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The relationship between serum vitamin D level with inflammatory markers and Total Antioxidant Capacity in Oral lichen planus

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ABSTRACT

Inflammatory markers play an important role in the pathophysiology of patients with oral problems such as oral lichen planus. This study aimed to investigate the relationship between vitamin D levels and inflammatory markers and total antioxidants in people with oral lichen planus. In this case-control study, 131 patients with oral lichen planus (67 in the lichen planus group and 54 in the control group) were examined. 8 cc of blood was taken from all participants to assess blood factors, inflammatory markers and antioxidant levels. Data were analyzed using SPSS statistical software. The mean age of subjects was 42 years. Vitamin D3 levels in the lichen planus group were lower than in the control group, but this decrease was not statistically significant (P> 0.05). According to statistical findings in the lichen planus group, there was a significant relationship between vitamin D3 levels, inflammatory markers and cellular stress factors (P≤0.05). It is concluded that vitamin D3 in people with oral lichen planus can play an important role in the pathogenesis of oral disease and increase inflammation. Because patients with oral lichen planus are affected by various inflammatory factors, paying attention to vitamin D levels in these patients can be effective in reducing inflammation caused by lichen planus.

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Introduction

Oral Lichen Planus (OLP) is a chronic autoimmune disease that causes many oral complications in the mouth with reticular, popular, plaque-like, erythematous (atrophic), and ulcerative lesions (1, 2). OLP is an immune reaction in which the fluid between the epithelial cells becomes inflamed, allowing T lymphocytes to penetrate the lamina propria. By increasing the immune response and oxidative stress at the site of inflammation, the cells are destroyed and more damage is done to the lining of the mouth (3, 4).

The mucous membranes of the mouth, tongue, and gums may be affected by OLP (5). Other areas such as the scalp, hair, nails, and mucous membranes of the genitals, esophagus, and conjunctiva may also be affected by OLP (6). OLP is found in all age groups, but adults are significantly more affected than children. It affects women more than men (7, 8).

The findings report an increase in oxidative stress and inflammatory factors in people with oral lichen planus. Other factors such as stress and hepatitis C virus also play a role in inducing oral lichen planus (9, 10). Oxidative stress is associated with an increase in free radicals and oxidants in the body, increasing inflammation and damage to many tissues in the body (11). The effect of oxidative stress on oral lichen planus is known as an important effective factor. Reports indicate that increased oxidative stress and immune response are associated with each other in this disease (12).

Studies show that vitamin D deficiency is associated with many oral diseases, and levels of this vitamin can affect the severity of oral lichen planus (13). In fact, studies show that many people with oral lichen planus suffer from vitamin D deficiency (14).

vitamin D plays an important role in the modulation of the inflammation system by regulating the production of inflammatory cytokines (15). Sheikh et al. observed that the stimulation of $CD4^+$ T cells with vitamin D suppresses proliferation capacity; diminished the percentage of pro-inflammatory cytokines, including IFN-γ, IL-17, and IL-22, except IL-4 in $CD4^+$ T cells (16). The role of vitamin D on

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inflammatory markers in many diseases, including Covid-19, has been identified and its deficiency has created many problems for the world (17).

Given that no definitive and complete treatment for OLP has been identified, and despite the many side effects of corticosteroids and the sedative role of this drug on this disease, it is necessary to provide a solution that can be effective in reducing OLP. Also, the relationship of this vitamin on inflammatory markers and cellular stress in patients with OLP has not been studied so far, and paying attention to this issue can be effective in further understanding this disease. Therefore, our study aimed to investigate the relationship between vitamin D levels and inflammatory markers, total antioxidants in people with oral lichen planus.

Materials and methods

Subjects and setting

This case-controlled study was done from July 2020 to July 2021 at a hospital in China. 131 People participated in this study in two groups (n = 70 for each group): Oral lichen planus (OLP) and control groups. The control group included healthy people who had undergone the common and same tests as the Oral lichen planus patients. Details including height and weight were recorded for all participants in a standard condition. Nearly 7 ccs of venous blood were taken from all participants for evaluating the levels of inflammatory markers, and then it was stored inside an EDTA + NaF tube.

Inclusion and exclusion criteria

The inclusion criteria of study groups included: 1) People with oral lichen planus between the ages of 15 and 70. 2) All participants signed informed consent before entering the study.

The exclusion criteria were having: 1) hyper-androgenism due to other reasons, 2) malignancies because of drug consumption such as glucocorticoids, anticonvulsants, vitamin and calcium supplements, 3) liver or kidney disease, 4) People with chronic diseases such as diabetes and high cholesterol and 5) Patients' reluctance to continue research. This study was approved by the ethics committee of our university.

Biochemical assessment

After instructing all patients to do not to eat, drink or smoke, the blood samples of all participants were gathered. During days 2-6 of the menstrual periods, a sample of 10 ccs of blood was collected within standard tubes with plain tops for conducting further analysis.

Through assessment of all blood samples before ovulation on the second day of the cycle, the normal values of 20–100 ng/dL were recognized to be preventive for other reasons of the abnormal condition of menstrual disorders.

Hydroxy vitamin D was measured with electron-chemiluminescence binding assays (ECLIA) (immune dingnostik, Bensheim). When the vitamin D3 serum level is higher than 30 ng/ml, it is defined as a normal level of vitamin D. Also, 20 ng/ml to 29 ng/ml level of vitamin D_3 was categorized as insufficient, 10 ng/ml to 19 ng/ml as moderately deficient and less than 10 ng/ml as severely deficient (18).

Measurement of inflammation markers and Total Antioxidant Capacity(TAC)

Blood samples (2 mL) were collected into anticoagulant-free tubes and centrifuged at 1000×g for 15 min.TAC was measured using the ferric reducing antioxidant power (FRAP) assay (ZellBio ELISA Kit, Germany), Serum hs-CRP was determined by immunologic turbidimetry (Orion Diagnostic, Espoo, Finland). Serum levels of TNF-α and IL-6 were measured in duplicate using a commercially available enzyme-linked immunosorbent assay kit (Human IL-6 and Quantikine HS TNF-α, Mannheim, Germany).

Statistical analysis

Analysis of variance and t-test were used to compare quantitative variables with normal distribution between the two groups. The Chi-square test was used to evaluate the qualitative variables. In the case of normal distribution, the relationship among the levels of vitamin D and metabolic variables was analyzed with Pearson’s coefficient. Spearman’s test was used for non-normal distribution. The statistical package for social sciences (SPSS) software version 25 was applied for carrying out all analyses. A P-value less than 0.05 was considered significant.

Results and discussion

The means of age were 39.24 ± 11.17 and 41.32 ± 8.51 years old in the control and OLP groups, respectively (P = 0.12). The means of body mass indexes were 28.13 ± 4.75 and 26.03 ± 5.40 kg/m^2 in the control and OLP groups, respectively (P = 0.09). There was no significant difference regarding waist circumferences between the two groups. (Table 1).

Table 1. Anthropometric parameters of the study

Group	OLP group (Mean ± SD)	Control group (Mean ± SD)	P value
Age	41.32 ± 8.51	39.24 ± 11.17	0.12
Weight	69.22±15.70	67.15±11.69	0.23
Body mass index	26.03 ± 5.40	28.13 ± 4.75	0.09

A total of 25.5% of participants were deficient in vitamin D. The percentages of vitamin D3 levels less than 10 ng/ml were 33.7 % in the OLP and 17.3% in the control groups. Also, percentages of vitamin D3 levels more than 30 ng/ml within the OLP group and control group were 31.4 % and 42.0% respectively (Table 2). But The different vitamin D3 deficiency between two groups was not significant (P = 0.07).

Table 2. Vitamin D3 levels in each study group

Vitamin D level (ng/ml)	< 10	10 - 30	> 30	P value
OLP group	%33.7	%31.4	%34.9	
Control group	%17.3	%42.0	%40.7	0.08
Total	%25.5	%36.2	%37.3	

Correlation between TAC level and vitamin D concentration, was a weak and positive relationship, although statistically were significant. TAC showed a significant increase in the control group (P =0.012). Evaluation of inflammatory markers shows that TNF-α, IL-6 and hs-CRP showed significant differences among the OLP group (Table 3). Results of TNF-α, IL-6 and hs-CRP showed an increase in the OLP group (P< 0.05).

Pearson's correlation was used to investigate the relationship between serum vitamin D levels and variables such as Total Antioxidant Capacity (TAC), TNF-α, IL-6 and hs-CRP in each group. Within the OLP group, there was a reverse, linear association among Total Antioxidant Capacity (TAC) and the level of vitamin D3 (P < 0.05). (Table 4). Within the control group association between, TNF-α, IL-6 and hs-CRP and the level of vitamin D3 (P < 0.05).

Table 3. Comparisions of serum **TAC** and IL-6, TNF-α, hs-CRP according to cognitive status

Groups Variables	OLP group (Mean ± SD)	Control group (Mean ± SD)	P value
TAC (µm/L)	402.3±72.5	448.3±96.5	0.012
TNF-α (pg/ml)	33.04 ±12.2	29.02 ±8.6	0.013
IL-6(pg/ml)	26.02 ±8.7	17.92 ±6.4	0.021
hs-CRP (mg/L)	2.40 ± 0.5	0.80 ± 0.4	< 0.001

Table 4. Correlation between vitamin D_3 and inflammatory markers and Total Antioxidant Capacity (Pearson's correlation)

Parameters		OLP group	Control group
TAC (µm/L)	Pearson's correlation	-.016	-.152
		.016	.022
TNF-α (pg/ml)	Pearson's correlation	.022	-.054
		.854	.662
IL-6(pg/ml)	Pearson's correlation	-.292	-.165
		.009	.047
hs-CRP (mg/L)	Pearson's correlation	.069	-.080
		.029	.027

Studies have shown that vitamin D plays an important role in reducing tissue inflammation by acting on antioxidant pathways. In fact, vitamin D has a beneficial effect on increasing the function of the immune system by increasing metabolism and reducing inflammation and accelerates the improvement of oral lichen planus (13, 14, 15, 16).

In our study, 25.5% of participants were deficient in vitamin D, but the deficiency was higher in the OLP group. Overall, the healthy group was better off in terms of vitamin D, with only 17% deficient in vitamin D; But the group of people with lichen planus were 33% deficient in vitamin D. Despite the difference in vitamin D levels in the lichen planus group, the difference between the two groups was not statistically significant. In fact, our findings show that vitamin D deficiency in both populations shows that Chinese health officials are paying more attention to this issue.

Few studies have been performed on vitamin D levels in people with oral lichen planus, and these

studies have found the role of this vitamin to be effective in oral lichen planus. These findings have shown that vitamin D deficiency is present in a high percentage of patients with OLP and the use of vitamin D supplements has significantly improved the effectiveness of treatment in these patients (19, 20, 21). A study by Gupta et al. In India also showed that vitamin D deficiency in lichen planus patients was lower than in the other group (22).

Findings show that paying attention to vitamin D levels in the population is an important issue. Research has shown that vitamin D plays important functions in the body, including regulating cell growth, the immune system, and reducing inflammation. Vitamin D is responsible for regulating genes that direct proteins that are responsible for cell proliferation, differentiation, and apoptosis (14, 15, 19, 21). Many healthy people are deficient in vitamin D, and a systematic review shows that targeting people with vitamin D deficiency can lead to clinically significant improvements in their bone density. In this study, the role of vitamin D in the better development of children has been reported (23).

The present study showed that the mean of oxidative stress was significantly higher among a group of people with OLP compared to the control group. In addition, our study shows that decreased antioxidant capacity in people with oral lichen planus may be associated with the disease. A systematic review of the role of oxidant levels and oxidative stress in the progression and development of lichen planus has been identified (24).

Various findings have been made regarding oral lichen planus, but the relationship between vitamin D levels and oxidative stress in patients with lichen planus has not yet been investigated. In cohort studies, levels of oxidative stress were compared between patients with OLPs and controls. The results of this study showed that the level of antioxidants in people with OLP is significantly lower than in healthy people, which is consistent with the results of the present study (25, 26).

Current evidence implicates various factors to develop oxidative stress and its severity in patients with OLPs. The antioxidant capacity of the serum based on both TAC and cTAS can help to provide better control of the disease in patients with OLPs (20,21,27).

We found a significant difference between the two groups regarding inflammatory markers.In the control group, the level of vitamin D3 less than 10 ng/ml could cause a considerable increment of inflammatory markers. This finding was consistent with similar studies on the association of vitamin D with inflammatory markers. Studies by Du et al. Show that vitamin D helps improve the immune response in patients with lichen planus by affecting the cellular pathway of inflammation (28). Recent findings suggest that vitamin D receptor genetic polymorphisms are associated with oral lichen planus in various populations in China (29).

Seif et al.'s study of vitamin D levels in people with oral lichen planus examined their vitamin D deficiency in patients. They suggested that vitamin D deficiency in these patients may have an effect on inflammatory pathways to increase inflammation. Oral and oral lichen planus helps (30).

Our findings were consistent with the above studies, which suggested that the inflammatory cytokine, IL-6 and hs-CRP might be a meaningful biological marker to link the cognitive impairment in OLP patients. Extensive studies have been performed to determine safe and effective drugs for the treatment of OLP; but so far, no highly effective drug has been developed that can be 100% responsive (31). As a result, it is important to look at ways to boost immune system function, such as taking vitamin D to prevent infection.

Findings show that the use of vitamin D as a drug supplement is effective in the treatment of oral lichen planus and certainly my levels of vitamin D are important in preventing this disease (32,33).

In the present study, we tried to take an important step in understanding the relationship between vitamin D and the factors contributing to occur OLP. Anyway, because of the prevalence of deficiency of vitamin D, the question of what is the cause of this deficiency in the population under study is of very high importance.

A limited number of foods naturally contain vitamin D. Meat, fatty fish and fish liver oil are among the best sources of vitamin D, and consumption of these foods by people with oral lichen planus can be effective in improving their vitamin D levels. Also, as mentioned, taking vitamin D supplements and sun exposure can also be effective in increasing vitamin D levels.

Conclusion

We investigated vitamin D3 deficiency and its relationship with inflammatory markers and Total Antioxidant Capacity. Due to the fact that various factors such as oxidative stress are effective in the development of OLP disease, therefore, pharmacological treatments along with diets containing antioxidants can be effective in relieving these metabolic disorders. The results of this study showed that vitamin D is one of the important causes for the treatment of oral lichen planus. Further studies are recommended on the response to vitamin D within the OLP group to investigate the fact that whether taking vitamin D3 supplements can be helpful or not. In addition, the effect of sun exposure can be compared to other treatments. It is hoped that the present study is an effective step in improving the health of most people.

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Interest conflict

The authors declare no conflict of interest.

Disclosure Statement

No potential conflict of interest was reported by the authors.

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