The profile of hematinic deficiencies in patients with oral lichen planus: a case-control study

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Abstract

Background: Oral lichen planus (OLP) is a relatively common mucocutaneous disorder, and its causative factors and pathogenesis are not fully understood. Existing studies on the association between hematinic deficiencies and OLP are limited and inconsistent. The aim of this study was to assess the hematinic deficiencies in a cohort of OLP patients and evaluate the correlation between hematinic deficiencies and OLP.

Methods: A total of 236 OLP patients and 226 age-and-gender-matched healthy controls were enrolled in this study. The levels of hemoglobin (Hb), serum folate, vitamin B12 and ferritin were measured and compared between OLP patients and healthy controls. An REU (reticular/hyperkeratotic, erosive/erythematous, ulcerative) scoring system was adopted and compared between the OLP patients with and without hematinic deficiencies. The correlation between hematinic deficiencies and OLP was analyzed.

Results: The frequencies of serum ferritin and vitamin B12 deficiency in OLP patients were both significantly higher than those of the healthy controls. According to gender and age, the profiles of hematinic deficiencies in OLP patients were significantly different. As for the REU score, no significant difference existed between OLP patients with and without hematinic deficiencies. Both serum ferritin deficiency and serum vitamin B12 deficiency were significantly correlated with OLP.

Conclusions: The present study suggested a significant association between hematinic deficiencies and OLP. Iron, folate, and vitamin B12 levels in OLP patients should be monitored routinely. Further studies are warranted to explore the interactions between OLP and hematinic deficiencies.

Background

Oral lichen planus (OLP) is a relatively common mucocutaneous disorder that affects 1%-2% of the population, mainly middle-aged adults, with a slight female predilection [1,2]. It is widely accepted that OLP is an immune-related disorder, in which aberrant T lymphocytes might play a vital role in the pathogenesis of this autoimmune disease [1,3]. Although abundant studies have been carried out, the exact causative factors and underlying pathogenesis of OLP are not fully understood and still require further investigation [1-3].

Iron, folate and vitamin B12 deficiency are the most common hematinic deficiencies, which have been closely associated to some common oral mucosal diseases, such as recurrent aphthous stomatitis (RAS) and atrophic glossitis (AG) [4,5]. In addition to affect the integrity of oral mucosa and increase the risk of secondary infection in oral cavity, hematinic deficiencies can also cause aberrant immune responses or contribute to psychological disorders, which may be involved in the pathogenesis of OLP [6-11]. However, the existing studies on the association between hematinic deficiencies and OLP have been lacking and the conclusions were inconsistent [12-15]. Wu et al proposed that OLP was one of the leading oral manifestations for iron deficiency anemia (IDA) patients and could be diagnosed in one-third patients with IDA in their oral mucosal disease clinic [16]. In another study, the same team reported that the frequencies of serum vitamin B12, iron deficiencies and anemia in OLP patients were significantly greater than that in controls [15]. By contrast, an earlier study showed the comparable frequencies of hematinic deficiencies and anemia between the OLP patients and the healthy controls, suggesting that hematinic deficiencies may not be a predisposing factor for OLP [13]. Therefore, further determination on the association between hematinic deficiencies and OLP are required, which would provide the foundation for nutritional supplements in the treatment of OLP and also give a new insight into the possible interaction between hematinic deficiencies and OLP.

The current study was undertaken to assess the hematinic deficiencies in a cohort of OLP patients and evaluate the association between hematinic deficiencies and OLP. Furthermore, whether the severity of OLP is related to the hematinic deficiencies was also analyzed.

Methods

A total of 236 OLP patients (41 males and 195 females, mean age 51.70±13.99 years) and 226 gender- and age-matched healthy controls (52 males and 174 females, mean age 49.46±17.26 years) were enrolled in this study (table 1). All patients were diagnosed consecutively from July 2015 to March 2019 at the Department of Oral Medicine, Shanxi Provincial People's Hospital, China. Healthy controls were recruited from December 2014 to August 2017 at the same hospital. The estimation of sample size was based on the formula provided by Machin D et al [17].

The diagnosis of OLP was established according to the recommended diagnostic criteria [3]. The characteristic clinical manifestations alone, such as bilateral grayish-white Wickham striae or papules may allow diagnosis, especially if classic skin or other extraoral lesions exist concomitantly. Besides the clinical examination, the biopsy for histopathological examination was routinely performed. When necessary, direct immunofluorescence (DIF) was employed to differentiate from autoimmune blistering diseases, such as pemphigus and pemphigoid. Specifically, any patient suspected of having oral lichenoid lesions, including contact hypersensitivity reactions, drug-induced reactions, paraneoplastic pemphigus and chronic graft-vs.-host disease, was not enrolled in the present study. A thorough medical history was obtained from each participant and standard clinical examination of the oral cavity was also performed. The duration of OLP, which was estimated from the first time noticing oral discomfort, was recorded for each patient. All patients who were histologically confirmed to have epithelial dysplasia, or diagnosed as having other oral mucosal lesions, such as leukoplakia, mucosal hyperkeratosis, erythema multiforme and discoid lupus erythematosus, were also excluded. In addition, patients who have concomitant systemic diseases, including benign or malignant tumors, HIV, rheumatic or autoimmune diseases, uncontrolled thyroid diseases, liver and kidney diseases, gastrointestinal diseases and a history of alcoholism were equally excluded from this study. None of the healthy controls exhibited any oral mucosal lesions and had relevant systemic diseases. None of the participants had taken any nutritional supplements, including (but not limited to) folate, vitamin B12 or iron supplements, at least 3 months prior to this study.

This study was conducted in accordance with the Helsinki Declaration and approved by the ethics committee of Shanxi Provincial People's hospital (No. 20190302) and informed consent was obtained from each of the participants.

Laboratory methods

After 12 hours of overnight fasting, blood samples were obtained from all participants. The serum was separated and tested immediately. After internal quality control, the serum levels of folate, vitamin B12 and ferritin were measured by chemiluminescence method (Beckman Coulter, DXI-800- Immunoassay system) in the department of clinical laboratory of Shanxi Provincial People's Hospital. The accepted normal serum folate level and serum vitamin B12 level was 4.0–18.7 ng/mL and 180–914 ng/L, respectively. Serum ferritin level was used to assess iron status with a normal level of 11.0–306.8 ng/mL for females and 15–336.2 ng/ml for males. Serum vitamin B12, folate, and ferritin deficiency was defined as a serum level below its lower cutoff value, respectively. The hemoglobin (Hb) level was also determined and anemia was diagnosed when the Hb level was lower than the lower cut-off value (male < 13 g/dL and female < 12 g/dL).

Clinical examination and scoring

REU (reticular/hyperkeratotic, erosive/erythematous, ulcerative) scoring system was adopted to evaluate the severity of OLP as previously reported [13,14]. Briefly, the oral cavity of each OLP patients was divided into 10 sites and the severity of the mucosal lesions in each site was scored according to the presence of reticular/hyperkeratotic, erosive/erythematous, and ulcerative lesions.

Data analysis

Statistical analyses were performed with SPSS software version 22.0 (SPSS Inc, Chicago, IL). Independent-sample t test was used for comparing age between OLP patients and healthy controls. The mean serum levels of vitamin B12, folate, ferritin, and hemoglobin were compared using Wilcoxon's rank-sum test. While the male-to-female ratio, the frequencies of hematinic deficiencies and anemia between OLP patients and health controls were compared using chi-square test. When the observed frequency was less than 1, the Fisher's exact test was applied. Wilcoxon-Mann-Whitney rank sum test (U test) was used for the statistical comparison of REU scores between the OLP patients with and without hematinic deficiencies. Logistic regression analysis was conducted to assess whether the age and gender are the significant factors related to the presence of hematinic deficiencies (ferritin, folate and vitamin B12 deficiency) in the OLP patients and healthy controls, respectively. Spearman's correlation coefficient was applied to evaluate the association between OLP and hematinic deficiencies. A p-value < 0.05 was accepted as statistically significant.

Results

1. Serum levels of vitamin B12, folate, ferritin, hemoglobin in OLP patients and healthy controls

Compared with healthy controls, OLP patients had significantly lower serum levels of vitamin B12 and folate (both $P < 0.001$, Table 1). For both males and females, the levels of ferritin and hemoglobin were not significantly different between the two groups (Table 1).

2. Hematinic deficiencies in OLP patients compared with healthy controls

Here, the frequency of overall hematinic deficiencies means the frequency of the subjects with at least one deficiency in serum folate, ferritin and vitamin B12. The frequency of overall hematinic deficiencies was 43.64% (103/236) in the OLP patients vs 12.39% (28/226) in the healthy controls. There was a statistically significant difference between the two groups ($P < 0.001$, Table 2). Similarly, the frequencies of serum ferritin deficiency and serum vitamin B12 deficiency in OLP patients were both significantly higher than those of the healthy controls (both $P < 0.001$, Table 2). However, no statistically significant difference was found in the frequency of serum folate deficiency between the OLP patients and healthy controls (4.24% *vs*1.33%, $P = 0.059$). Compared with the health controls, anemia was much more common in OLP patients (9.75% vs 3.98%, $P = 0.015$).

3. Hematinic deficiencies in OLP patients according to gender and age

Compared with the male OLP patients, the female OLP patients had a significantly higher frequency of serum ferritin deficiency (4.88% vs 24.62%, $P = 0.005$, Table 3). On the contrary, serum folate deficiency was more common in male OLP patients ($P < 0.001$). No significant differences in serum vitamin B12 deficiency or anemia were found between male and female OLP patients ($P = 0.511$ and $P = 0.148$, respectively).

The frequencies of hematinic deficiencies were also compared among age subgroups of the OLP patients. Significant differences in serum ferritin deficiency and anemia were revealed among age subgroups of OLP patients ($P < 0.001$, $P = 0.029$, respectively, Table 3). However, there was no significant difference among age subgroups in either serum folate or vitamin B12 deficiency ($P = 0.220$ and $P = 0.367$, respectively).

4. The correlation between two factors (age and gender) and presence of hematinic deficiencies in OLP patients

Both age and gender were significantly correlated with the presence of serum ferritin deficiency in the OLP patients (Table 4). However, neither age nor gender was significantly correlated with serum vitamin B12 deficiency in the OLP patients ($P = 0.413$ and $P = 0.623$, respectively, Table 4). Gender was significantly associated with serum folate deficiency in OLP patients ($P < 0.001$). Such significant correlation was also observed between age and anemia ($P = 0.021$, Table 4). For comparison, the same logistic regression analysis was conducted in the healthy controls and no significant correlation was found between the two factors and the presence of hematinic deficiencies (Table 4).

5. Analysis of disease duration in OLP patients

In this study, 63.56% of OLP patients (150/236) had not experienced oral symptoms more than 6 months. Among these patients, serum folate deficiency was detected in six patients, serum vitamin B12 deficiency in 44 patients and serum ferritin deficiency in 23 patients. A shorter duration, 1 month or less, was found in 30 OLP patients. Among these, seven patients, five patients and no patient had serum vitamin B12 deficiency, serum ferritin deficiency or serum folate deficiency, respectively.

6. Comparison of REU scores between OLP patients with and without hematinic deficiencies

The REU score (median ± IQR) of the OLP patients with one or more (two or three) deficiencies in serum folate, ferritin and vitamin B12 was 5 ± 3. For OLP patients without any deficiency, the REU score was 5 ± 3.75. The rank sum test revealed no significant difference between them ($P = 0.824$, Table 5). Separate analysis also showed that no significant difference existed between OLP patients with and without serum ferritin deficiency ($P = 0.783$), folate deficiency ($P = 0.173$), vitamin B12 deficiency ($P = 0.493$) or anemia ($P = 0.723$, Table 5).

7. Statistical analysis of the association between hematinic deficiencies and OLP.

Spearman's correlation coefficient revealed that both serum ferritin deficiency and serum vitamin B12 deficiency were significantly correlated with OLP ($R = -0.189$, $P < 0.001$; $R = -0.262$, $P < 0.001$, respectively). A borderline, but not statistically significant correlation was observed between serum folate deficiency and OLP ($R = -0.088$, $P = 0.059$). Moreover, anemia was not significantly associated with OLP ($R = -0.05$, $P = 0.284$).

Discussion

Consistent with previous studies [15, 18, 19], the present study exhibited a significantly higher frequency of hematinic deficiencies in OLP patients than in healthy controls. Moreover, a significant association between hematinic deficiencies and OLP was also demonstrated. However, this association should be interpreted with caution. Since OLP is a chronic condition with periods of exacerbation and remission, it may cause discomfort or pain and difficulty in eating and drinking [20–22]. A study on dietary changes in 48 patients with oral vesiculoulcerative diseases, of whom most were diagnosed with OLP, showed that even patients with mild forms of the disease would change their eating habits for extended periods, which might negatively affect the patients' nutritional status [22]. From this point of view, it seems reasonable to conclude that the hematinic deficiencies might

be the result of OLP. Nevertheless, it should be noted that the occurrence of hematinic deficiencies usually takes several months to years to appear [8, 9]. For example, due to the important hepatic stores and enterohepatic circulation, vitamin B12 deficiency would occur only if the daily intake has been insufficient for years [8]. In this study, we found that hematinic deficiencies had already existed in many of the enrolled patients, which were unlikely caused by OLP because of their rather short duration. Hence, we could speculate that, at least for some OLP patients, hematinic deficiencies occur earlier than the onset of OLP.

On the other hand, there is yet no direct evidence suggesting that hematinic deficiencies are involved in the pathogenesis of OLP, but several plausible mechanisms are proposed and need to be investigated in further studies. First, inadequate iron, folate or vitamin B-2 can significantly alter the immune response and affect cell-mediated immunity [10, 11, 23, 24]. For example, significant suppressed natural killer (NK) cell activity was noted in patients with vitamin B12 deficiency compared with control subjects and the decreased activity could be restored after vitamin B12 supplementation [25]. Coincidentally, significantly decreased NK cell activity was also found in LP patients [26, 27]. Second, the levels of vascular cell adhesion molecule-1 (VCAM-1), an inducible adhesion protein in endothelial cells, were significantly higher in patients with IDA compared with controls [28]. The expression of VCAM-1 in OLP was equally significantly increased [29]. Third, it has been demonstrated that psychological disorders, such as anxiety and depression, might play an important role as a trigger for OLP and might also be responsible for many relapses [6]. Notably, vitamin B12 deficiency or insufficiency might contribute to the etiopathogenesis of depression. Folic acid and vitamin B12 supplements were recommended for inclusion in treating depression [30]. However, the question remains to be elucidated since more than half of OLP patients did not have hematinic deficiencies in the present study. Prospective studies on the incidence of OLP in patients having hematinic deficiencies with large-sample and long-term follow-up are needed to provide more clinical evidence.

The REU scoring system is a semiquantitative method with less subjectivity and good reproducibility and has been validated to be much more accurate for comparing the severity of oral lichenoid lesions [31–33]. In a previous study, the inter- and intra-observer agreement of this scoring system was assessed with the finding that the Spearman correlation coefficient was 1.0 and 0.98, respectively [31]. To the best of our knowledge, this is the first report of using a disease scoring system (DSS) in investigating the association between the severity of OLP and hematinic deficiencies. In this study, no significant difference in REU scores was found between OLP patients with and without hematinic deficiencies, suggesting that the hematinic deficiencies may not directly correlate with the severity of OLP. This finding might be due to the well-recognized phenomenon that the clinical character of OLP can alleviate or aggravate even in a short time, especially when there are local irritations and trauma in the oral cavity [2, 3]. However, the levels of serum ferritin, folate and vitamin B12 in the human body could not fluctuate so rapidly. The biomarkers that can sensitively reflect the severity of OLP still warrant further investigation.

One limitation of the present study is that there are still no “gold standard” laboratory tests for hematinic deficiencies in routine clinical practice [34, 35]. The sensitivity and specificity of available assays still need to be improved [34, 35]. Some newer assays, such as measuring holotranscobalamin II (holoTC), and additional tests of serum total homocysteine (tHcy), methylmalonic acid (MMA), transferrin receptor and red blood cell folate (RBC folate) are recommended for further studies [35].

Based on our findings, hematological screening for hematinic deficiencies should be included in routine laboratory examination of OLP patients. Several studies have proposed that vitamin replacement may improve the general health of OLP patients and increase their healing ability [12, 36]. Therefore, compensation of hematinic deficiencies with adequate nutritional supplements or in combination with other drugs, is supposed to produce improved

therapeutic effects on OLP patients [12, 36]. In addition, dietary assessment and guidance to maintain adequate nutrition and optimal quality of life should be considered as a component of OLP management [20, 22].

Conclusion

In conclusion, the present study suggested a significant association between hematinic deficiencies and OLP. Folate, vitamin B12 and iron levels in OLP patients should be monitored routinely. Further studies are warranted to explore the interactions between OLP and hematinic deficiencies.

Declarations

Ethics approval and consent to participate

This study was approved by the ethics committee of Shanxi Provincial People's Hospital (No. 20190302) and written consent was obtained from each of the participants.

Consent for publication

Consent was obtained from each of the participants.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests related to this study.

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Authors' contributions

BZX, YXW and SJ collected the data for participants. BZX drafted the manuscript. YXW analyzed and interpreted the patient data. WYF conceived the idea and corrected the manuscript. All authors read and approved the final manuscript.

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Abbreviations

AG: atrophic glossitis; DIF: direct immunofluorescence; DSS: disease scoring system; Hb: hemoglobin; IDA: iron deficiency anemia; OLP: Oral lichen planus; RAS: recurrent aphthous stomatitis; REU: reticular/hyperkeratotic, erosive/erythematous, ulcerative.

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Tables

Table 1. Demographic data and the serum levels of vitamin B12, folate, ferritin, hemoglobin of the participants

	OLP (n=236)	Health controls (n=226)	*P*
Age [a]	51.70±13.99	49.46±17.26	0.127
Male-to-female ratio [b]	41:195	52:174	0.131
vitamin B12 (ng/L) [c]	261.00±171.75	306.50±220.5	<0.001
folate (ng/ml) [c]	9.38±5.90	11.50±30.04	<0.001
Ferritin (ng/ml) [c]			
Male	126.80±94.85	120.30±130.38	0.629 [c]
Female	54.20±101.8	38.65±65.62	0.458 [c]
Hemoglobin (g/dL) [c]			
Male	160.00±18.50	156.00±16.25	0.798 [c]
Female	136.00±16.00	136.00±14.25	0.845 [c]

a, Independent-sample t test; Mean ± SD; b, Chi-square test; c, Wilcoxon's rank-sum test, Median±QR.
SD= Standard deviation; QR= Quartile Range.

Table 2. Hematinic deficiencies in the OLP patients compared with the healthy controls

	OLP patients	Healthy controls	c^2	*p*
ıll hematinic deficiencies	43.64% (103/236)	12.39% (28/226)	52.366	<0.001
rum ferritin deficiency	21.19%(50/236	7.52% (17/226)	16.147	<0.001
erum folate deficiency	4.24% (10/236)	1.33% (3/226)	3.574	0.059
m vitamin B12 deficiency	27.54% (65/236)	7.52% (17/226)	31.696	<0.001
Anemia	9.75% (23/236)	3.98% (9/226)	5.949	0.015

Table 3. The statistical analysis of hematinic deficiencies in OLP patients according to gender and age

Hematinic deficiencies	OLP patients								
	Gender				Age				
	Male	Female	c^2	*P*	≤40	41-60	≥61	c^2	*P*
Serum ferritin deficiency	4.88% (2/41)	24.62% (48/195)	7.904	0.005	29.16% (14/48)	28.23% (35/124)	1.56% (1/64)	20.271	<0.001
Serum folate deficiency	17.07% (7/41)	1.54% (3/195	16.501	<0.001	8.33% (4/48)	2.42% (3/124)	4.68% (3/64)	3.027	0.220
Serum vitamin B12 deficiency	31.71% (13/41)	26.67% (52/195)	0.431	0.511	22.92% (11/48)	31.45% (39/124)	23.44% (15/64)	2.005	0.367
Anemia	2.44% (1/41)	11.28% (22/195)	2.090	0.148	10.42% (5/48)	13.71% (17/124)	1.56% (1/64)	7.112	0.029

Table 4. Logistic regression analysis on the correlation between the two factors (age and gender) and the presence of hematinic deficiencies.

	Factor	OLP patients				Healthy controls			
		β	OR	95%CI	P	β	OR	95%CI	P
Serum ferritin deficiency	Age	0.074	1.077	1.045-1.109	<0.001	0.029	1.029	0.998-1.061	0.066
	Gender	-2.798	0.061	0.012-0.300	0.001	-18.763	--	--	0.997
Serum folate deficiency	Age	0.002	1.002	0.962-1.043	0.933	0.057	1.059	0.986-1.136	0.115
	Gender	2.567	13.028	3.130-54.222	<0.001	2.437	11.435	0.928-140.956	0.057
Serum vitamin B12 deficiency	Age	0.009	1.009	0.988-1.030	0.413	-0.003	0.997	0.968-1.027	0.866
	Gender	0.187	1.205	0.572-2.541	0.623	0.339	1.403	0.456-4.322	0.555
Anemia	Age	0.040	1.041	1.006-1.077	0.021	0.015	1.015	0.976-1.056	0.460
	Gender	-2.010	0.134	0.017-1.079	0.059	-18.155	-	--	0.997

OLP patients		REU scores (median ± IQR)	*P*
Overall hematinic deficiencies	with	5±3	0.824
	without	5±3.75	
Serum ferritin deficiency	with	5.25±3.5	0.783
	without	5±3	
Serum folate deficiency	with	4.5±1.75	0.173
	without	5±3.5	
Serum vitamin B12 deficiency	with	5±3.25	0.493
	without	4.5±3	
Anemia	with	6±4	0.723
	without	5±3	

Table 5. The comparison of REU scores between the OLP patients with and without hematinic deficiencies
REU (reticular/hyperkeratotic, erosive/erythematous, ulcerative), IQR (Interquartile range).

Supplementary Files

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- STROBEchecklistv4combinedPlosMedicine.docx