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Original Article

Anemia, hematinic deficiencies, hyperhomocysteinemia, and serum gastric parietal cell antibody positivity in oral lichen planus patients with vitamin B12 deficiency

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Abstract *Background/purpose:* Our previous study found that 60 of 588 oral lichen planus (OLP) patients have vitamin B12 deficiency. This study assessed whether the vitamin B12-deficient OLP (B12D/OLP) patients had significantly higher frequencies of anemia, hematinic deficiencies, hyperhomocysteinemia, and serum gastric parietal cell antibody (GPCA) positivity than healthy control subjects and evaluated whether all B12D/OLP patients had pernicious anemia (PA).
Materials and methods: The blood hemoglobin (Hb) and serum iron, vitamin B12, folic acid, homocysteine, and GPCA levels in 60 B12D/OLP patients and 588 healthy control subjects were measured and compared.

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Results: We found that 60 B12D/OLP patients had significantly lower mean blood Hb and serum iron, vitamin B12, and folic acid levels as well as significantly higher mean corpuscular volume (MCV) and mean serum homocysteine level than 588 healthy control subjects (all *P*-values <0.01). Moreover, 60 B12D/OLP patients had significantly higher frequencies of macrocytosis (55.0 %), blood Hb (68.3 %) and serum iron (31.7 %) and vitamin B12 (100.0 %) deficiencies, hyperhomocysteinemia (91.7 %), and serum GPCA positivity (66.7 %) than 588 healthy control subjects (all *P*-values <0.001). The four most common types of anemia in 41 anemic B12D/OLP patients were PA (17 patients, 41.5 %), normocytic anemia (12 patients, 29.3 %), iron deficiency anemia (6 patients, 14.6 %), and macrocytic anemia other than PA (5 patients, 12.2 %).
Conclusion: The B12D/OLP patients have significantly higher frequencies of macrocytosis, blood Hb and serum iron and vitamin B12 deficiencies, hyperhomocysteinemia, and serum GPCA positivity than healthy control subjects. Only 17 (28.3 %) of 60 B12D/OLP patients have PA.
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Introduction

Oral lichen planus (OLP) is a chronic inflammatory oral mucosal disease. It predominantly affects the middle-aged and elderly women, with a prevalence of 0.2–3% in the population.[1–4] The buccal mucosa, gingiva, and tongue are the three most commonly affected oral sites. OLP typically shows bilateral and symmetrical oral mucosal lesions.[3,4] Clinically, OLP can present in six forms: reticular, papular, plaque-like, erosive/atrophic, ulcerative, and bullous.[2–4]

Our previous study found that 60 (10.2 %) of 588 OLP patients have vitamin B12 deficiency.[4] The etiologies of vitamin B12 deficiency include inadequate intake, food-bound vitamin B12 malabsorption, the presence of gastric parietal cell antibody (GPCA) or intrinsic factor autoantibody or both in sera of patients, ileal malabsorption in patients with enteritis or ileal resection, biologic competition including bacterial overgrowth and tapeworm infestation, and defective transport such as transcobalamin II deficiency.[5] The GPCA can destroy gastric parietal cells, resulting in lack of intrinsic factors and hypochlorhydria.[6] Intrinsic factor deficiency may lead to malabsorption of vitamin B12 from terminal ileum and finally the vitamin B12 deficiency.[5–9] Vitamin B12 and/or folic acid deficiencies may lead to macrocytic anemia or hyperhomocysteinemia in OLP patients.[10–13] In addition, decreased gastric secretion of hydrochloric acid may cause iron malabsorption and subsequent iron deficiency.[14–16] Thus, it is interesting to know whether the OLP patients with vitamin B12 deficiency (so-called B12D/OLP patients in this study) are prone to have significantly higher frequencies of anemia, serum iron, vitamin B12, and folic acid deficiencies, hyperhomocysteinemia, and serum GPCA positivity than healthy control subjects.

In our oral mucosal disease clinic, the patients with OLP, burning mouth syndrome (BMS), atrophic glossitis (AG), recurrent aphthous stomatitis (RAS), oral submucous fibrosis, or oral precancerous lesions are frequently encountered.[4,17–48] For patients with one of these six specific diseases, complete blood count, serum iron, vitamin B12, folic acid, homocysteine, GPCA, thyroglobulin antibody, and thyroid microsomal antibody (also called anti-thyroid peroxidase antibody or anti-TPO antibody) levels are frequently examined to assess whether these patients have anemia, hematinic deficiencies, hyperhomocysteinemia, and serum GPCA, thyroglobulin antibody, and thyroid microsomal antibody positivities.[4,17–48]

In this study, 60 B12D/OLP patients were retrieved from our previous study.[4] We tried to assess whether these 60 B12D/OLP patients had significantly higher frequencies of anemia, serum iron, vitamin B12, and folic acid deficiencies, hyperhomocysteinemia, and serum GPCA positivity than 588 healthy control subjects and evaluated whether all B12D/OLP patients had pernicious anemia (PA).

Materials and methods

Subjects

This study consisted of 60 (7 men and 53 women, age range 25–77 years, mean age 59.3 ± 12.5 years) B12D/OLP patients selected from 588 OLP patients in our previous study.[4] For each OLP patient, one healthy control subject, matched by age (within ±2 years) and sex, was chosen. Thus, 588 age- and sex-matched healthy control subjects (110 men and 478 women, aged 20–89 years, with a mean age of 56.0 ± 14.1 years) were included in this study.[4] All OLP patients and healthy control subjects were consecutively seen, diagnosed, and treated at the Department of Dentistry, National Taiwan University Hospital (NTUH) from July 2007 to June 2023. The 588 OLP patients were selected based on the following criteria: (i) the presence of characteristic clinical features, such as radiating grayish-white Wickham striae, papules, plaques, either individually or in combination, and erosion or ulceration on the oral mucosa; (ii) a bilateral and symmetric distribution of OLP lesions on the oral mucosa. For 25 patients with an uncertain clinical diagnosis of OLP, an incisional biopsy of the typical oral mucosal lesion was performed. The histopathological diagnosis of OLP was confirmed using the criteria described previously.[4,17–20] However, OLP patients with a history of betel quid chewing, heavy alcohol use, autoimmune diseases (such as systemic lupus erythematosus, rheumatoid

arthritis, Sjogren's syndrome, pemphigus vulgaris, or cicatricial pemphigoid), inflammatory diseases, malignancy, recent surgery, or systemic conditions like liver, kidney, coronary artery, or stroke diseases were excluded from this study.[17–20] The healthy control subjects were individuals without any oral mucosal or systemic illnesses and with no more than dental caries or mild periodontal disease. None of the OLP patients had received medication for OLP for at least three months before participating in the study.

Blood samples were collected from 60 B12D/OLP patients, 528 non-B12D/OLP patients, and 588 healthy control subjects to measure complete blood count, serum iron, vitamin B12, folic acid, homocysteine levels, and serum GPCA positivity. All OLP patients and healthy control subjects provided informed consent before participating in the study. The 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, as well as serum iron, vitamin B12, folic acid, and homocysteine concentrations, were measured using routine tests conducted in the Department of Laboratory Medicine at the NTUH.[17–48]

Determination of serum gastric parietal cell antibody level

The serum GPCA level was measured using the indirect immunofluorescence technique, with the rat stomach serving as the substrate, as previously described.[17–48] Sera were considered positive if they exhibited fluorescence at a dilution of 1:10 or more.

Statistical analysis

The mean corpuscular volume (MCV) and mean blood hemoglobin (Hb) and serum iron, vitamin B12, folic acid, and homocysteine levels were compared between any two of three groups of 60 B12D/OLP patients, 528 non-B12D/OLP patients, and 588 healthy control subjects by Student's *t*-test. The differences in frequencies of microcytosis (defined as MCV $<$80 fL),[14–16,41–43] macrocytosis (defined as MCV $\geq$100 fL),[44–48] blood Hb and serum iron, vitamin B12, and folic acid deficiencies, hyperhomocysteinemia, and serum GPCA positivity were compared between any two of three groups of 60 B12D/OLP patients, 528 non-B12D/OLP patients, and 588 healthy control subjects by chi-square test. Moreover, comparisons of frequencies of macrocytic, normocytic or microcytic anemia between 60 B12D/OLP patients and 528 non-B12D/OLP patients were also performed by chi-square test. The result was considered to be significant if the *P*-value was less than 0.05.

Results

Comparisons of MCV and mean blood Hb and serum iron, vitamin B12, folic acid, and homocysteine levels between any two of three groups of 60 B12D/OLP patients, 528 non-B12D/OLP patients, and 588 healthy control subjects are shown in Table 1. Because men usually had higher blood levels of Hb and iron than women, these two mean levels were calculated separately for men and women. We found significantly lower mean blood Hb (for men and women) and serum iron (for men and women), vitamin B12, and folic acid levels as well as significantly higher MCV and mean serum homocysteine level in 60 B12D/OLP patients than in 588 healthy control subjects (all *P*-values $<$0.01, Table 1).

Moreover, we discovered significantly lower MCV and lower mean blood Hb (for men and women) and serum iron (for women only), vitamin B12, and folic acid levels as well as significantly higher mean serum homocysteine level in the 528 non-B12D/OLP patients than in 588 healthy control subjects (all *P*-values $<$0.01, Table 1). Furthermore, 60 B12D/OLP patients also had significantly lower mean blood Hb (for men and women) and serum iron (for men and women) and vitamin B12 levels as well as significantly higher MCV and mean serum homocysteine level than those in 528 non-B12D/OLP patients (all *P*-values $<$0.05, Table 1).

Table 1 Comparisons of mean corpuscular volume (MCV) and mean blood hemoglobin (Hb) and serum iron, vitamin B12, folic acid, and homocysteine levels between any two of three groups of 60 oral lichen planus (OLP) patients with vitamin B12 deficiency (B12D/OLP patients), 528 non-B12D/OLP patients, and 588 healthy control subjects.

Group	MCV (fL)	Hb (g/dL)		Iron (μg/dL)		Vitamin B12 (pg/mL)	Folic acid (ng/mL)	Homocysteine (μM)
		Men	Women	Men	Women			
B12D/OLP patients (n = 60)	95.2 ± 10.7	13.3 ± 1.3 (n = 7)	11.7 ± 1.2 (n = 53)	66.6 ± 28.8 (n = 7)	74.7 ± 26.0 (n = 53)	176.5 ± 17.7	12.4 ± 5.8	15.4 ± 3.5
[a]*P*-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.004	<0.001
[b]*P*-value	<0.001	0.028	<0.001	0.015	0.039	<0.001	0.725	<0.001
Non-B12D/OLP patients (n = 528)	87.6 ± 7.9	14.6 ± 1.5 (n = 103)	12.9 ± 1.3 (n = 425)	101.3 ± 36.4 (n = 103)	83.8 ± 30.6 (n = 425)	617.9 ± 233.7	12.7 ± 6.3	8.6 ± 2.9
[a]*P*-value	<0.001	<0.001	<0.001	0.604	<0.001	<0.001	<0.001	0.006
Healthy control subjects (n = 588)	90.4 ± 3.8	15.2 ± 0.8 (n = 110)	13.5 ± 0.8 (n = 478)	103.6 ± 27.9 (n = 110)	96.7 ± 27.4 (n = 478)	682.6 ± 231.7	14.7 ± 5.8	8.2 ± 1.9

[a] Comparisons of means of parameters between 60 B12D/OLP patients or 528 non-B12D/OLP patients and 588 healthy control subjects by Student's *t*-test.

[b] Comparisons of means of parameters between 60 B12D/OLP patients and 528 non-B12D/OLP patients by Student's *t*-test.

According to the World Health Organization (WHO) criteria, microcytosis of erythrocyte was defined as having MCV <80 fL,[14–16,41–43] macrocytosis of erythrocyte was defined as having MCV ≥100 fL,[44–48] and men with Hb < 13 g/dL and women with Hb < 12 g/dL were defined as having Hb deficiency or anemia.[49] Furthermore, patients with the serum iron level <60 μg/dL,[50] the serum vitamin B12 level <200 pg/mL,[51] or the serum folic acid level <4 ng/mL[52] were defined as having 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, 13.3 %, 55.0 %, 68.3 %, 31.7 %, 100.0 %, 1.7 %, 91.7 % and 66.7 % of 60 B12D/OLP patients, and 13.3 %, 0.6 %, 20.3 %, 15.2 %, 0.0 %, 1.1 %, 13.1 % and 18.8 % of 528 non-B12D/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. We found that 60 B12D/OLP patients had significantly higher frequencies of microcytosis, macrocytosis, blood Hb and serum iron, and vitamin B12 deficiencies, hyperhomocysteinemia, and serum GPCA positivity than 588 healthy control subjects (all *P*-values <0.001, Table 2). We also discovered that 528 non-B12D/OLP patients had significantly higher frequencies of microcytosis, blood Hb and serum iron and folic acid deficiencies, hyperhomocysteinemia, and serum GPCA positivity than 588 healthy control subjects (all *P*-values <0.001 except $P = 0.029$ for folic acid deficiency, Table 2). In addition, 60 B12D/OLP patients also had significantly higher frequencies of macrocytosis and blood Hb and serum iron and vitamin B12 deficiencies, hyperhomocysteinemia, and serum GPCA positivity than those in 528 non-B12D/OLP patients (all *P*-values <0.001, Table 2).

We also found that 41 (68.3 %) of 60 B12D/OLP patients had anemia (defined as having an Hb concentration <13 g/dL for men and <12 g/dL for women).[49] Of the 41 anemic B12D/OLP patients, 17 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),[46–48] 5 had macrocytic anemia other than PA (defined as having anemia and an MCV ≥100 fL), 12 had normocytic anemia (NA, defined as having anemia and an MCV between 80.0 fL and 99.9 fL),[53–56] 6 had iron deficiency anemia (IDA, defined as having anemia, an MCV <80 fL, and a serum iron level <60 μg/dL),[14–16,49,50] and one had microcytic anemia other than IDA and thalassemia trait-induced anemia (defined as having anemia, a RBC count >5.0 M/μL, an MCV <74 fL, and a Mentzer index (MCV/RBC) < 13)[57] (Table 3). Thus, only 17 (41.5 %) of 41 anemic B12D/OLP patients or 17 (28.3 %) of 60 B12D/OLP patients had PA. In addition, 12 (29.3 %) of the 41 anemic B12D/OLP patients had NA.

Of the 107 anemic non-B12D/OLP patients, 2 had macrocytic anemia (defined as having anemia and an MCV ≥100 fL) other than PA, 56 had NA,[53–56] 26 had IDA,[14–16,49,50] 19 had thalassemia trait-induced anemia,[57] and 4 had microcytic anemia (defined as having anemia and an MCV <80 fL)[14–16,41–43] other than IDA and thalassemia trait-induced anemia. These findings indicate that by

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 any two of the three groups of 60 oral lichen planus (OLP) patients with vitamin B12 deficiency (B12D/OLP patients), 528 non-B12D/OLP patients, and 588 healthy control subjects.

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
B12D/OLP patients (n = 60)	8 (13.3)	33 (55.0)	41 (68.3)	19 (31.7)	60 (100.0)	1 (1.7)	55 (91.7)	40 (66.7)
[a]*P*-value	<0.001	<0.001	<0.001	<0.001	<0.001	0.160	<0.001	<0.001
[b]*P*-value	0.854	<0.001	<0.001	<0.001	<0.001	0.788	<0.001	<0.001
Non-B12D/OLP patients (n = 528)	70 (13.3)	3 (0.6)	107 (20.3)	80 (15.2)	0 (0.0)	6 (1.1)	69 (13.1)	99 (18.8)
[a]*P*-value	<0.001	0.211	<0.001	<0.001	ND	0.029	<0.001	<0.001
Healthy control subjects (n = 588)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	10 (1.7)	10 (1.7)

ND = not done.
[a] Comparisons of means of parameters between 60 B12D/OLP patients or 528 non-B12D/OLP patients and 588 healthy control subjects by chi-square test.
[b] Comparisons of means of parameters between 60 B12D/OLP patients and 528 non-B12D/OLP patients by chi-square test.

Table 3 Anemia types, iron and folic acid deficiencies, hyperhomocysteinemia, and gastric parietal cell antibody (GPCA) positivity in 41 anemic oral lichen planus (OLP) patients with vitamin B12 deficiency (B12D/OLP patients) and in 107 anemic non-B12D/OLP patients.

Anemia type	Patient number (%)					
	Patient number (%)	Mean corpuscular volume (fL)	Iron deficiency (<60 μg/dL)	Folic acid deficiency (<4 ng/mL)	Hyperhomo-cysteinemia (>12.0 μM)	GPCA positivity
B12D/OLP patients (n = 60)						
Pernicious anemia	17 (41.5)	≥100	2 (11.8)	0 (0.0)	17 (100.0)	17 (100.0)
Other macrocytic anemia	5 (12.2)	≥100	0 (0.0)	0 (0.0)	5 (100.0)	0 (0.0)
Normocytic anemia	12 (29.3)	80.0–99.9	8 (66.7)	1 (8.3)	10 (83.3)	3 (25.0)
Iron deficiency anemia	6 (14.6)	<80	6 (100.0)	0 (0.0)	4 (66.7)	5 (83.3)
Other microcytic anemia	1 (2.4)	<80	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)
Total	41 (100.0)		16 (39.0)	1 (2.4)	37 (90.2)	25 (61.0)
Non-B12D/OLP patients (n = 528)						
Other macrocytic anemia	2 (1.9)	≥100	0 (0.0)	0 (0.0)	2 (100.0)	2 (100.0)
Normocytic anemia	56 (52.3)	80–99.9	22 (39.3)	1 (1.8)	12 (21.4)	9 (16.1)
Iron deficiency anemia	26 (24.3)	<80	26 (100.0)	0 (0.0)	6 (23.1)	4 (15.4)
Thalassemia trait-induced anemia	19 (17.8)	<74	0 (0.0)	0 (0.0)	1 (5.3)	1 (5.3)
Other microcytic anemia	4 (3.7)	<80	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total	107 (100.0)		48 (44.9)	1 (0.9)	21 (19.6)	16 (15.0)

Table 4 Comparisons of frequencies of macrocytic, normocytic or microcytic anemia between 60 oral lichen planus (OLP) patients with vitamin B12 deficiency (B12D/OLP patients) and 528 non-B12D/OLP patients.

Anemia type	Patient number (%)		[a]*P*-value
	B12D/OLP patients (n = 60)	Non-B12D/OLP patients (n = 528)	
Macrocytic anemia	22 (36.7)	2 (0.4)	<0.001
Normocytic anemia	12 (20.0)	56 (10.6)	0.052
Microcytic anemia	7 (11.7)	49 (9.3)	0.715
Total	41 (68.3)	107 (20.3)	<0.001

[a] Comparison of frequencies of macrocytic, normocytic or microcytic anemia between 60 B12D/OLP patients and 528 Non-B12D/OLP patients by chi-square test.

the strict WHO criteria, the NA (52.3 %), IDA (24.3 %), and thalassemia trait-induced anemia (17.8 %) are the three most common types of anemia in our 107 anemic non-B12D/OLP patients (Table 3).

Comparisons of frequencies of macrocytic, normocytic or microcytic anemia between 60 B12D/OLP patients and 528 non-B12D/OLP patients are shown in Table 4. By the overall evaluation, 60 B12D/OLP patients had significantly higher frequencies of anemia (68.3 % or 41/60) than 528 non-B12D/OLP patients (20.3 % or 107/528; $P < 0.001$, Table 4). We also found that 60 B12D/OLP patients had significantly higher frequencies of macrocytic anemia (36.7 %) than 528 non-B12D/OLP patients (0.4 %, $P < 0.001$, Table 4).

Discussion

The major finding of this study was that 60 B12D/OLP patients had significantly higher frequencies of macrocytosis (55.0 %), blood Hb (68.3 %) and serum iron (31.7 %) and vitamin B12 (100.0 %) deficiencies, hyperhomocysteinemia (91.7 %), and serum GPCA positivity (66.7 %) than 588 healthy control subjects (all *P*-values <0.001). Our previous study demonstrated that 42 vitamin B12-deficient BMS (B12D/BMS) patients had significantly higher frequencies of macrocytosis (52.4 %), blood Hb (61.9 %) and serum iron (26.2 %) deficiencies, hyperhomocysteinemia (83.3 %), and serum GPCA positivity (42.9 %) than 442 healthy control subjects (all *P*-values <0.001).[23] Moreover, 56 vitamin B12-deficient AG (B12D/AG) patients had significantly higher frequencies of macrocytosis (53.6 %), blood Hb (64.3 %) and serum iron (26.8 %) deficiencies, hyperhomocysteinemia (89.3 %), and serum GPCA positivity (55.4 %) than 532 healthy control subjects (all *P*-values <0.005).[31] Furthermore, 32 vitamin B12-deficient RAS (B12D/RAS) patients had significantly higher frequencies of macrocytosis (37.5 %), blood Hb (62.5 %) and serum iron (25.0 %) deficiencies, hyperhomocysteinemia (78.1 %), and serum GPCA positivity (34.4 %) than 355 healthy control subjects (all *P*-values <0.001).[34] In addition, our previous study also found that 90 oral mucosal disease patients (including 34

with AG only, 21 with BMS only, 6 with OLP only, 15 with both OLP and AG, 9 with both RAS and AG, and 5 with both RAS and BMS) with vitamin B12 deficiency had significantly higher frequencies of macrocytosis (41.1 %), blood Hb (38.9 %) and serum iron (22.2 %) deficiencies, hyperhomocysteinemia (72.2 %), and serum GPCA positivity (47.8 %) than 180 healthy control subjects (all *P*-values <0.001).[47] These findings indicate that the frequencies of vitamin B12 deficiency-related macrocytosis, anemia, hyperhomocysteinemia, and serum GPCA positivity are slightly higher in OLP patients than in AG or BMS patients, and are relatively higher in OLP patients than in RAS patients or a mixed population of oral mucosal disease patients.[23,31,34,47]

Severe vitamin B12 deficiency can result in macrocytosis of erythrocytes.[44–48] Macrocytosis was found in 33 (55.0 %) of 60 B12D/OLP patients in the present study as well as in 22 (52.4 %) of 42 B12D/BMS patients,[23] in 30 (53.6 %) of 56 B12D/AG patients,[31] in 12 (37.5 %) of 32 B12D/RAS patients,[34] and in 37 (41.1 %) of 90 vitamin B12-deficient oral mucosal disease patients in our previous studies.[47] Based on the above findings, macrocytosis is detected in 37.5 %–55.0 % of these vitamin B12-deficient OLP, BMS, AG, and RAS patients.[23,31,34,47] Because both vitamin B12 and folic acid are involved in DNA synthesis, in patients with vitamin B12 or folic acid deficiency, decreased deoxynucleotide synthesis impairs S-phase progression of erythroblasts. Inhibition of erythroblast DNA replication can retard cell division, but it does not affect protein (Hb predominantly) synthesis rates, and therefore the relative sizes of the erythroblasts and their daughter cells increase.[55] This can explain why vitamin B12 deficiency results in macrocytosis of erythrocytes in oral mucosal disease patients (including OLP, BMS, AG, and RAS patients) with vitamin B12 deficiency.

The B12D/OLP patients are supposed to have macrocytic anemia or PA, because severe vitamin B12 deficiency can lead to macrocytosis or PA.[44–48] The present study found that 22 (36.7 %) of 60 B12D/OLP patients have macrocytic anemia, including 17 (28.3 %) with PA and 5 (8.3 %) with macrocytic anemia other than PA. Our previous studies discovered that 20 (47.6 %) of 42 B12D/BMS patients have macrocytic anemia, including 15 (35.7 %) with PA and 5 (11.9 %) with macrocytic anemia other than PA.[23] Our previous studies also showed PA in 22 (39.3 %) of 56 B12D/AG patients,[31] in 4 (12.5 %) of 32 B12D/RAS patients,[34] and in 17 (18.9 %) of 90 vitamin B12-deficient oral mucosal disease patients.[47] Taken these findings together, for overall OLP, BMS, AG, and RAS patients, PA is detected in 12.5 %–39.3 % of these vitamin B12-deficient patients.[23,31,34,47]

Homocysteine is formed during methionine metabolism.[11] Both vitamin B12 and folic acid act as coenzymes for the conversion of homocysteine to methionine.[12] Moreover, vitamin B6 is a coenzyme for the conversion of homocysteine to cysteine.[12] Therefore, patients with vitamin B12, folic acid, and/or vitamin B6 deficiencies may have hyperhomocysteinemia.[11–13] This study demonstrated hyperhomocysteinemia in 55 (91.7 %) of 60 B12D/OLP patients. Our previous study also showed hyperhomocysteinemia in 35 (83.3 %) of 42 B12D/BMS patients,[23] in 50 (89.3 %) of 56 B12D/AG patients,[31] in 25 (78.1 %) of 32B12D/RAS patients,[34] and in 65 (72.2 %) of 90 vitamin B12-deficient oral mucosal disease patients.[47] However, folic acid deficiency was detected in 1 (1.7 %) of 60 B12D/OLP patients, in 0 (0.0 %) of 42 B12D/BMS patients,[23] in 2 (3.6 %) of 56 B12D/AG patients.[31] in 0 (0.0 %) of 32 B12D/RAS patients,[34] and in 0 (0.0 %) of 90 vitamin B12-deficient oral mucosal disease patients.[47] These findings suggest that vitamin B12 deficiency plays a major role in causing hyperhomocysteinemia in 60 B12D/OLP patients, 42 B12D/BMS patients, 56 B12D/AG patients, 32 B12D/RAS patients, and 90 vitamin B12-deficient oral mucosal disease patients.[23,31,34,47]

GPCA can induce destruction of gastric parietal cells, resulting in failure of intrinsic factor production[6] and ileal malabsorption of vitamin B12 that finally leads to significantly higher frequencies of macrocytosis, anemia (including macrocytic, normocytic, and microcytic anemias), and vitamin B12 deficiency in our 60 B12D/OLP patients than in 588 healthy control subjects.[5–9] However, the serum GPCA positivity was found in only 40 (66.7 %) of our 60 B12D/OLP patients, in only 18 (42.9 %) of our 42 B12D/BMS patients,[23] in only 31 (55.4 %) of our 56 B12D/AG patients,[31] in only 11 (34.4 %) of our 32 B12D/RAS patients,[34] and in only 43 (47.8 %) of 90 vitamin B12-deficient oral mucosal disease patients.[47] Thus, for 20 B12D/OLP patients, 24 B12D/BMS patients, 25 B12D/AG patients, 21 B12D/RAS patients, and 47 vitamin B12-deficient oral mucosal disease patients without serum GPCA positivity, the vitamin B12 deficiency may be due to other factors such as inadequate intake, vitamin B12 malabsorption, biologic competition including bacterial overgrowth and tapeworm infestation, and transcobalamin II deficiency.[5,47]

The serum iron deficiency was found in 19 (31.7 %; 13 patients also had serum GPCA positivity) of 60 B12D/OLP patients. Our previous study discovered serum iron deficiency in 11 (26.2 %; 6 patients also had serum GPCA positivity) of 42 B12D/BMS patients,[23] in 15 (26.8 %; 12 patients also had serum GPCA positivity) of 56 B12D/AG patients,[31] in 8 (25.0 %; 4 patients also had serum GPCA positivity) of 32 B12D/RAS patients,[34] and in 20 (22.2 %; 11 patients also had serum GPCA positivity) of 90 vitamin B12-deficient oral mucosal disease patients.[47] These findings indicate that the GPCA-induced reduction of gastric hydrochloric acid secretion may play a major role in causing serum iron deficiency in our 60 B12D/OLP, 42 B12D/BMS, and 56 B12D/AG patients, although other factors such as decreased absorption (due to gastrectomy, duodenal bypass, *Helicobacter pylori* infection, celiac sprue, inflammatory bowel diseases, etc.), chronic blood loss (due to peptic ulcer, diverticulitis, angiodysplasia, hook worm infestation, etc.), and drug-related (glucocorticoids, nonsteroidal anti-inflammatory drugs, proton-pump inhibitors, etc.) iron deficiency may also play roles in causing iron deficiency in our 60 B12D/OLP, 42 B12D/BMS, and 56 B12D/AG patients.[14–16]

This study discovered that 60 B12D/OLP patients had significantly higher frequencies of macrocytosis (55.0 %), blood Hb (68.3 %) and serum iron (31.7 %) and vitamin B12 (100.0 %) deficiencies, hyperhomocysteinemia (91.7 %), and serum GPCA positivity (66.7 %) than 588 healthy control subjects. Of 41 anemic B12D/OLP patients, 17 (41.5 %) had PA, 5 (12.2 %) had macrocytic anemia other than PA, 12

(29.3 %) had normocytic anemia, 6 (14.6 %) had IDA, and one (2.4 %) had microcytic anemia other than IDA and thalassemia trait-induced anemia. We conclude that B12D/OLP patients have significantly higher frequencies of macrocytosis, blood Hb and serum iron and vitamin B12 deficiencies, hyperhomocysteinemia, and serum GPCA positivity than healthy control subjects. Although PA is the most common type of anemia in our B12D/OLP patients, only 17 (41.5 %) of 41 B12D/OLP patients have PA.

Declaration of competing interest

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

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None.

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