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Serum vitamin D and antinuclear antibody level in oral lichen planus patients: a cross-sectional study

Tahereh Nosratzehi

Background: Vitamin D is a secosteroid prohormone that regulates the immune system. Antinuclear antibody (ANA) is a protein antibody made against substances inside the nucleus of cells. Serum vitamin D and ANA levels progress to psoriasis and oral cancer. The present study aimed to measure the serum vitamin D and ANA levels in patients with oral lichen planus (OLP), an autoimmune and precancerous disease.

Methods: We conducted this cross-sectional study on patients with OLP ($n = 50$) and healthy individuals ($n = 50$). We used the enzyme-linked immunosorbent assay method to measure serum vitamin D and ANA levels and Mann–Whitney *U*-test and *t*-test to analyze data.

Results: The present study showed that 14 (28%) patients with OLP had vitamin D deficiency, and 18 (36%) had insufficient vitamin D. Further, 9 (18%) and 15 (30%) of the participants in the control group suffer from vitamin D deficiency and insufficient vitamin D level, respectively. Results showed a significant relationship between levels of serum vitamin D in both groups. The level of ANA positive in patients with OLP was 6 (12%). The results of the *t*-test showed no significant difference between the mean serum ANA levels in the two nodes with an 80% confidence interval ($P = 0.34$).

Conclusion: Researchers of the present study reported low serum vitamin D in many OLP patients. Due to the prevalence of vitamin D deficiency in society, we need to perform comprehensive studies to evaluate the deficiency's effects on pathogenesis.

Keywords: antinuclear antibody, oral lichen planus, vitamin D

Introduction

Lichen planus is a common chronic immunological skin-mucosal disease in the oral mucosa. The pathogenesis of OLP is unknown. OLP is ideal for studies on inflammation and autoimmunity by T cells. Many autoimmune features (such as a chronic disease, onset in adulthood and inclination to women, comorbidity with other autoimmune diseases, decreased immunosuppressive activity in OLP patients, and presence of autotoxicity cells in lichen planus lesions) supports an autoimmune role in the pathogenesis of the disease[1]. Since the first case of squamous cell carcinoma (SCC) evolved from lichen planus, the transformation of these lesions into SCC has gained more importance. Previous papers have suggested that patients with OLP are at risk of developing SCC, leading to classifying the disease as a precancerous lesion (WHO)[2].

HIGHLIGHTS

- The present study aimed to measure the serum vitamin D and antinuclear antibody (ANA) levels in patients with oral lichen planus (OLP), an autoimmune and precancerous disease.
- The present study showed no significant difference in the amount of ANA autoantibodies between the control and patient groups.
- The results showed no significant difference between the serum level of vitamin D_3 in the control and patient groups.

The underlying molecular mechanisms, which initiate the development of oral cancer in patients with OLP, are not well known. However, OLP lesions can develop from seemingly normal epithelium or precancerous lesions. Basement membrane ruptures may initiate keratinocyte apoptosis[3].

Various factors such as genetic predisposition, psychological states, and susceptibility to infectious factors may play a role in OLP development. Various systemic diseases (such as hypertension, diabetes, and thyroid disorders) may increase OLP risk[4]. The etiology of this disease remained undetermined. Similar lesions may appear in different systemic conditions, various autoimmune diseases, the use of drugs, and exposure to substances and infections. The exact relationship between factors and lesions has not yet been determined. However, the immune response allows the diseases to develop[5]. Researchers have discussed the possibility of malignant changes for many years. Despite the extensive studies and identification of a precancerous lesion, they wonder if the lesion is benign or malignant[6].

Vitamin D (sunlight vitamin D) is anticancer. It is a fat-soluble vitamin with two sources. The internal source (a precursor of

Department of Oral and Maxillofacial Medicine, School of Dentistry, Oral and Dental Disease Research Center, Zahedan University of Medical Sciences, Zahedan, Iran

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**Corresponding author. Oral Medicine Department, Faculty of Dentistry, Azadegan street, Sistan-va-Balouchestan, 981655534, Zahedan, Iran. Tel.: +985434113041 E-mail address: taherehnosratzehi@yahoo.com (T. Nosratzehi).*

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vitamin D in the skin) consists of 7-dehydrocholesterol converted to its highly active type (1 and 25 dihydroxycholecalciferols) depending on the body's needs and the influence of parathyroid hormone; maintains its known biochemical functions (calcium and phosphorus balance) and the hard tissues (bones and teeth)[7]. If the calcium and phosphorus are balanced, 24 and 25 dihydroxycholecalciferols with unknown effects are formed in the kidneys. Any defect in reception, absorption, and conversion of vitamin D to its active forms and the vitamin D responses in the body cause complications[8].

CD4 + T lymphocytes contain small amounts of vitamin D receptors (VDRs). Different forms of vitamin D can prevent or suppress autoimmune diseases in animal models, thus suppressing the immune system. The potential role of vitamin D in modulating immune responses with the discovery of VDRs in macrophages, cell dendrites, and active B, and T lymphocytes coincided with the discovery of the role of 1,25-dihydroxyvitamin D_3 in the proliferation, differentiation, and function stages. These cells can express the CYP27B1 gene for immune responses. Animal studies demonstrated the vital role of 1,25-dihydroxyvitamin D_3 in regulating the immune response due to the prevention of several autoimmune diseases[9,10].

ANA is a protein antibody made against substances inside the nucleus of cells and seen in autoimmune diseases. This test is featured by visibility before the onset of symptoms. It is positive in about 5% of healthy people and increases with aging, thus reaching 20% at 70 years of age. In systemic connective tissue diseases, the grade of the ANA test is more than 60%. In current scientific papers, the effect of autoantibodies (such as ANA) and autoimmune diseases in the development of neoplasms and precancerous lesions has gained importance. Some autoantibodies may play a role in the development of these lesions. Some studies consider OLP an autoimmune disease because of the high association with some of the causative agents of autoimmune diseases[11].

The present study compared this vitamin and ANA level in patients with lichen with those in healthy individuals.

Materials and methods

Patients with OLP ($n = 50$) and healthy individuals ($n = 50$) were referred to Zahedan Dental School. Their age and sex were matched with those of the experimental groups.

Researchers measured vitamin D and ANA in the blood of 50 patients and 50 healthy individuals using the enzyme-linked immunosorbent assay (ELISA) method (anti-vitamin D3 kit, made in Biorex Fars).

Normal values include values less than 10 ng/ml (deficient in vitamin D), 10–29 ng/ml (insufficient amounts of vitamin), 30–30 ng/ml (sufficient vitamin), and above 100 ng/ml (vitamin D toxicity). The titer in the ANA test was negative (> 1.20 U/l) and positive (< 1.20 U/l). In the calibration of vitamin D, the ANA of the patient or control sample was first added to the wells coated with ANA and antivitamin D antibodies; then the diluent was added to the wells to separate the ANA and vitamin D molecules from the carrier proteins. Adding the conjugated enzyme solution, competition for connecting to the site begins between the sample's specified concentrations of biotin and vitamin D.

After the specified incubation and washing, the added substrate produced dye with an intensity inversely related to the concentration of vitamin D and ANA. A curve of wavelength-concentration activity was drawn and measured with an unknown sample concentration curve. First, all standards, controls, and samples were placed in a room (25–25°C), 25 µl of each standard, control, and serum sample, and 75 µl of the diluted solution were added to all wells. They were vibrated using a plate at 200–400 rpm for 20 s, then incubated for 60 min at room temperature.

The researchers discarded the solution on the surface and washed the wells with 1× buffer at 350 µl three times. They added 100 µl of the conjugated enzyme solution of vitamin D, and ANA to all wells and incubated for 15 min at room temperature (25–28°C), 100 µl of the prepared substrate solution to all wells and incubated for 15 min at room temperature in the dark, then 100 µl of the stopper solution to all wells and read light absorption of vitamin D and ANA at 450 nm. They determined serum levels of vitamin D and ANA in each sample. First, researchers used Kolmogorov–Smirnov to measure the normal distribution of variables, the Mann–Whitney test to compare vitamin D and ANA between patients and healthy individuals, and different OLP.

The work has been reported in line with the STROCSS criteria[12].

Results

The sample population included 50 OLP patients (case group) and 50 healthy individuals (control group). Treated patients, those who had a recurrence or took supplements and vitamin D, left the study. Table 1 shows the demographic information of patients.

We compared vitamin D levels between patients with OLP and controls by the Mann–Whitney test. The results of Table 2 showed no significant difference between both groups regarding the mean of vitamin D ($P = 0.25$).

The type of ELISA kit defined normal and abnormal ranges of vitamins; less than 10 ng/ml of vitamin D had a deficiency.

Results showed that vitamin D_3 deficiency or insufficient amount was almost similar between erosive-atrophic and nonerosive types, and there was no significant difference ($P = 0.75$). These results are true for ANA levels ($P = 0.52$)

ANA positive in patients with OLP was 12% (six patients). The results of the *t*-test showed no significant difference between the mean levels of serum ANA in the two nodes, with a reliability of 80% ($P = 0.34$).

Discussion

The present study measured serum levels of vitamin D and ANA in patients with lichen planus and healthy individuals. The results

Table 1

Evaluation of age and sex distribution among patients with oral lichen planus and healthy individuals

				Sex		
Group	*N*	Age (mean ± SD)	*P*	Male	Female	*P*
Oral lichen planus	50	53.08 ± 10.61	0.028	17	33	0.183
Control	50	51.09 ± 7.51		17	33	

Table 2

The mean vitamin D levels between patients with oral lichen planus and controls

Group	N	Mean ± SD	P
Oral lichen planus	50	18.51 ± 15.25	0.25
Control	50	21.56 ± 16.21	

showed no significant difference between the serum level of vitamin D_3 and ANA in the control and patient groups.

Lichen planus is an inflammatory disease of the oral mucosa of the mouth and skin caused by etiological factors. However, its pathogenesis has not been fully elucidated[1]. Vitamin D is a steroid prohormone in diagnosing some diseases and has rarely been discussed in lichen planus.

Grimm and colleagues evaluated vitamin D levels in patients with oral SCC and VDR gene expression in precancerous lesions of the mouth. His study measured VDR gene expression and serum levels of vitamin D in five healthy individuals, 11 OLP patients, and 42 patients with oral SCC.

The results showed that VDR expression was more significant in the prognosis and incidence of oral SCC than that in normal individuals. VDR expression has significantly decreased in patients with oral SCC. They had severe vitamin D deficiency[9].

The present study showed a decrease in serum level of vitamin D, but if we examine the expression level of VDRs, we can determine its role in OLP.

Gupta *et al.*[10] assessed vitamin D levels in Indian patients with OLP. Despite vitamin D deficiency in Indian patients with OLP, they observed the association of vitamin D deficiency in lichen planus patients. Varma and colleagues conducted a case study on three patients with lichen planus. They found a relationship between vitamin D deficiency with OLP symptoms and the association of administrating vitamin D supplements with improvement in patients[11]. The present study has not found a significant difference between patient and control groups but found that 28% had vitamin D_3 and 36% had insufficient vitamin D_3. This result is consistent with that of Thum-Tyzo *et al.*[13], who showed that 84 and 15% of patients with lichen planus had vitamin D deficiency and insufficient vitamin D, respectively. So far, researchers have identified the suppressive and regulatory properties of vitamin D on immune cells. Vitamin D affects both T and B lymphocytes. Frequent expression of VDRs in many immune cells (such as active B and T cells) indicates the regulatory role of vitamin D in various types of immune system arms. Much evidence showed that vitamin D deficiency develops several malignancies, metabolic and cardiovascular diseases, neurological and immune disorders such as autoimmune diseases, and its known role in bone disorders[14].

Seif *et al.*[15] found that a high percentage of patients with OLP were deficient in vitamin D, so they suggested further studies due to the prevalence of such deficiency.

The present study found that the mean of vitamin D in patients with erosive and atrophic forms was lower than that in patients with nonerosive forms and fewer people had average amounts of vitamin D, but this difference was not significant. This difference will become more evident if more samples are examined, and the relationship between vitamin D and forms of lichen planus disease will be determined.

A significant number of in-vitro and in-vivo studies have shown that the most active metabolites include vitamins D, 1, and 25-dihydroxycalciferol or calcitriol with antiproliferative, proapoptotic, and antiangiogenic properties. The combination of calcitriol and a large number of cytotoxic drugs has synergistic effects[16].

Insufficient vitamin D is prevalent in the general population due to sunscreen use, indoor activities, and skin covered with clothing. The present study showed that nine patients in the control group (30%) were deficient in vitamin D, and 15 patients (50%) had insufficient vitamin D. One of the reasons for the insignificance was the difference between vitamin D levels in patients and controls. Thus, reducing skin cancer may have unintended consequences resulting from the development of vitamin D deficiency[17]. Accordingly, OLP may increase, and these two issues may be strongly related. Vitamin D deficiency matters the most at different ages. Vitamin D is stored in high doses in fat mass due to fat dissolution and increases intoxication after saturation. Vitamin D deficiency in older adults appears because of reduced fat mass and risk of accumulation, diminished skin potency in vitamin D synthesis, and inadequate nutrition. We can prescribe OLP to patients and healthy individuals with higher doses[18].

Mozaffari and colleagues identified 20 cases with OLP(based on WHO diagnosis criteria) and 20 cases in the control group of similar sex and age; 25-hydroxyvitamin D was measured with electrochemiluminescence immunoassay analyzer. Patients with OLP had vitamin D deficiency. This deficiency was statistically significant in both groups and proposed as a potential factor in OLP etiopathogenesis[19]. Razi *et al.*[20] considered vitamin D therapy efficient for treating lesions of OLP in women during perimenopause.

Ghaliani and colleagues performed a comparative study on the presence of autoantibodies [such as smooth muscle antibody (SMA), ANA, and rheumatoid factor (RF) serum], in patients with lichen planus, lichenoid drug reactions, and oral contact in 2010. They showed no difference between the presence or absence of autoantibodies (ANA, SMA, anti-DNA, RF) in lichen planus groups and lichenoid reaction[21].

Lin *et al.*[22] reported a significant reduction in ANA levels after 2–38 months of follow-up in OLP patients treated with levamisole. Pruktrakual *et al.*[23] reported higher ANA levels in OLP patients than in controls. Lundstrom[24] noted a significant difference between OLP patients and healthy individuals regarding RF, ANA, antimitochondrial antibody, and SMA serum. Shuttleworth *et al.*[25] reported no significant differences in autoimmune factors in both groups. Sun and colleagues performed a study on the presence of SMA autoantibodies and ANA in two groups of patients, including patients with OLP and patients with other mucosal diseases 1 (as a control group). They showed that ANA was present in 29% of patients with OLP and 5% of patients in the control group; this difference was significant[26]. Lukac *et al.*[27] examined the presence of autoantibodies against desmoglein 1 and 3 in patients with OLP by ELISA and showed that the presence of these two antibodies is higher in patients with erosive OLP.

The present study showed no significant difference in the amount of ANA autoantibodies between the two groups. Although ANAs were positive in seven patients (%) of lichen planus, we found no significant difference in the presence of

autoantibodies. We found no specific immunological difference between the two groups.

One of the limitations of the present study is the participation of the small number of patients and the matched control group. So, we recommend further studies with a larger sample size to understand the role of vitamin D in OLP. Further, we recommend evaluating vitamin serum, performing a molecular and immunohistochemical study of VDRs in OLP, and examining different types of OLP and pathological factors.

Provenance and peer review

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Ethical approval

The study is approved by the Ethics Committee of Zahedan University of Medical Sciences.

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Authors' contribution

T.N.: study design and concept, performing, literature review, and drafting.

Conflicts of interest disclosure

The authors declare that they have no financial conflict of interest with regard to the content of this report.

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