja se razvija usled prisustva drugih bolesti (akutni pankreatitis, apendicitis), kod trudnih žena koje ne boluju od dijabetes melitusa, kao i kod onih sa gestacionim dijabetes melitusom, još više naglašavaju potrebu uzimanja u obzir ovog entiteta u svakodnevnoj kliničkoj praksi. Cilj ovog rada je da se rasvetle uzroci i sam tok ove komplikacije, njene posledice po majku i fetus, kao i da se doprinese njenom celokupnom boljem razumevanju.

Ključne reči: dijabetes melitus, dijabetična ketoacidoza, trudnoća.

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Review article

COMPARISON OF CONVENTIONAL PAP SMEAR AND LIQUID-BASED CYTOLOGY IN DETECTING CERVICAL ABNORMALITIES

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Abstract: Cervical cancer represents one of the most common types of cancer in women, requiring early diagnosis to reduce prevalence and mortality rates. The Pap smear plays a crucial role in the early diagnosis of precancerous lesions. While the conventional Pap smear has been the standard method for lesion detection, liquid-based cytology (LBC) is emerging as an alternative with potential advantages.

Research comparing the conventional Pap smear to LBC has shown that LBC yields a higher percentage of satisfactory samples and demonstrates greater sensitivity and specificity in identifying various cervical abnormalities. Similar findings have been reported in Japanese research. However, some studies have shown conflicting results, emphasizing the specificity of the conventional method. While some studies suggest that the conventional Pap smear is better at detecting ASC-US, others show similar or favorable results for the LBC method.

The LBC method stands out for its higher diagnostic sensitivity, especially in detecting various types of cervical abnormalities, while the conventional Pap smear method maintains its specificity, particularly in diagnosing LSIL. The LBC method has the advantage of better sample representativeness and fewer unsatisfactory samples.

Keywords*:* cervical cancer, Pap smear, liquid-based cytology, precancerous lesions of cervical cancer, diagnosis of cervical cancer, Pap smear classification.

INTRODUCTION

Cervical cancer (lat. *Carcinoma cervicis*) is a malignant condition that develops slowly over several years, allowing for detection in its early stages. Compared to other types of cancer, the diagnosis of cervical cancer is relatively straightforward and effective. Timely diagnosis, through regular Pap smear tests, enables the detection of early stages of cervical cancer (1).

The Pap smear is the most effective method for the early diagnosis of cervical cancer. According to the study by Derya et al. (2), participants who underwent Pap tests were more aware of gynecological cancers than those who did not (2).

The Pap smear test and liquid-based cytology (LBC) are the methods most commonly used for the early detection of precancerous lesions of the cervix (Latin: neoplasma malignum cervicis uteri). Conventional Pap smear has been the gold standard for cervical cancer screening for many years, but limitations in its sensitivity and specificity have led to the development and increased use of LBC (3). The association of human papillomavirus (HPV) infection with the development of cervical cancer has been established and is considered one of the most common causes. Two types of HPV, 16 and 18, are responsible for the majority of high-grade cervical precancerous conditions (4).

According to data from 2020, cervical cancer is the fourth most commonly diagnosed cancer and the fourth leading cause of cancer death in women, with an estimated 604.000 new cases and 342.000 deaths worldwide. About 90% of new cases and deaths worldwide in 2020 occurred in low- and middle-income countries. Globally, more than 500.000 new cases of cancer and 250.000 fatal cases are registered annually (5, 6).

Pap smear – Papanicolaou method or exfoliative cytopathology is the study of normal and altered states of spontaneously exfoliated or mechanically displaced cells with the aim of detecting and diagnosing various infections, abnormal hormonal activities, and precancerous or cancerous lesions (7).

The classification of Pap smear has evolved gradually throughout history, with advancements in tech-

nology and understanding of the biological nature of cervical dysplasia and cancer. Previously, Pap smears were classified according to a system based on classes from I to V, according to the Papanicolaou classification (4). Its classification was based on the qualitative assessment of cell atypia. Although this classification was simple, it had the potential for different interpretations, which was its drawback. Nevertheless, the classification played a significant role in the history of cytology, as it was extremely important for saving a large number of lives. After numerous clinical studies worldwide, in 1954, the National Cancer Institute (NCI) recommended that the Papanicolaou classification should no longer be used for establishing a final diagnosis but continue to be used as a routine classification for triaging detected changes (5, 8).

The classification based on cervical intraepithelial neoplasia (CIN) is used to assess precancerous lesions in cervical cells. It is divided on a scale from 1 to 3 (9).

– CIN I (dysplasia gradus laevis) - indicates a mild precancerous lesion with a small number of altered cells of the squamous epithelium of the cervix. The cells have abundant, clear, well-defined cytoplasm. Based on type, the cells belong to superficial and intermediate squamous cells. The nucleus is enlarged compared to intermediate cells, and the chromatin is finely granular and moderately hyperchromatic (10).

– CIN II (dysplasia gradus medii) - moderate dysplasia with a variable number of altered cells. Large superficial, fewer intermediate, and small parabasal cells are present. They are mostly oval or round but can also be spindle-shaped. They exhibit surface maturation of cytoplasm, which stains cyanophilic, but there can also be many cells with eosinophilic cytoplasm. Nuclei are enlarged, and chromatin is moderately hyperchromatic. The N:C ratio is increased (11).

– CIN III (dysplasia gravis or carcinoma in situ) - severe dysplasia with a large number of abnormal cells or carcinoma in situ. Atypical parabasal cells occupy more than two-thirds of the total epithelium. They have scant cytoplasm forming a ring around the nucleus. Cells are round or oval, irregular, or elongated in shape. The nucleus is enlarged and hyperchromatic, with coarsely granular chromatin. The N:C ratio is extremely increased, making it easily recognizable (11).

The CIN classification is supplemented by dividing into low-grade squamous intraepithelial lesions (LSIL) and high-grade squamous intraepithelial lesion (HSIL) (4). Squamous intraepithelial lesion (SIL) is usually a result of sexually transmitted HPV, although SIL itself cannot be transmitted from person to person. SIL is also termed dysplasia or neoplasia. It is divided into high-grade and low-grade SIL. CIN is graded as I, II, and III (3, 12).

LSIL indicates the presence of mild abnormalities in squamous cells, indicating a precancerous condition. These abnormalities usually regress spontaneously. However, in some cases, these changes can progress and lead to cancer development. Changes are typically caused by infection with certain types of HPV and are detectable on Pap smear. Most detected HPV infections are low-grade and regress spontaneously within two years. However, some LSIL cases progress to HSIL within two years, which is more likely in older women. LSIL is also called mild dysplasia, encompassing CIN I (4, 13, 14).

Routine classification for triage of detected changes in LSIL:

a) Changes associated with HPV,

b) Mild dysplasia CIN I (4).

HSIL represents the presence of abnormalities in squamous cells associated with HPV. It includes terms CIN II, CIN III, moderate and severe dysplasia, and carcinoma in situ. HSILs are associated with persistent infection and a higher risk of progression to invasive cancer, especially if the persistent infection is a high-risk genotype such as HPV16 and/or HPV 18 (15).

Routine classification for triage of detected changes in HSIL:

a) Moderate dysplasia CIN II,

b) Severe dysplasia CIN III,

c) Carcinoma in situ CIN III (4).

Cervical cell sampling for the Pap smear is performed by scraping the endocervical and exocervical areas. The primary aim is to sample the entire transformation zone (TZ) with minimal trauma to the cervical and endocervical epithelium. This is crucial as most precancerous changes occur within the TZ, making cell collection from this area extremely significant. Various devices are available for collecting cells from the cervix, such as Ayer's spatula, Szalay, plastic spatulas of different sizes, and cotton swabs (16).

In the early 1980s, research was initiated aimed at improving cytological preparations, leading to the development of liquid-based cell collection before placing them on slides. The result was liquid-based preparations. Finally, in the 2000s, the liquid-based cytology (LBC) method was developed, which is now applied equally to the conventional Pap smear. The implementation of LBC technology in the Pap smear is one reason contributing to the decline in cervical cancer incidence (17-19).

This method differs from the conventional Pap smear because the cell sample is not immediately placed on a slide after collection but is instead placed in a liquid preservative to preserve and protect the cells from damage. Samples are processed in an automated device that uses centrifugal force to separate the cells

from the liquid, then place the cells on slides, staining them for better visibility. Additionally, LBC slides are suitable for automated analysis (17, 20, 21).

With LBC, testing for HPV, gonorrhea, and chlamydia from a single sample is possible. This method has the advantages of easier interpretation, clearer backgrounds reducing the likelihood of epithelial cell occlusion, fewer unsatisfactory results, and filtering of blood and debris (17, 20).

The LBC technique involves collecting cells from the TZ using a Cervex-Brush, which is then transferred to a bottle with a liquid preservative. This brush has the advantage of allowing simultaneous sampling of cells from the TZ and endocervical region. However, a drawback of the Cervex-Brush is that it may cause epithelial damage and bleeding and is also expensive. There are two main sample preparation methods for LBC: SurePath and ThinPrep. The U.S. Food and Drug Administration (FDA) approved the use of SurePath in 1999, while it approved ThinPrep as a replacement for cervicovaginal smears in 1966. These methods differ in principle but produce similar preparations (16).

DIAGNOSTIC SENSITIVITY AND SPECIFICITY OF THE CONVENTIONAL PAP SMEAR METHOD AND LIQUID-BASED CYTOLOGY METHOD

In a study conducted in India, involving 100 randomly selected subjects, the conventional Pap smear test was found to be less effective compared to LBC. Samples had a higher percentage of satisfactory results with the LBC method. Furthermore, the sensitivity for LSIL and HSIL, as well as overall, was higher compared to the conventional method, and the specificity was also higher, except for LSIL detection (22).

Similarly, in a Japanese study involving 312 subjects, LBC was found to be a more sensitive method, while the conventional method was more specific. For CIN I, the specificity of the LBC method was lower, at 25% compared to 32.1% for the conventional Pap smear test. LBC had higher sensitivity and negative predictive value for detecting CIN I and CIN II, as well as overall (23).

According to research by Shobana et al. (22), the conventional method proved to be more specific in diagnosing LSIL and HSIL, with a specificity estimate of 93% compared to 49% and 100% compared to 96%, retrospectively. On the other hand, the LBC method showed higher sensitivity in diagnosing LSIL and HSIL, with a sensitivity estimate of 66% compared to 40% and 100% compared to 50%, respectively (22).

The results of prospective, prospective-observational, and cross-sectional studies have shown that overall, the LBC method is more diagnostically sensitive and specific compared to the conventional Pap smear test (22-25). Conversely, research conducted by Dhananjayaet al. (25) showed that the conventional method is more sensitive compared to the LBC method, with a sensitivity estimate of 33.33% compared to 22.22%, while the specificity of both methods was the same, at 96.65% (25).

FINDINGS OF THE CONVENTIONAL PAP SMEAR TEST AND LBC IN DETECTING CERVICAL ABNORMALITIES

The most common findings obtained by the LBC method are negative for intraepithelial lesion or malignancy (NILM), normal, and nonspecific inflammation, 46%, 21.5%, and 13.5%, retrospectively. The LBC method proved superior in detecting Candida spp., while the difference between these two methods was minimal regarding Trichomonas vaginalis, with only one additional case detected by the conventional Pap smear test. In this study, the diagnostic consistency was 83.9%. The LBC method showed better results in detecting endocervical, epithelial, and atrophic cells (66.7%, 25.4%, 88.5%, 85.5%, 7.4%, 3.8%, retrospectively). There was a slight difference in favor of the conventional Pap smear test in detecting metaplastic cells, with 1.1% compared to 0.8%. The LBC method stands out as significantly better in terms of false-negative diagnoses, with one case compared to 14 cases with the conventional Pap smear test. The LBC method can improve sample quality and reduce the number of unsatisfactory samples (26). A study conducted by Shobana et al. (22) revealed fewer abnormalities compared to the LBC method, 22% versus 28%, retrospectively (22).

In contrast, in a Pakistani population of 3.929 participants, the conventional method detected a higher number of Candida spp. cases, while a smaller number of Trichomonas vaginalis infections were identified. However, this may be due to the conventional method analyzing a larger number of samples, nearly 1.000 more. The LBC method detected a higher number of LSIL, HSIL, and glandular epithelial lesions. For detecting NILM, the conventional Pap smear test performed better with 97.9% compared to 96.2% with LBC. The LBC method is cost-effective in mass screening for cervical cancer (27). In a study among the Indian population, the conventional Pap smear test was less effective compared to the LBC method, as a higher number of HSIL and squamous cell carcinoma cases were detected using the LBC method (28).

Among six studies investigating ASC-US (Atypical squamous cells of undetermined significance), the results showed similar trends. In three of these studies, the conventional method detected a higher number of cases (14.5% compared to 11.5%, 6% compared to 2.6%, 3.31% compared to 2%, retrospectively). In one study, both methods identified the same number of ASC-US cases, while differences in favor of the LBC method were minimal in the remaining studies, where only one additional case was recorded compared to the conventional method, and 1% compared to 0.6% in favor of the LBC method (23, 24, 27-30).

The results of three studies conducted in Japan, Thailand, and India, involving 312, 1206, and 97 participants, retrospectively, showed that the conventional method was better in diagnosing HSIL compared to LBC (23-25). Conversely, studies conducted in India, Pakistan, and Egypt, involving 200, 3929, and 150 participants, respectively, showed that the LBC method was better at diagnosing HSIL (27-29). It is important to note that studies supporting the LBC method included a larger number of participants, almost 2700 more.

According to a study by Ranjana et al. (30), the LBC method and the conventional Pap smear test show equal abilities in detecting the presence of LSIL and HSIL in younger participants (30).

QUALITY OF SAMPLE IN CONVENTIONAL PAP SMEAR AND LBC

A comparison of the quality and quantity of cervical tissue samples obtained by conventional Pap smear test and LBC method showed that components of TZ were present in 96.8% of samples by conventional Pap smear test and in 98.1% of LBC samples. Greater opacity was recorded with the conventional Pap smear test compared to LBC (24). LBC improves sample quality and reduces the likelihood of false-negative results, thus enhancing the effectiveness of screening programs (22).

One case of false-negative diagnosis was recorded with the LBC method, whereas with the conventional Pap smear test, there were 14 cases, highlighting the superiority of LBC in reducing the risk of false-negative diagnoses (26).

The conventional Pap smear test has an inadequacy rate ranging from 5% to 25% (31). Additionally, it has several shortcomings such as inadequate transfer of cells to slides, uneven cell distribution, and the presence of obscuring materials like inflamed cells, blood, and overlapping epithelial cells (32). LBC has the advantage of fewer unsatisfactory smears and fewer obscuring factors such as blood or mucus (28).

CONCLUSION

The LBC method stands out as a method with higher diagnostic sensitivity, especially in detecting LSIL, HSIL, and CIN. On the other hand, the conventional Pap smear test retains its specificity, especially in diagnosing LSIL. LBC demonstrates an advantage in terms of sample representativeness and a smaller number of unsatisfactory samples. This method provides greater reliability in diagnostic procedures, which is of exceptional importance for achieving high diagnostic accuracy and timely detection of potential abnormal changes. It is important to consider that the results of the study varied depending on the population, methodology, and sample size, suggesting the need for further research to confirm these findings and better understand the difference between these two methods.

Abbreviations

ASC-US - Atypical squamous cells of undetermined significance

CIN - Cervical intraepithelial neoplasia

FDA - U.S. Food and Drug Administration

HPV - Human papillomavirus

HSIL - high-grade squamous intraepithelial lesion

LBC - liquid-based cytology

LSIL - low-grade squamous intraepithelial lesion

SIL - squamous intraepithelial lesion

TZ - Transformation zone

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Sažetak

KOMPARACIJA KONVENCIONALNE METODE PAPA TESTA I TEČNE CITOLOGIJE U DETEKCIJI CERVIKALNIH ABNORMALNOSTI

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Rak cerviksa predstavlja jednu od najčešćih vrsta raka kod žena koji zahteva ranu dijagnostiku kako bi se smanjila prevalencija i smrtnost, a Papa test ima ključnu ulogu u ranoj dijagnostici prekanceroznih lezija. Konvencionalna metoda Papa testa se već dugo vremena koristi za rano otkrivanje lezija, dok se LBC metoda sve više koristi kao alternativna metoda sa potencijalnim prednostima.

U istraživanju, konvencionalni Papa test je pokazao manju efikasnost u poređenju sa LBC metodom. LBC metoda je imala veći postotak zadovoljavajućih uzoraka i pokazala je veću osetljivost i specifičnost za identifikaciju različitih abnormalnosti cerviksa. Slična otkrića su pronađena i u japanskom istraživanju. Međutim, postoje istraživanja koja su pokazala suprotne rezultate, naglašavajući specifičnost konvencionalne metode. Konvencionalna metoda Papa testa je u nekim istraživanjima pokazala veću sposobnost detekcije ASC-US-a, dok su u drugim istraživanjima rezultati bili slični ili u korist LBC metode.

LBC metoda se ističe po većoj dijagnostičkoj osetljivosti, posebno u otkrivanju različitih vrsta cervikalnih abnormalnosti, dok konvencionalna metoda Papa testa zadržava svoju specifičnost, posebno u dijagnostici LSIL-a. LBC metoda ima prednost zbog bolje reprezentativnosti uzoraka i manjeg broja nezadovoljavajućih uzoraka.

Ključne reči: rak grlića materice, Papa test, tečna citologija, prekancerozne lezije raka grlića materice, dijagnoza raka grlića materice, klasifikacija Papa testa.

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Review article

EPIDEMIOLOGY OF TRAFFIC TRAUMATISM

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Abstract: Through a review of relevant literature, we provide insight into the public health issue of traffic trauma both globally and locally. According to estimates by the World Health Organization (WHO), approximately 1.35 million people die annually in traffic accidents, while nearly 50 million suffer minor or severe injuries. Globally, traffic injuries account for about 2.37% of all deaths, ranking eighth among all causes of death. The Global Status Report on Road Safety 2018 indicates that the number of traffic deaths did not decrease in any low-income country between 2013 and 2016, although a reduction was observed in 48 middle- and high-income countries during that period. Despite the increase in absolute numbers, the global road traffic mortality rate has remained fairly constant at around 18 deaths per 100 000 inhabitants over the past 15 years, with the highest rate in the African region and the lowest in the European region. Currently, traffic accidents are the leading cause of death among children and young adults aged 5 to 29 years, with a higher incidence among males. Further research is needed to better understand the specific characteristics of traffic injuries in the local population and to identify the most effective intervention programs.

Keywords: Trauma, trauma registry, polytrauma, public health.

INTRODUCTION

According to estimates from the World Health Organization (WHO), approximately 1.35 million people die in traffic accidents worldwide each year. In addition, nearly 50 million people suffer minor or serious injuries as a result of traffic trauma, whether during the accident, transport, or hospitalization (1). Globally, of the 56.9 million deaths worldwide, road traffic injuries account for about 2.37% and rank eighth among all causes of death, including both communicable and non-communicable diseases. Traffic accidents are currently the leading cause of death among children and young adults aged 5 to 29 years, with a higher incidence among males (1, 2). The WHO estimates that by 2030, traffic injuries will become the fifth leading cause of death, with 2.4 million deaths per year, and the third leading cause of disability, significantly impacting individuals, families, and society as a whole (3). Over 90% of traffic deaths occur in low- and middle-income countries, which, despite having about 60% of the world's vehicles, suffer from poor road infrastructure, inadequate vehicle maintenance, and insufficient education (4, 5). Literature suggests that road traffic fatality rates are three times higher in low-income countries compared to high-income countries (27.5 versus 8.3 deaths per 100 000 population), highlighting a significant concern for public health systems in these regions.

Epidemiology of traffic trauma in the world

The Global Status Report on Road Safety 2018 indicates that the number of traffic deaths did not decrease in any low-income country between 2013 and 2016, though a reduction was observed in 48 middle- and high-income countries during that period (1). Despite an increase in absolute numbers, the global road traffic mortality rate has remained relatively constant at around 18 deaths per 100 000 inhabitants over the past 15 years (1, 6), with the highest rate in the African region and the lowest in the European region (1).

Figure 1 illustrates the incidence of traffic injuries and their trends from 1990 to 2019 across 204

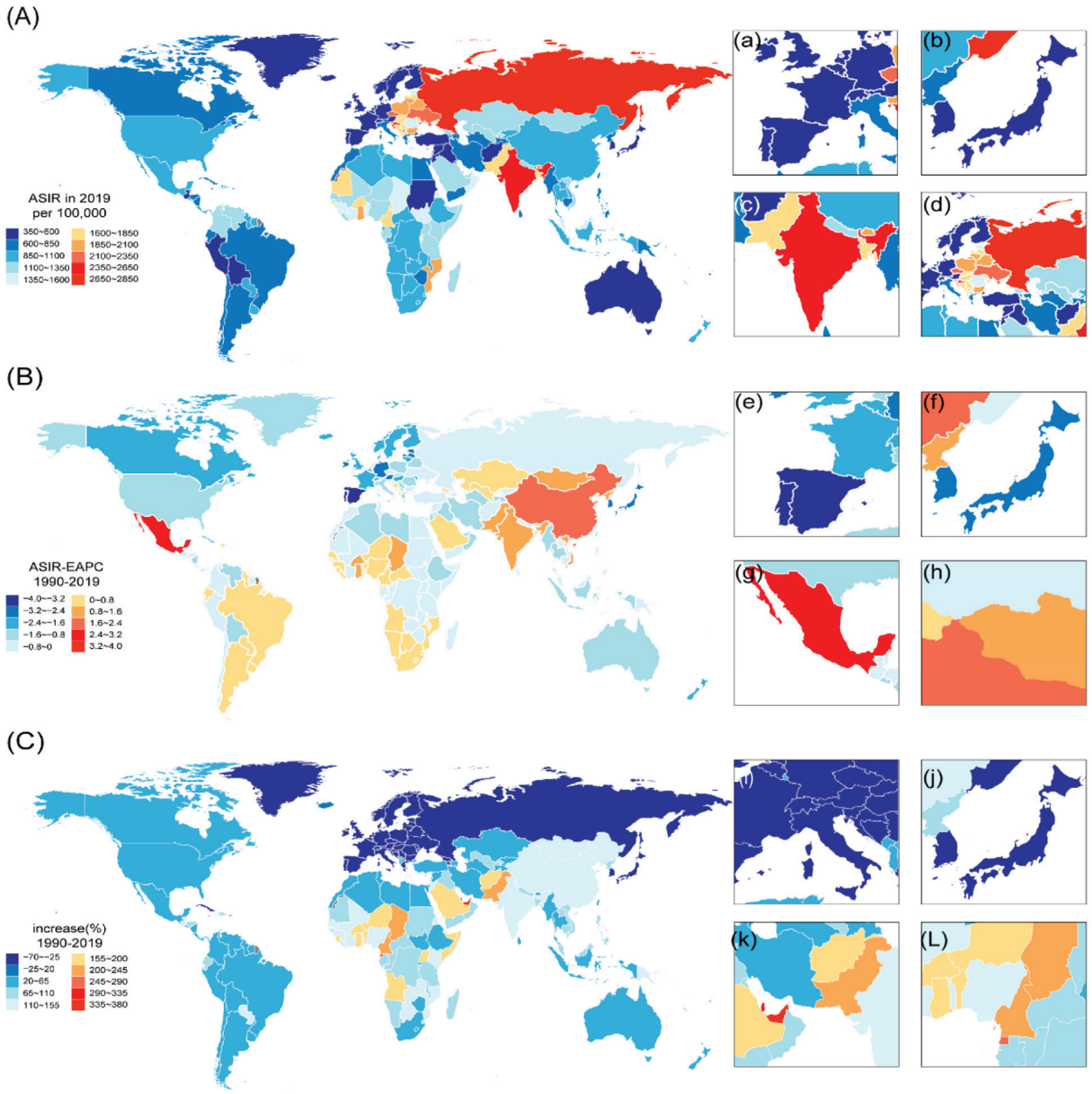

Figure 1. *Global burden of road injuries from 1990-2019*
(Source: Xu Y, Chen M, Yang R, Wumaierjiang M, Huang S. Global, Regional, and National Burden of Road Injuries from 1990 to 2019. Int J Environ Res Public Health. 2022; 19(24): 16479.)

countries, including Western Europe, South Asia, the Asia-Pacific Region, Eastern and Western Europe, East Asia, Central and Latin America, North Africa, the Middle East, and Sub-Saharan Africa (7). The results show a decrease in incidence after 2010, which is attributed to global improvements in road infrastructure and the implementation of traffic injury prevention regulations. The highest incidence is recorded in India, with South Asia showing the largest numerical value (7).

In all age groups, the annual rates of fatal injuries are higher in men compared to women (79 per 100 000 versus 34 per 100 000, respectively) (7). Traffic injuries are the leading cause of death for children and young people aged 5 to 29 years globally, with males being more likely to be involved in traffic accidents; 73% of all road deaths in this age group occur among young men (1).

The leading causes of mortality from traffic trauma worldwide are head injuries and bleeding. Bleeding is responsible for 80% of deaths that occur within the first hours after the accident, and 55% of those who die in the hospital succumb within the first 24 hours. Head injuries are more common among those who die between 1 and 6 days after hospitalization (8).

The economic costs of traffic accidents are substantial, with nearly two-thirds (60.7%) of fatal accidents involving the most productive segment of the

population, making traffic accidents a leading cause of disability (9). Additionally, the annual costs of caring for and treating the injured represent a significant economic burden on society (10). In 2019, the costs of care, treatment, and rehabilitation for traffic accident victims in the United States amounted to 340 billion dollars, leading to a tremendous economic burden, with one in ten hospital beds occupied by traffic accident patients (11). Ameratunga et al. predict that an increase in the number of vehicles per inhabitant will result in an 80% increase in the number of traffic-related injuries and deaths. They also forecast that medical costs due to traffic accidents will rise by 1.0% of gross national income in low-income countries, 1.5% in middle-income countries, and 2.0% in high-income countries (12).

Epidemiology of Traffic Trauma in Europe

In Europe, traffic accident mortality ranks 15th among all causes of death. Annually, approximately 120 000 people die in traffic accidents, and around 2.5 million sustain minor or severe injuries (13). Traffic accidents in Europe are the fourth leading cause of injury, with over 90% of fatal cases occurring among individuals under forty years of age (14, 15, 16). The traffic mortality rate in middle-income countries in Europe is 14.4 deaths per 100 000 inhabitants, nearly three times higher than in high-income countries, where the rate is 5.1 deaths per 100 000 inhabitants. Mortality is particularly high among the younger population in low- and middle-income countries (17, 18).

Except for the Eastern Mediterranean region, the rate of traffic deaths per 100.000 inhabitants generally decreases with increasing income (6). The geographical distribution of mortality from road traffic accidents reveals that Western European countries have much lower mortality rates compared to Central and Eastern European countries (19). Although there has been a significant reduction in traffic accident mortality over the past three decades due to improvements in road infrastructure, public education, and preventive campaigns, the reasons for the relatively high death rates in Central and Eastern Europe remain unclear, necessitating continued efforts to reduce mortality in these regions.

In 2022, Sweden reported the lowest traffic death rate in Europe at 21 deaths per million inhabitants, followed by Denmark with 26 deaths per million. In contrast, Romania (86/million) and Bulgaria (78/million) had the highest death rates. The average death rate in the EU due to traffic trauma was 46 road deaths per million inhabitants. Among Western Balkan countries, Montenegro had the highest fatality rate (88 per million inhabitants) in 2022, with Serbia in second place (75/million). Montenegro also experienced the largest increase in fatalities, rising by 33% compared to 2021.

In 2021, 52% of traffic deaths in the EU occurred on rural roads, 39% in urban areas, and 9% on highways. Men were responsible for three out of four deaths (78%). Car occupants (drivers and passengers) accounted for 45% of all road deaths, pedestrians 18%, users of two-wheeled motor vehicles (motorcycles and mopeds) 19%, and cyclists 9%. In urban areas, pedestrians, cyclists, and motorized two-wheeler users together accounted for slightly less than 70% of total fatalities (20).

Adolescents, dominated by the male population, aged 16 to 19 years are faced with the highest risk of traffic accidents due to the consumption of alcohol and psychoactive substances and the use of mobile phones, especially during the first year of driving, and teenagers in this age group have twice the mortality rate of girls (14, 4).

Epidemiology of Traffic Trauma in the Republic of Serbia

Traffic injuries in the Republic of Serbia rank among the top causes of death, following cardiovascular diseases and malignant neoplasms (21, 22, 23). According to data from the Agency for Traffic Safety of the Republic of Serbia, between 2017 and 2021, there were a total of 173360 traffic accidents, resulting in 2674 fatalities, 16474 severe injuries, and 83280 minor injuries. Among the participants in traffic accidents, the highest number of fatalities were drivers and passengers in passenger cars (45%), followed by pedestrians (26%), cyclists and motorcyclists (9% each), tractor drivers and passengers (5%), and drivers and passengers in heavy vehicles (3%) (22).

The statistical report on traffic safety for 2021 shows that 521 people died and 3347 sustained severe injuries during that year. Men accounted for 78% of fatalities and 61% of those injured. Among the deceased, the majority were drivers of passenger cars (47%), followed by passengers in motor vehicles (33%), pedestrians (13%), and cyclists (7%). The age distribution of fatalities indicates that the highest proportion of deceased individuals were aged 65 and older (33.6%), followed by those aged 15-30 years (19.4%), and 31-44 years (15.2%). The highest proportion of deceased drivers were in the 65 and older age group, followed by those aged 15-30 years. Among pedestrians and cyclists, those aged 65 and older were most frequently represented (22).

Analysis of fatalities and injuries by gender reveals that men are more likely to die as drivers and

pedestrians compared to women. Conversely, women are more likely to be injured as passengers and pedestrians, while men are more frequently injured as drivers of motor vehicles. The public risk in Serbia is significantly higher compared to EU countries, with an average of approximately 33 more fatalities per million inhabitants (24).

On September 1, 2018, Serbia established a trauma registry, initiated by the Resuscitation Council of Serbia (RSS). This registry enables the monitoring of epidemiological data related to trauma and is the first to record all injuries treated by Emergency Medical Services (25).

Epidemiology of Traffic Trauma in Montenegro

Montenegro, with a population of approximately 630,000 inhabitants, has an economy primarily based on services, with tourism as a key sector. The nominal GDP of Montenegro for 2015 was estimated at 3.4 billion euros, with projected annual GDP growth of 3% until 2025 and 2.5% until 2035 (26, 27).

Montenegro lacks a dedicated trauma registry, leading to limited data on traffic trauma. Available data is sourced from the "Statistical Office" and the "Ministry of Interior." In 2021, the Ministry of Interior reported 6,109 traffic accidents, marking an increase of 1517 accidents or 33.0% compared to 2020, which had 4592 accidents (28). The state of road traffic safety report indicates that 3,003 people were injured in traffic accidents in 2021, an increase of 916 or 43.9% from 2020 (Table 1).

Regarding fatalities, 55 people died in traffic accidents in 2021, compared to 48 in 2020, representing an increase of 7 fatalities or 14.6% (Table 2). According to the Ministry of Interior, the most frequent fatalities were drivers of motor vehicles, accounting for 50% of all deaths. Passengers made up 25% of fatalities, pedestrians about 17%, and motorcyclists approximately 8%. The majority of fatalities occurred on main roads (60%), followed by streets (20%), local roads (5%), regional roads (3%), and unclassified roads (2%).

Table 1. *Traffic Accidents with Injuries*

	2021	2020	Change (%)
Total injured persons	3,003	2,087	43.9%
Seriously injured persons	474	380	24.7%
Slightly injured persons	2,529	1,707	48.2%

(Source: Ministry of Interior of Montenegro, report on the state of road traffic safety for 2021. Available at: https://wapi.gov.me)

Table 2. *Traffic Accidents with Fatalities*

	2021	2020	Change (%)
Total fatalities	55	48	14.6%
Drivers	27	21	28.6%
Passengers	10	16	-37.5%
Cyclists	1	0	/
Motorcyclists	10	4	150.0%
Pedestrians	7	7	/

(Source: Ministry of Interior of Montenegro, report on the state of road traffic safety for 2021. Available at: https://wapi.gov.me)

Human factors are identified as the primary cause of most traffic accidents in Montenegro (28, 29). A study by Peličić and colleagues conducted from 2011 to 2020 highlights that the distance to the nearest hospital and specialized treatment centers, as well as transport time, significantly impact the outcomes of traffic accident injuries (30).

CONCLUSION

The literature review indicates that traffic injuries are a significant global public health issue, requiring coordinated efforts at both national and international levels. Implementation of preventive measures and strategies, including the establishment of trauma registries, is crucial for reducing the incidence of traffic injuries. This may involve driver education, enforcement of traffic safety measures, and infrastructure improvements. Prompt and efficient emergency medical assistance can significantly reduce mortality and long-term consequences following traffic accidents. Further research is necessary to better understand the specific characteristics of traffic injuries within local populations and to develop the most effective intervention programs.

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Sažetak

EPIDEMIOLOGIJA SAOBRAĆAJNOG TRAUMATIZMA

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Kroz pregled relevantne literature, pružili smo uvid u javnozdravstveni problem saobraćajnih povreda kako globalno, tako i lokalno. Prema procenama Svetske zdravstvene organizacije (SZO), oko 1,35 miliona ljudi godišnje umre u saobraćajnim nesrećama, dok skoro 50 miliona ljudi pretrpi manje ili teže povrede. Globalno gledano, saobraćajne povrede čine oko 2,37% od ukupnog broja smrtnih slučajeva širom sveta, što ih svrstava na osmo mesto među svim uzrocima smrti. Globalni izveštaj o stanju sigurnosti na putevima 2018. pokazuje da se broj smrtnih slučajeva u saobraćaju nije smanjio ni u jednoj zemlji s niskim prihodima između 2013. i 2016. godine, ali je u tom periodu uočeno smanjenje u 48 zemalja sa srednjim i visokim dohotkom. Uprkos porastu u apsolutnim brojevima, globalna stopa smrtnosti u saobraćaju je ostala prilično konstantna, na oko 18 smrtnih slučajeva na 100.000 stanovnika u proteklih 15 godina, sa najvišom stopom u afričkom regionu i najnižom u evropskom regionu. Trenutno su vodeći uzrok smrti među decom i mladima od 5 do 29 godina gde prednjači muška populacija. Potrebna su dalja istraživanja kako bi se bolje razumele specifične karakteristike saobraćajnih povreda u lokalnoj populaciji i identifikovali najefikasniji programi intervencija.

Ključne reči: Trauma, registar traume, politrauma, javno zdravlje.

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Spates ST, Mellette JR, Fitzpatrick J. Metastatic basal cell carcinoma. J Dermatol Surg. 2003; 29(2): 650–652.

2. **Knjiga:**

Sherlock S. Disease of the liver and biliary system. 8th ed. Oxford: Blackwell Sc Publ, 1989.

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Latković Z. Tumori očnih kapaka. U: Litričin O i sar. Tumori oka. 1. izd. Beograd: Zavod za udžbenike i nastavna sredstva, 1998: 18–23.

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