Journal of Dental Sciences 20 (2025) 1078–1085

Available online at www.sciencedirect.com

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journal homepage: www.e-jds.com

Original Article

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

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Received 7 December 2024; Final revision received 9 December 2024
Available online 17 December 2024

KEYWORDS
Oral lichen planus;
Gastric parietal cell antibody;
Hyperhomocysteinemia;
Iron deficiency;
Iron deficiency anemia;
Normocytic anemia

Abstract *Background/purpose:* Our previous study found that 99 of 588 oral lichen planus (OLP) patients have iron deficiency (ID). This study assessed whether all OLP patients with ID (so-called ID/OLP patients) had iron deficiency anemia (IDA) and evaluated whether the ID/OLP patients had significantly higher frequencies of anemia, hematinic deficiencies, hyperhomocysteinemia, and serum gastric parietal cell antibody (GPCA) positivity than healthy control subjects.
Materials and methods: The blood hemoglobin (Hb) and serum iron, vitamin B12, folic acid, homocysteine, and GPCA levels in 99 ID/OLP patients and 588 healthy control subjects were measured and compared.
Results: We found that 99 ID/OLP patients had significantly lower mean blood Hb and serum

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https://doi.org/10.1016/j.jds.2024.12.009

iron, vitamin B12, folic acid levels as well as significantly higher mean serum homocysteine level than 588 healthy control subjects (all *P*-values <0.001). Moreover, 99 ID/OLP patients had significantly higher frequencies of blood Hb (64.7 %) and serum vitamin B12 (19.2 %), and folic acid (2.0 %) deficiencies, hyperhomocysteinemia (34.3 %), and serum GPCA positivity (33.3 %) than 588 healthy control subjects (all *P*-values <0.05). Furthermore, of 64 anemic ID/OLP patients, 2 (3.1 %) had pernicious anemia, 30 (46.9 %) had normocytic anemia, and 32 (50.0 %) had IDA.

Conclusion: ID/OLP patients have significantly higher frequencies of blood Hb and serum vitamin B12 and folic acid deficiencies, hyperhomocysteinemia, and serum GPCA positivity than healthy control subjects. Although the IDA (50.0 %) is the most common type of anemia in our 64 anemic ID/OLP patients, there are still 46.9 % of the 64 anemic ID/OLP patients who had the normocytic anemia.

Introduction

Oral lichen planus (OLP) is a chronic inflammatory disease of the oral mucosa, primarily affecting 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 most commonly affected oral sites. OLP lesions typically appear bilaterally and symmetrically on the oral mucosa.[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 99 (16.8 %) and 139 (23.6 %) of 588 OLP patients have iron deficiency (ID) and gastric parietal cell antibody (GPCA) positivity, respectively.[4] The etiologies of ID may include a reduced intake of iron, a decreased absorption of iron in patients who have gastrointestinal diseases (such as inflammatory bowel disease, *Helicobacter pylori* infection, or gastric or colonic carcinoma) or who take drugs that inhibit the secretion of hydrochloric acid, and chronic blood loss from the body.[5–7] Additionally, the serum GPCA can damage gastric parietal cells, leading to a deficiency in the secretion of intrinsic factor and hydrochloric acid.[8] A lack of intrinsic factor can result in impaired absorption of vitamin B12 in the terminal ileum, ultimately causing the vitamin B12 deficiency.[8–11] Moreover, the reduced gastric hydrochloric acid secretion can hinder iron absorption, resulting in iron deficiency.[5–7] In addition, hepcidin is the master regulator of systemic iron homeostasis. High hepcidin levels block intestinal iron absorption and macrophage iron recycling, causing the iron deficiency and anemia.[12] Therefore, it is interesting to know whether all OLP patients with ID (so-called ID/OLP patients in this study) have ID anemia (IDA), and whether ID/OLP patients are prone to have significantly higher frequencies of anemia, hematinic deficiencies, hyperhomocysteinemia, and serum GPCA positivity than healthy control subjects.

In our oral mucosal disease clinic, patients with OLP, burning mouth syndrome, atrophic glossitis, recurrent aphthous stomatitis, oral submucous fibrosis, or oral precancerous lesions are frequently encountered.[4,13–43] For patients diagnosed with any of these six specific diseases, it is common to test complete blood count, as well as serum levels of iron, vitamin B12, folic acid, homocysteine, GPCA, thyroglobulin antibody, and thyroid microsomal antibody (anti-thyroid peroxidase antibody or anti-TPO antibody). These tests help assess whether the patients have anemia, hematinic deficiencies, hyperhomocysteinemia, and positive serum levels of GPCA, thyroglobulin antibody, and thyroid microsomal antibody.[4,13–43]

In this study, 99 ID/OLP patients and 489 non-IDA/OLP patients were selected from the 588 OLP patients reported in our previous study.[4] The aim was to determine whether all ID/OLP patients had IDA and primarily to assess whether the 99 ID/OLP patients had significantly higher frequencies of anemia, serum iron, vitamin B12, and folic acid deficiencies, hyperhomocysteinemia, and serum GPCA positivity compared to 588 healthy control subjects.

Materials and methods

Subjects

This study included 99 ID/OLP patients (11 men and 88 women, aged 24–87 years, with a mean age of 53.0 ± 15.4 years) and 489 non-ID/OLP patients (99 men and 390 women, aged 20–88 years, with a mean age of 56.4 ± 13.8 years), all selected from the 588 OLP patients reported in our previous study.[4] For each OLP patient, one healthy control subject, matched by age (within ±2 years) and sex, was chosen. As a result, 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.[4,13–16] 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.[13–16] 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 99 ID/OLP patients, 489 non-ID/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.[13–43]

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.[13–43] Sera were considered positive if they exhibited fluorescence at a dilution of 1:10 or higher.

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 99 ID/OLP patients or 489 non-ID/OLP patients and 588 healthy control subjects as well as between 99 ID/OLP patients and 489 non-ID/OLP patients by Student's *t*-test. The differences in frequencies of microcytosis (defined as MCV $<$80 fL),[5–7,35–37] macrocytosis (defined as MCV $\geq$100 fL),[38–42] blood Hb and serum iron, vitamin B12, and folic acid deficiencies, hyperhomocysteinemia, and serum GPCA positivity between 99 ID/OLP patients or 489 non-ID/OLP patients and 588 healthy control subjects as well as between 99 ID/OLP patients and 489 non-ID/OLP patients were compared by chi-square test. Moreover, comparisons of frequencies of macrocytic, normocytic or microcytic anemia between 99 ID/OLP patients and 489 non-ID/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 99 ID/OLP patients or 489 non-ID/OLP patients and 588 healthy control subjects as well as between 99 ID/OLP patients and 489 non-ID/OLP patients 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 MCV and 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 mean serum homocysteine level in the 99 ID/OLP patients than in 588 healthy control subjects (all *P*-values $<$0.001, Table 1). Moreover, we discovered significantly lower MCV and 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 mean serum homocysteine level in the 489 non-ID/OLP patients than in 588 healthy control subjects (all *P*-values $<$0.01, Table 1). Furthermore, 99 ID/OLP patients also had significantly lower MCV and lower mean blood Hb (for men and women) and serum iron (for men and women) and vitamin B12 levels as well as significantly higher mean serum homocysteine level than those in 489 non-ID/OLP patients (all *P*-values $<$0.01, Table 1).

According to the World Health Organization (WHO) criteria, microcytosis of erythrocyte was defined as having MCV $<$80 fL,[5–7,35–37] macrocytosis of erythrocyte was defined as having MCV $\geq$100 fL,[38–42] and men with Hb $<$ 13 g/dL and women with Hb $<$ 12 g/dL were defined as having Hb deficiency or anemia.[44] Furthermore, patients with the serum iron level $<$60 μg/dL,[7,45] the serum vitamin B12 level $<$200 pg/mL,[46] or the serum folic acid level $<$4 ng/mL[47] 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, 36.4 %, 3.0 %, 64.7 %, 100.0 %, 19.2 %, 2.0 %, 34.3 % and 33.3 % of 99 ID/OLP patients, and 8.6 %, 6.8 %, 17.2 %, 0.0 %, 8.4 %, 1.0 %, 18.4 % and 21.7 % of 489 non-ID/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 99 ID/OLP patients had significantly higher frequencies of microcytosis, macrocytosis, blood Hb and serum iron, vitamin B12, and folic acid deficiencies, hyperhomocysteinemia, and serum GPCA positivity than 588 healthy control subjects (all *P*-values $<$0.001 except $P = 0.015$ for folic acid deficiency, Table 2). We also discovered that 489 ID/OLP patients had significantly higher frequencies of microcytosis, macrocytosis, blood Hb and serum vitamin B12 and folic acid deficiencies, hyperhomocysteinemia, and serum GPCA positivity than 588 healthy control subjects (all *P*-values $<$0.001 except $P = 0.045$ for folic acid deficiency, Table 2). In addition, 99 ID/OLP patients also had significantly higher frequencies of microcytosis and blood Hb and serum iron and vitamin B12 deficiencies, hyperhomocysteinemia, and

Table 1 Comparisons of mean corpuscular volume (MCV) and mean blood levels of hemoglobin (Hb), iron, vitamin B12, folic acid, and homocysteine between 99 oral lichen planus (OLP) patients with iron deficiency (ID/OLP patients) or 489 non-ID/OLP patients and 588 healthy control subjects as well as between 99 ID/OLP patients and 489 non-ID/OLP patients.

Group	MCV (fL)	Hb (g/dL)		Iron (μg/dL)		Vitamin B12 (pg/mL)	Folic acid (ng/mL)	Homocysteine (μM)
		Men	Women	Men	Women			
ID/OLP patients (n = 99)	83.8 ± 9.5	12.9 ± 1.8 (n = 11)	11.6 ± 1.5 (n = 88)	38.1 ± 17.1 (n = 11)	41.9 ± 13.2 (n = 88)	505.8 ± 285.3	11.6 ± 6.1	10.3 ± 4.5
[a]*P*-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
[b]*P*-value	<0.001	<0.001	<0.001	<0.001	<0.001	0.005	0.057	0.003
Non-ID/OLP patients (n = 489)	89.3 ± 8.0	14.7 ± 1.4 (n = 99)	13.0 ± 1.1 (n = 390)	88.5 ± 7.8 (n = 99)	92.0 ± 24.9 (n = 390)	586.5 ± 251.2	12.9 ± 6.2	9.1 ± 3.4
[a]*P*-value	0.003	0.002	<0.001	<0.001	0.009	<0.001	<0.001	<0.001
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 99 ID/OLP patients or 489 non-ID/OLP patients and 588 healthy control subjects by Student's *t*-test.
[b] Comparisons of means of parameters between 99 ID/OLP patients and 489 non-ID/OLP patients by Student's *t*-test.

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 99 oral lichen planus (OLP) patients with iron deficiency (ID/OLP patients) or 489 non-ID/OLP patients and 588 healthy control subjects as well as between 99 ID/OLP patients and 489 non-ID/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
ID/OLP patients (n = 99)	36 (36.4)	3 (3.0)	64 (64.7)	99 (100.0)	19 (19.2)	2 (2.0)	34 (34.3)	33 (33.3)
[a]*P*-value	<0.001	<0.001	<0.001	<0.001	<0.001	0.015	<0.001	<0.001
[b]*P*-value	<0.001	0.239	<0.001	<0.001	0.002	0.744	<0.001	0.018
Non-ID/OLP patients (n = 489)	42 (8.6)	33 (6.8)	84 (17.2)	0 (0.0)	41 (8.4)	5 (1.0)	90 (18.4)	106 (21.7)
[a]*P*-value	<0.001	<0.001	<0.001	NA	<0.001	0.045	<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)

NA = not assessed.
[a] Comparisons of means of parameters between 99 ID/OLP patients or 489 non-ID/OLP patients and 588 healthy control subjects by chi-square test.
[b] Comparisons of means of parameters between 99 ID/OLP patients and 489 non-ID/OLP patients by chi-square test.

serum GPCA positivity than those in 489 non-ID/OLP patients (all *P*-values <0.05, Table 2).

We also found that 64 (64.7 %) of 99 ID/OLP patients had anemia (defined as having an Hb concentration <13 g/dL for men and <12 g/dL for women).[44] Of the 64 anemic ID/OLP patients, 2 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),[38–42] 30 had normocytic anemia (NA, defined as having anemia and an MCV between 80.0 fL and 99.9 fL),[48–51] and 32 had IDA (defined as having anemia, an MCV <80 fL, and a serum iron level <60 μg/dL) (Table 3).[5–7,44] Thus, only 32 (50.0 %) of 64 anemic ID/OLP patients or 32 (32.3 %) of 99 ID/OLP patients had IDA. There were still 30 (46.9 %) of the 64 anemic ID/OLP patients who had NA.

Of the 84 anemic non-ID/OLP patients, 15 had PA,[38–42] 7 had macrocytic anemia (defined as having anemia and an MCV ≥100 fL) other than PA, 38 had NA,[48–51] 19 had 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),[52] and 5 had microcytic anemia (defined as having anemia and an MCV <80 fL)[5–7,35–37] other than IDA and thalassemia trait-induced anemia. These findings indicate that by the strict WHO criteria, the NA (45.2 %), thalassemia trait-induced anemia (22.6 %), and PA (17.9 %) are the three most common types of anemia in our 84 anemic non-ID/OLP patients (Table 3).

Comparisons of frequencies of macrocytic, normocytic or microcytic anemia between 99 ID/OLP patients and 489 non-ID/OLP patients are shown in Table 4. By the overall evaluation, 99 ID/OLP patients had significantly higher frequencies of anemia (64.7 % or 64/99) than 489 non-ID/OLP patients (17.2 % or 84/489; $P < 0.001$, Table 4). We also found that 99 ID/OLP patients had significantly higher frequencies of NA and microcytic anemia than 489 non-ID/OLP patients (both *P*-values <0.001, Table 4).

Discussion

In this study, three key findings emerged. Firstly, our research revealed that 99 ID/OLP patients exhibited significantly lower MCV, mean blood Hb, serum iron, vitamin B12, and folic acid levels, along with notably higher mean serum homocysteine levels compared to the 588 healthy control subjects (all *P*-values <0.001). Secondly, the 99 ID/OLP patients showed significantly higher frequencies of blood Hb (64.7 %) and serum iron (100.0 %), vitamin B12 (19.2 %), and folic acid (2.0 %) deficiencies, hyperhomocysteinemia (34.3 %), and serum GPCA positivity (33.3 %) compared to the healthy control group (all *P*-values <0.001, except for folic acid deficiency which had a *P*-value of 0.015, as shown in Table 2). Thirdly, it is noteworthy that while IDA was the predominant form of anemia in 50.0 % of the 64 anemic ID/OLP patients, 46.9 % (30 individuals) of the 64 anemic ID/OLP patients presented with NA, indicating a relatively high frequency of NA in our cohort of ID/OLP patients.

Iron plays a crucial role as a structural component of heme, which consists of protoporphyrin IX and iron. In individuals with ID, heme synthesis is impaired. A decrease in

Table 3 Anemia types, vitamin B12 and folic acid deficiencies, hyperhomocysteinemia, and gastric parietal cell antibody (GPCA) positivity in the 64 anemic oral lichen planus (OLP) patients with iron deficiency (ID/OLP patients) and in the 84 anemic non-ID/OLP patients.

Anemia type	Patient number (%)	Mean corpuscular volume (fL)	Vitamin B12 deficiency (<200 pg/mL)	Folic acid deficiency (<4 ng/mL)	Hyperhomo-cysteinemia (>12.0 μM)	GPCA positivity
ID/OLP patients (n = 99)						
Pernicious anemia	2 (3.1)	≥100	2 (100.0)	0 (0)	2 (100.0)	2 (100.0)
Normocytic anemia	30 (46.9)	80.0–99.9	8 (26.7)	1 (3.3)	11 (36.7)	7 (23.3)
Iron deficiency anemia	32 (50.0)	<80	6 (18.8)	0 (0)	10 (31.3)	9 (28.1)
Total	64 (100.0)		16 (25.0)	1 (1.6)	23 (35.9)	18 (28.1)
Non-ID/OLP patients (n = 489)						
Pernicious anemia	15 (17.9)	≥100	15 (100.0)	0 (0)	15 (100.0)	15 (100.0)
Other macrocytic anemia	7 (8.3)	≥100	5 (71.4)	0 (0.0)	7 (100.0)	2 (28.6)
Normocytic anemia	38 (45.2)	80–99.9	4 (10.6)	1 (2.6)	11 (29.0)	5 (13.2)
Thalassemia trait-induced anemia	19 (22.6)	<74	0 (0.0)	0 (0.0)	1 (5.3)	1 (5.3)
Other microcytic anemia	5 (6.0)	<80	1 (20.0)	0 (0.0)	1 (20.0)	0 (0.0)
Total	84 (100.0)		25 (29.8)	1 (1.2)	35 (41.7)	23 (27.4)

Table 4 Comparisons of frequencies of macrocytic, normocytic or microcytic anemia between 99 oral lichen planus (OLP) patients with iron deficiency (ID/OLP patients) and 489 non-ID/OLP patients.

Anemia type	Patient number (%)		[a]*P*-value
	ID/OLP patients (n = 99)	Non-ID/OLP patients (n = 489)	
Macrocytic anemia	2 (2.0)	22 (4.5)	0.391
Normocytic anemia	30 (30.3)	38 (7.8)	<0.001
Microcytic anemia	32 (32.3)	24 (4.9)	<0.001
Total	64 (64.7)	84 (17.2)	<0.001

[a] Comparison of frequencies of macrocytic, normocytic or microcytic anemia between 99 ID/OLP patients and 489 non-ID/OLP patients by chi-square test.

the intracellular heme levels within erythroblasts activates heme-regulated inhibitor kinase (HRI), leading to the phosphorylation of the α-subunit of the eukaryotic translational initiation factor 2 (eIF2α). This phosphorylated eIF2α inhibits the synthesis of proteins, particularly Hb, resulting in the production of hypochromic and microcytic red blood cells.[50] As a consequence, it is expected that the ID/OLP patients would present with microcytic anemia.[5–7,35–37] Despite this expectation, 2 (2.0 %) of the 99 ID/OLP patients in our study exhibited macrocytosis, along with vitamin B12 deficiency and serum GPCA positivity concurrently. These two patients were diagnosed as having PA based on the established criteria for PA.[38–42] GPCA in the serum can target gastric parietal cells, leading to reduced secretion of intrinsic factors, impaired vitamin B12 absorption, and ultimately, the vitamin B12 deficiency.[8] Consequently, we propose that GPCA-induced vitamin B12 deficiency plays a pivotal role in the development of macrocytosis and PA in these two ID/OLP patients with diagnosed PA.[38–42]

In this study, the IDA was identified in 32 (32.3 %) of the 99 ID/OLP patients. Among these 32 ID/OLP patients with IDA, 6 exhibited vitamin B12 deficiencies, 10 had hyperhomocysteinemia, and 9 tested positive for serum GPCA. Consequently, the iron malabsorption resulting from GPCA-induced hypochlorhydria could account for the ID in only 9 out of the 32 ID/OLP patients with IDA.[5–7] The ID in the rest of 23 ID/OLP patients with IDA could be attributed to the causes of ID such as a reduced intake of iron, a decreased absorption of iron in patients who have gastrointestinal diseases or who take drugs that inhibit the secretion of hydrochloric acid, and chronic blood loss from the body.[5–7]

In this study, the NA was detected in 30 (46.9 %) of the 64 anemic ID/OLP patients. Among these 30 NA/ID/OLP patients, all had ID, 8 had vitamin B12 deficiency, one had folic acid deficiency, and 7 tested positive for serum GPCA. These results indicate that the ID may be the primary factor contributing to the NA in these 30 NA/ID/OLP patients, whereas vitamin B12/folic acid deficiency played a lesser role in causing the NA in this group. Vitamin B12/folic acid deficiency is typically associated with macrocytosis or macrocytic anemia.[38–42] However, when both ID and vitamin B12/folic acid deficiency coexist in the OLP patients, the NA may occur.[48–51] The GPCA-induced hypochlorhydria and consequent impaired iron absorption could elucidate the ID in the 7 GPCA-positive NA/ID/OLP patients.[5–8] Alongside the ID, NA may stem from various factors such as chronic illnesses, inflammatory conditions, infections, bone marrow hypoplasia, reduced erythropoietin production or inadequate response to erythropoietin, hemolytic disorders, subtle yet persistent gastrointestinal blood loss, and cytokine-mediated suppression of erythropoiesis.[48–51]

This study identified anemia in 64 (64.7 %) out of 99 ID/OLP patients, while the remaining 35 ID/OLP patients did not exhibit anemia. The diagnosis of ID was established based on the observation of the low iron levels (<60 μg/dL) in the sera of OLP patients.[7,45] Serum iron is not the sole source of iron used for Hb production in the body, as iron is also stored in serum ferritin (indicative of total body iron stores), bone marrow, and hepatocytes (acting as a long-term iron reservoir).[5,6] Moreover, iron is crucial for Hb in red blood cells and myoglobin in muscles, which contain approximately 60 % of total body iron. The iron within erythrocytes can be recycled for Hb synthesis.[6,12] A low serum iron level does not necessarily indicate insufficient total body iron stores. Therefore, even when the serum iron levels are low (<60 μg/dL), if patients still possess adequate body iron stores, some stored iron can be utilized for Hb synthesis over several weeks or months.[5,6] This may partly elucidate why the 35 ID/OLP patients in our study had serum ID but did not present with anemia. It is essential to remember that a low serum ferritin level (<30 μg/L) is the most sensitive and specific indicator of ID.[5,6] However, serum ferritin levels can be elevated independently of iron status in acute and chronic inflammatory disorders, malignant diseases, and liver diseases, posing a significant diagnostic challenge. In such cases, individuals with a ferritin concentration of 50 μg/L or higher may still be deficient in iron. For patients with chronic kidney disease, a serum ferritin level of 100 μg/L has been proposed as a diagnostic cutoff for ID, while a level of 200 μg/L has been suggested for hemodialysis patients.[5,6] Additionally, assessing serum ferritin, transferrin saturation (where a saturation <16 % indicates IDA), serum soluble transferrin receptors (increased synthesis in ID leads to higher levels of soluble transferrin receptors), and the serum soluble transferrin receptors-ferritin index can provide more accurate diagnoses of IDA compared to the traditional red cell indices.[5,6]

This research identified hyperhomocysteinemia in 34 (34.3 %) out of 99 ID/OLP patients. Among these 34 patients, 21 were positive for serum GPCA, 14 had vitamin B12 deficiency, and two had folic acid deficiency. One of the folic acid-deficient ID/OLP patients also had concurrent vitamin B12 deficiency. Thus, we propose that folic acid and vitamin B12 deficiencies could account for the hyperhomocysteinemia observed in 15 out of the 34 ID/OLP patients with hyperhomocysteinemia.[53] The remaining 19 ID/OLP patients with hyperhomocysteinemia may attribute it to other factors such as chronic alcohol or tobacco consumption, abnormalities in enzymes and cofactors involved in homocysteine biosynthesis, excessive methionine intake, specific medical conditions (chronic renal failure, hypothyroidism, anemia, and malignancies), and adverse effects

of certain medications (including cholestyramine, metformin, methotrexate, nicotinic acid, and fibric acid derivatives).[54]

In this study, we observed that the 99 ID/OLP patients had significantly higher frequencies of blood Hb (64.7 %) and serum iron (100.0 %), vitamin B12 (19.2 %), and folic acid (2.0 %) deficiencies, hyperhomocysteinemia (34.3 %), and serum GPCA positivity (33.3 %) compared to the 588 healthy control individuals. Among the 64 anemic ID/OLP patients, IDA was the most prevalent type of anemia at 50.0 %, followed by NA at 46.9 %, and PA at 3.1 %. Our findings indicate that the 99 ID/OLP patients exhibited significantly higher frequencies of blood Hb and serum iron, vitamin B12, and folic acid deficiencies, as well as hyperhomocysteinemia and serum GPCA positivity compared to the 588 healthy control individuals. It is important to note that not all ID/OLP patients present with anemia. While IDA was the predominant form of anemia among the 64 anemic ID/OLP patients at 50.0 %, 46.9 % of these patients had NA. Furthermore, the ID/OLP patients could also potentially have PA if they exhibit both serum GPCA positivity and vitamin B12 deficiency concurrently.

Declaration of competing interest

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

Acknowledgments

None.

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