ACTA
STOMATOLOGICA
CROATICA
www.ascro.hr

Acta stomatol Croat. 2023;57(3):265-272.
DOI: 10.15644/asc57/3/7

ORIGINAL SCIENTIFIC PAPER
IZVORNI ZNANSTVENI RAD

Ana Družijanić[1], Livia Cigić[1,2*], Ana Glavina[3], Mirna Draganja[2], Dinko Martinović[4], Mare Ković[2]

Serum Concentration of Vitamin D in Patients with Oral Lichen Planus

Koncentracija vitamina D u serumu oboljelih od oralnoga lihen planusa

[1] Department of Dental Medicine, Department of Maxillofacial Surgery, University Hospital of Split, Split, Croatia
Odjel za dentalnu medicinu Zavoda za maksilofacijalnu kirurgiju KBC-a Split, Hrvatska
[2] Study of Dental Medicine, School of Medicine, University of Split, Split, Croatia
Studij dentalne medicine Medicinskog fakulteta Sveučilišta u Splitu, Hrvatska
[3] Outpatient Clinic Split, Split, Croatia
Stomatološka poliklinika Split, Hrvatska
[4] Department of Maxillofacial Surgery, University Hospital of Split, Split, Croatia
Zavod za maksilofacijalnu kirurgiju KBC-a Split, Hrvatska

Abstract

Objectives: The vitamin D receptor is involved in immunologically mediated diseases such as oral lichen planus. Some studies suggest an association between lower vitamin D concentrations and a higher risk of inflammatory conditions such as oral lichen planus. The aim of this study was to investigate 1) whether there is a difference in serum vitamin D concentration in patients with oral lichen planus compared to patients in the control group, 2) whether there is a difference in serum vitamin D concentration in patients with erosive compared to non-erosive forms of oral lichen planus, 3) whether there is a difference in serum vitamin D concentration in patients with developed oral cancer and those without cancer. **Material and Methods:** The study included 68 patients, 34 with oral lichen planus and 34 healthy controls. Fasting venous blood was taken from each participant to determine serum concentrations of vitamin D. **Results:** The T-test results confirmed a statistically significant lower serum vitamin D concentration in patients with oral lichen planus compared to the control group (p=0.001). According to the results, a statistically significant lower serum vitamin D concentration was found in patients with erosive form OLP. All five patients with oral cancer, which was developed from erosive OLP, had low serum vitamin D concentrations. **Conclusions:** Determination of serum vitamin D concentration could be important for monitoring OLP patients to prevent the development of severe clinical manifestations of erosive OLP and the conversion of symptomatic lesions to oral cancer.

Received: March 13, 2023
Accepted: July 11, 2023

Address for correspondence
Livia Cigic
University of Split
School of Medicine
Study of Dental Medicine
Soltanska 2, Split, Croatia
liviacigic@yahoo.com, lcigic@mefst.hr

MeSH Terms: Serum; Vitamin D Deficiency; Calcitriol Receptors; Mouth Neoplasms
Author Keywords: Oral Lichen Planus; Vitamin D; Oral Cancer

Ana Družijanić – 0000-0002-2049-7136
Livia Cigić – 0000-0003-4134-1103
Ana Glavina – 0000-0003-2137-9665
Mirna Draganja – 0009-0004-2643-5376
Dinko Martinović – 0000-0003-2060-5130
Mare Ković – 0009-0008-2968-8423

Introduction

Lichen planus (LP) is a common chronic, immune-mediated, inflammatory disease of the mucous membranes. Skin lesions have a unique clinical presentation, are usually self-limiting, and occur for a year or less. The papular and plaque-like, livid, pruritic lesions commonly appear on sites such as the flexor sides of the wrists, forearms, and legs, as well as in the sacral region. Oral lichen planus (OLP) presents a range of morphologic manifestations, persists over a long period, and is chronic. On average, oral lesions last 4.5 years longer than skin lesions (1-4). Studies have shown that skin lesions can manifest together with oral lesions, and oral lesions occur by themselves in 30%-70% of cases (3). The prevalence of OLP is 1.9% to 2.5%. According to current knowledge, OLP affects women (60%-65%) more than men (3, 5).

Uvod

Lihen planus (LP) česta je kronična imunosna upalna mukokutana bolest. Kožne lezije imaju jedinstvenu kliničku sliku, obično su samoograničavajuće i traju do godinu dana ili kraće. Klinički se očituju u obliku lividnih papula i plakova koji svrbe, a pojavljuju se na fleksornim površinama ručnih zglobova, podlaktica, bedara, koljena i sakralnog područja. Oralne promjene manifestiraju se različitim kliničkim slikama, traju dulje i kroničnog su tijeka (1 – 4). Istraživanja su pokazala da se kožne lezije mogu pojavljivati zajedno s oralnim manifestacijama, a oralne lezije nastaju zasebno u 30 do 70 % slučajeva (3). Prevalencija oralnoga lihena planusa (OLP) iznosi od 1,9 do 2,5 %. Češće obolijevaju žene (60 do 65 %) (3, 5).

Vitamin D može znatno utjecati na homeostazu usne šupljine zbog snažnoga protuupalnog i imunomodulirajućeg

Vitamin D may significantly impact oral homeostasis due to its potent anti-inflammatory and immunomodulatory effects. Its modulatory role in various oral diseases has been thoroughly studied: recurrent aphthous stomatitis (RAS), syndromes such as Behçet's syndrome and PFAPA syndrome (periodic fever, aphthous stomatitis, pharyngitis, and adenitis), Sjöegren's disease (SD), periodontitis, oral squamous cell carcinoma (OSCC) (6). Vitamin D receptor (VDR) is involved in immune-mediated conditions such as OLP. Research suggests a possible link between lower vitamin D levels and a higher risk of some inflammatory diseases, such as OLP and inflammatory bowel disease (IBD). OLP patients had nearly 50% lower VDR levels in the oral mucosa, which may be caused by immune responses. There is evidence that a nucleotide polymorphism in vitamin D-related genes may affect the properties of vitamin D, such as its anticarcinogenic activity. Since OLP is considered a potentially malignant disease of the oral cavity (PMOD), specific polymorphisms in a nucleotide in the VDR or vitamin D gene may play a significant role in the development of oral cancer (7).

Therefore, the aim of this study was to investigate whether there is a difference in vitamin D serum concentration in in OLP patients compared to the control group, whether there is a difference in vitamin D serum concentration in patients with erosive OLP compared to patients with non-erosive OLP, whether there is a difference in vitamin D serum concentration in OLP patients who had already developed oral cancer compared to other OLP patients (non-erosive and erosive forms) and the control group.

Material and Methods

Subjects

The study was conducted from September 2020 to May 2021 at the Department of Dental Medicine, Department of Maxillofacial Surgery, University Hospital of Split, in Split, Croatia and at the Department of Oral Medicine and Periodontology, Study of Dental Medicine, School of Medicine, University of Split, Split, Croatia. The Ethics Committee approved the study protocols of the School of Medicine, University of Split, Split, Croatia (reference number 003-08/21-03/0003, 2181-198-03-04-21-0061). All participants voluntarily participated in the study by signing the informed consent form.

A random sample was used. The participants were patients referred to the Department of Dental Medicine, Department of Maxillofacial Surgery, University Hospital Split, Split, Croatia, for an initial specialist examination. The same experienced specialist (L. C.) performed the clinical oral examination of all participants. The test group comprised 34 patients (29 women and five men).The control group consisted of 34patients (26 women and eight men) who had been referred for an initial specialist examination during the same period. No pathological changes in the oral mucosa were observed in the control group.

djelovanja. Autori mnogih istraživanja bavili su se modificirajućom ulogom vitamina D u različitim oralnim bolestima, kao što su recidivirajući aftozni stomatitis (RAS), srodni sindromi poput Behçetove bolesti i PFAPA sindroma (periodična groznica, aftozni stomatitis, faringitis, cervikalni adenitis), Sjögrenov sindrom, parodontitis i planocelularni karcinom usne šupljine (PCC) (6). Receptor vitamina D (VDR) uključen je u imunosno posredovane poremećaje u koje se ubraja i OLP. Provedena istraživanja upućuju na moguću povezanost snižene koncentracije vitamina D i povećanog rizika za neke upalne bolesti poput OLP-a i upalnih bolesti crijeva (UBC). Bolesnici s OLP-om imali su gotovo 50 % nižu razinu VDR-a u oralnoj sluznici, što može biti prouzročeno imunoreakcijama. Više dokaza upućuje na to da polimorfizam jednog nukleotida u vitaminu D povezanim genima može utjecati na svojstva toga vitamina, poput antikancerogenog učinka. Budući da se OLP smatra potencijalno zloćudnim oralnim poremećajem (PMOP), specifični polimorfizmi u jednom nukleotidu u VDR-u ili vitaminu D genu mogu biti važni u nastanku karcinoma usne šupljine (7).

Zato je svrha istraživanja bila ispitati: 1) postoji li razlika u serumskoj koncentraciji vitamina D između bolesnika s OLP-om i zdravih ispitanika u kontrolnoj skupini; 2) postoji li razlika u serumskoj koncentraciji vitamina D između bolesnika s erozivnim oblikom OLP-a u odnosu na neerozivni oblik; 3) postoji li razlika u serumskoj koncentraciji vitamina D između bolesnika s OLP-om kojima se pojavio karcinom usne šupljine u odnosu prema ostalim ispitanicima iz skupine bolesnika s OLP-om (neerozivni i erozivni oblik) te zdravim ispitanicima iz kontrolne skupine.

Materijali i postupci

Ispitanici

Istraživanje je provedeno u Ambulanti za oralnu medicinu Odjela za dentalnu medicinu Zavoda za maksilofacijalnu kirurgiju Kliničkoga bolničkog centra Split i Katedri za oralnu medicinu i parodontologiju Medicinskog fakulteta Sveučilišta u Splitu između rujna 2020. i svibnja 2021. godine. Za ovo istraživanje dobivena je dozvola Etičkog povjerenstva Medicinskog fakulteta Sveučilišta u Splitu (Klasa: 003-08/21-03 /OOO3, Ur.br.: 2181-198-03-04-21-0061.). Svi ispitanici pristali su dobrovoljno sudjelovati u istraživanju te su potpisali informirani pristanak.

Uzorak je bio prikladan. Sastojao se od ispitanika koji su došli na prvi specijalistički pregled u Ambulantu oralne medicine Odjela za dentalnu medicinu Zavoda za maksilofacijalnu kirurgiju Kliničkoga bolničkog centra Split. Isti specijalist oralne medicine obavio je klinički oralni pregled svih ispitanika. Ispitna skupina sastojala se od 34 ispitanika (29 žena i 5 muškaraca, medijan 56,5 godina, raspon godina 21 do 84). Kontrolna skupina sastojala se također od 34 ispitanika (26 žena i 8 muškaraca, medijan 47 godina, raspon godina 18 do 79) koji su došli na prvi specijalistički pregled u Ambulantu oralne medicine Odjela za dentalnu medicinu Zavoda za maksilofacijalnu kirurgiju Kliničkoga bolničkog centra Split u istom razdoblju. Oni nisu imali patološke promjene na sluznici usne šupljine.

Data collection

A detailed medical history was obtained from all participants. We recorded the following data: age (in years), gender (male/female), the diagnosis of oral cancer (yes/no, type, location), and smoking habits (yes/no). Incisional biopsy was performed in all patients with a clinical diagnosis of OLP. Each patient signed an informed consent form before the procedure. Patients were informed about the process and its possible complications (discomfort, swelling, infection, bleeding and transient or chronic paraesthesia), the occurrence of reactive oral lesions, and the potential need for a rebiopsy. The same specialist performed an incisional biopsy of the oral mucosa in all OLP patients to confirm the final diagnosis. The most representative sample was sent to the Department of Pathology, Forensic Medicine and Cytology, University Hospital Split, Split, Croatia. Histopathological analysis confirmed the OLP diagnosis according to the World Health Organization (WHO) modified criteria Van der Meij and van der Waal (8). The OLP patients were divided into two groups based on clinical manifestations and histopathologic analysis: a group with non-erosive lesions (papular, reticular, plaque OLP) and a group with erosive lesions (bullous, erosive, atrophic OLP).

The exclusion criteria are: Vitamin D substitution therapy, vitamin D absorption disorders associated with diseases of the digestive tract, pancreas and biliary tract, disorders of vitamin D metabolism (functional damage to liver and kidneys), diet with insufficient amounts of vitamin D (lactose intolerance, vegetarian and vegan diets).

Laboratory tests

All serological tests were performed in the same laboratory at the Department of Medical Biochemical Diagnostics, University Hospital Split, Split, Croatia. Fasting venous blood was taken from each participant to determine serum concentrations of vitamin D. Serum concentration of the active form of vitamin D, 25(OH)D, was measured using the commercially available Elecsys® Vitamin D (Roche Diagnostics International Ltd., Rotkreuz, Switzerland), which uses a reliable method to accurately measure electrochemiluminescence immunoassay (ECLIA) in the sample. Reference values are expressed in nmol/L. This assay detects values between 7.5 and 250 nmol/L 25(OH) D.

Statistical analysis

Data were entered into spreadsheets, and statistical analysis was performed using STATISTICA 11.0. software package. The incidence and percentages were calculated for each categorical variable. Continuous variables were described using basic statistical parameters (average, standard deviation, median, minimum and maximum values). The normal distribution of the data was checked using the Shapiro-Wilks W test, and the equality of variances was checked using the Levene's test. In the case of normality deviation, the results

Prikupljanje podataka

Svim ispitanicima uzeta je detaljna anamneza tijekom prvoga specijalističkog pregleda. Na temelju anamneze dobili smo podatke o dobi (u godinama), spolu (muški/ženski), preboljenom karcinomu usne šupljine (da/ne, vrsta, lokalizacija) i navici svakodnevnog pušenja cigareta (da/ne). Svim bolesnicima kojima je poslije detaljnoga kliničkog oralnog pregleda postavljena klinička dijagnoza OLP-a, učinjena je incizijska biopsija sluznice usne šupljine za patohistološku dijagnostiku. Prije incizijske biopsije od svakog bolesnika dobiven je potpisani informirani pristanak. Bolesnik je obaviješten o postupku, mogućim komplikacijama (nelagoda, oticanje, infekcija, krvarenje, prolazne ili trajne parestezije) i pojavi reaktivnih lezija, ali i da će možda biti potrebna rebiopsija. Isti specijalist obavio je incizijsku biospiju oralne sluznice svih bolesnika s OLP-om kako bi se postavila konačna dijagnoza. Najreprezentativniji uzorak oralne sluznice poslan je u Klinički zavod za patologiju, sudsku medicinu i citologiju Kliničkoga bolničkog centra Split. Patohistološkom analizom potvrđena je dijagnoza OLP-a prema van der Meijovim i van der Waalovim modificiranim kriterijima Svjetske zdravstvene organizacije (SZO) (8). Bolesnike s OLP-om podijelili smo prema kliničkoj slici i patohistološkom nalazu u dvije skupine: skupinu s neerozivnim (papularni, retikularni, plakozni) i erozivnim (bulozni, erozivni, atrofični) oblikom bolesti.

Kriteriji za isključivanje bili su: supstitucijska terapija vitaminom D; poremećaj apsorpcije vitamina D u slučaju bolesti probavnih organa, gušterače, žučnih putova, stanja poslije kirurških zahvata na probavnim organima, poremećaji metabolizma vitamina D (funkcionalna oštećenja jetre i bubrega), prehrana koja ne sadržava dovoljnu količinu vitamina D (intolerancija na laktozu, vegetarijanstvo, veganstvo), druge kronične upalne i autoimune bolesti.

Laboratorijske analize

Sve serološke pretrage obavljene su u istom laboratoriju Zavoda za medicinsko-biokemijsku dijagnostiku Kliničkoga bolničkoga centra Split. Svakom ispitaniku izvađen je uzorak venske krvi natašte za određivanje serumske koncentracije vitamina D. Serumska razina aktivnoga oblika vitamina D 25(OH)D izmjerena je uporabom komercijalno dostupnoga vitamina D Elecsys® (Roche Diagnostics International Ltd., Rotkreuz, Switzerland) za čiju se proizvodnju koristi pouzdana imunokemijska metoda preciznog mjerenja elektrokemiluminiscencije (ECLIA) uzorka. Referentne vrijednosti izražene su u nmol/L. Ovim testom mogu se otkriti razine između 7,5 i 250 nmol/L 25(OH)D.

Statistička analiza

Podatci su uneseni u proračunske tablice i učinjena je statistička analiza upotrebom programskog paketa STATISTICA 11.0. Za svaku kategoričku varijablu izračunati su učestalost i postotak. Kontinuirane varijable opisane su s pomoću osnovnih statističkih parametara (srednja vrijednost, standardna devijacija, medijan, minimalna i maksimalna vrijednost). Normalnost podataka provjerena je Shapiro-Wilksovim W testom, a homogenost varijance Leveneovim testom. U slučaju odstupanja od normalnosti rezultati su matema-

were mathematically normalized. The difference between the two groups of continued normally distributed variables was checked with the t-test in case of more than two groups with the one-way analysis of variance, and then, in case of a significant difference in the analysis of variance, with the Newman-Keuls test. To confirm the possible influence of predictor variables on selected dependent variables (the occurrence of oral cancer, OLP), polynomial regression analysis and a general regression model were used. The results are presented in the form of a Pareto chart with the t-values. The significance value for all analyzes was set at $p < 0.05$.

Results

Age and sex

A total of 68 participants took part in this study. The test group consisted of 34 participants with non-erosive or erosive OLP (mean age 56.5 years, age range 21 to 84 years). The control group consisted of 34 control subjects (mean age 47 years, age range 18 to 79 years). Women predominated in both groups, 85.3%in the test group and76.5% in the control group.

Vitamin D serum concentration

Table 1 shows the frequency (N, %) of participants where vitamin D serum concentrations in OLP patients were considered as well as those in patients without pathological oral mucosa changes in the control group. Only two OLP patients (5.9%) had adequate serum vitamin D concentration (> 75 nmol/L), ten OLP patients (29.4%) had inadequate levels, and 22 OLP patients (64.7%) had vitamin D deficiency with levels below 50 nmol/L. In the control group, 23.5% of the participants had adequate levels, four times more compared with the OLP group. However, ten patients in the control group (29.4%) had a deficit in serum concentration of vitamin D compared with 22 OLP patients (64.7%), i.e., twice less, Table 1.

Serum vitamin D concentration in the OLP patients and in the control group, in which no pathological changes of the oral mucosa were observed, is shown in Table 2. Serum vita-

tički normalizirani. Razlika između dviju skupina kontinuiranih, normalno raspodijeljenih varijabli provjerena je t-testom, a za više od dvije skupine jednosmjernom analizom varijance, a zatim Newman-Keulsovim testom u slučaju da je analiza varijance pokazala značajnu razliku. Za potvrđivanje potencijalnog utjecaja prediktorskih varijabli na odabrane zavisne varijable (pojava karcinoma usne šupljine, oblik OLP-a) korištena je višestruka regresijska analiza i generalni regresijski model čiji su rezultati prikazani u formi Paretova dijagrama t-vrijednosti. U svim analizama značajnost je postavljena na $p < 0,05$.

Rezultati

Dob i spol

U istraživanju je sudjelovalo ukupno 68 ispitanika. U ispitnoj skupini bila su 34 ispitanika s neerozivnim ili erozivnim oblikom OLP-a (medijan 56,5 godina, raspon godina 21 do 84), a u kontrolnoj 34 zdrava ispitanika (medijan 47 godina, raspon godina 18 do 79). U objema skupinama prevladavale su žene koje su u skupini bolesnika s OLP-om bile zastupljene s 85,3 %, a u kontrolnoj sa 76,5 %.

Serumska koncentracija vitamina D

U tablici 1. prikazana je učestalost (N, %) ispitanika s obzirom na serumsku razinu vitamina D kod bolesnika s OLP-om i u kontrolnoj skupini bez patoloških promjena na sluznici usne šupljine. Kod bolesnika s OLP-om, adekvatnu serumsku koncentraciju vitamina D (>75 nmol/L) imala su samo dva ispitanika (5,9 %), kod deset (29,4 %) utvrđena je insuficijencija, a kod 22 ispitanika (64,7 %) deficit s vrijednostima manjima od 50 nmol/L. U kontrolnoj skupini odgovarajuća razina utvrđena je kod 23,5 % ispitanika, što je četiri puta više u odnosu prema skupini bolesnika s OLP-om. Deficit je ustanovljen kod dvostruko manje ispitanika (29,4 %) u odnosu prema skupini bolesnika s OLP-om.

Serumska razina vitamina D u skupini bolesnika s OLP-om i u kontrolnoj skupini prikazana je u tablici 2. U skupini bolesnika s OLP-om kretala se od 13,6 do 89,6 nmol/L (47,5 ± 16,3), a u kontrolnoj skupini od 35,7 do 113,0 nmol/L

Table 1 The frequency of subjects (N,%) considering vitamin D serum concentration in the OLP and control group
Tablica 1. Učestalost ispitanika (N, %) s obzirom na serumsku razinu vitamina D u ispitnoj i kontrolnoj skupini

Serum level of Vitamin D • Serumska razina vitamina D	OLP		Control • Kontrola	
	N	%	N	%
Adequate • Adekvatna (>75 nmol/L)	2	5,9	8	23,5
Insufficiency • Insuficijencija (51-75 nmol/L)	10	29,4	16	47,1
Deficit (<50 nmol/L)	22	64,7	10	29,4

Table 2 Serum levels of vitamin D in the OLP and control group
Tablica 2. Serumska razina vitamina D u ispitnoj i kontrolnoj skupini

Serum levels of vitamin D • Serumska razina vitamina D (nmol/ L)	Group • Skupina	
	OLP	Control • Kontrola
X	47.5	63.1
SD	16.3	21.0
Minimum	13.6	35.7
Maximum • Maksimum	89.6	113.0
Median • Medijan	48.1	57.7

*X – mean value • srednja vrijednost, SD- standard deviation • standardna devijacija

min D concentrations in the OLP group ranged from 13.6 to 89.6 nmol/L (47.5 ± 16.3), and in the control group from 35.7 to 113.0 nmol/L (63.1 ± 21.0). The T-test results confirmed the lower serum vitamin D concentration in the OLP patients compared with the control group (t=3.4; p=0.001), Table 2.

The means and standard deviations of serum vitamin D concentrations in patients with non-erosive and erosive forms of OLP and the control group are shown in Figure 1.

OLP forms and the development of oral cancer

Of the 34 OLP patients, 82.4% were diagnosed with the erosive form of the disease. Five patients with the erosive disease (14.7%) had developed oral cancer: tongue (the same patient, twice), base of tongue (two patients),*carcinoma in situ* of the cheek (one patient), gingiva and vestibule (the same patient, twice). The basic statistical parameters of vitamin D serum concentration in the OLP group, in which the development of oral cancer was considered, and in the control group are shown in Table 3.

(63,1 ± 21,0). Rezultati t-testa potvrdili su statistički znatno nižu serumsku razinu vitamina D u bolesnika s OLP-om u odnosu prema zdravim ispitanicima iz kontrolne skupine (t = 3,4; p = 0,001).

Srednje vrijednosti i standardne devijacije serumske razine vitamina D kod bolesnika s neerozivnim i erozivnim oblikom OLP-a i u kontrolnoj skupini ispitanika kod kojih nisu uočene patološke promjene na sluznici usne šupljine prikazane su na slici 1.

Oblik OLP-a i pojava karcinoma usne šupljine

Od 34 bolesnika s OLP-om kod 82,4 % dijagnosticiran je erozivni oblik bolesti. Pet bolesnika s erozivnim oblikom OLP-a (14,7 %) oboljelo je od karcinoma usne šupljine i to od karcinoma jezika (isti ispitanik, dva puta), karcinoma baze jezika (dva ispitanika), *carcinoma in situ* obraza (jedan ispitanik, jedanput), vestibuluma i gingive (isti ispitanik, dvaput). Osnovni statistički parametri serumske razine vitamina D u skupini s dijagnosticiranim OLP-om, ovisno o tome jesu li razvili karcinom usne šupljine i u kontrolnoj skupini ispi-

Table 3 Basic statistical parameters of vitamin D serum concentration
Tablica 3. Osnovni statistički parametri serumske razine vitamina D

Cancer • Karcinom	Level of vitamin D • Razina vitamina D (nmol/ L)				
	X	SD	Minimum	Maximum • Maksimum	Median • Medijan
Yes - OLP • Da – OLP	28.7	7.0	21.7	36.8	28.1
No - OLP • Ne – OLP	50.0	15.5	13.6	89.6	49.1
No - healthy population • Ne – zdrava populacija	63.1	21.0	35.7	113.0	57.7

*X – mean value • srednja vrijednost, SD – standard deviation • standardna devijacija

1

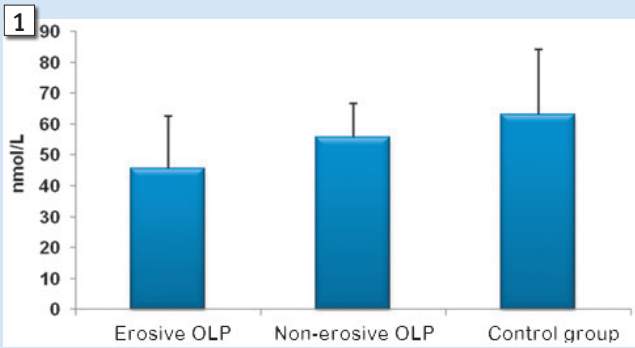
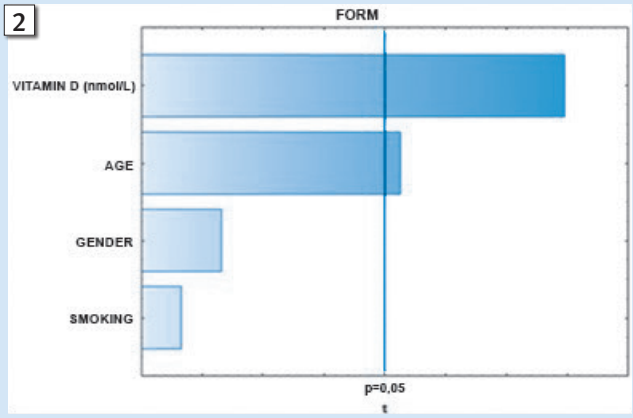


Figure 1 Vitamin D serum concentration in patients with non-erosive and erosive forms of OLP.
Slika 1. Serumska razina vitamina D kod bolesnika s neerozivnim i erozivnim oblikom OLP-a
Figure 2 Pareto diagram of the dependence of the t-values: a) of OLP type on selected predictor variables; b) of the occurrence of oral cancer on selected predictor variables.
Slika 2. Paretov dijagram t-vrijednosti

2



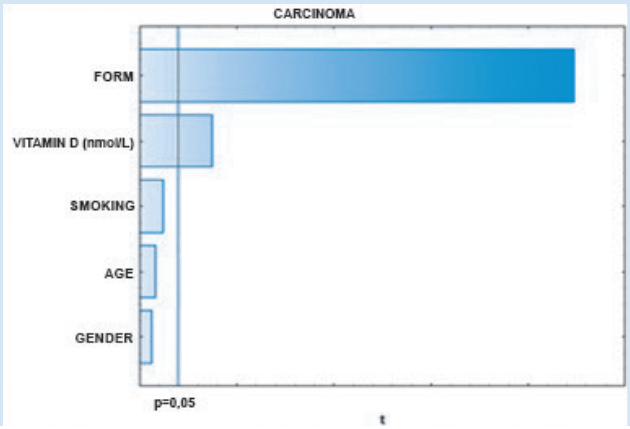


Figure 2 shows the Pareto diagram of the dependence of the t-values: a) of OLP type on selected predictor variables; b) of the occurrence of oral cancer on selected predictor variables. The variables that cross the red line have a statistically significant benefit at the significance level of $p < 0.05$.

xxx

The medical history data revealed the smoking habits of the participants. Three smokers were in the OLP group (8.8%), and four participants were in the control group (11.8%).

Discussion

Vitamin D may play a role in the development or severity of the clinical picture of symptomatic forms of OLP by regulating immune system function (9). Active forms of vitamin D have a regulatory role in the expression of several genes. There is evidence of an association with many autoimmune and malignant diseases. Vitamin D deficiency decreases the number of Th2 cells, particularly those involved in inflammatory pathways such as Th1 and Th17. It also leads to significant inflammation in immune-mediated diseases such as OLP. Therefore, it is essential to investigate serum vitamin D levels in the population, especially in chronic inflammatory autoimmune diseases such as OLP (9).

The results of this study show that the average serum vitamin D concentration in the OLP patients was 48.1 nmol/L, while in the control group it was 57.7 nmol/L. The minimum serum vitamin D concentration in the OLP group was 13.6 nmol/L and in the control group it was 35.7 nmol/L (11, 12, 13). The T-test results showed a statistically significantly lower serum vitamin D concentration in the OLP group compared to the control group ($p=0.001$). These results confirm the study of Sadeghi et al., who found a statistically significantly lower vitamin D serum concentration in OLP patients (9). On the other side, the research by Bahramian et al. (10) and the systematic review and meta-analysis by Sakthivel et al. (11) found no statistically significant difference in serum vitamin D concentration between the OLP patients and the control group.

It is widely accepted in the scientific community that 25-(OH) D3 concentrations ranging between 75 and 150 nmol/L are desirable, and that concentrations below 50 nmol/L are inadequate for musculoskeletal health (9). However, in the OLP group, only two patients had adequate serum vitamin D concentrations (5.88%), whereas eight participants had adequate serum vitamin D concentrations in the control group (23.53%).

This study showed a statistically significant ($p < 0.05$) lower serum vitamin D concentration in the erosive OLP compared to the non-erosive. These results confirm the study of Ahmed et al., who found a statistically significant ($p=0.002$) lower serum vitamin D concentration in the symptomatic OLP compared to the non-symptomatic patients (12).

According to the available literature, the annual rate of

tanika kod kojih nisu uočene patološke promjene na sluznici usne šupljine, prikazani su u tablici 3.

Slika 2.: Paretov dijagram t-vrijednosti ovisnosti: a) oblika OLP-a o odabranim prediktorskim varijablama; b) pojave karcinoma usne šupljine o odabranim prediktorskim varijablama. Varijable koje prelaze crvenu crtu imaju statistički znatan doprinos na razini značajnosti $p < 0,05$.

Navike ispitanika

Anamnestički podatci uzeti su i o navici svakodnevnog konzumiranja cigareta. U ispitnoj skupini troje bolesnika s OLP-om (8,8 %) svaki dan je pušilo, a u kontrolnoj skupini to je činilo njih četvero (11,8 %), što statistički nije bila značajna razlika.

Rasprava

Vitamin D može imati ulogu u pokretanju ili jačini kliničke slike simptomatskih oblika OLP-a tako što regulira funkcije imunosnog sustava. Aktivni oblik vitamina D sudjeluje u regulaciji ekspresije mnogih važnih gena. Dokazana je i njegova povezanost s mnogim autoimunim i malignim bolestima. Nedostatak vitamina D rezultira smanjenjem broja Th2 stanica, posebno onih koje sudjeluju u upalnim putovima poput Th1 i Th17. To rezultira naglašenijim upalnim stanjima u imunosno posredovanim bolestima poput OLP-a. Zato je potrebno obratiti pozornost na serumsku razinu vitamina D u populaciji, a posebno kod oboljelih od kroničnih upalnih autoimunih bolesti kao što je OLP (9).

Rezultati ovog istraživanja pokazali su da je prosječna serumska vrijednost vitamina D kod bolesnika s OLP-om 48,1 nmol/L, a prosječna vrijednost kod zdravih ispitanika u kontrolnoj skupini iznosila je 57,7 nmol/L. Minimalna serumska vrijednost vitamina D u skupini bolesnika s OLP-om iznosila je 13,6 nmol/L, a kod onih zdravih u kontrolnoj skupini 35,7 nmol/L. Rezultati t-testa pokazali su statistički znatno nižu koncentraciju vitamina D kod oboljelih od OLP-a u usporedbi sa zdravim ispitanicima iz kontrolne skupine ($p = 0,001$). Dobiveni podatci u skladu su s istraživanjem Sadeghija i suradnika koji su utvrdili statistički značajno nižu koncentraciju serumskog vitamina D kod bolesnika s OLP-om (9). Istraživanje Bahramiana i suradnika (10) te sustavni pregled i metaanaliza Sakthivela i suradnika (11) nisu pokazali statistički znatnu razliku serumske koncentracije vitamina D kod bolesnika s OLP-om u odnosu prema zdravim ispitanicima iz kontrolne skupine.

Stručnjaci su suglasni da su koncentracije 25-(OH)D3 od 75 do 150 nmol/L poželjne u populaciji, a one niže od 50 nmol/L nedostatne su za zdravlje muskuloskeletnog sustava (9). Adekvatnu razinu vitamina D kod bolesnika s OLP-om imalo je u našem istraživanju samo dvoje ispitanika (5,88 %), a u kontrolnoj skupini razinu veću od 75 nmol/ L imalo je čak osam ispitanika (23,53 %).

Ovo istraživanje pokazalo je statistički značajno ($p < 0,05$) nižu koncentraciju serumskog vitamina D kod bolesnika s erozivnim oblikom OLP-a u odnosu na neerozivni. Rezultati su u skladu s istraživanjem Ahmeda i suradnika koje je pokazalo statistički značajno ($p = 0,002$) nižu koncentraciju serumskog vitamina D kod bolesnika sa simptomatskim obli-

malignant transformation from OLP to OSCC ranges from 0.04% to 1.74% with the erosive and ulcerative forms of the disease having a greater tendency to malignant transformation (13). In our test group, five patients had developed OSCC of the oral cavity, and all had the erosive OLP form. In addition, all five patients who had undergone surgery for oral cancer had lower serum vitamin D concentrations than the rest of the OLP group (with erosive and non-erosive forms of OLP) and the control group.

In a study by Anand et al., VDR expression was found to be increased in patients with PMOP and oral cancer. Vitamin D serum insufficiency and deficiency were also common in PMOP patients (14).

There was no statistically significant difference between the groups regarding smoking habits. In our study, most OLP patients did not smoke (91.2%). Although this habit is not directly associated with the development of OLP, some oral mucosal lesions may have a similar clinical and histopathological appearance to OLP and yet be caused by chemical and thermal toxicity, which can be manifested by a burning sensation in the oral cavity (15). In addition, every dentist should educate the patient about the harmful effects and risks of smoking, including the impact of smoking on the development of oral cancer as a proven etiological factor. Since OLP lesions are defined as PMOP, it is of utmost importance to inform patients about these risks. Barbosa et al. could not establish an association between smoking habits and OLP. Most of their OLP patients were non-smokers (97.3%), comparable to our study (16).

There are several limitations of our study. The reliability of the results obtained should be confirmed by using a larger sample. The relatively small sample and the nature of the sample (convenience sample) compared to the population are also limitations. It would be beneficial to continue to recruit new subjects, paying attention to the homogeneity of the groups. Longitudinal studies are also needed to observe and monitor the clinical picture of the disease and relapses after patients have started vitamin D supplementation. Nevertheless, this study is helpful because it adds to previous knowledge about serum vitamin D concentrations in OLP patients.

Further studies are needed to determine the efficacy of vitamin Don oral mucosa and its therapeutic effect in OLP patients.

Conclusion

This study showed that the serum concentration of vitamin D was statistically significantly lower in the OLP patients, the erosive OLP, and the patients who developed oral cancer from the erosive OLP compared with the control group. Measuring serum vitamin D concentration is important for monitoring OLP patients in order to prolong the period of remission, prevent the occurrence of more severe clinical picture of erosive OLP and transformation of symptomatic forms into oral cancer.

kom bolesti u odnosu na asimptomatski (12).

Prema dostupnoj literaturi godišnja stopa prelaska OLP-a u PCC usne šupljine kreće se između 0,04 i 1,74 %, sa zapaženom većom tendencijom zloćudne alteracije erozivnih i ulceroznih oblika bolesti (13). U našoj ispitnoj skupini petero bolesnika imalo je PCC usne šupljine i svi su nastali iz erozivnog oblika OLP-a. Kod svih pet ispitanika koji su operirani zbog karcinoma usne šupljine zabilježene su snižene serumske koncentracije vitamina D. Istraživanje Ananda i suradnika pokazalo je da je ekspresija receptora za vitamin D povećana u slučaju PMOP-a i karcinoma usne šupljine. Insuficijencija i deficit vitamina D u navedenom istraživanju prevladavaju kod bolesnika s PMOP-om (14).

Kad je riječ o svakodnevnom pušenju cigareta, nije bilo statistički znatne razlike među ispitivanim skupinama. U našem istraživanju većina bolesnika s OLP-om nije svaki dan pušila cigarete (91,2 %). Iako se spomenuta navika ne dovodi izravno u vezu s razvojem OLP-a, promjene sličnoga kliničkog i histopatološkog izgleda katkad mogu biti prouzročene kemijskim i toplinskim toksičnim učinkom cigareta na oralnu sluznicu, a mogu se manifestirati uz osjećaj pečenja usne šupljine (15). Doktor dentalne medicine također je dužan informirati pacijente o štetnosti pušenja kao dokazanog etiološkog čimbenika u razvoju karcinoma usne šupljine, a s obzirom na to da su lezije u sklopu OLP-a definirane kao PMOP. Barbosa i suradnici u svojem istraživanju ne povezuju OLP i navedenu naviku. Većina njihovih bolesnika s OLP-om bili su nepušači (97,3 %), što je u skladu s rezultatima našeg istraživanja (16).

Naše istraživanje ima nekoliko ograničenja. Jedno od njih je pouzdanost dobivenih rezultata pa bi se ponovnim mjerenjima trebali potvrditi na većem uzorku. Razmjerno mali uzorak ispitanika (prikladan uzorak) u odnosu na ukupnu populaciju također bi mogao biti jedno od ograničenja. Korisno bi bilo nastaviti prikupljati podatke te pritom paziti na homogenost skupina. Također bi bilo važno dulje pratiti klinički razvoj bolesti i učestalost recidiva, nakon što bolesnici kojima je dokazan manjak koncentracije vitamina D počnu sa suplementacijom. Ipak, ovo istraživanje je korisno jer je upotpunilo dosadašnje spoznaje o serumskoj koncentraciji vitamina D kod oboljelih od OLP-a. Istodobno, ono upozorava da su potrebna daljnja istraživanja o učinku vitamina D na oralnu sluznicu te o njegovim terapijskim dobrobitima u praćenju bolesnika s OLP-om.

Zaključak

Ovim istraživanjem dokazana je statistički znatno niža serumska koncentracija vitamina D kod bolesnika s OLP-om, kod bolesnika s erozivnim oblikom bolesti i onih kojima se pojavio karcinom usne šupljine iz erozivnog oblika bolesti u odnosu prema zdravim ispitanicima iz kontrolne skupine. Određivanje serumske koncentracije vitamina D važno je u praćenju bolesnika s OLP-om kako bi se produljilo razdoblje remisije te prevenirala teža klinička slika erozivnih oblika bolesti i moguća maligna transformacija.

Conflict of interest

There are no conflicts to declare.

Author's contribution: **A.D. and L.C.** - conceptualization, **L.C. and D.M.** – validation; **A.D., L.C., M.D.** and **M.K.** – investigation; **M.D, M.K.** and **L.C.** - Writing—original draft preparation; **A.D., A.G.** and **L.C.** - Writing—review and editing; **A.G., A.D.** and **L.C.** – visualization; **D.M.** - Statistical analysis; **A.D., L.C.** and **D.M.** – supervision.

Sukob interesa

Autori nisu bili u sukobu interesa.

Doprinos autora: **A. D. i L. C.** – konceptualizacija; **L. C. i D. M.** – validacija; **A. D., L. C., M. D. i M. K.** – istraživanje; **M. D, M. K. i L. C.** – pisanje teksta, pregled i uređivanje; **A. G., A. D. i L. C.** – vizualizacija; **D. M.** – statistička analiza; **A. D., L. C. i D. M.** – nadzor

Sažetak

Svrha rada: Vitamin D receptor je uključen u imunosno posredovane poremećaje kojima pripada i oralni lihen planus (OLP). Provedena istraživanja sugeriraju moguću povezanost smanjene koncentracije vitamina D i povećanog rizika za neke upalne bolesti, pa tako i za oralni lihen planus. Svrha istraživanja bila je ispitati postoji li razlika u serumskoj koncentraciji vitamina D između bolesnika s različitim kliničkim oblicima oralnoga lihen planusa (erozivni vs. neerozivni) i zdravih ispitanika u kontrolnoj skupini. **Ispitanici i postupci:** U istraživanje je bilo uključeno ukupno 68 ispitanika koji su došli na prvi specijalistički pregled u Ambulantu oralne medicine Odjela za dentalnu medicinu Zavoda za maksilofacijalnu kirurgiju Kliničkoga bolničkoga centra Split. Ispitnu skupinu činila su 34 ispitanika s neerozivnim ili erozivnim oblikom OLP-a, a kontrolnu skupinu također 34 ispitanika bez patoloških promjena na oralnoj sluznici. **Rezultati:** Rezultati t-testa potvrdili su statistički znatno nižu serumsku razinu vitamina D kod bolesnika s OLP-om u usporedbi sa zdravim ispitanicima iz kontrolne skupine (p = 0,001). Rezultati su pokazali i statistički znatno nižu serumsku koncentraciju vitamina D kod bolesnika s erozivnim oblikom OLP-a. Kod svih pet ispitanika kojima se pojavio karcinom usne šupljine iz erozivnog oblika OLP-a zabilježene su snižene serumske vrijednosti vitamina D. **Zaključak:** Određivanje serumske razine vitamina D važno je u praćenju bolesnika s OLP-om kako bi se prevenirao mogući utjecaj njegova deficita na razvoj teže kliničke slike erozivnih oblika bolesti.

Zaprimljen: 13. ožujka 2023.
Prihvaćen: 11. srpnja 2023.

Adresa za dopisivanje
Livia Cigić
Medicinski fakultet Sveučilišta u Splitu
Studij dentalne medicine
Šoltanska 2, Split, Hrvatska
liviacigic@yahoo.com, lcigic@mefst.hr

MeSH pojmovi: serum; nedostatak vitamina D; kalcitriol receptor; oralne novotvorine
Autorske ključne riječi: oralni lihen planus; vitamin D; oralni karcinom

References

1. Greenberg MS, Glick M, editors. Burket's Oral Medicine, Diagnosis and Treatment. 10th ed. Zagreb: Medicinska naklada; 2006.
2. Biočina-Lukenda D. Oral lihen ruber. In: Cekić-Arambašin A, editor. Oral Medicine. Zagreb: Školska knjiga; 2005.
3. Biočina-Lukenda D. Oralni lichen ruber – I Etiologija i patogeneza. Acta Stomatol Croat. 2002;36(4):451-463.
4. Krupaa RJ, Sankari SL, Masthan KM, Rajesh E. Oral lichen planus: An overview. J Pharm Bioallied Sci. 2015 Apr;7(Suppl 1):S158-61.
5. Vučević Boras V. Manual/atlas of oral diseases from diagnosis to therapy. 2nd ed. Zagreb: Medicinska naklada; 2019.
6. Ślebioda Z, Szponar E, Dorocka-Bobkowska B. Vitamin D and Its Relevance in the Etiopathogenesis of Oral Cavity Diseases. Arch Immunol Ther Exp (Warsz). 2016 Oct;64(5):385-97.
7. Shen H, Liu Q, Huang P, Fan H, Zang F, Liu M, et al. Vitamin D receptor genetic polymorphismsare associated with oral lichen planus susceptibilityin a Chinese Han population. BMC Oral Health. 2020 Jan 30;20(1):26.
8. van der Meij EH, van der Waal I. Lack of clinicopathologic correlation in the diagnosis of oral lichen planus based on the presently available diagnostic criteria and suggestions for modifications. J Oral Pathol Med. 2003;32(9):507-512.
9. Sadeghi M, Zarabadipour M, Azmodeh F, Mirzadeh M, Golezari AS. Association of serum level of 25-hydroxy vitamin D with Oral Lichen Planus. A case-control study. J Oral Res. 2020; 9(5):400-404.
10. Bahramian A, Bahramian M, Mehdipour M, Falsafi P, Khodadadi S, Dabaghi Tabriz F, et al. Comparing Vitamin D Serum Levels in Patients with Oral Lichen Planus and Healthy Subjects. J Dent (Shiraz). 2018 Sep;19(3):212-216.
11. Sakthivel S, Gunasekaran S, Castelino RL, Babu GS, Ajila V, Buch SA. Serum vitamin D levels in patients with oral lichen planus: A systematic review and meta-analysis. Gulhane Med J. 2021;63:267-273.
12. Ahmed SA. The Role of Serum Vitamin D Deficency in oral Lichen Planus Case Control Study. Diyala Journal of Medicine. 2019;17(2):189-198.
13. Van der Meij EH, Schepman K, van der Waal I. The possible premalignant character of oral lichen planus and oral lichenoid lesions: a prospective study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2003 Aug;96(2):164-71.14.
14. Anand A, Singh S, Sonkar AA, Husain N, Singh KR, Singh S, et al. Expression of vitamin D receptor and vitamin D status in patients with oral neoplasms and effect of vitamin D supplementation on quality of life in advanced cancer treatment. Contemp Oncol (Pozn). 2017;21(2):145-151.
15. Cvijetić M, Topić B, Plančak D. Quantitative Analysis of Candidiasis and Pyrosis in Oral Lichen Ruber. Acta stomatol Croat. 2000;34(1):79-81.
16. Barbosa NG, Silveira ÉJD, Lima ENdA, Oliveira PT, Soares MSM, de Medeiros AMC. Factors associated withclinical characteristics andsymptomsin a case series of orallichen planus. Int J Dermatol. 2015 Jan;54(1):e1-6.