Journal of Dental Sciences 20 (2025) 1052–1059

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.e-jds.com

Original Article

Anemia, hematinic deficiencies, and hyperhomocysteinemia in male and female oral lichen planus patients

Julia Yu-Fong Chang [a,b,c†], **Yu-Hsueh Wu** [d,e†], **Yi-Pang Lee** [f,g], **Yi-Ping Wang** [a,b,c], **Andy Sun** [a,b,c**], **Chun-Pin Chiang** [a,b,c,f,g*]

[a] *Department of Dentistry, National Taiwan University Hospital, College of Medicine, National Taiwan University, Taipei, Taiwan*
[b] *Graduate Institute of Oral Biology, School of Dentistry, National Taiwan University, Taipei, Taiwan*
[c] *Graduate Institute of Clinical Dentistry, School of Dentistry, National Taiwan University, Taipei, Taiwan*
[d] *Department of Stomatology, National Cheng Kung University Hospital, College of Medicine, National Cheng Kung University, Tainan, Taiwan*
[e] *Institute of Oral Medicine, School of Dentistry, National Cheng Kung University, Tainan, Taiwan*
[f] *Department of Dentistry, Hualien Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, Hualien, Taiwan*
[g] *Institute of Oral Medicine and Materials, College of Medicine, Tzu Chi University, Hualien, Taiwan*

Received 30 November 2024; Final revision received 3 December 2024
Available online 12 December 2024

KEYWORDS
Oral lichen planus;
Hemoglobin;
Iron;
Vitamin B12;
Folic acid;
Homocysteine

Abstract *Background/purpose:* Our previous study found that 21.9 %, 13.6 %, 7.1 %, 0.3 %, and 14.8 % of 352 oral lichen planus (OLP) patients have anemia, serum iron, vitamin B12, and folic acid deficiencies, and hyperhomocysteinemia, respectively. This study mainly evaluated the anemia, hematinic deficiencies, and hyperhomocysteinemia in 110 male and 478 female OLP patients.
Materials and methods: The blood hemoglobin (Hb) and serum iron, vitamin B12, folic acid, and homocysteine levels in 110 male and 478 female OLP patients were measured and compared with the corresponding levels in 110 male and 478 female healthy control subjects, respectively.
Results: We found that 110 male OLP patients had significantly lower mean blood Hb and serum folic acid levels than 110 male healthy control subjects. Moreover, 478 female OLP patients had significantly lower mean blood Hb and serum iron, vitamin B12, and folic acid levels

* Corresponding author. Department of Dentistry, Hualien Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, and Institute of Oral Medicine and Materials, College of Medicine, Tzu Chi University, No. 707, Section 3, Chung-Yang Road, Hualien 970, Taiwan.
** Corresponding author. Department of Dentistry, National Taiwan University Hospital, No. 1, Chang-Te Street, Taipei 10048, Taiwan.
E-mail addresses: andysun7702@yahoo.com.tw (A. Sun), cpchiang@ntu.edu.tw (C.-P. Chiang).
† These two authors contributed equally to this work.

https://doi.org/10.1016/j.jds.2024.12.002

and significantly higher mean serum homocysteine level than 478 female healthy control subjects. In addition, 110 male OLP patients had significantly higher mean blood Hb and serum iron levels, significantly lower mean serum vitamin B12 and folic acid levels, a significantly higher frequency of folic acid deficiency, and significantly lower frequencies of blood Hb and serum iron deficiencies than 478 female OLP patients.

Conclusion: The male OLP patients do have significantly higher mean blood Hb and serum iron levels, significantly lower mean serum vitamin B12 and folic acid levels, a significantly higher frequency of folic acid deficiency, and significantly lower frequencies of blood Hb and serum iron deficiencies than female OLP patients.

Introduction

Oral lichen planus (OLP) is a chronic inflammatory condition of the oral mucosa that is more commonly observed in middle-aged and elderly female patients.[1–3] OLP frequently manifests on the buccal mucosa, tongue, and gingiva. The OLP lesions always display a bilateral and symmetric distribution on the oral mucosa.[3] Clinically, OLP can present in various forms, including reticular, papular, plaque-like, erosive/atrophic, ulcerative, and bullous types.[2,3] Patients with erosive/atrophic or ulcerative OLP may experience oral symptoms, such as pain and a burning sensation in the oral mucosa when consuming irritating foods or drinks. These discomforting oral symptoms can disrupt the eating habits of OLP patients, leading to reduced food intake. Consequently, this reduced food intake can result in anemia, hematinic deficiencies, and hyperhomocysteinemia in a certain percentage of OLP patients.[3]

Our previous studies investigated whether there are significant disparities in anemia, hematinic deficiencies, and hyperhomocysteinemia between male and female atrophic glossitis (AG) or burning mouth syndrome (BMS) patients.[4,5] Our findings revealed that the male AG patients indeed exhibit significantly higher mean blood Hb and serum homocysteine levels, significantly lower mean serum vitamin B12 and folic acid levels, and significantly higher frequencies of Hb, vitamin B12, and folic acid deficiencies and hyperhomocysteinemia than their female AG counterparts.[4] Additionally, the male BMS patients also demonstrated significantly higher mean blood Hb and serum homocysteine levels, significantly lower mean serum vitamin B12 and folic acid levels, and significantly higher frequencies of folic acid deficiency and hyperhomocysteinemis than their female BMS counterparts.[5] To the best of our knowledge, none of previous studies compared the complete blood count data, serum iron, vitamin B12, folic acid, homocysteine, and gastric parietal cell antibody (GPCA) levels between a large group of male and female OLP patients. Therefore, in this study, we divided the 588 OLP patients into 110 male and 478 female OLP patients. We mainly evaluated whether 110 male OLP patients had significantly lower frequencies of blood hemoglobin (Hb) and serum iron, vitamin B12, and folic acid deficiencies, hyperhomocysteinemia, and serum GPCA positivity than 478 female OLP patients. We also assessed whether there were significantly higher frequencies of blood Hb and serum iron, vitamin B12, and folic acid deficiencies, hyperhomocysteinemia, and serum GPCA positivity in 110 male and 478 female OLP patients than in 110 male and 478 female healthy control subjects, respectively.

Materials and methods

Subjects

This study included 110 male OLP patients (age range 25–87 years, mean 50.4 ± 15.3 years) and 478 female OLP patients (age range 20–88 years, mean 57.1 ± 13.5 years). For each OLP patients, one age- (±2 years of each patient's age) and sex-matched healthy control subject was selected. Thus, 110 male (age range 24–87 years, mean 50.7 ± 15.4 years) and 478 female (age range 20–89 years, mean 57.2 ± 13.5 years) age- and sex-matched healthy control subjects were selected and included in this study. All the patients and control subjects were seen consecutively, diagnosed, and treated in the Department of Dentistry, National Taiwan University Hospital (NTUH) from July 2007 to June 2023. The 588 OLP patients were selected according to the following criteria: (i) a typical clinical presentation of radiating grayish-white Wickham striae, papules and plaques, separately or in combination, and erosion or ulceration on the oral mucosa; (ii) the OLP lesions had a bilateral and symmetric distribution on the oral mucosa. In 25 OLP patients the clinical diagnosis of OLP was uncertain, thus, an incisional biopsy of the typical oral mucosal lesion was performed. When the biopsy specimens showed the characteristic features of OLP, that is, hyperkeratosis or parakeratosis, a slightly acanthotic epithelium with liquefaction degeneration of the basal epithelial cells, a pronounced band-like lymphocytic infiltrate in the lamina propria, and the absence of epithelial dysplasia, the histopathological diagnosis of OLP was confirmed.[3,6–9] However, all OLP patients with areca quid chewing habit, autoimmune diseases (such as systemic lupus erythematosus, rheumatoid arthritis, Sjogren's syndrome, pemphigus vulgaris, and cicatricial pemphigoid), inflammatory diseases, malignancy, or recent surgery were excluded. In addition, all OLP patients with serum creatinine concentrations indicative of renal dysfunction (ie, men, >131 μmol/L; women, >115 μmol/L), and who reported a history of stroke, heavy alcohol use, or diseases of the liver, kidney, or coronary arteries were also excluded.[6–9] Healthy control subjects had either dental caries or mild periodontal diseases but did not have any oral mucosal or systemic diseases. None of the OLP patients had taken any prescription medication for OLP at least 3 months before entering the study.

The blood samples were drawn from 588 OLP patients and 588 healthy control subjects for the measurement of complete blood count, serum iron, vitamin B12, folic acid, and homocysteine concentrations, and the serum GPCA positivity. All OLP patients and healthy control subjects signed the informed consent forms before entering the study. This study was reviewed and approved by the Institutional Review Board at the NTUH (202402086RINC).

Determination of blood hemoglobin, iron, vitamin B12, folic acid, and homocysteine concentrations

The complete blood count and serum iron, vitamin B12, folic acid, and homocysteine concentrations were determined by the routine tests performed in the Department of Laboratory Medicine, NTUH.[6–30]

Determination of serum gastric parietal cell antibody level

The serum GPCA level was detected by the indirect immunofluorescence technique with rat stomach as a substrate as described previously.[6–30] Sera were scored as positive when they produced fluorescence at a dilution of 10-fold or more.

Statistical analysis

Comparisons of the mean corpuscular volume (MCV) and mean blood Hb and serum iron, vitamin B12, folic acid, and homocysteine levels between 110 male or 478 female OLP patients and 110 male or 478 female healthy control subjects, respectively, as well as between 110 male and 478 female OLP patients were performed by Student's *t*-test. The differences in frequencies of microcytosis (MCV <80 fL),[23–25] macrocytosis (MCV ≥100 fL),[26–30] blood Hb and serum iron, vitamin B12, and folic acid deficiencies, hyperhomocysteinemia, and serum GPCA positivity between 110 male or 478 female OLP patients and 110 male or 478 female healthy control subjects, respectively, as well as between 110 male and 478 female OLP patients were compared by chi-square test. In addition, the differences in frequencies of 6 different types of anemia between 110 male and 478 female OLP patients were also compared by chi-square test. The result was considered to be significant if the *P*-value was less than 0.05.

Results

The MCV, mean blood Hb and serum iron, vitamin B12, folic acid, and homocysteine levels in 110 male and 478 female OLP patients and in 110 male and 478 female healthy control subjects are shown in Table 1. We found that 110 male OLP patients had significantly lower mean blood Hb and serum folic acid levels than 110 male healthy control subjects (both *P*-values <0.05, Table 1). Moreover, 478 female OLP patients had significantly lower MCV and mean blood Hb and serum iron, vitamin B12, and folic acid levels, and significantly higher mean serum homocysteine level than 478 female healthy control subjects (all *P*-values <0.001, Table 1). In addition, 110 male OLP patients had significantly higher mean blood Hb and serum iron levels and significantly lower mean serum vitamin B12 and folic acid levels than 478 female OLP patients (all *P*-values <0.05, Table 1). The 110 male OLP patients also had higher mean serum homocysteine level than 478 female OLP patients (marginal significance, $P = 0.066$). However, no significant difference in the MCV was found between 110 male and 478 female OLP patients (Table 1).

According to the World Health Organization (WHO) criteria, microcytosis of erythrocyte was defined as having MCV <80 fL,[23–25] macrocytosis of erythrocyte was defined as having MCV ≥100 fL,[26–30] and men with Hb < 13 g/dL and women with Hb < 12 g/dL were defined as having Hb

Table 1 Comparisons of mean corpuscular volume (MCV) and mean blood hemoglobin (Hb) and serum iron, vitamin B12, folic acid, and homocysteine levels between 110 male or 478 female OLP patients and 110 male or 478 female healthy control subjects, respectively, as well as between 110 male and 478 female OLP patients.

Group	MCV (fL)	Hb (g/dL)	Iron (μg/dL)	Vitamin B12 (pg/mL)	Folic acid (ng/mL)	Homocysteine (μM)
Male OLP patients (n = 110)	88.4 ± 8.0	14.6 ± 1.5	99.1 ± 36.8	525.3 ± 215.5	10.6 ± 5.5	9.9 ± 2.5
[a]*P*-value	0.08	<0.001	0.584	0.112	0.011	0.497
[b]*P*-value	0.911	<0.001	<0.001	0.032	<0.001	0.066
Female OLP patients (n = 478)	88.3 ± 8.6	12.7 ± 1.3	82.8 ± 30.3	583.9 ± 266.8	13.2 ± 6.3	9.2 ± 3.8
[a]*P*-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Male healthy control subjects (n = 110)	89.9 ± 4.0	15.2 ± 0.8	103.6 ± 27.9	570.1 ± 200.9	12.5 ± 5.5	9.7 ± 1.8
Female healthy control subjects (n = 478)	90.5 ± 3.7	13.5 ± 0.8	96.7 ± 27.4	708.5 ± 230.8	15.2 ± 5.8	7.9 ± 1.8

[a] Comparisons of means of parameters between 110 male or 478 female OLP patients and 110 male or 478 female healthy control subjects by Student's *t*-test, respectively.
[b] Comparisons of means of parameters between 110 male and 478 female OLP patients by Student's *t*-test.

deficiency or anemia.[31] Furthermore, patients with the serum iron level <60 μg/dL,[32,33] the serum vitamin B12 level <200 pg/mL,[34] or the folic acid level <4 ng/mL[35] were defined as having serum iron, vitamin B12 or folic acid deficiency, respectively. In addition, patients with the blood homocysteine level >12.0 μM (which was the mean serum homocysteine level of healthy control subjects plus two standard deviations) were defined as having hyperhomocysteinemia. By the above-mentioned definitions, 9.1 %, 4.6 %, 16.4 %, 10.0 %, 6.4 %, 3.6 %, 20.9 %, and 18.2 % of 110 male OLP patients, and 14.2 %, 6.5 %, 27.2 %, 18.4 %, 11.1 %, 0.6 %, 21.3 %, and 24.9 % of 478 female OLP patients were diagnosed as having microcytosis, macrocytosis, blood Hb and serum iron, vitamin B12, and folic acid deficiencies, hyperhomocysteinemia, and serum GPCA positivity, respectively (Table 2). Moreover, 110 male OLP patients had significantly higher frequencies of microcytosis, blood Hb and serum iron and vitamin B12 deficiencies, hyperhomocysteinemia, and serum GPCA positivity than 110 male healthy control subjects (all *P*-values <0.05, Table 2). Furthermore, 478 female OLP patients had significantly higher frequencies of microcytosis, macrocytosis, blood Hb and serum iron and vitamin B12 deficiencies, hyperhomocysteinemia, and serum GPCA positivity than 478 female healthy control subjects (all *P*-values <0.001, Table 2). In addition, 110 male OLP patients had a significantly higher frequency of folic acid deficiency and significantly lower frequencies of blood Hb and serum iron deficiencies than 478 female OLP patients (all *P*-values <0.05, Table 2).

In this study, 18 (16.4 %) of 110 male and 130 (27.2 %) of 478 female OLP patients had anemia (defined as having an Hb concentration <13 g/dL for men and <12 g/dL for women).[31] Thus, the 478 female OLP patient had a significantly higher frequency of anemia than the 110 male OLP patients ($P = 0.025$, Table 3). Of the 18 anemic male OLP patients, two had pernicious anemia (PA, defined as having anemia, an MCV ≥100 fL, a serum vitamin B12 level <200 pg/mL, and the presence of serum GPCA positivity),[26–30,36] one had macrocytic anemia (defined as having anemia and an MCV ≥100 fL) other than PA,[26–30,36] 11 had normocytic anemia (defined as having anemia and an MCV between 80 fL and 99.9 fL),[37–40] one had iron deficiency anemia (IDA, defined as having anemia, an MCV <80 fL, and a serum iron level <60 μg/dL),[31,32] and three had thalassemia trait-induced anemia (defined as having anemia, a red blood cell count >5.0 M/μL, an MCV <74 fL, and a Mentzer index (MCV/RBC) < 13).[41] Thus, by the strict WHO criteria the normocytic anemia (61.1 %, 11/18) was the most common type of anemia in our18 anemic male OLP patients (Table 3).

Of the 130 anemic female OLP patients, 15 had PA,[26–30,36] 6 had macrocytic anemia other than PA,[26–30,36] 57 had normocytic anemia,[37–40] 31 had IDA,[31,32] 16 had thalassemia trait-induced anemia,[41] and 5 had microcytic anemia[23–25,33] other than IDA and thalassemia trait-induced anemia. Therefore, by the strict WHO criteria the normocytic anemia (43.9 %, 57/130), IDA (23.9 %, 31/130), and thalassemia trait-induced anemia (12.3 %, 16/130) were the three most common types of anemia in our 130

Table 2 Comparisons of frequencies of microcytosis (mean corpuscular volume or MCV <80 fL), macrocytosis (MCV ≥100 fL), blood hemoglobin (Hb) and serum iron, vitamin B12, and folic acid deficiencies, hyperhomocysteinemia, and gastric parietal cell antibody (GPCA) positivity between 110 male or 478 female OLP patients and 110 male or 478 female healthy control subjects, respectively, as well as between 110 male and 478 female OLP patients.

Group	Patient number (%)							
	Microcytosis (MCV <80 fL)	Macrocytosis (MCV ≥100 fL)	Hb deficiency (Men <13 g/dL, women <12 g/dL)	Iron deficiency (<60 μg/dL)	Vitamin B12 deficiency (<200 pg/mL)	Folic acid deficiency (<4 ng/mL)	Hyperhomocysteinemia (>12.0 μM)	GPCA positivity
Male OLP patients (n = 110)	10 (9.1)	5 (4.6)	18 (16.4)	11 (10.0)	7 (6.4)	4 (3.6)	23 (20.9)	20 (18.2)
[a]*P*-value	0.004	0.070	<0.001	0.002	0.021	0.130	<0.001	<0.001
[b]*P*-value	0.202	0.586	0.025	0.047	0.193	0.033	0.937	0.171
Female OLP patients (n = 478)	68 (14.2)	31 (6.5)	130 (27.2)	88 (18.4)	53 (11.1)	3 (0.6)	101 (21.3)	119 (24.9)
[a]*P*-value	<0.001	<0.001	<0.001	<0.001	<0.001	0.247	<0.001	<0.001
Male healthy control subjects (n = 110)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	5 (4.6)	2 (1.8)
Female healthy control subjects (n = 478)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	5 (1.1)	8 (1.7)

[a] Comparisons of frequencies of parameters between 110 male or 478 female OLP patients and 110 male or 478 female healthy control subjects by chi-square test, respectively.
[b] Comparisons of frequencies of parameters between 110 male and 478 female OLP patients by chi-square test.

Table 3 Comparison of frequencies of 6 different types of anemia between 110 male OLP patients and 478 female OLP patients.

Anemia type	Patient number (%)		[a]*P*-value (Chi-square test)
	Male OLP patients (n = 110)	Female OLP patients (n = 478)	
Pernicious anemia	2 (1.8)	15 (3.1)	0.668
Other macrocytic anemia	1 (0.9)	6 (1.3)	0.853
Normocytic anemia	11 (10.0)	57 (11.9)	0.686
Iron deficiency anemia	1 (0.9)	31 (6.5)	0.036
Thalassemia trait-induced anemia	3 (2.7)	16 (3.4)	0.974
Other microcytic anemia	0 (0.0)	5 (1.1)	0.616
Total anemic OLP patients	18 (16.4)	130 (27.2)	0.025

[a] Comparison of frequencies of 6 different types of anemia between 110 male OLP patients and 478 female OLP patients by chi-square test.

anemic female OLP patients (Table 3). In addition, 478 female OLP patients had a significantly higher frequency of IDA than 110 male OLP patients ($P = 0.036$, Table 3).

Discussion

This study found that the 478 female OLP patients had significantly lower mean blood Hb and serum iron levels and significantly higher frequencies of blood Hb and serum iron deficiencies than the 110 male OLP patients. The higher prevalence of anemia and serum iron deficiency in female than in male OLP patients could be explained as follows. First, the menstrual blood loss is an important factor causing a higher susceptibility to anemia in female than in male OLP patients. Each month, during their reproductive years, women experience menstrual bleeding, which results in a loss of iron and red blood cells (containing Hb). Although menstrual blood losses are highly variable, these blood losses range from 10 to 250 mL (containing 4—100 mg of iron) per period.[42] A man must absorb about 1 mg of iron daily to maintain equilibrium of the body iron. During childbearing years, an adult female loses an average of 2 mg of iron daily and must absorb a similar quantity of iron in order to maintain equilibrium of the body iron.[42] Therefore, this monthly blood loss can lead to IDA, one of the most common types of anemia, in the female OLP patients.

Second, the pregnancy is also a major factor leading to anemia and iron deficiency in the female OLP patients. Pregnant women have an increased demand for iron and other nutrients to support the growth and development of the fetus. If the mother's diet does not provide sufficient iron, she can develop IDA, which can affect both the female subject and the baby. In general, each healthy pregnancy depletes the mother of approximately 500 mg of iron.[42] In addition, the child-bearing women may experience significant blood loss during labor and delivery, further depleting their iron stores and resulting in IDA.

Third, the difference in sex hormone between men and women is another factor causing more anemia and iron deficiency in the female OLP patients. Sex hormones exert a substantial impact on the red blood cell counts, Hb levels, and serum iron levels.[42—48] Among these hormones, androgens play a role in promoting erythropoiesis and elevating the quantities of red blood cells, Hb, and hematocrit. Androgens stimulate the hematopoietic system through diverse mechanisms, such as stimulating the release of erythropoietin, enhancing bone marrow activity, and facilitating the incorporation of iron into red blood cells.[43] Erythropoietin, a hormone produced by the kidneys, plays a vital role in promoting the generation and upkeep of red blood cells.[45] However, it's noteworthy that in individuals with chronic mountain sickness (Monge's disease), estrogens have a pronounced and dose-dependent adverse impact on the erythropoietic response. When estrogen is added at concentrations of 5—10 nM (within the physiological range of 0.1—10 nM) to erythroid cells isolated from females with chronic mountain sickness, a significant reduction in red blood cell production occurs. Furthermore, the introduction of estrogen at concentrations of 50—100 nM results in a substantial decrease in red blood cell production.[46] In postmenopausal women, estrogen levels decline due to the cessation of ovarian functions, while iron levels increase because of a decrease in menstrual periods. Nevertheless, estrogen deficiency leads to an upregulation of hepcidin, which, in turn, inhibits the absorption of iron in the intestines, ultimately causing a decrease in serum iron levels.[47] Consequently, the variation in sex hormone levels between men and women can offer partial insight into why men typically exhibit higher levels of blood Hb and serum iron.

Fourth, another contributing factor to the higher prevalence of anemia and iron deficiency among female OLP patients is the difference in dietary habits compared to men. Women typically consume fewer calories than men, making it necessary for them to be over twice as efficient as men in iron absorption to prevent iron deficiency. As a result, anemia is twice as common in females compared to males.[48] This disparity becomes even more significant during the childbearing years due to the impact of pregnancies and menstrual cycles.[48] Furthermore, women often have a preference for diets rich in vegetables and fruits, driven by concerns about body weight. Since non-heme iron from plant-based sources is less readily absorbed by the body than heme iron from animal-based sources, this dietary choice leads to a higher incidence of iron deficiency and anemia in female OLP patients when compared to their male OLP counterparts.

We expanded upon the rationale for observing that 110 male OLP patients exhibited a lower mean in their serum vitamin B12 and folic acid levels, along with a higher occurrence of serum folic acid deficiency, when compared to 478 female OLP patients. A previous study demonstrated that there were notably reduced average folate levels in both buccal mucosal cells and sera in a group of 25 smokers as compared to a cohort of 34 non-smokers.[49] In another study, Pivathilake et al.[50] also confirmed the presence of lower concentrations of buccal mucosal cell folate and vitamin B12 in 39 current smokers in comparison to 60 individuals who were not current smokers. Our prior investigation of serum vitamin B12 and folic acid levels among oral precancer patients, we also identified a significant discrepancy. We observed that the mean serum folic acid levels were notably lower in 87 individuals who smoked cigarettes compared to the 44 non-smokers. Furthermore, within the group of smokers, those who consumed more than 20 cigarettes per day (26 individuals) exhibited lower mean serum folic acid levels than the 61 smokers who consumed 20 or fewer cigarettes per day.[51] Additionally, we noted a lower mean serum folic acid level in 52 oral precancer patients who chewed betel quid compared to the 79 non-chewers.[51] Altogether, the outcomes of these aforementioned studies underscore the presence of vitamin B12 and folic acid deficiencies in both the sera and oral mucosal cells of individuals who smoke and those who chew betel quids. We hypothesize that the mechanisms behind vitamin B12 and folic acid deficiencies may stem from an increased demand for these nutrients as a response to the accelerated tissue growth or repair caused by the irritation or damage inflicted on oral mucosal cells by carcinogens found in tobacco or betel quid.[52,53] The present study found that the 110 male OLP patients had significantly lower mean serum vitamin B12 and folic acid levels and a significantly higher frequency of folic acid deficiency than the 478 female OLP patients. Notably, our study did not include an evaluation of the prevalence of cigarette smoking and betel quid chewing habits among our 110 male and 478 female OLP patients. However, in the general population of Taiwan, males aged 18 and above had a significantly higher prevalence of smoking habits (23.1 % for men compared to 2.9 % for women) and betel quid chewing habits (16.8 % for men compared to 1.2 % for women).[54] This regional disparity indicates that the higher prevalence of smoking or betel quid chewing habits in men, as compared to women in Taiwan, may strongly contribute to the substantially lower mean serum vitamin B12 and folic acid levels and the notably increased frequency of serum folic acid deficiency observed in the 110 male OLP patients in contrast to the 478 female OLP patients.[54]

In this study, the normocytic anemia (61.1 %, 11/18) was the most common type of anemia in our 18 anemic male OLP patients, followed by the thalassemia trait-induced anemia (16.7 %, 3/18) and PA (11.1 %, 2/18) (Table 3). Furthermore, the normocytic anemia (43.9 %, 57/130), IDA (23.9 %, 31/130), and thalassemia trait-induced anemia (12.3 %, 16/130) were the three most common types of anemia in our 130 anemic female OLP patients (Table 3). From the anemia data of male and female OLP patients in Table 3, we discovered that regardless of the normocytic anemia, both the male and female OLP patients tended to have more patients with microcytic anemia (IDA, thalassemia trait-induced anemia, and other microcytic anemia) than patients with macrocytic anemia (PA and other macrocytic anemia). As stated before, our 478 female OLP patients had the significantly lower mean blood Hb and serum iron levels and significantly higher frequencies of blood Hb and serum iron deficiencies than 110 male OLP patients. These results could easily explain why the female OLP patients were prone to have microcytic anemia, especially the IDA, in this study.

Homocysteine is produced as a byproduct in the process of methionine metabolism.[55] Both vitamin B12 and folic acid serve as coenzymes essential for the conversion of homocysteine into methionine.[56] Consequently, individuals with deficiencies in either vitamin B12, folic acid, or both may experience elevated homocysteine levels, a condition known as hyperhomocysteinemia. A previous study has confirmed that the administration of folic acid and vitamins B12 and B6 can effectively lower blood homocysteine levels.[57] In our earlier research, we also provided evidence that supplementation with vitamin B complex and vitamin C capsules, combined with the corresponding deficient vitamin B12 and/or folic acid, is capable of reducing abnormally elevated serum homocysteine levels to a significantly lower range in patients afflicted with either AG or BMS.[58,59] In this study, significantly lower mean serum vitamin B12 and folic acid levels and a significantly higher frequency of serum folic acid deficiency in 110 male OLP patients than in the 478 female OLP patients could explain why our male OLP patients had notably higher mean serum homocysteine level than our 478 female OLP patients (Table 1, $P = 0.066$, marginal significance). However, our 478 female OLP patients had a relatively higher frequency of GPCA positivity (24.9 %) than the 110 male OLP patients (18.2 %) (Table 2). This finding can further explain why our male and female OLP patients had approximately equal frequencies of hyperhomocysteinemia (20.9 % for male and 21.3 % for female OLP patients) (Table 2).

The results of this study conclude that the male OLP patients do have significantly higher mean blood Hb and serum iron levels, significantly lower mean serum vitamin B12 and folic acid levels, a significantly higher frequency of folic acid deficiency, and significantly lower frequencies of blood Hb and serum iron deficiencies than female OLP patients.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

Acknowledgements

None.

References

1. Sugerman PB, Savage NW, Walsh LJ, et al. The pathogenesis of oral lichen planus. *Crit Rew Oral Biol Med* 2002;13:350—65.
2. Scully C, Beyli M, Ferreiro MC, et al. Update on oral lichen planus: etiopathogenesis and management. *Crit Rev Oral Biol Med* 1998;9:86—122.

3. Chiang CP, Chang JYF, Wang YP, Wu YH, Lu SY, Sun A. Oral lichen planus — differential diagnoses, serum autoantibodies, hematinic deficiencies, and management. *J Formos Med Assoc* 2018;117:756—65.
4. Wu YH, Wu YC, Chang JYF, Lee YP, Chiang CP, Sun A. Significantly higher frequencies of macrocytosis, anemia, serum vitamin B12 and folic acid deficiencies, and hyperhomocysteinemia in male than in female atrophic glossitis patients. *J Dent Sci* 2022;17:1371—7.
5. Wu YH, Jin YT, Wu YC, Chang JYF, Chiang CP, Sun A. Anemia, hematinic deficiencies, and hyperhomocysteinemia in male and female burning mouth syndrome patients. *J Dent Sci* 2022; 17:935—41.
6. Chen HM, Wang YP, Chang JYF, Wu YC, Cheng SJ, Sun A. Significant association of deficiencies of hemoglobin, iron, folic acid, and vitamin B12 and high homocysteine level with oral lichen planus. *J Formos Med Assoc* 2015;114:124—9.
7. Chang JYF, Chiang CP, Hsiao CK, Sun A. Significantly higher frequencies of presence of serum autoantibodies in Chinese patients with oral lichen planus. *J Oral Pathol Med* 2009;38: 48—54.
8. Chang JYF, Chen IC, Wang YP, Wu YH, Chen HM, Sun A. Anemia and hematinic deficiencies in gastric parietal cell antibody-positive and antibody-negative erosive oral lichen planus patients with thyroid antibody positivity. *J Formos Med Assoc* 2016;115:1004—11.
9. Chang JYF, Wang YP, Wu YH, Su YX, Tu YK, Sun A. Hematinic deficiencies and anemia statuses in anti-gastric parietal cell antibody-positive or all autoantibodies-negative erosive oral lichen planus patients. *J Formos Med Assoc* 2018;117:227—34.
10. Chiang CP, Wu YH, Wu YC, Chang JYF, Wang YP, Sun A. Anemia, hematinic deficiencies, hyperhomocysteinemia, and serum gastric parietal cell antibody positivity in 884 patients with burning mouth syndrome. *J Formos Med Assoc* 2020;119: 813—20.
11. Chiang CP, Wu YC, Wu YH, Chang JYF, Wang YP, Sun A. Gastric parietal cell and thyroid autoantibody in patients with burning mouth syndrome. *J Formos Med Assoc* 2020;119:1758—63.
12. Chiang ML, Jin YT, Chiang CP, Wu YH, Chang JYF, Sun A. Anemia, hematinic deficiencies, hyperhomocysteinemia, and gastric parietal cell antibody positivity in burning mouth syndrome patients with vitamin B12 deficiency. *J Dent Sci* 2020; 15:34—41.
13. Chiang ML, Chiang CP, Sun A. Anemia, hematinic deficiencies, and gastric parietal cell antibody positivity in burning mouth syndrome patients with or without hyperhomocysteinemia. *J Dent Sci* 2020;15:214—21.
14. Jin YT, Wu YH, Wu YC, Chang JYF, Chiang CP, Sun A. Anemia, hematinic deficiencies, and hyperhomocysteinemia in serum gastric parietal cell antibody-positive burning mouth syndrome patients without serum thyroid autoantibodies. *J Dent Sci* 2021;16:1110—6.
15. Wu YH, Jin YT, Wu YC, Chang JYF, Chiang CP, Sun A. Anemia, hematinic deficiencies, hyperhomocysteinemia, and gastric parietal cell antibody positivity in burning mouth syndrome patients with normocytosis. *J Dent Sci* 2022;17:35—41.
16. Jin YT, Wu YH, Wu YC, Chang JYF, Chiang CP, Sun A. Higher gastric parietal cell antibody titer significantly increases the frequencies of macrocytosis, serum vitamin B12 deficiency, and hyperhomocysteinemia in patients with burning mouth syndrome. *J Dent Sci* 2022;17:57—62.
17. Jin YT, Wu YC, Wu YH, Chang JYF, Chiang CP, Sun A. Anemia, hematinic deficiencies, and hyperhomocysteinemia in burning mouth syndrome patients with thyroglobulin antibody/thyroid microsomal antibody positivity but without gastric parietal cell antibody positivity. *J Dent Sci* 2022;17:106—12.
18. Wu YH, Wu YC, Chang JYF, Lang MJ, Chiang CP, Sun A. Anemia, hematinic deficiencies, and hyperhomocysteinemia in younger and older burning mouth syndrome patients. *J Dent Sci* 2022; 17:1144—50.
19. Wu YH, Wu YC, Chang JYF, Lee YP, Chiang CP, Sun A. Significantly lower mean serum vitamin B12 and folic acid levels and a significantly higher frequency of serum iron deficiency in younger than in older atrophic glossitis patients. *J Dent Sci* 2022;17:1487—93.
20. Wu YH, Lee YP, Chang JYF, Wang YP, Chiang CP, Sun A. Higher frequencies of anemia, hematinic deficiencies, and gastric parietal cell antibody positivity in vitamin B12-deficient Taiwanese male oral submucous fibrosis patients. *J Dent Sci* 2023;18:367—73.
21. Wu YH, Lee YP, Chang JYF, Wang YP, Chiang CP, Sun A. High frequencies of vitamin B12 and folic acid deficiencies and hyperhomocysteinemia in Taiwanese male patients with oral submucous fibrosis. *J Dent Sci* 2023;18:407—13.
22. Wu YH, Lee YP, Chang JYF, Wang YP, Chiang CP, Sun A. Higher frequencies of anemia, vitamin B12 deficiency, and gastric parietal cell antibody positivity in folic acid-deficient Taiwanese male oral submucous fibrosis patients. *J Dent Sci* 2023;18: 801—7.
23. Chiang CP, Chang JYF, Wang YP, Wu YC, Wu YH, Sun A. Anemia, hematinic deficiencies, hyperhomocysteinemia, and serum gastric parietal cell antibody positivity in atrophic glossitis patients with or without microcytosis. *J Formos Med Assoc* 2019;118:1401—7.
24. Jin YT, Wu YC, Wu YH, Chang JYF, Chiang CP, Sun A. Anemia, hematinic deficiencies, hyperhomocysteinemia, and gastric parietal cell antibody positivity in burning mouth syndrome patients with or without microcytosis. *J Dent Sci* 2021;16: 608—13.
25. Lin HP, Wu YH, Wang YP, Wu YC, Chang JYF, Sun A. Anemia and hematinic deficiencies in gastric parietal cell antibody-positive and —negative oral mucosal disease patients with microcytosis. *J Formos Med Assoc* 2017;116:613—9.
26. Jin YT, Wu YH, Wu YC, Chang JYF, Chiang CP, Sun A. Anemia, hematinic deficiencies, hyperhomocysteinemia, and gastric parietal cell antibody positivity in burning mouth syndrome patients with macrocytosis. *J Dent Sci* 2021;16:1133—9.
27. Chiang CP, Wu YH, Chang JYF, Wang YP, Wu YC, Sun A. Hematinic deficiencies, hyperhomocysteinemia, and gastric parietal cell antibody positivity in atrophic glossitis patients with macrocytosis. *J Formos Med Assoc* 2019;118:1515—21.
28. Sun A, Wang YP, Lin HP, Jia JS, Chiang CP. Do all the patients with gastric parietal cell antibodies have pernicious anemia? *Oral Dis* 2013;19:381—6.
29. Sun A, Chang JYF, Wang YP, Cheng SJ, Chen HM, Chiang CP. Do all the patients with vitamin B12 deficiency have pernicious anemia? *J Oral Pathol Med* 2016;45:23—7.
30. Chang JYF, Wang YP, Wu YC, Cheng SJ, Chen HM, Sun A. Hematinic deficiencies and pernicious anemia in oral mucosal disease patients with macrocytosis. *J Formos Med Assoc* 2015; 114:736—41.
31. WHO/UNICEF/UNU. *Iron deficiency anemia assessment, prevention, and control: a guide for programme managers*. Geneva, Switzerland: World Health Organization, 2001.
32. Wu YC, Wang YP, Chang JYF, Cheng SJ, Chen HM, Sun A. Oral manifestations and blood profile in patients with iron deficiency anemia. *J Formos Med Assoc* 2014;113:83—7.
33. Shine JW. Microcytic anemia. *Am Fam Physician* 1997;55: 2455—62.
34. Morris MS, Jacques PF, Rosenberg IH, Selhub J. Folate and vitamin B-12 status in relation to anemia, macrocytosis, and cognitive impairment in older Americans in the age of folic acid fortification. *Am J Clin Nutr* 2007;85:193—200.
35. de Benoist B. Conclusions of a WHO technical consultation on folate and vitamin B12 deficiencies. *Food Nutr Bull* 2008; 29(suppl):S238—44.

36. Lahner E, Annibale B. Pernicious anemia: new insights from a gastroenterological point of view. *World J Gastroenterol* 2009;15:5121—8.
37. Brill JR, Baumgardner DJ. Normocytic anemia. *Am Fam Physician* 2000;62:2255—63.
38. Koury MJ, Rhodes M. How to approach chronic anemia. *Hematology Am Soc Hematol Educ Program* 2012;2012:183—90.
39. Koury MJ. Abnormal erythropoiesis and the pathophysiology of chronic anemia. *Blood Rev* 2014;28:49—66.
40. Means Jr RT. Pathogenesis of the anemia of chronic disease: a cytokine-mediated anemia. *Stem Cell* 1995;13:32—7.
41. Wang YP, Chang JYF, Wu YC, Cheng SJ, Chen HM, Sun A. Oral manifestations and blood profile in patients with thalassemia trait. *J Formos Med Assoc* 2013;112:761—5.
42. Medscape. *Why is iron deficiency anemia more common in women than in men?*. Available at: https://www.medscape.com/answers/202333-153111/why-is-iron-deficiency-anemia-more-common-in-women-than-in-men. [Accessed 12 November 2021].
43. Shahani S, Braga-Basaria M, Maggio M, Basaria S. Androgens and erythropoiesis: past and present. *J Endocrinol Invest* 2009;32:704—16.
44. Calado RT, Yewdell WT, Wilkerson KL, et al. Sex hormones, acting on the TERT gene, increase telomerase activity in human primary hematopoietic cells. *Blood* 2009;114:2236—43.
45. Spivak JL. The mechanism of action of erythropoietin. *Int J Cell Clon* 1986;4:139—66.
46. Azad P, Villafuerte FC, Bermudez D, Patel G, Haddad GG. Protective role of estrogen against excessive erythrocytosis in Monge's disease. *Exp Mol Med* 2021;53:125—35.
47. Yang Q, Jian J, Katz S, Abramson SB, Huang X. 17β-Estradiol inhibits iron hormone hepcidin through an estrogen responsive element half-site. *Endocrinology* 2012;153:3170—8.
48. Medscape. *How does the prevalence of anemia vary between males and females?*. Available at: https://www.medscape.com/answers/198475-155034/how-does-the-prevalence-of-anemia-vary-between-males-and-females. [Accessed 12 November 2021].
49. Piyathilake CJ, Hine RJ, Dasanayake AP, et al. Effect of smoking on folate levels in buccal mucosal cells. *Int J Cancer* 1992;52:566—9.
50. Pivathilake CJ, Macaluso M, Hine RJ, Richards EW, Krumdieck CL. Local and systemic effects of cigarette smoking on folate and vitamin B-12. *Am J Clin Nutr* 1994;60:559—66.
51. Wu YH, Wu YC, Chu FY, Cheng SJ, Sun A, Chen HM. Significantly higher frequencies of hematinic deficiencies and hyperhomocysteinemia in oral precancer patients. *J Formos Med Assoc* 2019;118:1299—307.
52. Hecht SS. Tobacco smoke carcinogens and lung cancer. *J Natl Cancer Inst* 1999;91:1194—210.
53. Li WC, Lee PL, Chou IC, Chang WJ, Lin SC, Chang KW. Molecular and cellular cues of diet-associated oral carcinogenesis—with an emphasis on areca-nut-induced oral cancer development. *J Oral Pathol Med* 2015;44:167—77.
54. Health Promotion Administration, Ministry of Health and Welfare. *The prevalences of smoking, betel quid chewing, and alcohol drinking habits in the population equal to or greater than 18 years old*. Taipei, Taiwan: Ministry of Health and Welfare, 2021.
55. Spence JD. Homocysteine-lowering therapy: a role in stroke prevention? *Lancet Neurol* 2007;6:830—8.
56. Chanarin I, Deacon R, Lumb M, Perry J. Cobalamin-folate interrelations. *Blood Rev* 1989;3:211—5.
57. Lonn E, Yusuf S, Arnold MJ, et al. Homocysteine lowering with folic acid and B vitamins in vascular disease. *N Engl J Med* 2006;354:1567—77.
58. Sun A, Wang YP, Lin HP, Chen HM, Cheng SJ, Chiang CP. Significant reduction of homocysteine level with multiple B vitamins in atrophic glossitis patients. *Oral Dis* 2013;19:519—24.
59. Sun A, Lin HP, Wang YP, Chen HM, Cheng SJ, Chiang CP. Significant reduction of serum homocysteine level and oral symptoms after different vitamin supplement treatments in patients with burning mouth syndrome. *J Oral Pathol Med* 2013;42:474—9.