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Josipa Vaništu, Kompozicija IX iz 1962. godine
Ulje na platnu
MLU-S-753

Josip Juraj Strossmayer University of Osijek
Faculty of Medicine

Josipa Vaništu, Kompozicija IX iz 1962. godine. Ulje na platnu, 13x98,5 cm. MLU-S-753

Tražio sam pravo na pogrešku, na kontradikciju, na metamorfozu – zapisao je u Knjizi zapisa (2001.) Josip Vaništa (1924. – 2018.), univerzalna umjetnička ličnost i paradigma hrvatske kulture (Igor Zidić). U vidu poetike apsurda njegovi crteži su i svojevrsni ne-crteži, slikarstvo ne-slikarstvo, a djelovanje u Gorgoni ne-djelovanje. Kao osnivač i član umjetničke grupe Gorgona (1959. – 1966.), koju unutar mentalne izolacije čine umjetnici zajedničkog duhovnog srodstva, zalagao se za neoavangardni duh, slobodu umjetnosti i uma, što je anticipiralo kasnije sadržaje nove umjetničke prakse. Diplomirao je na ALU-u u Zagrebu 1950. (M. Tartaglia), a od 1951. do 1994. profesor je na Arhitektonskom fakultetu. Uz oris figure, u crtežu mu je važna bjelina iz koje je iznjedren motiv. Nastavljajući tradiciju moderne (J. Račić, M. Steiner), stvorio je motivsko meditativno slikarstvo u kojem je bjelina metafora obojene svjetline. Od 1961. reducira svoj slikarski sadržaj serijom monokromnih slika. (…) Minimalnom slikarskom metodom anticipira buduće tendencije, a svjesnošću o konceptualnosti slikarstva, Vaništa faktičnost nadomješta verbalnošću kojom zamjenjuje proces slikanja preciznim opisom likovnog postupka. Nakon 70-ih opet se približava realističkoj poetici. Iz tog razdoblja su najvažniji akvareli. Bavio se ilustracijom, opremom knjiga i scenografijom, a autor je i više knjiga. Redoviti je član HAZU-a od 1994. i dobitnik je Nagrade „Vladimir Nazor" za životno djelo 2006.

– izvor, Željko Marciuš, muzejski savjetnik Nacionalnog muzeja moderne umjetnosti

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Case report

Cervical Lymph Node Metastases from Unknown Primary Tumor

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Abstract

Introduction: Metastases of the lymph nodes of the neck originating from cancers of an unknown primary site represent a diagnostic and therapeutic challenge. Metastatic carcinomas of unknown primary site (CUPS) account for about 3-5% of all malignant disease diagnoses. Planocellular carcinomas account for 90% of cancers of unknown site, while the remaining 10% are poorly differentiated and adenocarcinomas.

Materials and methods: This paper presents three case reports of patients with surgical treatment at the Department of Maxillofacial Surgery, Clinical Hospital, Stip, who had metastases of the lymph nodes of the neck with the unknown primary tumor site.

Results: We follow diagnostic protocols which include a detailed clinical examination, radiological diagnostics, fine needle biopsy (FNAB) of the tumor change in the neck, esophagogastroduodenoscopy, detailed examination of the naso, oro and hypo pharynx, evaluation of the laryngeal structures. In all patients, after the clinical evaluation, ultrasonography, otorhinolaryngological examination, neck CT and fine needle biopsy were performed. FNAB findings showed metastatic deposits from squamous cell carcinomas. The therapy of metastases from CUPS includes surgical treatment (neck dissection) and the use of radiotherapy (RT), and some authors also recommend chemoradiotherapy, in cases with advanced regional disease.

Conclusion: Significant advances in diagnostic and operative techniques have increased the probability of identifying the primary tumor, as well as its regional and systemic spread. If CT or MRI does not identify a primary site, PET/CT scans should be performed before surgical biopsy. Although high-quality data on treatment protocols are lacking, patients with more advanced stages of regional disease require combined treatment that includes neck dissection, and postoperative radiotherapy with or without chemotherapy.

(Milosev V. Cervical Lymph Node Metastases from Unknown Primary Tumor. SEEMEDJ 2024; 8(2); 1-10)

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KEYWORDS: carcinoma, unknown primary origin site (CUPS), metastases, lymph nodes, neck

Introduction

Carcinoma of unknown primary site (CUPS) is a heterogeneous group of cancers defined by the presence of metastatic disease with no identified primary tumor at presentation.

One of the first symptoms of the existence of a malignant process whose localization has not been identified is a painless swelling in the neck area due to metastatic changes in the lymph nodes. Very few oncological diseases pose such difficult diagnostic and therapeutic dilemmas for surgeons as metastatic tumors with unknown primary site (Cancer Unknown Primary Site – CUPS).

The purpose of imaging for lymph node (LN) metastasis from an unknown primary site is to identify the primary tumor or detect its absence, which leads to the correct diagnosis and optimal treatment. The authors discuss diagnostic imaging approaches for identifying the primary tumor in cases of unknown primary cervical LN metastases. The distribution and characteristics of LN metastases may help locate the primary site. Unknown primary LN metastasis often occurs at nodal levels II and III, and in recent reports, these were mostly related to human papillomavirus (HPV)–positive squamous cell carcinoma of the oropharynx. Another characteristic imaging finding suggestive of metastasis from HPV-associated oropharyngeal cancer is a cystic change in LN metastases. Other characteristic imaging findings such as calcification may help predict the histologic type and locate the primary site. In cases of LN metastases at nodal levels IV and VB, a primary lesion located outside the head and neck region must also be considered. One clue for detecting the primary lesion at imaging is the disruption of anatomic structures, which can help in identifying small mucosal lesions or submucosal tumors at each subsite. Additionally, fluorine 18 fluorodeoxyglucose PET/ CT may help identify a primary tumor. These imaging approaches for identifying primary tumors enable prompt identification of the primary site and assist clinicians in making the correct diagnosis.

The definition of metastatic tumor of unknown primary site - Cancer of unknown primary site (CUPS) implies a histologically proven metastatic malignant epithelial disease, without an identified primary localization, despite detailed investigations, which include: a detailed clinical examination of the head, face, ears and oral cavity, furthermore, fiberoptic examination of the oral cavity, pharynx, larynx, and sinonasal cavity, including areas not visible at clinical inspection, should also be performed, gynecological and rectal examination, complete biochemical analyses, CT of the head, chest and abdomen, immunohistochemical analysis of the histological material. Fine-needle aspiration of a neck mass is necessary to establish the histologic diagnosis. It is recommended to use p16 testing for cervical LNs with carcinoma of an unknown primary site, especially for LN metastasis at nodal levels II or III, while EBV testing is used for p16-negative metastases, which indicate nasopharyngeal cancer (1).

The exact incidence is difficult to estimate because these patients are often treated under other diagnoses and thus are not adequately represented in tumor registries. Various authors report an incidence of these diseases of about 3-5% of all malignant diseases of the head and neck (2). The average age limit of the patients is about 60 years, with a somewhat more frequent occurrence in men (3).

The most common primary sites for SCC cervical LN metastasis from an unknown primary tumor are reported to be the oropharynx (palatine tonsil, 45%; base of the tongue, 44%), hypopharynx, and nasopharynx (4). The frequency of unknown primary SCC has recently shown an increasing trend, and 60%–90% of unknown primary SCCs are p16 positive, which is strongly suggestive of a primary site in the palatine or lingual tonsils (5). For histologic types other than SCC, the possible primary sites in the head and neck region are the thyroid gland and salivary glands. Primary sites outside the head and neck region may include the lung, breast, uterine cervix, and stomach (6).

The predominant histological type is squamous cell carcinoma, with representation in 75-90% of

cases, while a smaller percentage is represented by undifferentiated carcinomas and adenocarcinomas (7). Identification of the primary location of the tumor allows better planning of surgical treatment, which is associated with a better prognosis and survival time. In the last decade, several national guidelines have been presented recommending strategies for diagnosis and treatment (8,9), but a consensus on the diagnostic approach of head and neck CUPS has not yet been reached.

CUPS is characterized by a silent primary focus, a high degree of aggressiveness and early metastasis in regional lymph nodes. The primary tumor has a slow growth, unlike the secondary deposits, that is, it becomes involuted, and therefore more difficult for early detection. Clinically, it presents with symptoms caused by the metastases, as well as general symptoms, such as anorexia, weight loss, and fatigue.

Treatment of head and neck CUP prioritizes loco-regional control. Initial recommendations include surgical treatment (neck dissection) with radiotherapy (RT) (10,11). Chemotherapy is recommended for emphasized N2,N3 and metastases with extracapsular extension (12,13).

However, the treatment of these diseases remains heterogeneous and is based on clinical experience and institutional policies.

The distribution of cervical LN metastases can be broadly divided into the following three areas, which are useful for predicting the primary site: (a) nodal levels II, III, and VA;(b) nodal levels IV and VB; and (c) other LNs, including level I, parotid LNs, and superficial lateral, facial, and occipital LNs (Fig 1, Table 1) (14).

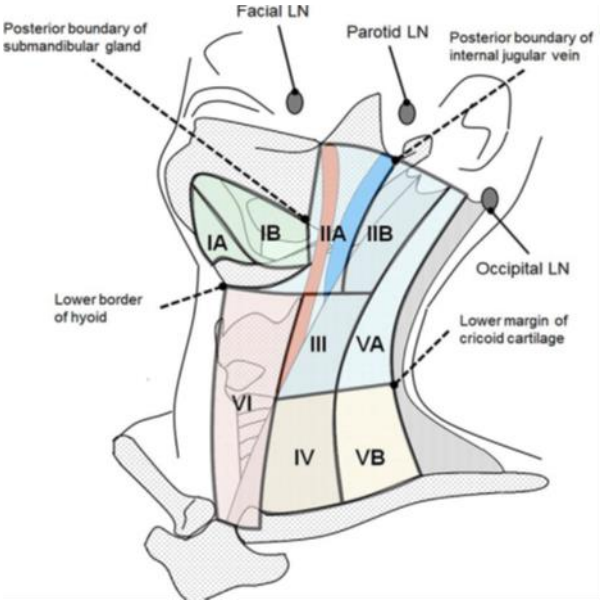

Figure 1. Anatomic boundaries of the neck levels and sublevels according to the American Head and Neck Society classification system. Adapted from Robbins et al. (14).

Table 1 . Primary Site Predicted from Distribution of Cervical LN Metastases

Distribution of LN Metastases	Potential Primary Site
Levels II, III, VA	Oropharynx(palatine tonsil and the base of tongue), nasopharynx and hypopharynx
Levels IV, VB , VI	Hypoprahynx , supraglottic, cervical esophagus , thyroid gland, thorax, abdomen (lung, breast, esophagus, gastric, gynecologic ,etc)
Other LNs	
Parotid and superfitial lateral LNs	Parotid gland, cutaneous face, scalp, sinonasal cavity
Levels IA , IB	Floor of mouth, submandibular gland, anterior oral cavity , sinonasal cavity, lips , periorbital issues, cutaneous face
Facial LNs	Cutaneous face, oral cavity
Occipital LNs	Scalp, cutaneous face

The prognosis of this disease is poor, and survival is 4-13 months, despite treatment. The extreme variety in the manifestation, the metastatic character of the disease at the time of diagnosis and the poor response to therapy have resulted in little interest of researchers in this problem. This is the main reason why we were motivated to investigate this problem and to present case reports from our clinical practice.

Materials and Methods

Case reports from our clinical practice

The presented case reports were diagnosed and treated at the Department of Maxillofacial Surgery, Clinical Hospital, Stip, North Macedonia. In order to present the data in this paper, we followed the ethical rules, and each respondent and his companion signed an informed consent.

Case Report 1

A 72-year-old patient came to the Department of Maxillofacial Surgery, Clinical Hospital, Stip, because of a painless swelling in the left parotid region. Anamnestic data for its existence is about 4 months. On examination the patient did not report any other relevant head and neck symptoms or any comorbidities. He was a smoker, up to 60 cigarettes per day, for the last 10 years with reduced intensity. Clinically, on inspection, a tumor mass is evident, painless, fixed to the substrate, the size of an orange, without changes in the covering skin. After the ultrasonography, a tumor formation was found, with a heterogeneous structure, with a diameter of about 4 cm. A careful otorhinolaryngological examination was performed, with flexible naso-pharyngo-laryngoscopy. The clinical ORL finding was normal. Before admission and surgical treatment, head, neck and chest CT was performed with intravenous contrast. CT findings indicated metastatic disease in the right parotid region, with central necrosis and infiltration of surrounding structures. A histopathological diagnosis of metastatic squamous cell carcinoma (SCC) was obtained by fine needle biopsy (FNAB). Intraoperative findings showed infiltration of the superficial lobe of the parotid gland, and the posterior bellows of the digastric muscle. A complete removal of the metastatic deposit was performed unblock with the superficial lobe of the right parotid gland and the posterior bellows of the digastric muscle. The pathohistological finding was consistent with metastatic SCC, confirming parotid lymph node metastases. With the decision of the oncology board, the patient underwent postoperative RT of the neck (from the skull base to the cricoid cartilage), including ipsilateral neck level II, III, IV and parotid gland. Regular controls were made at the Department of Maxillofacial Surgery, Clinical Hospital, Stip, in the first two years after the operation, every six months. After two years of regular oncological controls, the patient was lost to follow-up.

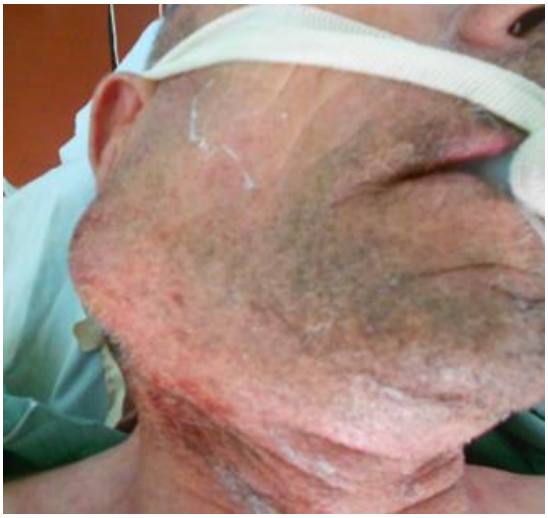

Figure 2. Preoperative status

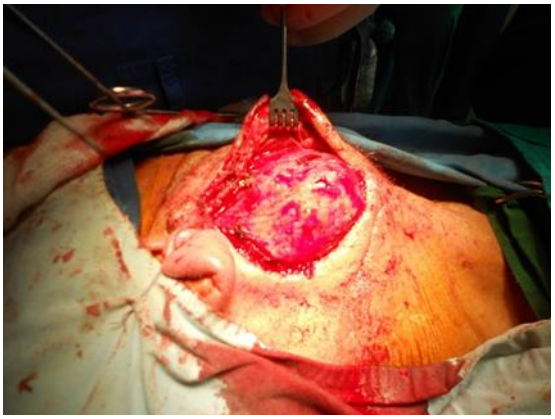

Figure 3. Intraoperative finding

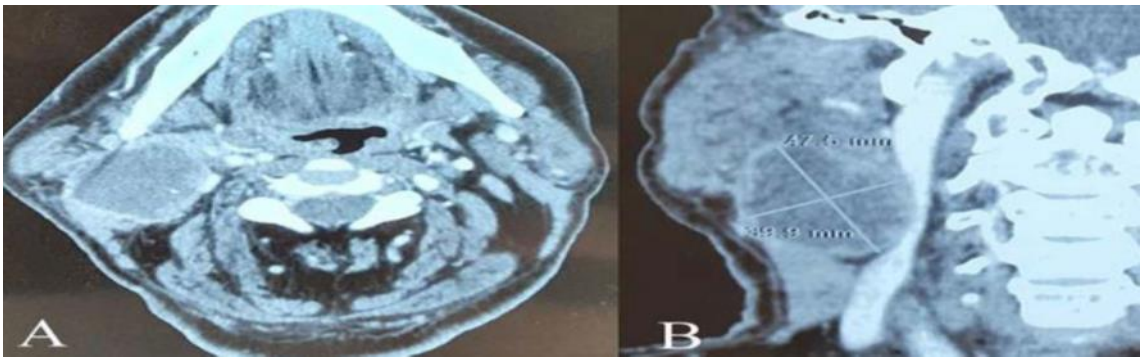

Figure 4. and 5. CT finding of the neck with intravenous contrast

Two years after the operation, the patient developed difficulties in swallowing. On additional otorinolaryngological examination, with indirect laryngoscopy, a pharyngeal tumor was found on the left lateral oro-hypo-pharyngeal wall, with extension to the piriform fossa and involvement of the right larynx. Biopsy with histopathological examination confirmed the diagnosis of SCC. CT scan of the head, neck and chest showed no signs of regional or distant disease spread. The patient underwent total pharyngolaryngectomy, tracheostomy and left selective neck dissection. The patient received postoperative RT (60 Gy in 30 fractions). Five months after RT, the patient was free of recurrent disease.

Case Report 2

A 77-year-old patient was referred to the Department of Maxillofacial Surgery, Clinical Hospital, Stip, due to the presence of an infiltrative-ulcerative change in the right parotid region, which also extends retroauricularly. According to information from a family member, the change appeared about eight months ago as a subcutaneous spherical formation, with gradual growth. Due to no adherence to the therapeutic process, patient refused to stay in the hospital. As result of this, during the long period of time, there is a rapid growth of the change and the appearance of ulceration in the parotid region. Echotomography on the right side of the neck revealed a large infiltrative, ulcerated lesion, with central necrosis, and pathologically enlarged lymph nodes on the neck. An incisional biopsy of the change was made, which pathohistologically showed that it was a metastatic deposit from carcinoma of epithelial origin. Considering the comorbidities present (two previous strokes, decompensated cardiac condition) after the preoperative anesthesiological assessment, the patient was refused surgical treatment and was referred to Oncology for further treatment.

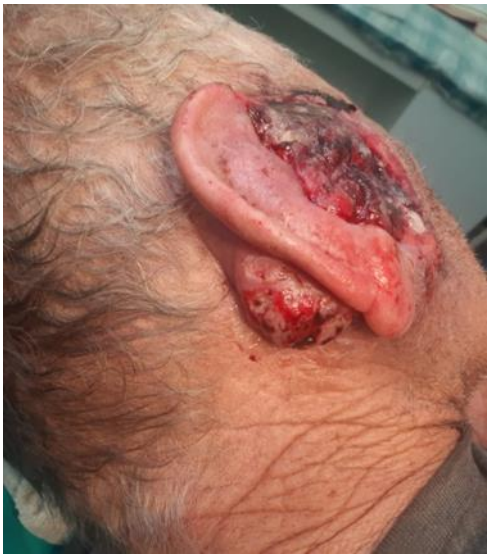

Figure 6. Clinical picture

Case report 3

A 72-year-old patient came for an examination at the Department of Maxillofacial Surgery, Clinical Hospital, Stip due to swelling on the left side of the neck. The swelling is painless, with a hard consistency, fixed. An ultrasonographic examination and a fine needle biopsy were

performed. The result of the histological examinations is a metastatic deposit of cancer of epithelial origin. A CT scan of the head, neck and lungs was performed. The finding of the lung parenchyma is without focal diseases.

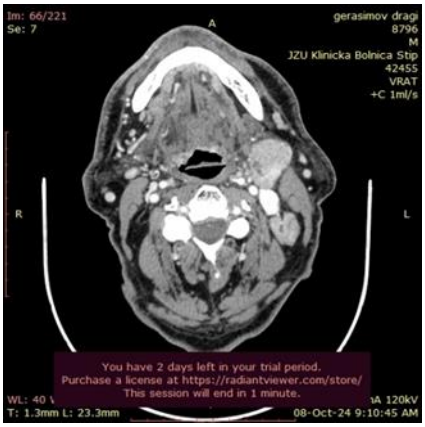

Figure 7. CT finding of the neck with contrast - preoperative

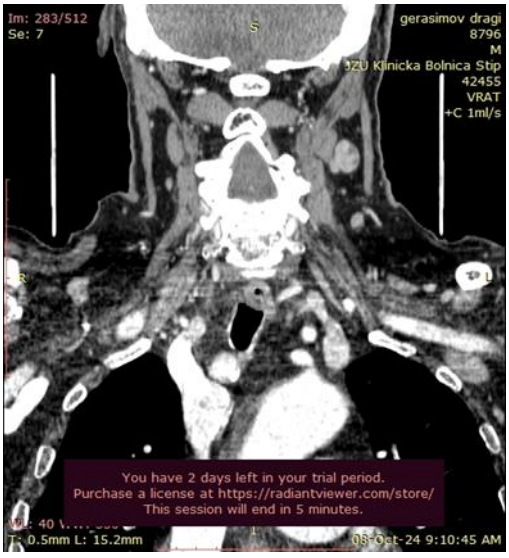

Figure 8. CT finding of the neck with contrast - preoperative

An otorinolaryngology examination was also performed, with a nasoropharyngolaryngeal evaluation, during which no pathological changes were detected. The findings of the esophago-gastro-duodenoscopy were normal. An indication for surgical treatment has been established. The operation was performed in endotracheal anesthesia. A complete removal of the metastatic deposit was performed. The postoperative course was uneventful. The patient was released for home treatment 5 days after the operation. An immunohistochemical analysis was performed on the preparation, which showed involvement of lymph nodes on the neck with a metastatic deposit of moderately differentiated invasive squamous cell carcinoma, which penetrates the capsule with the presence of emboli from malignant cells in the lymphocytes around the capsule of the lymph node.

Immunohistochemical profile:

1.CK 5/6 – negative
2.CK MNF 116 +++ positive
3. p63 ++++ strongly positive

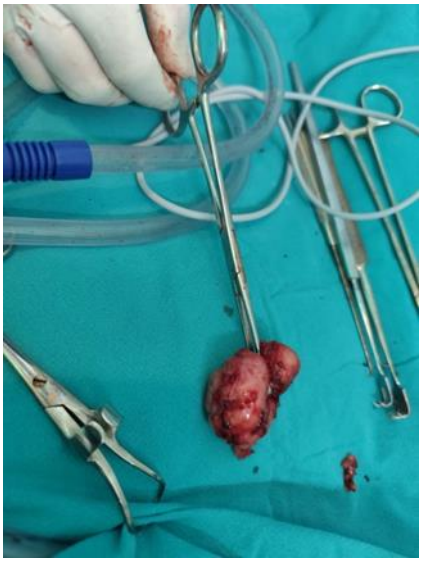

Figure 9. Preparate for histopathological examination

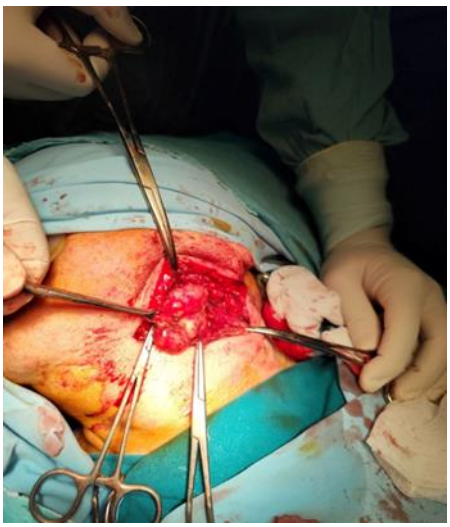

Figure 10. Intraoperative finding

Two weeks after the operation, the patient is referred to Oncology, where radiotherapy is prescribed.

Results and Discussion

The inability to detect the location of the primary tumor in a patient with metastatic CUPS represents a clinical challenge that can affect the course of treatment and the prognosis of the disease. After clinical examination and diagnostic imaging (ultrasonography, CT), aspiration biopsy (FNAB) is crucial in the assessment of neck tumor mass in CUPS. The American Joint Committee on Cancer (AJCC) recommends adding HPV assessment (staining) to diagnostic procedures (15).

With positive immunohistochemical staining of the specific HPV marker - p16, it would indicate a potential oropharyngeal primary tumor (palatine tonsils and base of tongue). Lymph node metastases with CUPS were positive for HVP in 7.8% to 30% of patients (16).

PET/CT is recommended in those patients in whom conventional imaging has failed to identify the primary tumor site, PET/CT has a high sensitivity (up to 88.3%) making it an excellent additional diagnostic procedure (17,18). Diagnostic protocols using preoperative PET/CT, which would precede EGD (esophago-gastro-duodenoscopy) with directed biopsies, have resulted in detection of the primary lesion in over 90% of patients (19-21). The National Comprehensive Cancer Network (NCCN) provides recommendations for endoscopic examination (nasopharyngoscopy), inspection and palpation of the oral cavity and oropharynx, laryngoscopy, bronchoscopy. Oropharyngeal locations, particularly the tonsils and base of the tongue, are the most common sites for primary occult tumors.

Treatment of patients with unknown primary cancer with neck metastases includes neck dissection, followed by postoperative RT or consideration of chemo-RT 14. According to some reports, patients undergoing bilateral RT did not have a significant increase in survival and regional recurrences, compared to patients treated with unilateral RT of the neck and mucosal surfaces. On the other hand, some studies favor bilateral nodal and mucosal irradiation (22).

The NCCN recommends combined chemo-RT in cases of N2/N3 neck lymph nodes present with extracapsular extension (23,24), although it is noteworthy that no randomized trials have demonstrated the superiority of this treatment over RT alone. Due to the low incidence of the disease and the lack of high-quality data, there are still no clear clinical protocols for these diseases.

Conclusion

A focused search for the primary tumor is recommended in CUP cases. Identifying patients with prognostically favorable disease is important, since they may have substantial benefit from directed treatment and experience prolonged survival.

Significant advances in diagnostic and operative techniques and the application of digital technology have increased the probability of identifying the primary tumor, as well as its regional and systemic spread. If CT or MRI does not identify a primary site, PET/CT scans should be performed before surgical endoscopy and biopsy. Although high-quality evidence-based treatment protocol data are lacking, patients with more advanced stages of regional disease require combined treatment that includes neck dissection and postoperative radiotherapy with or without chemotherapy.

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Disclosure

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Competing interests. None to declare.

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Author contribution. Single author article

Metastaze limfnih čvorova vrata od karcinoma nepoznatoga primarnog sijela

Sažetak

Uvod: Metastaze limfnih čvorova vrata koje potječu od karcinoma nepoznatog primarnog sijela predstavljaju dijagnostički i terapijski izazov. Metastatski karcinomi nepoznatog primarnog sijela (CUPS) čine otprilike 3 – 5 % svih dijagnoza malignih bolesti. Planocelularni karcinomi čine 90 % karcinoma nepoznatoga sijela, dok preostalih 10 % otpada na slabo diferencirane i adenokarcinome.

Materijali i metode: Ovaj rad prikazuje tri prikaza slučajeva pacijenata kirurški liječenih na Odjelu za maksilofacijalnu kirurgiju, Klinička bolnica Štip, koji su imali metastaze limfnih čvorova vrata s nepoznatim primarnim sijelom tumora.

Rezultati: Pratili smo dijagnostičke protokole koji uključuju detaljan klinički pregled, radiološku dijagnostiku, citološku punkciju tankom iglom (FNAB) tumorske promjene na vratu, ezofagogastroduodenoskopiju, detaljan pregled nazo-, oro- i hipofarinksa te procjenu struktura larinksa. Kod svih pacijenata, nakon kliničke evaluacije, provedeni su ultrazvuk, otorinolaringološki pregled, CT vrata i FNAB. FNAB nalazi su pokazali metastatske naslage planocelularnih karcinoma. Terapija metastaza od CUPS-a uključuje kirurško liječenje (disekcija vrata) i primjenu radioterapije (RT), dok neki autori preporučuju i kemoradioterapiju kod slučajeva s uznapredovalom bolešću regije.

Zaključak: Značajan napredak u dijagnostičkim i operativnim tehnikama povećao je vjerojatnost identifikacije primarnog tumora, kao i njegovog regionalnog i sistemskog širenja. Ako CT ili MRI ne identificiraju primarno sijelo, potrebno je napraviti PET/CT pretrage prije kirurške biopsije. Iako nedostaju visokokvalitetni podaci o protokolima liječenja, pacijenti s uznapredovalim stadijima regionalne bolesti zahtijevaju kombinirano liječenje koje uključuje disekciju vrata te postoperativnu radioterapiju, s ili bez kemoterapije.

Original article

Prognostic Value of Hemoglobin to Red Cell Distribution Width Ratio for Patients with Multiple Myeloma

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Abstract

Aim: Multiple myeloma (MM) is a malignant hematological disorder characterized by clonal plasma cell proliferation and is often associated with renal impairment, anemia, and increased mortality. This study aims to determine whether the hemoglobin-to-red cell distribution width ratio (HRR) at the time of diagnosis acts as an independent prognostic factor for overall survival (OS).

Subjects and methods: This study included patients diagnosed with MM between 2017 and 2023 at the University Hospital Center Osijek. Data were obtained from medical records, and statistical analysis was conducted using SPSS 23 and MedCalc Statistical Software version 22.018 with significance set at $\alpha = 0.05$.

Results: A total of 56 patients with MM were included in the study, consisting of 29 (52%) males and 27 (48%) females. Male patients demonstrated significantly higher HRR values (P = 0.04), with notable variations related to the International Staging System (ISS). Patients with elevated HRR (HR = 0.63) had significantly longer survival rates. The cut-off HRR value for predicting mortality was ≤ 5.09. Receiver operating characteristic (ROC) analysis revealed that 42 patients (75%) had HRR values above 5.09, while 14 (25%) had values ≤ 5.09. Patients with HRR values above 5.09 demonstrated significantly better survival outcomes.

Conclusion: There is a statistically significant difference in HRR values based on sex, ISS, and outcome, in male patients, patients in ISS stage I and survivors exhibiting higher HRR levels. Reduced HRR is associated with poorer outcomes and OS in MM patients, establishing HRR as a straightforward and valuable prognostic indicator for long-term survival in MM.

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KEYWORDS: hemoglobine, multiple myeloma, prognostic factor

Introduction

Multiple myeloma (MM) is a malignant hematopoietic disorder characterized by the clonal proliferation of plasma cells within the bone marrow, resulting in the overproduction of monoclonal immunoglobulins or their light chains. This neoplastic process leads to severe complications, including osteolytic bone lesions, renal insufficiency, anemia, and hypercalcemia, all of which profoundly affect the morbidity and mortality of patients (1). Although MM remains an incurable malignancy, advancements in therapeutic modalities have significantly improved overall survival and quality of life (2). Prognosis in MM is influenced by a range of clinical and laboratory parameters. The hemoglobin-to-red cell distribution width ratio (HRR) has recently been identified as a novel prognostic biomarker. Anemia, commonly observed in MM, is reflected by decreased hemoglobin levels, while elevated red cell distribution width (RDW) is often indicative of anisocytosis, inflammation, and oxidative stress, all of which are associated with advanced disease (3–8). Emerging evidence suggests that HRR, as a readily available and cost-effective biomarker, holds potential in predicting patient outcomes. The focus of this study was to examine the prognostic value of HRR in patients with MM, with an emphasis on its association with disease progression and OS. Furthermore, the integration of HRR with other prognostic markers such as the International Staging System (ISS), may enhance risk stratification and guide individualized therapeutic strategies for MM patients.

Patients and methods

This retrospective study included 56 patients diagnosed with MM over a six-year period (December 2017 to November 2023) at the Department of Hematology, University Hospital Center Osijek. Basic patient data, including sex, disease outcome and laboratory results such as hemoglobin, RDW, albumin and beta-2-microglobulin were collected from medical records. The HRR ratio was calculated, and the ISS score was determined based on the beta-2-microglobulin to albumin value.

Statistical methods

Categorical data were presented as absolute and relative frequencies, while the normality of numerical variables was tested using the Shapiro–Wilk test. Continuous data were described using the median and interquartile range (IQR). Differences between two groups were tested with the Mann–Whitney U test (including the Hodges-Lehmann difference and a 95% confidence interval), while differences across three or more independent groups were assessed using the Kruskal–Wallis test (with post hoc Conover analysis). Cox regression analysis was used to assess the prognostic value of the HRR on survival, which was visualized with Kaplan–Meier curves. The diagnostic value of HRR for OS was evaluated using Receiver operating characteristic (ROC) analysis based on sensitivity and specificity. All P-values were two-sided, with the significance level set at $\alpha = 0.05$. Data analysis was performed using MedCalc® Statistical Software version 22.018 (MedCalc Software Ltd, Ostend, Belgium) and SPSS 23 (IBM Corp, Armonk, NY, 2015).

Results

This study included 56 patients diagnosed with MM, with 29 (52%) being male and 27 (48%) female. According to the ISS, 27 patients (48%) were classified as stage III, 18 patients (32%) as stage II, and 11 patients (20%) as stage I. Moreover, 37 patients (66%) had a positive treatment outcome (survived), while 19 patients (34%) did not survive. The median follow-up time for patients was 24.5 months, with a maximum of 76 months and a minimum of 2 months. The two-year OS rate was calculated on a sample of 29 patients who met the criteria, as the remaining 27 patients had not reached the two-year follow-up mark. Of these, 20 patients (69%) survived for two years post-diagnosis, while 9 patients (31%) passed away within two years of their MM diagnosis. Male patients exhibited significantly higher HRR values compared to females (Mann–Whitney U test, P = 0.04).

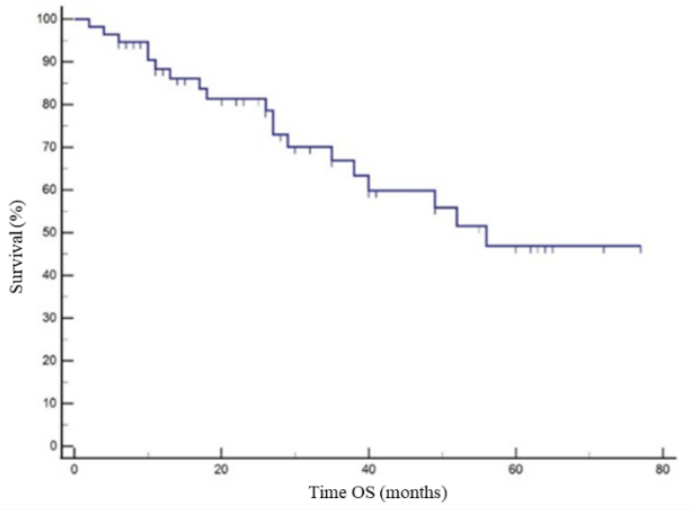

Figure 1. Kaplan-Meier`s overall survival curve

Overall survival (OS) is shown by Kaplan Meier curve (Figure 1).

Additionally, patients classified as ISS stage I had the highest HRR values, while those in stage III showed the lowest values (Kruskal–Wallis test, P < 0.001). Furthermore, patients with a negative treatment outcome (death) had significantly lower HRR values than those who survived (Mann–Whitney U test, P = 0.01) (Table 1).

Cox regression analysis confirmed that a higher HRR is associated with better overall survival (OS) (HR = 0.63) (Table 2).

Table 1. The differences in the hemoglobin/RDW ratio concerning sex and clinical characteristics

	Median (interquartile range) Hemoglobin/RDW ratio	§Difference (95 % confidence interval)	*P*
Gender			
Male	7,23 (5,29 – 9,26)	-1,29 (-2,74 to -0,08)	**0,04***
Female	6,12 (4,36 – 7,20)		
International Staging System			
I	10,32 (8,51 – 10,94)		**<0,001†‡**
II	7,16 (5,14 – 8,50)		
III	5,32 (4,30 – 6,67)		
Outcome			
Survived	7,13 (5,42 – 9,26)	-1,67 (-2,99 to -0,36)	**0,01***
Died	5,28 (4,26 – 7,30)		

IQR – interquartile range; *Mann–Whitney U test; §Hodges-Lehmann median difference
†Kruskal–Wallis test (post-hoc Conover);
‡at the level of P < 0,05 there are significant differences between all values

Table 2. Overall survival estimate for HRR ratio values (Cox regression)

	ß	*P*	HR (95% Confidence interval)
Overall survival			
hemoglobin/RDW ratio (HRR)	-0,457	**0,004**	0,63 (0,47 to 0,86)

ß – regression coefficient, HRR – hemoglobin/red cell distribution width, RDW – red blood cell distribution width, HR – hazard ratio

The diagnostic value of HRR was assessed using a ROC curve, which evaluated sensitivity and specificity, adjusting the cut-off points to differentiate between survival and death outcomes. The cut-off value for predicting death was determined to be ≤ 5.09 (Table 3, Figure 2).

Table 3. Values of the ROC analysis of the HRR ratio in the assessment of OS outcomes

Factor	AUC	95 % CI	Sensitivity	Specificity	*cut- off*	Youden index	*P*
HRR ratio	0,708	0,572 – 0,822	47	86	≤ 5,09	0,339	**0,004**

AUC – area under the curve; CI – confidence interval; HRR – hemoglobin/red cell distribution width; ROC – receiver operating characteristics; OS – overall survival

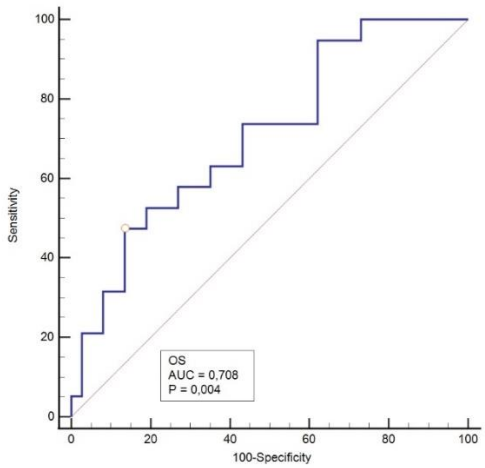

Figure 2. Hemoglobin to red cell distribution width ratio as diagnostic indicator of overall survival (ROC-analysis)

Based on this threshold (derived from the ROC analysis), 42 (75%) patients had an HRR value greater than 5.09, while 14 (25%) had values ≤ 5.09. The two-year OS rate varied based on HRR values. Among patients with an HRR values ≤ 5.09, the two-year OS rate was 50%, whereas for patients with an HRR above 5.09, the two-year OS rate was 72%. Patients with HRR values above 5.09 demonstrated significantly longer survival (Figure 3, Table 4).

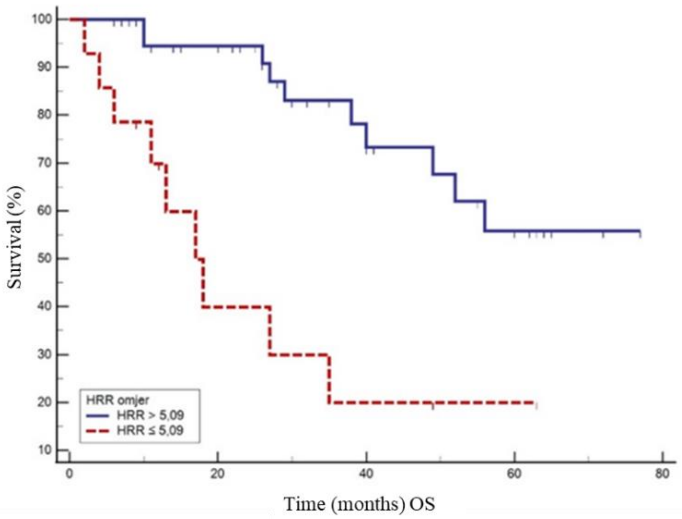

Figure 3. Kaplan-Meier curves of overall survival (OS) versus hemoglobin to red cell distribution width ratio (HRR)

Table 4. Overall survival (OS) in terms of HRR ratio

HRR ratio	Outcome		Arithmetic mean (months)	95 % Confidence interval	Median	95 % Median confidence interval	P value (Log rank test)
	Died	Alive					
> 5,09	10	32	59,6	51,0 do 68,2	-	-	**<0,001**
≤ 5,09	9	5	25,4	13,0 do 37,7	17	6 do 35	

HRR – hemoglobin/red cell distribution width

Discussion

The distribution of participants in this study aligns with epidemiological data showing a higher prevalence of male cases (9). HRR values were significantly higher in men compared to women, which is consistent with previous research on esophageal carcinoma (3). This difference may be attributed to higher baseline hemoglobin levels in men, likely influenced by factors such as body composition, testosterone levels, and hormonal regulation (10). One of the key findings of this study is the association between lower HRR values and more advanced stages of disease according to the ISS. Patients classified as ISS stage III had the lowest HRR values, while those in stage I had the highest. This relationship between lower HRR values and higher disease stages has been reported in other studies, further supporting the relevance of HRR as a prognostic indicator in MM (11). Understanding this association is crucial for assessing prognosis and informing treatment decisions. Patients with lower HRR values and higher ISS scores likely represent a subset of individuals with a more aggressive disease course and an increased risk of mortality. Integrating HRR with established prognostic markers such as ISS may enhance risk stratification, enabling more personalized therapeutic approaches and potentially improving patient outcomes (12 – 14). This study's findings demonstrate that HRR is an independent prognostic marker for OS and disease outcome, with lower HRR values indicating worse prognosis and higher mortality risk. Previous research on other malignancies, including cancers of the esophagus, head and neck, lung, and hematological malignancies, has shown similar associations between lower HRR values and advanced disease stages, highlighting the potential of HRR as a reliable prognostic marker (3,11,15 – 18). Despite these promising results, it is important to acknowledge the limitations of this study. The small sample size and single-center design may limit the generalizability of the findings, and the retrospective nature of the study introduces the possibility of bias. Larger, prospective studies are needed to validate the prognostic significance of HRR in MM and to explore its potential role in guiding clinical management. Nevertheless, these results suggest that HRR holds promise as a valuable marker for understanding disease progression and personalizing treatment strategies in MM.

Conclusion

The results of this study confirm the utility of HRR as a simple and routinely accessible prognostic marker that could be integrated into standard diagnostic and monitoring protocols for MM patients.

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Disclosure

Funding. No specific funding was received for this study.
Competing interests. None to declare.

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Analysis and interpretation of data: NM, VP, JSP
Conception and design: NM, VP, JSP
Critical revision of the article for important intellectual content: NM, VP, JSP
Drafting of the article: NM, VP, JSP
Final approval of the article: NM, VP, JSP
Guarantor of the study: NM, VP, JSP

Prognostička vrijednost omjera vrijednosti hemoglobina i širine distribucije eritrocita u bolesnika s multiplim mijelomom

Sažetak

Cilj: Multipli mijelom (MM) je zloćudni hematološki poremećaj karakteriziran klonalnom proliferacijom plazma stanica i često je povezan s bubrežnim oštećenjem, anemijom i povećanom smrtnošću. Ova studija ima za cilj utvrditi djeluje li omjer hemoglobina i širine distribucije eritrocita (HRR) u trenutku postavljanja dijagnoze kao neovisni prognostički faktor za ukupno preživljenje (OS).

Ispitanici i metode: Studija je uključila pacijente kojima je dijagnosticiran MM između 2017. i 2023. godine u Kliničkom bolničkom centru Osijek. Podaci su prikupljeni iz medicinske dokumentacije, a statistička analiza provedena je pomoću SPSS-a 23 i MedCalc statističkog softvera verzije 22.018, uz razinu značajnosti postavljenu na α =0,05.

Rezultati: U studiju je bilo uključeno ukupno 56 pacijenata s MM-om, od kojih je 29 (52%) bilo muškaraca, a 27 (48%) žena. Muški pacijenti pokazali su značajno više vrijednosti HRR-a (P = 0,04), s primjetnim razlikama vezanim uz Međunarodni sustav stadija (ISS). Pacijenti s povišenim HRR-om (HR = 0,63) imali su značajno dulje stope preživljenja. Granična vrijednost HRR-a za predviđanje smrtnosti iznosila je ≤ 5,09. Analiza krivulje radne karakteristike primatelja (ROC) otkrila je da je 42 pacijenta (75%) imalo HRR vrijednosti iznad 5,09, dok je 14 (25%) imalo vrijednosti ≤ 5,09. Pacijenti s HRR vrijednostima iznad 5,09 pokazali su značajno bolje ishode preživljenja.

Zaključak: Postoji statistički značajna razlika u vrijednostima HRR-a s obzirom na spol, ISS i ishod, pri čemu muški pacijenti, pacijenti u ISS stadiju I te preživjeli imaju više razine HRR-a. Smanjeni HRR povezan je s lošijim ishodima i ukupnim preživljenjem kod pacijenata s MM-om, što HRR čini jednostavnim i vrijednim prognostičkim pokazateljem za dugoročno preživljenje kod MM-a.

Review article

Cognitive Reactivity to Sad Mood as a Risk Factor for Depressive Recurrence

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Abstract

According to cognitive theories of depression, vulnerability for onset and recurrence lies in some kind of cognitive dysfunction, or maladaptive cognitive information processing. This literary review paper aims to clarify the role of cognitive reactivity to sad mood as a risk factor to depressive recurrence, mechanism and measurement in order to provide tailor treatment and prevention for depression. Comprehensive searches of PsycInfo, PubMed and Web of Science were conducted. Teasdale's differential activation hypothesis suggests that the initial depressive episode establishes specific dysfunctional patterns of processing that lie latent after recovery but that can be reactivated by depressed mood. This activation would in turn strengthen the dysfunctional processing patterns and thereby create a vicious loop of depression recurrence. Teasdale calls this concept cognitive reactivity. Cognitive Reactivity (CR) is the extent to which an individual experiences a negative shift in cognitive content and processes during a sad mood. Finding from our review suggests that CR can be conceptualized as a risk factor that is present in vulnerable individuals before depression-onset, that distinguishes between vulnerable and non-vulnerable groups even when in remission, and that predicts depression relapse.There also research evidence that the duration of the first depressive episode, regardless of its intensity, is of crucial importance in the formation of cognitive reactivity to sad mood.Based on findings from different countries we conclude that Index for Depression sensitivity, LEIDS-R is a promising test for measuring cognitive reactivity to sad mood (CR). The results obtained from previous research indicate the importance of an interactive approach when examining the factors that contribute to the recurrent course of depression.

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KEYWORDS: cognitive reactivity; sad mood; risk factor; depressive recurrence

Introduction

Major Depressive Disorder (MDD) is a heavy burden, for vulnerable individuals and for society as a whole.The prognosis of affective disorders, including depression, is generally good, and a large number of patients achieve satisfactory or complete remission. One of the disabling aspects of major depressive disorder (MDD) is its recurrent nature (1). In about a third of patients, MDD becomes recurrent (2) and the risk of recurrences increases after each episode (3). Recurrent depressive disorder often results in complete remission, but cases with persistent limiting dysfunctional symptomatology are also common. Impairment and suffering result both from the initial episode and later recurrence, and identifying risk factors for depressive recurence is therefore pivotal for decreasing its detrimental effects through effective personalized treatment and prevention strategies.

During the last decades, research has been presented that focused on various demographic risk factors for the occurrence, but not recurrence of depression (marital status, gender, negative life events, and Socio-Economic Status (SES) (4). On the other hand, another group of factors, such as : age of onset, number of episodes, and episode severity, together with comorbidity and family history of psychopathology are all implicated in the recurrence of depression (4). But what they all have in common is that these risk factors are either unchangeable or hard to modify, and it may be difficult to tailor treatment and prevention efforts according these variables.

According to cognitive theories of depression, vulnerability for onset and recurrence lies in some kind of cognitive dysfunction, maladaptive cognitive information processing.

This literary review paper aims to clarify the role of cognitive reactivity to sad mood as a risk factor to depressive recurrence, mechanism and measurement in order to provide tailored treatment and prevention for depression.

Method: Comprehensive searches of PsycInfo, PubMed and Web of Science were conducted. We decided on a broader search to not miss out on any potentially eligible studies by frequent use of "or" rather than strict search terms. The keywords searched were "Cognitive AND (reactivity OR dysfunction*) AND depress* AND (predict* OR relapse OR recur*).

Defining cognitive reactivity to sad mood (CR)

Decades of research into the role of cognitive factors in depression have provided support for many aspects of cognitive theories of depression (5). The theoretical-empirical framework from which we started when postulating the problem of this review paper is the paradigm of maladaptive information processing. Despite the revolution in the theory, research, and treatment of depression, existing theories have been insufficient to explain how cognitive patterns, which are latent in nature, contribute to the recurrent course of the disorder. In the last decades, research has focused on examining the role of dysfunctional thought patterns in the development of depression, and a number of findings have resulted in contradictory interpretations of the status of cognitive factors as causative factors or consequences of their depressive state (5,6,7). It became clear that the mechanisms responsible for maintaining depression should not be sought at the level of content, but at the level of processes. In response to these criticisms, studies and models followed that emphasized less "what" and more began to consider "how" those contents become active.

Different theories and hypothesis have been proposed to explaine activation of cognitive schemas. We would like to emphasise the hypothesis of differential activation (8) by which Teasdale, in addition to the stressor as an activator of the schema, proposes another way how the schema can be activated.

He builds on Beck's cognitive model (9) but proposes that the presence of negative affectivity that existed at times when the depressive schema was active can reactivate the schema.

The mentioned hypothesis assumes that during the early episodes of depression, certain patterns of processing are formed, thus forming a link between dysphoria and negative cognitions. It refers to the interaction of affect and cognition and their role as mechanisms that explain the recurrent course of depressive disorder. Teasdale's differential activation hypothesis (10) suggests that the initial depressive episode establishes specific dysfunctional patterns of processing that lie latent after recovery but that can be reactivated by depressed mood. This activation would in turn strengthen the dysfunctional processing patterns and thereby create a vicious loop of depression recurrence (11).

Teasdale calls this concept cognitive reactivity (12). Cognitive Reactivity is defined as the negative change in cognitive processes and content during a sad mood state (CR) (13). Cognitive Reactivity (CR) is the extent to which an individual experiences a negative shift in cognitive content and processes during a sad mood.

The differential activation hypothesis has been tested by correlational studies as well as studies with the induction of depressed affect in depressed patients in remission (14). Also, affect-induction experiments confirm the existence of latent depressive patterns in individuals who have gone through a depressive episode, but it remains unclear how after the depressive episode, the pattern becomes latent again. It was assumed that with the change of the conditions in which the person is, the depressive cognitions withdraw and eventually become inactive again, thus enabling a return to a normal emotional state.

The mechanisms of the recurrent course of depression

Contemporary cognitive-behavioral theories have explained these findings by arguing that dysfunctional cognitions do not disappear during the remission of a depressive episode, but rather remain "latently present" and that they can easily be activated by a sad/dysphoric mood that occurs on the occasion of an unfavorable event (15). An important dilemma in the field of cognitive vulnerability to depression concerns whether such vulnerability is related to the initial episode of depression and/or to the recurrent course of the disorder. Behind the notion of cognitive vulnerability, there is an implicit idea that cognitive vulnerability participates both in the development of the first symptoms and in the development of repeated episodes.

Research on cognitive reactivity to sadness arises as a direct consequence of negative research findings on the stability hypothesis of dysfunctional attitudes and beliefs. According to the stability hypothesis, dysfunctional attitudes and beliefs represent a stable cognitive trait, so it is logical to expect that in vulnerable persons they are registered before the episode of depression, as well as after the symptoms of acute depression disappear. However, the results of research on these hypotheses show that individuals who later entered an episode of clinical depression did not have more dysfunctional attitudes before the episode, than people who did not enter a depressive episode. Endorsement of dysfunctional attitudes declines after depressed individuals enter remission (16).

The above-mentioned research indicated the existence of cognitive reactivity, defined as a person's tendency to react to sadness by producing negative thoughts that are characteristic of depression.

Cognitive reactivity scores are higher in patients treated with antidepressants compared to those treated with cognitive therapy. Furthermore, high cognitive reactivity predicted depressive relapse, independent of prior treatment modality, and a low level of cognitive reactivity to sad mood and a high level of decentering ability was associated with the lowest recurrence rate during the 18-month follow-up (17).

While the contribution of cognitive reactivity to depressive relapses has been empirically investigated, little is still known about the origins and generation of this reactivity. Some authors (18) suggest that it is a remnant of previous depression, which is activated by the

sad/dysphoric mood. In people who are not prone to depression, the cognitive-affective activity quickly subsides, but in people who are vulnerable to depression, the negative spiral continues, in which there is a wider and more elaborate processing of depressive thoughts and intensification of symptoms characteristic of clinical depression.

In this regard, the duration of the first depressive episode, regardless of its intensity, is of crucial importance in the formation of cognitive reactivity to sad mood (16).The summary of meta-analysis conducted by Duaas Nymoen (11) suggests that CR can be conceptualized as a risk factor that is present in vulnerable individuals before depression-onset, that distinguishes between vulnerable and non-vulnerable groups even when in remission, and that predicts depression relapse. The 23 experiments that she analyzed in the meta-analyses on cross-sectional studies vary widely in methodological as well as participant variables, strengthening the generalizability of the findings and supporting the conclusion that there is a moderate CR difference between euthymic ND and FD participants. The 7 longitudinal studies as a part of this meta-analysis, suggested that CR is predictive of depression relapse, with no significant moderators (11).

Measuring Cognitive Reactivity (CR) to sad mood

Cognitive reactivity as a central term of the differential activation hypothesis refers to the idea that once established, negative thought patterns can be easily reactivated through very small triggers, such as subtle changes in mood. Dysfunctional attitudes are considered important causal and maintaining factors of depression, and for that reason most research to date investigates the potential relationship between cognitive reactivity and first episode or depressive relapse based on the Dysfunctional Attitudes Scale (DAS) (19).

The operationalization of cognitive reactivity offered by Van der Does (20), which actually followed Teasdale's formulation of cognitive reactivity, replaced the DAS, which was the "golden standard" until then. Using the Depression Sensitivity Index-Revised (LEIDS-R), respondents report how their typical behaviors and cognitions change while experiencing negative mood.

Research has shown that the LEIDS-R predicts the onset of first symptoms (21), and that it discriminates well between subjects with a prior history of a depressive episode from those without a prior history of depressive symptoms (22). The findings of Sokić, (22) suggest that general cognitive reactivity could be a good predictor of the first episode, but also that specific cognitive reactivity (eg, rumination, dysfunctional attitudes) are related to the recurrent course of the disorder. The results support the concept of cognitive reactivity at a more specific level, in the form of negative automatic thoughts and ruminations in response to current negative mood.

Numerous findings support the notion of vulnerability through the cognitive stress diathesis, yet empirical studies that directly examine whether it actually contributes to the onset, recurrence, or recurrent course of symptoms are rare. In one such study that examined cognitive reactivity, operationalized through Dysfunctional Attitudes Scales (DAS), to negative mood induction as a predictor of depressive relapse, Segal et al. (23) showed that the level of cognitive reactivity at baseline successfully predicted relapse 13-48 months later. These findings were replicated in a similar but methodologically more rigorous study (24).

Comparing whether dysfunctional beliefs (DAS) or the reactivity of such beliefs to mild dysphoric states (i.e., cognitive reactivity assessed with the LEIDS-R) represent a key factor predicting relapse, a group of researchers (25) showed that cognitive reactivity, especially the items related to rumination - predict depressive relapse in the long term. The main finding in the research conducted by Figueroa et al. (25) is that cognitive reactivity (CR) is a risk factor of depressive recurrence. They state that the current measurement of CR, by assessing change on the Dysfunctional Attitudes Scale (DAS) after mood-induction, is not reliable and that the Leiden

Index Depression Sensitivity-Revised (LEIDS-R) is an alternative CR measure. In contrast to mood-induction, it reliably assesses depression vulnerability.

From 2003 until today, a large number of researchers in Europe and other continents have translated and validated this instrument. For example, Senín-Calderón et al. (26) on a Spanish population extracted only 5 factors and subscales with satisfactory psychometric characteristics, similar to Solis et al. (27).

Himle et al. (28) on a Norwegian population confirmed the validity of 6 factor subscales, but also proposed a better psychometric model with only five subscales. Ostovar et al. (29) on a population from Iran confirmed the psychometric characteristics of the LEIDS-R, and 6 separate factors (subscales), with a satisfactory reliability coefficient. The Japanese version of the LEIDS-R was shown to have reasonable reliability and validity (30). The modified Chinese version of the Leiden Index of Depression Sensitivity (LEIDS-RR-CV) is a 26-item self-report measure of CR to sad mood, which contains 5 subscales, including hopelessness/suicidality, acceptance coping, aggression, control/perfectionism, and avoidant coping (31).

Participants with recurrent major depressive episodes showed more repetitive thoughts about negative issues and avoidance from internal and external aversive events when depressive mood was induced, compared to participants with only a single episode of depression. These results suggest that the characteristics of cognitive reactivity are important considerations for preventing relapse of depression. Currently, we are working on validation of LEIDS-R on non-clinical and clinical population in Republic of North Macedonia.

Conclusion and future directions

In recent years, a promising line of research has highlighted the role of CR in the development, maintenance, and relapse/recurrence of depressive symptoms or clinical depression (32). Thus, the relevance of CR to depression should be further explored. Our paper reveals enough evidence to support our hypothesis that cognitive reactivity to sad mood is a risk factor for depressive reccurence. There is also research evidence for the importance of the first depressive episode. While the contribution of cognitive reactivity to depressive relapses has been empirically investigated, little is still known about the origins and generation of this reactivity. The literature review confirmed the lack of standardization of methodologies applied in the research and their great diversity. Even though, DAS proves its good psychometric characteristics in many research studies, according to the psychometric characteristics of the Index for Depression sensitivity, LEIDS-R in various countries in Europe, and countries (Japanes, China, Iran etc.) from other continents, in which it has been translated and validated, we conclude that it is a promising test for measuring cognitive reactivity to sad mood (CR).

The results obtained from previous research indicate the importance of an interactive approach when examining the factors that contribute to the recurrent course of depression. Furthermore, the elaboration of the answer to these questions, through research that takes into account the mentioned aspects, would contribute to understanding the situational, dispositional, emotional and cognitive mechanisms within the framework of recurrent depressive symptoms. Additionaly, understanding the neural mechanism responsible for the biased processing of emotional stimuli in depression might bring important clinical benefits, including predicting, detecting and treating depression (33).

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Kognitivna reaktivnost na tužno raspoloženje kao čimbenik rizika za ponovno pojavljivanje depresije

Sažetak

Prema kognitivnim teorijama depresije, ranjivost za nastanak i ponavljanje depresije leži u određenoj vrsti kognitivne disfunkcije ili neprilagođenoj obradi kognitivnih informacija. Ovaj pregledni rad ima za cilj razjasniti ulogu kognitivne reaktivnosti na tužno raspoloženje kao čimbenika rizika za ponovno pojavljivanje depresije, njezin mehanizam i mjerenje, kako bi se omogućilo prilagođeno liječenje i prevencija depresije. Provedena je sveobuhvatna pretraga baza podataka PsycInfo, PubMed i Web of Science. Teasdaleova hipoteza diferencijalne aktivacije sugerira da prvi depresivni epizoda uspostavlja specifične disfunkcionalne obrasce obrade informacija koji ostaju latentni nakon oporavka, ali se mogu ponovno aktivirati depresivnim raspoloženjem. Ta aktivacija dodatno jača disfunkcionalne obrasce obrade i tako stvara začarani krug ponavljanja depresije. Teasdale taj koncept naziva kognitivna reaktivnost.

Kognitivna reaktivnost (KR) definira se kao razina do koje pojedinac doživljava negativnu promjenu u kognitivnom sadržaju i procesima tijekom tužnog raspoloženja. Nalazi našeg pregleda sugeriraju da se KR može konceptualizirati kao čimbenik rizika prisutan kod ranjivih osoba prije pojave depresije, koji razlikuje ranjive od neranjivih skupina čak i u remisiji, te koji predviđa povratak depresije. Također postoje istraživački dokazi da je trajanje prve depresivne epizode, bez obzira na njezin intenzitet, od ključne važnosti za formiranje kognitivne reaktivnosti na tužno raspoloženje.

Na temelju rezultata iz različitih zemalja zaključujemo da je Indeks osjetljivosti na depresiju, LEIDS-R, obećavajući test za mjerenje kognitivne reaktivnosti na tužno raspoloženje (KR). Rezultati prethodnih istraživanja ukazuju na važnost interaktivnog pristupa pri ispitivanju čimbenika koji doprinose ponavljajućem tijeku depresije.

Original article

Connection between Sleeping Disorders among Students and Academic Success

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Abstract

Aim of the study: Objective of this research was to examine whether sleep disturbances affect the academic success of students of the Faculty of Medicine in Osijek.

Methods: The respondents conducted a self-assessment using a sociodemographic questionnaire and the Pittsburgh Sleep Quality Index (PSQI).

Results: It is observed that there is no significant correlation between age, overall academic success and months of work (if the respondents were employed) with the sleep quality index. Respondents from lower years of studies have a higher sleep quality index, therefore worse sleep quality compared to higher years respondents, whereas daily average studying time has proportional values to the PSQI sleep quality index. In other words, the more hours they spend studying, the higher the sleep quality index. Therefore, the quality of sleep is lower. There are no significant differences in the PSQI sleep quality index in relation to the general characteristics, except in the case of treatment by a psychiatrist. Subjects who were treated by a psychiatrist had significantly worse sleep quality compared to other subjects.

Conclusion: There is no significant correlation between sleep disorders and academic success among students. Subjects from lower years of study had higher values of the PSQI and their sleep quality was inversely proportional to the time they spent studying. Subjects who had a psychiatric diagnosis had worse quality of sleep.

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KEYWORDS: academic success; correlation; sleep disorders

Introduction

Sleep is a physiological state involving temporary loss of consciousness. Although we spend a third of our lives sleeping the exact reason for sleep is unclear. As we age sleep becomes shorter and shallower. Newborns sleep 17 hours daily, middle-aged adults 9, and older adults 7-8 hours, often with naps. Sleep deprivation, particularly lack of REM sleep causes fatigue, confusion, memory issues and cognitive decline. It also disrupts hormonal balance, contributing to obesity (1, 2, 3, 4, 5, 6).

Sleep is divided into non-REM and REM stages. Non-REM sleep consists of four phases distinguished by EEG patterns. In the first phase brain wave frequency decreases while amplitude increases. In the second phase the frequency continues to decrease and amplitude increases, with the appearance of sleep spindles and high-frequency sharp waves. In the third phase deep sleep spindles decrease and brain wave frequency continues to drop with an increase in amplitude. The fourth phase, the deepest level of sleep, is characterized by delta waves—slow, high-amplitude, low-frequency waves. REM sleep on EEG resembles the waking state. Before REM phase muscle tone decreases leading to rapid eye movements. In coma patients EEG shows no non-REM or REM patterns while vegetative state patients display circadian elements on the EEG (1, 5).

Sleep disorders can be triggered by general medical conditions, psychiatric disorders and environmental factors. General medical conditions that cause sleep disturbances are categorized into cardiac, neurological, endocrinological, pulmonary and hematological disorders. Psychiatric conditions associated with sleep disturbances include depression and post-traumatic stress disorder (PTSD) while anxiety disorders may contribute to their onset. Environmental factors that lead to sleep disturbances include stressful life events, shift work, and frequent time zone changes. Conditions such as obstructive sleep apnea, type 1 narcolepsy, idiopathic hypersomnia, and delayed and advanced circadian rhythm disorders can have a genetic basis (1, 4).

Sleep disorders can be classified into primary, caused by endogenous imbalances, including parasomnias and dyssomnias, and secondary, associated with other psychiatric conditions. Sleep disorders can also be categorized as quantitative and qualitative. Quantitative disorders are more common while qualitative disorders often indicate an underlying psychiatric condition. A more recent classification according to the third edition of the International Classification of Sleep Disorders (2014) divides them into six groups: insomnia, sleep-related breathing disorders, central hypersomnias, circadian rhythm disorders, parasomnias and sleep movement disorders (1, 2, 7, 8).

Insomnia is diagnosed when sleep onset takes longer than 45 minutes, total sleep duration is less than six hours or there are at least four nocturnal awakenings. These symptoms must occur at least four times per week for one month. Insomnia is categorized as short-term, often triggered by stress or lifestyle changes, or long-term, frequently associated with medications or comorbidities such as schizophrenia or mood disorders. Initial treatment should focus on non-pharmacological approaches including sleep hygiene education or cognitive-behavioral therapy. Mild insomnia may be managed with antihistamines or melatonin while more severe cases may require benzodiazepines, antidepressants (SSRIs, TCAs) or antipsychotics. Non-benzodiazepine medications are preferred due to a lower incidence of side effects but treatment should be short-term to prevent tolerance and rebound insomnia (1, 2, 9, 10).

Sleep-related breathing disorders include obstructive and central sleep apnea as well as hypoventilation and hypoxemic syndromes. Obstructive sleep apnea occurrs when muscle tone decreases during deeper sleep causing airway obstruction. This leads to reduced oxygen levels and increased carbon dioxide. As a result patients experience fatigue due to less time in slow-wave and REM sleep. Diagnosis is made through polysomnography. Continuous positive airway pressure (CPAP) is recommended to keep the airways open (1, 4).

Central hypersomnias are characterized by excessive daytime sleepiness and may result from prolonged insomnia, atypical depression or psychiatric disorders. They include type 1 and type 2 narcolepsy, drug-induced hypersomnia and idiopathic hypersomnia. Narcolepsy is marked by sudden transitions to REM sleep. Diagnosis requires the tetrad of symptoms: excessive daytime sleepiness, cataplexy, hypnagogic hallucinations and sleep paralysis. Low CSF hypocretin levels confirm type 1. Treatment includes sleep hygiene and medications like modafinil for narcolepsy and sodium oxybate, TCAs, SSRIs, or SNRIs for cataplexy (2, 4, 7, 11).

Circadian rhythm disorders are classified into delayed, advanced and irregular sleep-wake patterns, as well as the non-24-hour sleep-wake disorder which is most common in blind individuals. Shift workers and those with certain psychiatric conditions are more prone to circadian rhythm disruptions while frequent travelers are at increased risk due to crossing multiple time zones. Diagnosis is made through a sleep diary. Treatment depends on the specific disorder. Delayed sleep phase disorder is treated with melatonin before bedtime while advanced sleep phase disorder is managed with evening light exposure (4).

Parasomnias are defined as awakening associated with amnesia. They are classified into non-REM and REM parasomnias based on the timing of occurrence. Diagnosis is made through polysomnography with extended EEG under video monitoring. Benzodiazepines or TCAs are prescribed for non-REM parasomnias while clonazepam and melatonin are used for REM parasomnias (4).

Sleep movement disorders involve repetitive, stereotypic movements during sleep. The two most common are bruxism and restless legs syndrome (Wittmaack-Ekbom syndrome). Patients with restless legs syndrome experience an uncontrollable urge to move their legs and tingling sensations which may spread to the entire leg. To alleviate symptoms mild cases are treated with warm baths or leg massages while more severe cases may require benzodiazepines. Bruxism is treated with oral appliances or clonazepam (1, 2, 4).

Due to the complexity of the system that controls the circadian rhythm, sleep phases and neurotransmitter levels it can be expected that many medications will affect the quality and duration of sleep. Additionally, a general rule is that drugs that enhance aminergic activity over cholinergic activity tend to induce insomnia while those that increase cholinergic activity over aminergic activity tend to cause hypersomnia (1, 5).

Antihistamines through their mechanism of action undesirably block histaminergic neurons in the tuberomammillary nucleus. By blocking these histaminergic neurons modulation of the locus coeruleus and raphe nuclei is impaired leading to the undesirable sedative effect of antihistamines (1, 12).

SSRIs, which are REM suppressors, shorten sleep duration, increase REM latency and may disrupt sleep continuity. However, even with a reduction in REM sleep phase a clinical picture of daytime insomnia is not typically developed (1, 7, 13).

In the Republic of Croatia there is a trend of increased prescribing of benzodiazepines, a medication used to manage various sleep disorders. Although they facilitate falling asleep and shorten the time required to reach deeper sleep, they may reduce the duration of slow-wave sleep and REM sleep and patients may develop anterograde amnesia. Sudden discontinuation of benzodiazepines can trigger a 'rebound' insomnia phenomenon. Prolonged use of benzodiazepines may lead to the development of tolerance (1, 4, 14, 15).

Sigmund Freud proposed that dreams are a reflection of daily conflicts and events, playing a crucial role in memory. Freud's hypothesis was supported among other studies, by research showing that rodents when required to remember spatial arrangements activate the same groups of hippocampal neurons during both the task and sleep. There is also a dual hypothesis emphasizing that during non-REM and REM phases a series of neurological events occur resulting in memory and memory integration. Declarative memory is formed during non-REM sleep, while procedural memory is consolidated during REM sleep. In addition to memory the REM phase plays a key

role in mood regulation and the organization of cognitive abilities (1, 4, 16).

Material and methods

This research got the approval of an appropriate ethics committee.

The study is structured as a cross-sectional study.

The study included 100 randomly selected students from the Faculty of Medicine in Osijek, representing all years of study. The research was conducted from February 1, 2024, to May 1, 2024. Data collection was performed electronically using an online survey questionnaire.

A sociodemographic questionnaire and the Pittsburgh Sleep Quality Index (PSQI) were used in the study. The sociodemographic questionnaire collected data on age, sex, year of study, cumulative grade point average (CGPA), current place of residence, number of siblings, presence of psychiatric disorders, presence of physical diseases, positive psychiatric family history, duration of employment during studies, average daily study time, presence of roommates, and whether the participant had repeated an academic year. The PSQI was used to assess sleep quality and collect the following data: bedtime, time taken to fall asleep, wake-up time, sleep duration, presence and frequency of sleep difficulties in the past month, use of sleep-affecting medications in the past month, frequency of problems maintaining wakefulness in the past month, frequency of lack of enthusiasm in the past month, and self-assessment of sleep quality over the past month. Categorical data were presented as absolute and relative frequencies. The normality of the distribution of numerical variables was tested using the Shapiro-Wilk test. Due to non-normal distribution data were described using the median and interquartile range. Differences in the sleep quality index between two independent groups were tested using the Mann-Whitney U test (with Hodges-Lehmann's median difference and 95% confidence interval) and differences between three or more groups were tested using the Kruskal-Wallis test. The strength of associations was assessed using Spearman's rank correlation coefficient (Rho). All p-values were two-tailed and statistical significance was set at alpha = 0.05. The data were analyzed using MedCalc® Statistical Software version 22.018 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2024).

Results

General Characteristics of the Participants

The study was conducted on 100 participants, consisting of 45 (45%) males and 55 (55%) females. The majority of participants, 51 (51%) were in their 5th and 6th years of study. The median age of the participants was 23 years with an age range from a minimum of 19 to a maximum of 41 years. A total of 58 (58%) participants live with their families and for 62 (62%) participants the current place of residence is shared with family members. Thirteen (13%) participants have no siblings, while the largest group 55 (55%) has only one sibling. Five (5%) participants have four siblings (Table 1, Figure 1).

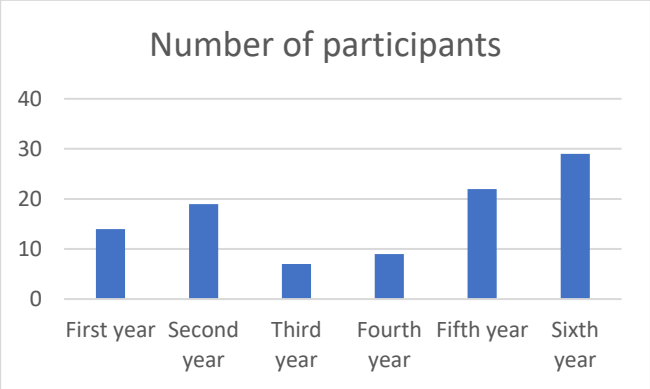

Figure 1. Distribution of participants by year of study

Twenty-three participants (23%) repeated the academic year. The median overall weighted average grade of the participants was 4.10, ranging from a minimum of 3.0 to a maximum of 4.95 (Table 2).

Ten participants (10%) suffer from physical illnesses while six participants (6%) have been examined by a psychiatrist. More pronounced conditions include anxiety-depressive disorders,

obsessive-compulsive disorder and mixed behavioral and emotional disorders (Table 3).

Table 1. Participants according to general characteristics

	Number (%) of participants
Sex	
Male	45 (45)
Female	55 (55)
Age of Participants (years) [Median (Interquartile Range)]	23 (20 – 25)
Who do you currently live with?	
Alone	22 (22)
With roommate	13 (13)
With family	58 (58)
With brother/sister	4 (4)
With grandparents	1 (1)
With partner	1 (1)
With own family as a father/mother	1 (1)
Place of current residence	
Coexistence with family	62 (62)
Tenancy	26 (26)
Dorm	10 (10)
Own apartment	2 (2)
How many brothers/sisters do they have in total?	
None	13 (13)
One brother/sister	55 (55)
Two brothers/sisters	21 (21)
Three brothers/sisters	6 (6)
Four or more	5 (5)

Table 2. Participants repeating an academic year and central tendency measure of the cumulative grade point average

Repeated academic year [n (%)]	23 (23)
Cumulative Grade Point Average (CGPA) [Median (Interquartile Range)]	4,10 (3,84 – 4,40)

Table 3. Frequency of physical and psychiatric diseases in participants

	Number (%) of participants
Has physical Illness	10 (10)
Treated by psychiatris	6 (6)
Psychiatric diagnoses of participants (n = 6)	
Anxiety-depressive disorder	2 / 6
Depression, anxiety	1 / 6
Obsessive-compulsive disorder	2 / 6
Behavioral disorder	1 / 6
Mixed behavioral and emotional disorders	2 / 6
Anorexia	1 / 6

Twenty-five participants (25%) reported having a family member who received psychiatric treatment with the most common psychiatric diagnoses in the family being depression in 8 participants (8%) and PTSD in 4 participants (4%) (Table 4).

Table 4. Frequency of psychiatric disorders within the family

	Number (%) of participants
Someone in the family has been treated by a psychiatrist	25 (25)
Psychiatric diagnoses in the family (n = 25)	
Alcoholism	1 / 25
Anxiety-depressive disorder	2 / 25
Bipolar disorder	2 / 25
Dementia	3 / 25
Alzheimer's disease	1 / 25
Suicidality	2 / 25
Delusion	1 / 25
Depression	8 / 25
Insomnia	1 / 25
Recurrent depression	2 / 25
PTSD	4 / 25

During the studies 42 participants (42%) were employed with a median of 7 months ranging from a minimum of half an hour to a maximum of 10 hours (Table 5).

Table 5. Participants based on employment during studies, average months worked and average time spent studying daily

Employed during studies [n (%)]	42 (42)
Duration of employment (months) [Median (Interquartile Range)]	7 (3 – 13)
Average time spent studying daily (hours) [Median (Interquartile Range)]	3 (2 – 5)

Sleep Quality (PSQI)

The Sleep Quality Scale is determined by seven areas: subjective sleep quality, sleep onset latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of pharmacological agents and daytime functioning. The possible range of scores is from 0 to 21 with higher scores indicating poorer sleep quality. The arithmetic mean of the PSQI scale is 4.8 (standard deviation (SD) 2.89). The median total sleep scale score for the participants is 4, ranging from 0 to a maximum of 13 (Table 6).

Table 6. Measures of central tendency and dispersion of the Pittsburgh Sleep Quality Index (PSQI)

	Possible range	Median (Interquartile Range)	Range from minimum to maximum value
Sleep Quality Index (PSQI)	0 - 21	7 (3 - 7)	0 - 13

Association between Sleep Quality and Participant Characteristics

The Spearman correlation coefficient was used to examine the relationship between the sleep quality index and participant characteristics including age, overall weighted average grade, number of months employed and the average daily number of hours spent studying. It was observed that there were no significant correlations between age, overall weighted average grade and months of employment (for those employed) and the sleep quality index. Participants in earlier years of study had a higher

sleep quality index indicating poorer sleep quality compared to those in later years of study (Rho = -0.285). Additionally, the average daily number of hours spent studying was proportional to the sleep quality index (PSQI) meaning that the greater the average number of daily study hours the higher the sleep quality indeks and consequently, the poorer the sleep quality (Rho = 0.212) (Table 7).

Table 7. Correlation of sleep quality index (PSQI) with age, year of study, grade point average, months of employment and average daily study hours

	Spearman's correlation coefficient Rho	
	Sleep quality index (PSQI)	**P value**
Age	-0,197	0,05
Year of study	**-0,285**	**0,004**
Cumulative grade point average	-0,063	0,53
Months of employment	0,089	0,58
Average daily study hours	**0,212**	**0,04**

There were no significant differences in the PSQI sleep quality index based on general characteristics except in the case of psychiatric treatment. Participants who had received psychiatric treatment had significantly poorer sleep quality compared to the other participants (median 7 vs. 4) (Mann-Whitney U test, P = 0.04) (Table 8).

Table 8. Correlation of psqi sleep quality index with gender, repeating year, place of residence, and psychiatric diagnosis

	Sleep quality index (PSQI)	**Difference (95% confidence interval)**	***P****
Sex			
Male	4 (3 – 6)	1 (0 do 2)	0,12
Female	5 (3 – 8)		
Repeating year			
No	4 (3 – 6)	1 (-1 do 2)	0,41
Yes	5 (3 – 8)		
Place of residence			
Living with family	5 (3 – 7)		
Renting	4 (3 – 6)	-	0,72[‡]
Student dorm	5 (1 – 8)		
Own apartment	4 (3 – 4)		
Psychiatric diagnosis			
No	4 (3 – 6)	3 (0 do 6)	**0,04**
Yes	7 (5 – 11)		

Discussion

We examined the relationship between academic performance and sleep quality in the student population. Sleep quality was assessed using the Pittsburgh Sleep Quality Index (PSQI) which evaluates seven domains: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleep medications and daytime dysfunction. The possible range of the index is from 0 to 21, with higher scores indicating poorer

sleep quality. A PSQI score greater than 5 indicates poor sleep quality.

The median total PSQI score of the participants in our study was 4, ranging from 0 to 13 (Table 6). The mean PSQI score was 4.8 (SD 2.89).

Given the demanding nature of medical studies, results indicating poorer sleep quality were expected which is also supported by a larger study conducted on 540 medical students in Botucatu, Brazil, where 87.1% of students had a PSQI score greater than 5. Similar results were found in a study conducted in Iran where the mean PSQI score was 6.18 (SD 3.42). Corrêa et al. suggested that the poor sleep quality results, particularly among Brazilian students in the earlier years of their studies may be attributed to the transition from a high school to a university lifestyle which likely influenced the results in our study as well. Waqas et al. conducted a study on medical students in Pakistan revealing an even worse sleep quality with an average PSQI score of 8.1 (SD 3.12). Their primary focus was to demonstrate the relationship between academic stress and sleep quality noting that their student population had a high rate of misuse of sleep medications. Ten percent of their respondents admitted to developing sleep difficulties due to the use of sleep medications which Waqas et al. attributed to the fact that pharmacies in Pakistan sell these medications without a prescription. In conclusion, all three studies showed that medical students in the aforementioned countries had poorer sleep quality compared to medical students in Osijek, likely due to greater awareness of the importance of sleep within the student population in Osijek (17, 18, 19).

Our research involved 100 participants 45 (45%) of whom were male and 55 (55%) female. The median PSQI score for male participants was 4, while the median PSQI score for female participants was 5 with no statistically significant correlation between sex and the PSQI sleep index (P = 0.12).

Considering the well-known link between the female sex and sleep disorders we expected female participants to have higher PSQI scores, indicating poorer sleep quality. Similar results were found by Tang et al., who conducted a study in China involving 26,851 participants. Although women (n = 12,551) had higher values for almost all PSQI components, it is important to note that the difference in sleep disturbances between men and women was not statistically significant (P = 0.232). It is important to consider that the PSQI test is based on self-evaluation and men and women may perceive the overall quality of their sleep differently, which could significantly affect research results. Morris et al. noted that female participants associate sleep quality with external factors that disturb sleep and the consequences of sleep deprivation, such as lack of concentration and daytime sleepiness while male participants associate sleep quality with sleep efficiency and duration. These findings suggest that we should focus on more objective sleep quality assessments to obtain more reliable results in future studies (20, 21).

The largest group of participants in our study consisted of 51 (51%) students from the 5th and 6th years of study. The median age of participants was 23 years with a range from 19 to 41 years. No significant correlation between age and sleep quality index was observed. Sleep quality tends to worsen with age as demonstrated by Minjung et al. They showed that the decline in sleep quality associated with older age is also influenced by the number of comorbidities and long-term medication use. Medications most disruptive to sleep include antihypertensives, bronchodilators, diuretics, beta-blockers, corticosteroids and antidepressants such as SSRIs. These medications are more commonly used by older populations so it can be expected that younger populations will have better sleep quality as they use them less. Since our sample size was relatively small and the study was limited to a student population with a median age of 23 years, with 51 (51%) of participants being in just two consecutive years of study we conclude that the age range of the participants was too narrow to observe a statistically significant decline in sleep quality related to age (22).

In our study 23 (23%) participants had repeated an academic year. The median cumulative grade

point average (GPA) of participants was 4.10, ranging from 3.0 to 4.95 (Table 2). No significant correlation between GPA and sleep quality index was found (P = 0.53). Previous studies have shown that sleep disturbances impair memory and cognitive abilities leading to a decrease in academic performance. This was not proven in our study likely due to the small number of participants (n = 100) (1, 23).

In our study 10 (10%) participants had physical illnesses and 6 (6%) had been examined by a psychiatrist. More prominent were anxiety-depressive disorders, obsessive-compulsive disorder and mixed behavioral and emotional disorders (Table 3). Participants who had been treated by a psychiatrist had significantly poorer sleep quality compared to others (median 7 vs. 4) (Mann Whitney U test, P = 0.04) (Table 8).

The statistically significant association between depression and sleep difficulties was expected as it has been well documented. One study conducted on physicians in China involving 1,378 participants showed that physicians with poor sleep hygiene were at higher risk for developing depressive symptoms. A similar study conducted in the United States found a statistically significant correlation between higher global PSQI scores and the risk of developing depressive symptoms. Despite the clear association it cannot be conclusively stated whether poorer sleep quality (higher global PSQI) contributes to the development of depressive symptoms or whether clinical depression promotes the development of poorer sleep quality. Segalàs et al. conducted a study on the relationship between sleep difficulties and obsessive-compulsive disorder (OCD) and found a significant decline in sleep quality in patients with OCD and depression compared to those with OCD alone although both groups had worse sleep quality than the control group. They also concluded that the severity of anxiety and depressive symptoms correlates with poorer sleep quality. This may contribute to the conclusion that each of these two conditions, through their pathophysiology, contributes to the deterioration of sleep quality likely mediated by changes in the balance of monoaminergic neurotransmitters which disrupt the circadian rhythm (24, 25, 26).

The relationship between mixed behavioral and emotional disorders and sleep disturbances was demonstrated by Hosokawa et al. in a study using the Strengths and Difficulties Questionnaire (SDQ) to assess the presence of emotional symptoms, behavioral problems, hyperactivity, attention issues and peer problems. As in the study by Chang et al. it is possible that the symptoms assessed by this questionnaire arose as a consequence of sleep disturbances. Although we found a statistically significant relationship between psychiatric disorders and sleep quality, the small number of participants with psychiatric disorders suggests that further research is needed to confirm the hypothesis of a connection between psychiatric disorders and sleep quality (24, 27).

Participants in the earlier years of study had a higher sleep quality index indicating poorer quality compared to those in later years (Rho = -0.285). Additionally, the daily average number of hours spent studying was proportional to the PSQI sleep quality index, meaning the greater the average number of hours spent studying the higher the sleep quality index, indicating poorer sleep quality (Rho = 0.212) (Table 7). Although the sample size was small this result was unexpected as age is typically proportional to sleep quality. This can be explained by the fact that first-year students, who are still adjusting to the study program, may sacrifice sleep in favor of studying. Since sleep duration is a key factor in the PSQI evaluation these students are likely to have higher PSQI scores which indicate poorer sleep quality. (17, 22).

We should also note that there are several chronotypes that are polygenetically inherited and play a significant role in determining an individual's circadian rhythm. Kalmbach et al. divided participants into early and late chronotypes in their genetic research. Early chronotypes are prone to falling asleep and waking up earlier, with peak productivity during the earlier parts of the day while late chronotypes tend to fall asleep and wake up later, with productivity increasing at night. Since

the academic system is structured around early waking hours this can cause chronic fatigue in students with late chronotypes contributing to concentration loss and daytime sleepiness which can promote the development of sleep disorders (16, 28).

Verweij et al. confirmed that sleep deprivation structurally alters neuronal circuits in the prefrontal region leading to a neural network that loses its optimal information-processing capability resulting in reduced working memory. We can conclude that students with late chronotypes will not only have difficulty maintaining concentration but also experience a decline in working memory capacity over time, negatively impacting their academic performance (29).

Conclusion

There is no significant correlation between sleep disorders and academic success among students. Subjects from lower years of study had higher values of the PSQI and their sleep quality was inversely proportional to the time they spent studying. Subjects who had a psychiatric diagnosis had worse quality of sleep.

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Final approval of the article: AM, DD
Guarantor of the study: AM, DD
Provision of study materials or patients: AM, DD
Statistical expertise: AM, DD

Povezanost smetnje spavanja u studenata s uspješnosti studiranja

Sažetak

Cilj istraživanja: Cilj ovog istraživanja bio je ispitati utječu li poremećaji spavanja na akademski uspjeh studenata Medicinskog fakulteta u Osijeku.

Metode: Ispitanici su proveli samoprocjenu koristeći sociodemografski upitnik i Pittsburški indeks kvalitete spavanja (PSQI).

Rezultati: Uočeno je da ne postoji značajna korelacija između dobi, ukupnog akademskog uspjeha i mjeseci rada (ako su ispitanici bili zaposleni) s indeksom kvalitete spavanja. Ispitanici iz nižih godina studija imaju viši indeks kvalitete spavanja, odnosno lošiju kvalitetu spavanja u usporedbi s ispitanicima iz viših godina, dok je prosječno dnevno vrijeme učenja proporcionalno indeksu kvalitete spavanja prema PSQI. Drugim riječima, što više sati provode u učenju, to je viši indeks kvalitete spavanja, odnosno kvaliteta spavanja je lošija. Nema značajnih razlika u PSQI indeksu kvalitete spavanja u odnosu na opće karakteristike, osim u slučaju liječenja kod psihijatra. Ispitanici koji su se liječili kod psihijatra imali su značajno lošiju kvalitetu spavanja u usporedbi s ostalim ispitanicima.

Zaključak: Ne postoji značajna korelacija između poremećaja spavanja i akademskog uspjeha među studentima. Ispitanici iz nižih godina studija imali su više vrijednosti PSQI-a, a njihova kvaliteta spavanja bila je obrnuto proporcionalna vremenu provedenom u učenju. Ispitanici s psihijatrijskom dijagnozom imali su lošiju kvalitetu spavanja.

Original article

The Effect of Continuous Positive Airway Pressure on Middle Ear Pressure

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Abstract

Aim of the study: The study investigated the effects of continuous positive airway pressure (CPAP) on middle ear pressure.

Methods: Forty-two patients with obstructive sleep apnea (OSA) were assigned to the study group. The patients underwent standard tympanometry before starting CPAP therapy and six months after regular CPAP therapy.

Results: The average pressure range of the CPAP device (cm H2O) was 4,80 – 13,50. Middle ear pressure (MEP) was between -146,00 and 64,00 daPa before therapy and between -103,00 and 40,00 daPa after treatment. The results showed that the subjects experienced an increase in middle ear pressure after the CPAP therapy.

Conclusion: This study demonstrated that the appropriate use of CPAP therapy leads to a statistically significant increase in pressure in the middle ear.

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KEYWORDS: OSA, middle ear pressure, tympanometry, CPAP

Introduction

Obstructive sleep apnea is a breathing disorder characterized by repeated episodes of partial or complete obstruction of the upper airways during sleep lasting at least 10 seconds, with consequent partial or complete cessation of breathing. (1,2). It is estimated that one-seventh of the adult population worldwide suffers from this disorder (3). A recent study found that 936 million adults worldwide aged 30 to 69 have OSA and 425 million adults aged 30 to 69 have moderate to severe OSA (5). According to the Wisconsin Sleep Cohort Study, 17.4% of women and 33.9% of men in the United States aged 30 to 70 have at least mild obstructive sleep apnea (1). The diagnosis is made by polysomnography, a method that represents the gold standard in diagnosing the disease. Continuous positive airway pressure (CPAP) is the first and most common choice of conservative therapy in patients with OSA (1). The principle of action is to prevent the closure and narrowing of the structures of the upper respiratory tract during sleep by applying positive pressure. CPAP involves a mask that covers the nose and/or mouth, a tube that connects the mask to the monitor, and a monitor that blows air into the tube. A pressure ranging from 4 to 20 cm H2O is applied, depending on the individual needs (5). Many patients have difficulties adapting to the use of the device, and due to phenomena such as allergic rhinitis, dryness of the mucous membrane of the upper respiratory tract, dysfunction of the Eustachian tube, as well as the noise of the device, they give up treatment.

The middle ear communicates with the nasal part of the pharynx through the Eustachian tube. Inside the middle ear, there is air under pressure that should be equal to atmospheric pressure. It is through the Eustachian tube that the pressure is equalized and maintained in the middle ear (6). Given that continuous positive pressure in the airways prevents the collapse of the pharyngeal musculature, this study aimed to examine the effect of such therapy on the middle ear, which communicates with the pharynx via the Eustachian tube. We hypothesize that middle ear pressure will have a directly proportional relationship to CPAP pressure in patients not only during therapy but also after the application of CPAP therapy. Earlier studies mainly examined the pressure in the middle ear during CPAP therapy. In this study, we measured the pressure in the middle ear in patients treated with CPAP therapy who did not wear CPAP during tympanometry.

Tympanometry (Ty) is a method that tests the compliance of the eardrum and the auditory chain. In a normal healthy ear, middle ear pressure is equal on both sides of the eardrum and then there is the lowest resistance to sound transmission and the compliance is the highest. In a normal, healthy ear, this pressure ranges from - 150 to + 50 deka Pascals (daPa), and compliance is from 0.18 to 1.8 milliliters (mL). A probe with three channels is placed in the patient's ear canal: a speaker, a microphone, and a channel that changes pressure. This is an objective method, but the movement during the examination and swallowing can affect the result. The result of the test is a curve that shows how many sounds released into the external ear canal bounced off the eardrum and returned to the microphone. There are three basic types of curves: Type A curve with pressure ranging from - 150 to + 50 daPa which is a regular finding; type C with a shift to negative at ≥ - 150 daPa in which, due to negative pressure, usually due to dysfunction of the Eustachian tube, the mobility of the chain of auditory ossicles is lessened. Type B curve usually has the appearance of a flat line, without a characteristic peak, mostly in favor of the rigidity of the chain of auditory ossicles or the presence of fluid in the middle ear (7).

With this research, we wanted to investigate the additional effects of CPAP therapy on other organ systems (in this example, the middle ear) with the aim of better understanding possible side effects that would lead to a decrease in cooperation in treatment.

Methods

The study was conducted in the Clinical Hospital Center Osijek in Croatia, where 42 adults with a new diagnosis of moderate to severe OSA (defined as an apnea-hypopnea index; AHI ≥15/h) were prospectively followed. The study was approved by the Ethics Committee of the

Clinical Hospital Center Osijek (R1/6414/2021, Osijek 2021) and Faculty of Medicine Osijek (No. 602-04/21-08/07; 2158-61-07-21-149, Osijek, 2021) and all participants signed an informed consent. All participants received CPAP therapy and agreed to participate in a 6-month follow-up. The inclusion criteria were age between 29 and 69, participants with normal hearing threshold and sensorineural hearing impairment, and participants with type A curves. The middle ear pressure was evaluated after 6 months of CPAP therapy. The exclusion criteria were middle ear disease, non-cooperation in the application of CPAP therapy (good co-operation is defined by the use of CPAP for 4 hours of sleep during the night, at least 70% of the night (8,9) and withdrawal from further monitoring at one's request.

Statistical analysis

Categorical data are presented with absolute and relative frequencies. Numerical variables are described by arithmetic mean, standard deviation, median, range, and interquartile range. A Shapiro-Wilk test was conducted to determine whether the differences between the two related groups are normally distributed. Since the assumption of normality was met and the sample size was adequate, a paired-samples t-test was used to determine if there was a significant difference in the middle ear pressure between the pre- and post-therapy results. Statistical significance was set at the level of P<0,05. Statistical analysis was performed using IBM SPSS (IBM Corp. Released 2020. IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp.) and MedCalc Statistical Software version 22.023 (MedCalc Software Ltd, Ostend, Belgium; https: //www.medcalc.org; 2024).

Results

The study included 42 patients with OSA, a total of 84 ears. The age of the participants ranged between 29 and 69; the mean age was 52,43 years (Table 1). Table 1 also shows descriptive statistics of AHI in patients with OSA and average pressure of CPAP.

Table 2 shows the distribution of patients according to the degree of apnea to those with moderate and those with severe apnea. In the study, 11 subjects had moderate OSA, and 31 had severe OSA.

Table 1. Descriptive statistics of age, AHI and average pressure of CPAP

Descriptive statistics	OSA group Age	OSA group AHI	OSA group after 6 months of therapy Average pressure of CPAP (cm H_2O)
N	42	42	42
Mean (SD)	52,43 (10,73)	47,50 (23,30)	7,54 (1,64)
Range	29,00 – 69,00	18,20 – 99,70	4,80 – 13,50

N – Number; SD – Standard deviation: OSA – Obstructive sleep apnea; AHI – Apnea-Hypopnea Index; CPAP – Continuous positive airway pressure

Table 2. Distribution of patients according to the degree of apnea

Variable		OSA group
AHI	Moderate OSA (15 – 30)	11 (26,2 %)
	Severe OSA (> 30)	31 (73,8 %)
Total		42 (100,0 %)

OSA – Obstructive sleep apnea; AHI – Apnea-Hypopnea Index

After 6 months, all subjects had their cooperation checked by a neurologist at a follow-up examination and the pressure value of the CPAP device was read from the SD card. The average pressure range of the CPAP device (cm H2O) was 4,80 – 13,50. All subjects had well-titrated CPAP devices (Table 1).

After the follow-up, they all underwent a control otoscopic examination and repeated tympanometry. Of the total number of subjects, none had a subjective feeling of fullness in the ear during or after CPAP therapy.

Middle ear pressure (MEP) was between -146,00 and 64,00 daPa before therapy and between -103,00 and 40,00 daPa after treatment. The results showed that the subjects experienced an increase in middle ear pressure after the CPAP therapy and that the difference in pressure before and after the therapy can be considered statistically significant (Table 3).

Table 3. Results of tympanometry analysis

Variable	Descriptive statistics and results of testing	OSA group before CPAP therapy		OSA group after 6 months of CPAP therapy
Middle ear pressure (daPa)	N	84		84
	Mean (SD)	-19,90 (36,57)		-0,58 (21,78)
	Range	-146,00 – 64,00		-103,00 – 40,00
	Difference		-19,32	
	95% Confidence Interval of the Difference	Lower: -25,04		Upper: -13,61
	P-value		*P* < 0,001*	

** Paired-sample t-test*

Discussion

It has been recorded in the literature that during CPAP therapy, the pressure in the middle ear can increase. What the literature does not tell us is how much this pressure is elevated, whether the values are still within the reference intervals, and whether this affects the subjective experience of the patient. In a study by authors Sivri et al. on 78 patients with moderate and severe OSA who were treated with CPAP therapy, an increase in middle ear pressure was recorded after 6 months of therapy, but tympanometrically it was still a regular type A curve (10). Ma et al analyzed the results of seven articles with a total of 664 subjects using a CPAP device. They demonstrated that short-term use of CPAP is associated with a transient increase in middle ear pressure in adults and that long-term use of CPAP could produce beneficial changes in the middle ear, particularly in patients with OSA and Eustachian tube dysfunction (11). An increase in pressure in the middle ear after CPAP treatment was also shown by Cheung et al (12). Mc Cormick et al presented a case of a patient in whom middle ear barotrauma occurred due to inadequate self-titration of the CPAP device (13). In this study, all subjects had an adequate titration of the CPAP device with a median of 7.10 cm H2O and a range of 4.8 to 13.5 cm H2O. During monitoring, no deviation from normal values of pressure in the middle ear was noted. A significant increase in pressure in the middle ear after CPAP treatment was recorded, which corresponds to data from the literature, but none of the subjects felt subjective complaints such as a feeling of fullness or pressure in the ears.

The limitations of this study are the small number of respondents and the fact that the research was conducted in only one center.

In conclusion, this study demonstrated that the appropriate use of CPAP therapy leads to a statistically significant increase in pressure in the middle ear during 6 months. Increased pressure in the middle ear does not cause subjective disturbances in patients who are on CPAP therapy, and it is not one of the reasons for abandoning treatment

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Statistical expertise: MGČ, DD

Učinak kontinuiranoga pozitivnog tlakana tlak u srednjem uhu

Sažetak

Cilj studije: Studija je ispitivala učinke kontinuiranog pozitivnog tlaka u dišnim putovima (CPAP) na tlak u srednjem uhu.

Metode: Četrdeset i dva pacijenta s opstruktivnom apnejom u snu (OSA) uključena su u istraživačku skupinu. Pacijenti su podvrgnuti standardnoj timpanometriji prije početka CPAP terapije i šest mjeseci nakon redovite CPAP terapije**.**

Rezultati: Prosječni raspon tlaka CPAP uređaja (cm H_2O) bio je od 4,80 do 13,50. Tlak u srednjem uhu (MEP) kretao se između -146,00 i 64,00 daPa prije terapije te između -103,00 i 40,00 daPa nakon terapije. Rezultati su pokazali da su ispitanici nakon CPAP terapije imali povećanje tlaka u srednjem uhu.

Zaključak: Ova studija pokazala je da odgovarajuća primjena CPAP terapije dovodi do statistički značajnog povećanja tlaka u srednjem uhu.

Original article

Viral Infection in Glioblastoma: Immunohistochemistry in Detection of Cytomegalovirus, Epstein-Barr and Herpes Simplex – 1 Virus

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Abstract

Introduction: Glioblastoma (GB) is the most aggressive glial tumor of the brain with a dismal prognosis. Studies conducted during the last two decades highlighted neurotropic viruses as a risk factors involved in development of glioblastoma. Authors present an immunohistological study conducted in a single center on sixty-three archive paraffin-embedded samples of GB.

Patients and methods: The tissues were tested using immunohistochemistry in a homogenous group of sixty-three glioblastoma paraffin-embedded tissues for the presence of Cytomegalovirus (CMV), Epstein-Barr virus (EBV) and herpes simplex virus type 1 (HSV-1).

Results: Three species of herpes viruses were tested: HSV-1, Epstein-Barr virus (EBV) and Cytomegalovirus using the standard automatized immunohistochemistry. According to the IRS score, there were six samples of HSV-1 regarded as IRS 2 and five IRS 1 samples of the same virus. EBV and CMV were negative.

Conclusion: The result of our study identified HSV-1 as the most prominent neurotropic virus among population surgically treated of GB. Further studies are necessary to confirm its possible oncomodulatory role.

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KEYWORDS: cytomegalovirus; Epstein-Barr virus; Herpes simplex virus; glioblastoma; neurotropic viruses; oncogenic viruses

Introduction

Glioblastoma (GB) is an aggressive primary tumor of the central nervous system (CNS) with median overall survival of fifteen months and the relative five years survival rate of 5%. Current treatment options may be considered ineffective due to aggressive course of glioblastoma. The most common anatomical predilection sites for glioblastoma development are the frontal lobe (25%), the temporal lobe (20%) and the parietal lobe (13%). The occipital lobe (3%) is the rarest supratentorial site and other predilection sites for GB development are medulla oblongata, cerebellum, and the spinal cord (1-3).

The fifth, revised WHO classification of CNS tumors published in 2021 classified gliomas, glioneural tumors and neuronal tumors into six subfamilies. Certain changes have been proposed in division of diffuse gliomas into adult and pediatric type according to its clinical and molecular distinctions. Simplification of the classification of diffuse gliomas in adults includes only three types of tumors: astrocytoma, isocitrate dehydrogenase (IDH) - mutant; oligodendroglioma, IDH mutant and 1p/19q deleted and glioblastoma IDH wildtype (4).

The mechanisms of development of glioblastoma are not yet clearly understood and viral infection presents a possible risk factor in tumor development. It is known that several first tier neurotropic viruses may reach CNS such as John Cunningham virus (JCV), BK virus and simian virus 40 (SV40). The second tier viruses include Herpes simplex virus type 2 (HSV-2), West Nile virus (WNV), Ebola, rabies virus and cause a wider spectrum of symptoms, therefore the role of neurotropic viruses (NTV) in the development of GB has been recently exploited. Recent pandemic recognizes SARS-CoV-2 as a neurotropic virus presenting with neuropathological conditions with yet unclear long term effects (5).

During the last two decades the focus of research has been set on the impact of neurotropic viruses, mostly human herpes viruses (HHV) in the development of glioblastoma. HHV viruses share similar structural and genetic characteristics, consisting of long, linear double-stranded DNA containing up to two hundred genes within the icosahedral capsid. Based on their genetic and biological characteristics, herpes viruses are divided into three subgroups: alpha, beta and gamma. The characteristic of neurotropic HHV viruses is latent viral infection, i.e. the presence of the virus in host cells at rest, but with the maintained potential of reactivation and replication. According to the site of viral latency, the sensory ganglia are the characteristic site for the alpha subgroup. Latency of the beta subgroup HHV is maintained in lymphocytes, kidneys, and secretory glands, while gamma HHVs maintain latency in lymphocyte B and T cells (6).

Recently conducted studies recognized cytomegalovirus (CMV) as the most commonly presented viral pathogenic factor, also the presence of Epstein-Barr virus (EBV) in tumor tissue of glioblastoma has been recently investigated. EBV is a DNA virus which is associated with CNS disorders; its presence in B-cells is well established, resulting mostly in B-cell lymphomas.

In contrast to aforementioned viruses, HSV-1 is best known as oncolytic virus due to its neurotropism and genomic modifiability which result in cellular transcription and translation damage. Viral infections are significantly more common than all bacterial, fungal and protozoal infections according to annual incidence and are most often manifested by milder symptoms, nevertheless more severe infections include death (7).

The aim of our study was to detect the presence of NTV in archive glioblastoma tumor tissue samples surgically treated in a single center in a time period of five years.

Patients and methods

The study was conducted on archival paraffin blocks of sixty-three surgically treated glioblastoma in adults during a time span of five years, from January 1, 2012 until December 31, 2017 at Osijek University Hospital Centre, Osijek, Croatia. The study was approved by the institutional Ethics Committee.

Immunohistochemistry was performed using Ultraview DAB detection kit on automated immunohistochemical Ventana BenchMARK Ultra (Roche®) staining system.

The formalin fixed, paraffin embedded tissue blocks were sectioned at a thickness of 4 μm and then were deparaffinized and rehydrated in graded alcohol and then incubated with HRP multimer. The procedure was continued by hematoxylin counterstaining at room temperature and impregnation in ULTRA LCS (Ultra liquid Coverslip) oil solution.

The staining signal was visualized with 3,3'-diaminobenzidine (DAB) chromogen (Ultra View DAB Copper).

The presence of neurotropic viruses (Epstein-Barr virus (EBV), cytomegalovirus (CMV) and herpes simplex virus (HSV)) were analyzed immunohistochemically with the following antibodies:

- CMV mouse monoclonal antibody (8B1.2, 1G5.2, 2D4.2) - Roche® (Basel, Switzerland) Ready-to-use, part number 06597190001
- Herpes Simplex Virus (10A3) rabbit polyclonal antibody - Dako® (Glostrup, DK-2600, Denmark) Ready-to-use, Type I, part number IR 521, 20068570
- Epstein-Barr Virus, mouse monoclonal antibody, Clones CS. 1-4 - Dako® (Glostrup, DK-2600, Denmark), Ready-to-use, part number 20019280. At each slide we used positive control (infected tissue) and negativne control (buffer, no primary antibody).

All the immunostained slides were scanned and analyzed using the Olympus® CX40 microscope. A positive finding is considered to be staining of the cytoplasm and nucleus in CMV, the cytoplasm and membrane in EBV, and a positive staining of the nucleus in HSV. The interpretation of staining is always associated with the evaluation of positive controls.

The results of immunohistochemical reactions were assessed by the IRS method (immunoreactive score of Remmele and Stegner) according to the percentage of tumor cells in the high-magnification (40x) microscopic field of view. Staining intensity was graded as 0 (negative), 1 (weak), 2 (moderate), and 3 (strong); percentage of positive cells examined was scored as 0 (negative), 1 (≤ 10%), 2 (11-50%), 3 (51-80%), and 4 (≥81%). The two scores were multiplied and the IRS (values from 0-12) was determined. According to this, cases were categorized into four groups: 0-1 as negative (0), 2-3- as weak positive (1), 4-8 as moderate (2) and 9-12 as strongly positive (8).

Results

Immunohistochemistry was conducted on sixty-three archive glioblastoma tumor samples.

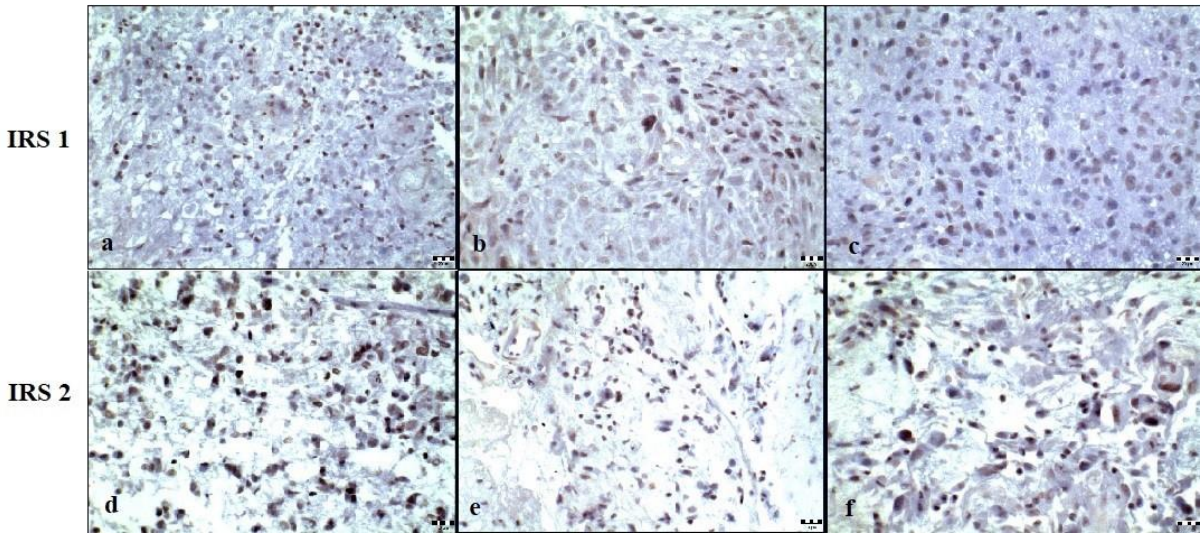

Figure 1. Representative slides of immunohistochemical staining of formalin-fixed paraffin-embedded glioblastoma tissues on HSV-1 antigen graded as IRS 1 (a – c) and IRS 2 (d – f), magnification 400 x.

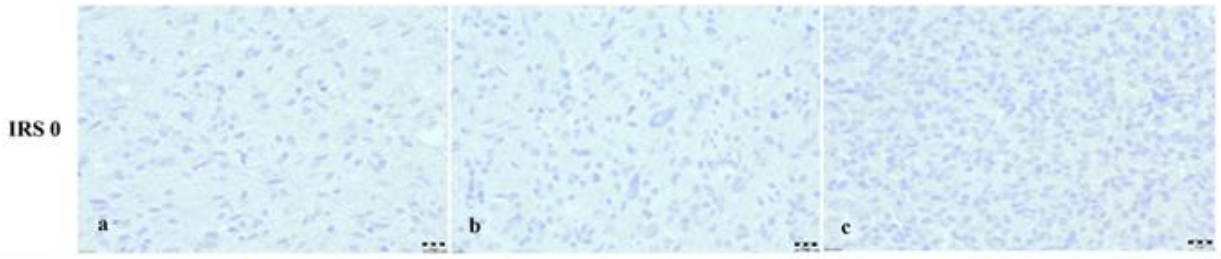

Figure 2. Representative slides of immunohistochemical staining of formalin-fixed paraffin-embedded glioblastoma tissues on CMV (a), EBV (b) and HSV-1 antigen (c) graded as IRS 0, magnification 400 x.

Use of polyclonal antibody HSV 10A3 determined the number of positively stained HSV - 1 nuclei per square millimeter of tumor tissue. The standard automatized immunohistochemistry revealed 5 mildly positive samples which were evaluated as IRS 1 and 6 moderately positive samples evaluated as IRS 2 according to the same semiquantitative assessment (Figure 1.). Use of monoclonal ready-to-use murine EBV antibodies (Clones CS. 1-4) and CMV antibodies (8B1.2, 1G5.2, 2D4.2) excluded the presence of these viruses from paraffin-embedded glioblastoma tissue. The semiquantitative value for CMV and EBV samples was therefore rated as IRS 0 (Figure 2.); additional microscopic evaluation did not reveal the expected staining of the cytoplasm and nucleus in CMV and the cytoplasm and membrane in EBV.

The total percentage of positively stained samples was 17.5%. Semiquantitative results supported the dominant presence of HSV – 1 based on IRS scoring..

Discussion

Glioblastoma still remains an unsolvable problem with an average survival of only 15 months despite numerous technological possibilities and the development of various treatment methods (9). Therefore, current treatment of these tumors can be considered as palliative one with an aim of maintaining the best possible quality of life in these patients. The vast majority of patients are diagnosed with glial tumors in its advanced stage due to the rapid growth of glial tumors and its sudden and progressive symptoms, expressed as headache, focal neurological deficit and epileptic seizures (10).

Extensive studies of the role of neurotropic viruses in glioblastoma have been conducted more intensively over the past two decades with the aim to establish a firm connection between the presence of neurotropic viruses and oncogenesis of glial tumors. The results of all studies are incoherent and do not show a clear insight into the role of neurotropic viruses in oncogenesis and development of glioblastoma.

Research on the role of CMV in glioma tumors began in 2002 with a study conducted by Cobbs et al. The presence of cytomegalovirus gene material in all tested samples was detected by immunohistochemistry and in situ hybridization methods (11). Further research by Scheurer et al. in 2008 and Slinger et al. in 2010 obtained similar results with more than 90% positive samples for cytomegalovirus presence by the same research methods, albeit with a relatively small samples (12,13). Over the next two decades, the results of CMV detection in tumor tissue samples revealed significant discrepancies both in the number and homogeneity of the tested samples and in the research results. Another significant study was enrolled by Libard and co-workers in 2014. They achieved a result of 90% positive samples of glioma tumors for the presence of CMV exclusively by immunohistochemistry. It should be emphasized that out of 469 samples, 219 of them were of glioma origin of all grades. Although various methods of detecting CMV have been used in dozens of studies, no firm consensus has been reached on the role of CMV in the development of glial tumors. Other studies have not

demonstrated the presence of CMV protein or gene material in glial tumor samples despite the use of highly sensitive detection methods, thus also questioning the oncomodulatory potential of cytomegalovirus (14-16). According to the opposite results among studies further need to optimize detection techniques and CMV diagnostics was discussed.

Recent studies were mostly focused on CMV, but novel studies were conducted to elucidate the possible role of EBV in gliomagenesis. EBV is widely spread among the child population and young adults with the possibility of lifelong persistence. Its role is primarily known in pathogenesis of Burkitt's lymphoma and epithelial cell cancers, although presence of EBV in CNS is presented in symptoms such as cerebellar ataxia and disseminated encephalomyelitis or CNS lymphoma which is commonly seen in both immunodeficient and immunocompetent patients. During the last decade a dozen studies were conducted on the role and presence of EBV in glial tumors. Reportedly, EBV DNA is mostly found in high-grade gliomas (type III and IV) in researches that include PCR, IHC and sera testings, although researches based on NGS doubted the connection between EBV and high grade gliomas (17, 18). Less than 5% of primary EBV infections lead to CNS diseases that are clinically manifested by meningitis, encephalitis, cerebelitis, cranial and peripheral neuropathies, as well as polyradiculomyelitis. Mononuclear inflammatory infiltration is characterized by leptomeningeal spread of inflammation with the development of perivascular demyelination. The most common CNS predilection sites for EBV infections are the cerebellum, basal ganglia and less commonly both cerebral hemispheres. Therefore, the most common symptoms are predominantly caused by infection of the thalamic region of the brain, while the highest mortality rate of patients with EBV infection relate to the brainstem (19, 20).

Our study excluded the presence of CMV and EBV, but confirmed the presence of HSV-1 which is the best known as an oncolytic virus (oHSV) with reduced neurotoxicity and retained neurovirulence (21).

A study similar to ours was conducted by Zavala-Vega et al. where authors included CMV, EBV and HSV. They have employed more methods of detection, although their sample group was smaller compared to ours. There was only one case of single HSV infection detected by IHC but at least 50% of HSV infections in their study were mixed infections (HSV and CMV or HSV and EBV) within the group. Overall, more than 70% of their samples were positive for single or mixed infection which was expected given the high seroprevalence of HHV infections among the Mexican population. A clear advantage of their study was a wider array of methods used to increase detection (22).

A possible infection of CNS caused by HSV-1 in adults is presented as herpes simplex encephalitis (HSE); the devastating nature is characterized by brain hemorrhage, brain edema and necrosis mostly affecting the frontal and temporal lobes and the lymbic system. HSV-1 may enter the CNS affecting peripheral neurons or the bloodstream, i.e through the blood-brain barrier (BBB).

Most commonly accepted mechanism of HSV-1 infection of the CNS is its retrograde transport and latent infection of trigeminal ganglia (TG) after peripheral epithelial cells infection. This route of infection is considerably alleviated compared to HSV-1 infection via the bloodstream due to protective cellular barriers between BBB and blood-cerebro-spinal fluid barriers. Besides TG, HSV-1 may also invade other parts of CNS, such as olfactory bulb and orbitofrontal lobe, brainstem, medial temporal lobes and cortex, the limbic system, but it is not correlated to infection of the higher brain areas and projection pathways. Primary infections include mucocutaneous tissue as the "gateway" of infection with consequent distribution of the viral particle. Primary infections occur in the second or third decade of life, with the possibility of reactivation regardless of further age. After primary infection the spread of the viral particle through the neural tissue takes place through the ends of the axon, after which it is

permanently located in the dorsal root ganglion (DRG) (23). Experimental evidence in animal models supports viral transmission via the olfactory or trigeminal nerve, suggesting the possibility of viral spread through the anterior commissure thus achieving viral dissemination of the contralateral temporal lobe (24). Moreover, unlike other cranial nerves with sensory function, olfactory nerve pathways do not pass through the thalamus but are projected directly toward the frontal and temporal lobes (25). Meningeal route of viral dissemination should be taken into account as the consequence of trigeminal innervation of meninges, therefore this route of viral dissemination in HSV-1 infection is not excluded. In addition to direct routes of spreading, reactivation of latent HSV infection of the trigeminal ganglion is another type of pathogenic mechanism of viral activation (26 - 28).

Intermittent reactivation of HSV-1 is mostly caused by immunosuppression, injury of tissues or fever which are conditions frequently found in patients with GB. According to Larjavaara et al., GB is mostly found in the frontal (40%) and the temporal lobe (30%), respectively, which correlates to aforementioned pathways of spreading of HSV-1 through the CNS (29). Therefore, taking into account the anatomical spreading and reactivation of HSV-1 in patients with GB, our results might be explained by the possibility of a high seroprevalence in our population and viral reactivation.

Results of our study can be only partially compared with the results of previously conducted studies in terms of the type of NTV, methods, techniques and the number of patients.

Dominating presence of HSV-1 in our study indicates a possible geographical predisposition and seropositivity among our population and its ethnic groups. The presence of HSV-1 in its latent state in patients with GB and its consequent reactivation is considered to be a result of immunodeficiency in malignant development of GB, although this claim cannot be firmly connected as a role of NTV in development or progression of GB.

Comparing our study to recently enrolled studies, its major limitations were retrospective formalin-fixed paraffin-embedded (FFPE) samples, impossibility of serological testing and limitations in methodology. Even though the total number of samples used in our study was larger comparing to other aforementioned studies, also we used a homogeneous group of histologically confirmed GB. Results from previous studies revealed certain inconsistencies according to different preparation and processing of fresh frozen tissue samples or retrospective samples. Also, genetic variability of population, geographical and racial differences, tumor sample heterogenity and methodologies might explain the different results among studies. Our study was enrolled during the Covid-19 pandemic and furthermore the other possibilities of detecting viruses, such as molecular testing were not included due to the lack of human and technical resources.

Assuming a fact of possible presence of HSV-1 in our population, we have to emphasize that further studies have to be designed to confirm a possible viral influence in the role of development of brain tumors. This claim is confirmed by recent analyses of NTV detection which revealed certain discrepancies related to technical issues and denoted the importance of optimization of staining protocols to obtain unbiased results (30 - 34).

In conclusion, many studies in the last two decades revealed excellent results in their endeavors to clarify the role of NTV in GB, although these results have opened many other inquiries. The importance of further studies is necessary to elucidate the viral oncomodulatory role.

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Virusna infekcija i glioblastom: Imunohistokemijsko otkrivanje citomegalovirusa, Epstein-Barrovog virusa i herpes simpleks virusa tipa 1

Sažetak

Uvod: Glioblastom (GB) najagresivniji je glijalni tumor mozga s lošom prognozom. Studije provedene tijekom posljednja dva desetljeća istaknule su neurotropne viruse kao čimbenike rizika uključene u razvoj glioblastoma. Autori predstavljaju imunohistološku studiju provedenu u jednom centru na šezdeset i tri arhivska parafinska uzorka GB-a.

Pacijenti i metode: Tkiva su ispitana metodom imunohistokemije u homognoj skupini od šezdeset i tri parafinski ugrađena uzorka glioblastoma na prisutnost citomegalovirusa (CMV), Epstein-Barrovog virusa (EBV) i herpes simpleks virusa tipa 1 (HSV-1).

Rezultati: Testirane su tri vrste herpes virusa: HSV-1, Epstein-Barrov virus (EBV) i citomegalovirus (CMV) primjenom standardizirane automatizirane imunohistokemije. Prema IRS skoru, šest uzoraka HSV-1 ocijenjeno je kao IRS 2, dok je pet uzoraka istog virusa imalo IRS 1. EBV i CMV nisu bili prisutni.

Zaključak: Rezultati naše studije identificirali su HSV-1 kao najistaknutiji neurotropni virus među populacijom kirurški liječenom od GB-a. Potrebna su daljnja istraživanja kako bi se potvrdila njegova moguća onkomodulatorna uloga.

Original article

Vitamin D Deficiency as a Risk Factor for Colorectal Cancer

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Abstract

Introduction: Colorectal cancer is the third most common diagnosis and cause of death in both sexes in highly developed countries. It is assumed that environmental factors are involved in the development of the disease, with strong evidence favoring lifestyle as well as the influence of diet. There are also many studies that indicate that low vitamin D levels are a significant risk factor for the occurrence of colorectal cancer.

Objective: To evaluate and compare serum concentrations of 25-hydroxyvitamin D (25(OH)D) between people diagnosed with colorectal cancer and a control group of healthy subjects.

Materials and methods: A total of 30 people with colorectal cancer and a control group of 30 healthy subjects were analyzed in the study.

Results: The comparison of the colorectal cancer group and control group in terms of serum vitamin D concentration showed that lower values were measured in the group with colorectal cancer. The mean vitamin D concentration in the colorectal cancer group was 16.6 ± 7.8, while in the control group it was 28.7 ± 10.3; the difference of 12.1 was statistically significant, for p=0.001.

Conclusion: The results of the presented study indicate significantly lower serum concentrations of 25(OH)D in individuals with colorectal cancer compared to the control group.

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KEYWORDS: colorectal cancer, cancer, vitamin D

Introduction

Colorectal cancer (CRC) is the third most common diagnosis and cause of death in both genders in highly developed countries (1). The incidence rate is decreasing in Western countries, mainly due to endoscopic screening, but the incidence in the population younger than 50 years is increasing (2). Environmental factors are assumed to be involved in the development of the disease, with strong evidence favoring lifestyle as well as the influence of nutrition (3). A nutrition rich in red meat and calories, and low in fiber, fruits and vegetables, as well as physical inactivity, increase the risk of CRC. There are also many studies that indicate that low levels of vitamin D3 are a significant risk factor for the occurrence of CRC (4). Hypovitaminosis of this vitamin is very widespread worldwide and is associated with several chronic diseases, including malignant diseases. Vitamin D3 is a fat-soluble vitamin, the main function of which is to regulate the metabolism of calcium, magnesium and phosphate along with numerous other biological functions (5). As anti-inflammatory, immunomodulatory, pro-apoptotic and anti-antiangiogenic functions (6). Many studies suggest that it also acts as an inhibitor of carcinogenesis slowing tumor progression by promoting cell differentiation and inhibiting the proliferation of cancer cells (4). This vitamin also affects the activity of the systemic and mucosal immune system, generally with its regulatory and anti-inflammatory properties (6).

25-hydroxyvitamin D (25(OH)D) is a metabolite of vitamin D in the human body and is indicative of the total level of vitamin D. Measuring the serum concentration of (25(OH)D) is a common test performed to determine vitamin D status and to indicate vitamin D deficiency or sufficiency. Laboratories generally report (25(OH)D) levels in ng/mL. Normal values of serum vitamin D concentrations are those above 30 ng/mL, insufficient or reduced from 20-30 ng/mL and deficient or low values below 20 ng/mL (7,8).

The purpose of this study is to evaluate and compare serum 25(OH)D concentrations between patients with diagnosed colorectal cancer (CRC) and a control group of healthy subjects (CG)(9).

Material and methods

The study analyzed a total of 30 subjects with CRC and a CG of 30 healthy subjects. The study was conducted in the period from June 2022 to January 2024 in Skopje - North Macedonia.

Inclusion criteria for the study were: age 40-80 years, positive medical history, elevated tumor markers CEA and CA 19-9, and pathohistologically detected colorectal cancer.

Serum concentrations of 25(OH)D in the subjects were determined with an Access 2 BeckmanCoulter immunoassay analyzer using the CLIA (Chemiluminiscence Immunoassay) method. Normal values of serum vitamin D concentrations were taken as those above 30 ng/mL, insufficient or reduced from 20-30 ng/mL and deficient or low values below 20 ng/mL.

Statistical analysis of data: The statistical analysis of the data obtained from the study was performed in the statistical programs Statistica for Windows 7.0 and SPSS 23.0. The obtained data are presented in tables and pictures.

Categorical (attributive) variables are presented with absolute and relative numbers. Numerical (quantitative) variables are presented with average, standard deviation, minimum and maximum values.

For comparison of the analyzed groups, in terms of gender, age, serum concentrations of vitamin D, non-parametric (Pearson Chi square test, Fischer exact test) and parametric tests for independent samples (Student t-test) were used.

Statistical significance was defined at the level of $p<0.05$.

Results

The gender structure of the subjects who participated in the study consisted of 26 (43 %) male and 34 (57%) female subjects. Female subjects were more frequently represented in both the CRC group and the CG. The difference in the distribution of male and female subjects between the study and control groups was statistically insignificant (p=0.71).

Table 1. Gender of subjects with colorectal cancer and control group

Variable	groups			p-value
	n	CRC	CG	
		n (%)	n (%)	
gender				
male	26	12 (40)	14 (47)	
female	34	18 (60)	16 (53)	p=0.71

CRC – colorectal cancer; 2 (Pearson Chi-square); CG - healthy subjects

Comparison of the CRC and CG groups in terms of serum vitamin D concentrations showed that lower values were measured in the group with diagnosed CRC. The mean vitamin D concentration in the CRC group was 16.6 ± 7.8, while in CG it was 28.7 ± 10.3; the difference of 12.1 was statistically significant, for p=0.001.

The data are shown in Table 2.

Table 2. Serum vitamin D concentrations in subjects with CRC and CG

groups	Descriptive statistics vitamin D (ng/mL)		p-value
	mean SD	min-max	
CRC	16,6 ±7.8 ng/mL	5.4-34.1 ng/mL	
CG	28,7 ±10.3 ng/mL	12.5-47.7 ng/mL	p=0.001**

*CRC-colorectal cancer; t(Student t-test); **p; CG- control group*

Normal vitamin D values were registered only in 4 (13%) subjects with CRC, and in 14 (47%) healthy subjects. Insufficient values were recorded in 10 (33%) subjects, all from the CG, while deficient vitamin D values were found in 26 (87%) subjects with CRC and 6 (20%) healthy subjects. For p < 0,001, a statistically significant difference in the distribution of normal, insufficient and deficient vitamin D values between subjects from the CRC group and the CG of subjects was confirmed.

The data are shown in Table 3 and Figure 1.

Table 3. Number (%) of cases with CRC and CG with normal, insufficient or deficient serum concentrations of vitamin D

Vitamin D	group		p-value
	n	CRC n(%)	CG n(%)	
Normal values	18	4 (13)	14 (47)	Fisher exact p < 0,001
Insufficient values	10	0	10 (33)	
Deficiency values	32	26 (87)	6 (20)	

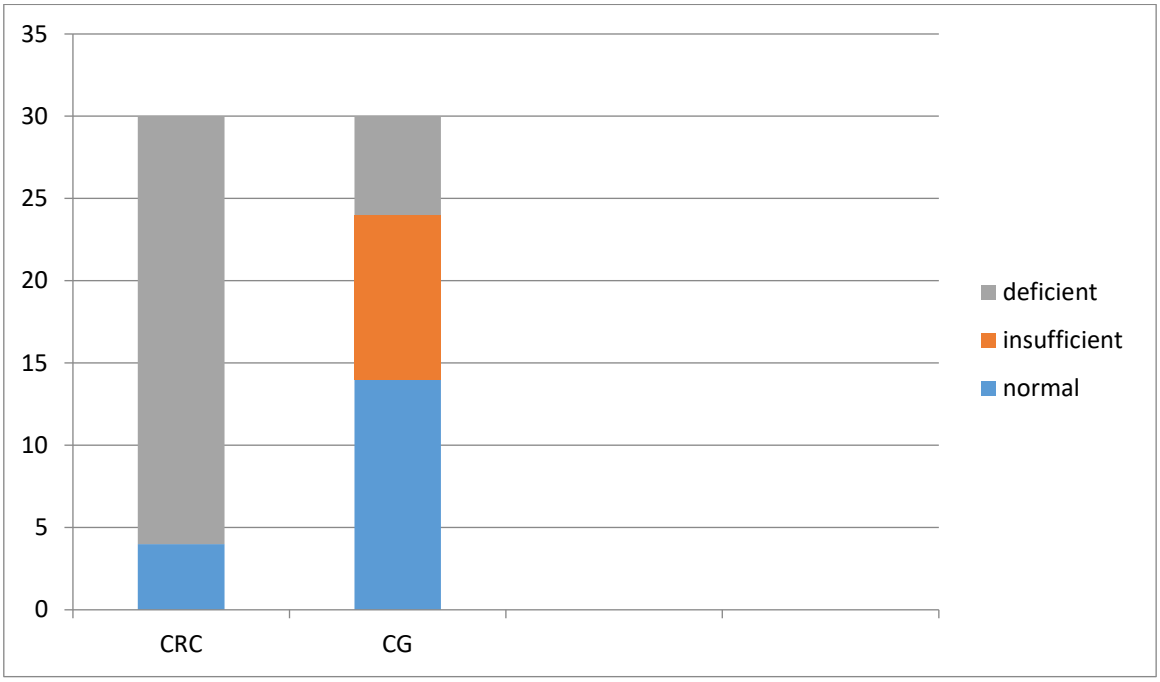

Figure 1. Number (%) of CRC and CG cases with normal, insufficient or deficient serum vitamin D concentrations

Discussion

The results of our study indicate that patients with CRC have lower mean serum concentrations of vitamin D compared to the CG. There was a statistically significant difference in the distribution of normal, insufficient and deficient values of vitamin D between the CRC group and the CG. In the literature, there are conflicting views on the relationship between vitamin D deficiency and CRC. Our study identified vitamin D deficiency in 86.7% of patients with CRC. Our subjects have lower serum concentrations of vitamin D than reported so far [11] for CRC, where Huncharek M et al. [11] reported that vitamin D deficiency was not associated with CRC, but another study [10] reported that there was a correlation. The results of our study indicate that serum concentrations of vitamin D are strongly associated with CRC.

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Disclosure

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Competing interests. None to declare.

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Author contribution. single author article

Manjak vitamina D kao čimbenik rizika za kolorektalni karcinom

Sažetak

Uvod: Kolorektalni karcinom treća je najčešća dijagnoza i uzrok smrtnosti kod oba spola u visoko razvijenim zemljama. Pretpostavlja se da su čimbenici okoliša uključeni u razvoj bolesti, pri čemu postoje snažni dokazi koji upućuju na utjecaj načina života i prehrane. Također, brojne studije ukazuju na to da su niske razine vitamina D značajan čimbenik rizika za nastanak kolorektalnog karcinoma.

Cilj: Procijeniti i usporediti serumske koncentracije 25-hidroksivitamina D (25(OH)D) kod osoba s dijagnosticiranim kolorektalnim karcinomom i kontrolne skupine zdravih ispitanika.

Materijali i metode: U studiji je analizirano ukupno 30 osoba s kolorektalnim karcinomom te kontrolna skupina od 30 zdravih ispitanika.

Rezultati: Usporedba skupine s kolorektalnim karcinomom i kontrolne skupine u pogledu serumske koncentracije vitamina D pokazala je da su niže vrijednosti izmjerene u skupini s kolorektalnim karcinomom. Prosječna koncentracija vitamina D u skupini s kolorektalnim karcinomom iznosila je 16,6 ± 7,8, dok je u kontrolnoj skupini bila 28,7 ± 10,3; razlika od 12,1 bila je statistički značajna (p=0,001).

Zaključak: Rezultati prikazane studije ukazuju na značajno niže serumske koncentracije 25(OH)D kod osoba s kolorektalnim karcinomom u usporedbi s kontrolnom skupinom.