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Original Article

Anemia, hematinic deficiencies, and gastric parietal cell antibody positivity in oral lichen planus patients with or without hyperhomocysteinemia

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Abstract *Background/purpose:* Our previous study found that 124 of 588 oral lichen planus (OLP) patients have hyperhomocysteinemia. This study assessed whether these 124 OLP patients with hyperhomocysteinemia (hyperhomocysteinemia/OLP patients) had significantly higher frequencies of anemia, hematinic deficiencies, and serum gastric parietal cell antibody (GPCA) positivity than 464 OLP patients without hyperhomocysteinemia (non-hyperhomocysteinemia/OLP patients) or 588 healthy control subjects.
Materials and methods: The blood hemoglobin (Hb) and serum iron, vitamin B12, folic acid, homocysteine, and GPCA levels in 124 hyperhomocysteinemia/OLP patients, 464 non-hyperhomocysteinemia/OLP patients, and 588 healthy control subjects were measured and compared.

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Results: We found that 124 hyperhomocysteinemia/OLP patients had significantly higher frequencies of macrocytosis, blood Hb and serum iron and vitamin B12 deficiencies, and serum GPCA positivity than 588 healthy control subjects (all *P*-values <0.001) or 464 non-hyperhomocysteinemia/OLP patients (all *P*-values <0.001). Anemia was found in 58 of 124 hyperhomocysteinemia/OLP patients and in 90 of 464 non-hyperhomocysteinemia/OLP patients. Normocytic anemia (22 cases), pernicious anemia (17 cases), and iron deficiency anemia (10 cases) were the three most common types of anemia in 124 hyperhomocysteinemia/OLP patients. Moreover, normocytic anemia (46 cases), iron deficiency anemia (22 cases), and thalassemia trait-induced anemia (18 cases) were the three most common types of anemia in 464 non-hyperhomocysteinemia/OLP patients. Moreover, the 124 hyperhomocysteinemia/OLP patients had significantly higher frequencies of macrocytic and normocytic anemia than the 464 non-hyperhomocysteinemia/OLP patients.
Conclusion: Hyperhomocysteinemia/OLP patients had significantly higher frequencies of macrocytosis, anemia, serum iron and vitamin B12 deficiencies, and serum GPCA positivity than healthy control subjects or non-hyperhomocysteinemia/OLP patients.
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Introduction

Oral lichen planus (OLP) is a chronic inflammatory oral mucosal disease that occurs more frequently in middle-aged and elderly female patients and affects 0.2–3 % of the population.[1] Our previous study found that 124 (21.1 %) of 588 OLP patients have hyperhomocysteinemia.[1] Serum homocysteine level is a biomarker of cardiovascular diseases. Higher serum homocysteine levels are associated with increased rates of coronary heart disease and stroke, because previous studies have demonstrated that high serum homocysteine level can cause oxidative stress, damage endothelium, and enhance thrombogenicity.[2–7] Homocysteine is formed during methionine metabolism.[2] Both vitamin B12 and folic acid act as coenzymes for the conversion of homocysteine to methionine.[3] Moreover, vitamin B6 is a coenzyme for the conversion of homocysteine to cysteine.[3] Therefore, patients with vitamin B12, folic acid, and/or vitamin B6 deficiencies may have hyperhomocysteinemia.[3,4] Furthermore, the gastric parietal cell antibody (GPCA) can destroy gastric parietal cells, resulting in lack of intrinsic factors and hypochlorhydria.[8] Intrinsic factor deficiency may lead to malabsorption of vitamin B12 from terminal ileum and finally the vitamin B12 deficiency.[8–12] In addition, decreased gastric secretion of hydrochloric acid may cause iron malabsorption and subsequent iron deficiency.[8,13] Thus, it is interesting to know whether OLP patients with hyperhomocysteinemia (so-called hyperhomocysteinemia/OLP patients in this study) are prone to have significantly higher frequencies of anemia, serum iron and vitamin B12 deficiencies, and serum GPCA positivity than OLP patients without hyperhomocysteinemia (so-called non-hyperhomocysteinemia/OLP patients in this study) or healthy control subjects.

In this study, we divided the 588 OLP patients into two groups: one group consisting of 124 hyperhomocysteinemia/OLP patients and the other group containing 464 non-hyperhomocysteinemia/OLP patients.[1] We tried to find out whether the 124 hyperhomocysteinemia/OLP patients had significantly higher frequencies of macrocytosis, anemia, serum iron and vitamin B12 deficiencies, and serum GPCA positivity than 464 non-hyperhomocysteinemia/OLP patients or 588 healthy control subjects.

Materials and methods

Subjects

This study consisted of 124 hyperhomocysteinemia/OLP patients (23 men and 101 women, age range 25–87 years, mean age 59.6 ± 13.2 years) and 464 non-hyperhomocysteinemia/OLP patients (87 men and 377 women, age range 20–88 years, mean age 54.8 ± 14.2 years).[1] 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, age range 20–89 years, mean age of 56.0 ± 14.1 years) were included in this study.[1] 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.[1,14–20] However, OLP patients with a history of betel quid chewing, heavy alcohol use, autoimmune diseases (such as systemic lupus erythematosus, rheumatoid arthritis, pemphigus vulgaris, etc.), inflammatory diseases, malignancy, recent surgery, or systemic conditions like

liver, kidney, coronary artery, or stroke diseases were excluded from this study.[1,14–20] 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 124 hyperhomocysteinemia/OLP patients, 464 non-hyperhomocysteinemia/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.[1,14–48]

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.[1,14–48] 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 124 hyperhomocysteinemia/OLP patients, 464 non-hyperhomocysteinemia/OLP patients, and 588 healthy control subjects by Student's *t*-test. The differences in frequencies of microcytosis (defined as MCV $<$80 fL),[13,41–43] 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 124 hyperhomocysteinemia/OLP patients, 464 non-hyperhomocysteinemia/OLP patients, and 588 healthy control subjects by chi-square test. Moreover, comparisons of frequencies of macrocytic, normocytic or microcytic anemia between 124 hyperhomocysteinemia/OLP patients and 464 non-hyperhomocysteinemia/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 124 hyperhomocysteinemia/OLP patients, 464 non-hyperhomocysteinemia/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 mean serum homocysteine level in 124 hyperhomocysteinemia/OLP patients than in 588 healthy control subjects (all *P*-values $<$0.01, Table 1). Moreover, we also found significantly lower mean blood Hb (for women only) and serum iron (for men and women) and vitamin B12 levels as well as significantly higher MCV and mean serum homocysteine level in 124 hyperhomocysteinemia/OLP patients than in 464 non-hyperhomocysteinemia/OLP patients (all *P*-values $<$0.05, Table 1). Furthermore, 464 non-hyperhomocysteinemia/OLP patients also had significantly lower MCV, mean blood Hb (for men and women) and serum iron (for women only), vitamin B12, folic acid, and homocysteine levels than 588 healthy control subjects (all *P*-values $<$0.005, Table 1).

According to the World Health Organization (WHO) criteria, microcytosis of erythrocyte was defined as having MCV $<$80 fL,[13,41–43] macrocytosis of erythrocyte was defined as having MCV $\geq$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, 11.3 %, 27.4 %, 46.8 %, 27.4 %, 44.4 %, 2.4 %, and 61.3 % of 124 hyperhomocysteinemia/OLP patients and 13.8 %, 0.4 %, 19.4 %, 14.0 %, 1.1 %, 0.9 %, and 13.6 % of 464 non-hyperhomocysteinemia/OLP patients were diagnosed as having microcytosis, macrocytosis, blood Hb and serum iron, vitamin B12, and folic acid deficiencies, and serum GPCA positivity, respectively. We found that 124 hyperhomocysteinemia/OLP patients had significantly higher frequencies of microcytosis, macrocytosis, blood Hb and serum iron, vitamin B12, and folic acid deficiencies, and serum GPCA positivity than 588 healthy control subjects (all *P*-values $<$0.005) and significantly higher frequencies of macrocytosis, blood Hb and serum iron and vitamin B12 deficiencies, and serum GPCA positivity than 464 non-hyperhomocysteinemia/OLP patients (all *P*-values $<$0.001). Moreover, 464 non-hyperhomocysteinemia/OLP patients had significantly higher frequencies of microcytosis, blood Hb and serum iron and vitamin B12 deficiencies, and serum GPCA positivity than 588 healthy control subjects (all *P*-values $<$0.05, Table 2).

We also found that 58 (46.8 %) of 124 hyperhomocysteinemia/OLP patients and 90 (19.4 %) of 464 non-hyperhomocysteinemia/OLP patients had anemia (defined as having an Hb concentration $<$13 g/dL for men and $<$12 g/dL for women).[49] Of the 58 anemic hyperhomocysteinemia/OLP patients, 17 had pernicious anemia (PA, defined as

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 124 oral lichen planus (OLP) patients with hyperhomocysteinemia (hyperhomocysteinemia/OLP patients), 464 OLP patients without hyperhomocysteinemia (non-hyperhomocysteinemia/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			
Hyperhomocysteinemia/OLP patients (n = 124)	91.3 ± 9.9	14.0 ± 1.6 (n = 23)	12.0 ± 1.6 (n = 101)	85.5 ± 36.0 (n = 23)	76.0 ± 29.4 (n = 101)	333.3 ± 221.2	12.0 ± 6.0	14.9 ± 3.0
[a]*P*-value	0.091	<0.001	<0.001	0.008	<0.001	<0.001	<0.001	<0.001
[b]*P*-value	<0.001	0.052	<0.001	0.046	0.011	<0.001	0.154	<0.001
Non-hyperhomocysteinemia/OLP patients (n = 464)	87.6 ± 8.0	14.7 ± 1.5 (n = 87)	12.9 ± 1.2 (n = 377)	102.7 ± 36.4 (n = 87)	84.6 ± 30.3 (n = 377)	636.9 ± 229.0	12.9 ± 6.3	7.8 ± 1.9
[a]*P*-value	<0.001	0.003	<0.001	0.844	<0.001	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 124 hyperhomocysteinemia/OLP patients or 464 non-hyperhomocysteinemia/OLP patients and 588 healthy control subjects by Student's *t*-test.
[b] Comparisons of means of parameters between 124 hyperhomocysteinemia/OLP patients and 464 non-hyperhomocysteinemia/OLP patients by Student's *t*-test.

having anemia, an MCV ≥100 fL, a serum vitamin B12 level <200 pg/mL, and the presence of serum GPCA positivity),[44–48] 7 had macrocytic anemia (defined as having anemia and an MCV ≥100 fL) other than PA,[44–48] 22 had normocytic anemia (defined as having anemia and an MCV between 80.0 fL and 99.9 fL),[53–56] 10 had iron deficiency anemia (IDA, defined as having anemia, an MCV <80 fL, and a serum iron level <60 μg/dL),[13,49,50] one 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),[57] and one had microcytic anemia (defined as having anemia and an MCV <80 fL) other than IDA and thalassemia trait-induced anemia (Table 3).[41–43] Of the 90 anemic non-hyperhomocysteinemia/OLP patients, 46 had normocytic anemia, 22 had IDA, 18 had thalassemia trait-induced anemia, and 4 had microcytic anemia other than IDA and thalassemia trait-induced anemia (Table 3).

Comparisons of frequencies of macrocytic, normocytic or microcytic anemia between 124 hyperhomocysteinemia/OLP patients and 464 non-hyperhomocysteinemia/OLP patients are shown in Table 4. By overall evaluation, 124 hyperhomocysteinemia/OLP patients had a significantly higher frequency of anemia than 464 non-hyperhomocysteinemia/OLP patients. We also discovered that 124 hyperhomocysteinemia/OLP patients had significantly higher frequencies of macrocytic anemia ($P < 0.001$) and normocytic anemia ($P = 0.024$) than 464 non-hyperhomocysteinemia/OLP patients (Table 4).

Discussion

In this study, hyperhomocysteinemia was discovered in 124 (21.1 %) of 588 OLP patients.[1] Our previous studies also found hyperhomocysteinemia in 127 (11.9 %) of 1064 atrophic glossitis (AG) patients,[31] in 170 (19.2 %) of 884 burning mouth syndrome (BMS) patients,[21] in 21 (7.7 %) of 273 recurrent aphthous stomatitis (RAS) patients,[34] and in 29 (22.1 %) of 131 oral precancer patients.[39] These findings suggest that among the five kinds of oral mucosal disease patients reported, the prevalence of hyperhomocysteinemia is highest in oral precancer patients (22.1 %) and lowest in RAS patients (7.7 %).[21,31,34,39]

This study found that of the 124 hyperhomocysteinemia/OLP patients, 34 (27.4 %) had macrocytosis, 58 (46.8 %) had anemia, 34 (27.4 %) had serum iron deficiency, 55 (44.4 %) had vitamin B12 deficiency, and 76 (61.3 %) had serum GPCA positivity. The above findings indicate that the hyperhomocysteinemia is a pretty good biomarker for screening the OLP patients with anemia, serum vitamin B12 deficiency, and serum GPCA positivity, because it can detect 46.8 %, 44.4 %, and 61.3 % of 124 hyperhomocysteinemia/OLP patients with anemia, serum vitamin B12 deficiency, and serum GPCA positivity, respectively. In other words, when the OLP patients are discovered to have hyperhomocysteinemia, they have a high probability to suffer from anemia, serum vitamin B12 deficiency, and serum GPCA positivity. Moreover, the above findings also imply that when a physician wants to check whether an OLP patient has anemia, serum vitamin B12 deficiency, and serum GPCA positivity, concomitant examination of the serum homocysteine level is necessary and helpful. Thus, for the

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, and gastric parietal cell antibody (GPCA) positivity between any two of three groups of 124 oral lichen planus (OLP) patients with hyperhomocysteinemia (hyperhomocysteinemia/OLP patients), 464 OLP patients without hyperhomocysteinemia (non-hyperhomocysteinemia/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)	GPCA positivity
Hyperhomocysteinemia/OLP patients (n = 124)	14 (11.3)	34 (27.4)	58 (46.8)	34 (27.4)	55 (44.4)	3 (2.4)	76 (61.3)
[a]*P*-value	<0.001	<0.001	<0.001	<0.001	<0.001	0.003	<0.001
[b]*P*-value	0.561	<0.001	<0.001	<0.001	<0.001	0.340	<0.001
Non-hyperhomocysteinemia/OLP patients (n = 464)	64 (13.8)	2 (0.4)	90 (19.4)	65 (14.0)	5 (1.1)	4 (0.9)	63 (13.6)
[a]*P*-value	<0.001	0.378	<0.001	<0.001	0.038	0.080	<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)

[a] Comparisons of means of parameters between 124 hyperhomocysteinemia/OLP patients or 464 non-hyperhomocysteinemia/OLP patients and 588 healthy control subjects by chi-square test.
[b] Comparisons of means of parameters between 124 hyperhomocysteinemia/OLP patients and 464 non-hyperhomocysteinemia/OLP patients by chi-square test.

Table 3 Anemia types, hematinic deficiencies, and gastric parietal cell antibody (GPCA) positivity in 58 anemic oral lichen planus (OLP) patients with hyperhomocysteinemia (hyperhomocysteinemia/OLP patients) and in 90 anemic OLP patients without hyperhomocysteinemia (non-hyperhomocysteinemia/OLP patients).

Anemia type	Patient number (%)					
	Patient number (%)	Mean corpuscular volume (fL)	Iron deficiency (<60 μg/dL)	Vitamin B12 deficiency (<200 pg/mL)	Folic acid deficiency (<4 ng/mL)	GPCA positivity
Hyperhomocysteinemia/OLP patients (n = 124)						
Pernicious anemia	17 (29.3)	≥100	2 (11.8)	17 (100.0)	0 (0.0)	17 (100.0)
Other macrocytic anemia	7 (12.1)	≥100	0 (0.0)	5 (71.4)	0 (0.0)	2 (28.6)
Normocytic anemia	22 (37.9)	80–99.9	11 (50.0)	10 (45.5)	2 (9.1)	7 (31.8)
Iron deficiency anemia	10 (17.2)	<80	10 (100.0)	4 (40.0)	0 (0.0)	7 (70.0)
Thalassemia trait-induced anemia	1 (1.7)	<74	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)
Other microcytic anemia	1 (1.7)	<80	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)
Total	58 (100.0)		23 (39.7)	37 (63.8)	2 (3.5)	34 (58.6)
Non-hyperhomocysteinemia/OLP patients (n = 464)						
Normocytic anemia	46 (51.1)	80–99.9	19 (41.3)	2 (4.4)	0 (0.0)	5 (10.9)
Iron deficiency anemia	22 (24.4)	<80	22 (100.0)	2 (9.1)	0 (0.0)	2 (9.1)
Thalassemia trait-induced anemia	18 (20.0)	<74	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Other microcytic anemia	4 (4.4)	<80	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total	90 (100.0)		41 (45.6)	4 (4.4)	0 (0.0)	7 (7.8)

hyperhomocysteinemia/OLP patients, the vitamin B12 and/or folic acid supplement treatments are necessary to correct the hyperhomocysteinemia condition and to prevent the patients from anemia, especially the macrocytic anemia.[2–4,22] Our previous study used relatively high doses of hematinic supplement treatments for 399 BMS patients with or without hyperhomocysteinemia, we found that supplementations with vitamin BC capsules plus corresponding deficient hematinics (iron, vitamin B12, and folic acid) for those BMS patients with specific hematinic deficiencies or with vitamin BC capsules only for those BMS patients without hematinic deficiencies can reduce the abnormally high serum homocysteine levels to normal levels in BMS patients.[22] In addition, complete regression of oral symptoms (such as burning sensation of oral mucosa, dry mouth, numbness of the oral mucosa, and loss or dysfunction of taste) is found in 177 (44.4 %) of 399 BMS patients treated with the above-mentioned regimens.[22]

Table 4 Comparisons of frequencies of macrocytic, normocytic or microcytic anemia between 124 oral lichen planus (OLP) patients with hyperhomocysteinemia (hyperhomocysteinemia/OLP patients) and 464 OLP patients without hyperhomocysteinemia (non-hyperhomocysteinemia/OLP patients).

Anemia type	Patient number (%)		[a]*P*-value
	Hyperhomocysteinemia/OLP patients (n = 124)	Non-hyperhomocysteinemia/OLP patients (n = 464)	
Macrocytic anemia	24 (19.4)	0 (0.0)	<0.001
Normocytic anemia	22 (17.7)	46 (9.9)	0.024
Microcytic anemia	12 (9.7)	44 (9.5)	0.915
Total	58 (46.8)	90 (19.4)	<0.001

[a] Comparison of frequencies of macrocytic, normocytic or microcytic anemia between 124 hyperhomocysteinemia/OLP patients and 464 non-hyperhomocysteinemia/OLP patients) by chi-square test.

Both the vitamin B12/folic acid deficiency and serum GPCA positivity contribute to cause hyperhomocysteinemia in OLP patients.[4,8,9] The GPCA is a gastric autoantibody that can damage the gastric parietal cells, resulting in lack of intrinsic factor production and ileal malabsorption of vitamin B12 that finally leads to vitamin B12 deficiency and hyperhomocysteinemia in OLP patients.[4,8,9] In addition, the GPCA-induced destruction of gastric parietal cells that in turn results in decreased secretion of hydrochloric acid may subsequently cause iron malabsorption and subsequent iron deficiency.[8,13] Thus, the high frequency of serum GPCA positivity (61.3 %) in the 124 hyperhomocysteinemia/OLP patients could relatively well explain why these 124 hyperhomocysteinemia/OLP patients had high frequency of vitamin B12 deficiency (44.4 %), anemia (46.8 %), macrocytosis (27.4 %), and iron deficiency (27.4 %), as well as why the 124 hyperhomocysteinemia/OLP patients had significantly higher frequencies of macrocytosis, anemia, and serum iron and vitamin B12 deficiencies than the 464 non-hyperhomocysteinemia/OLP patients in the present study.

In this study, when the three hyperhomocysteinemia-inducing factors, vitamin B12 deficiency, folic acid deficiency, and the serum GPCA positivity, were considered together to analyze the distribution of these three factors in the 124 hyperhomocysteinemia/OLP patients, we found 95 hyperhomocysteinemia/OLP patients had at least one of the above-mentioned three factors. Of these 95 hyperhomocysteinemia/OLP patients, 38 had the serum GPCA positivity only, 38 had both the serum GPCA positivity and vitamin B12 deficiency, one had both the vitamin B12 and folic acid deficiencies, 16 had the vitamin B12 deficiency only, and two had the folic acid deficiency only. For the 38 hyperhomocysteinemia/OLP patients with the serum GPCA positivity but without the vitamin B12 deficiency (vitamin B12 $<$ 200 pg/mL by the strict WHO definition),[51] they tended to have lower normal levels of vitamin B12 (mean, 463.6 $\pm$ 236.3 pg/mL). Moreover, for the 17 GPCA-negative vitamin B12-deficient hyperhomocysteinemia/OLP patients, their vitamin B12 deficiency was probably due to insufficient intake and malabsorption.[51] Taken together, the hyperhomocysteinemia in the aforementioned 95 hyperhomocysteinemia/OLP patients are considered to be attributed to the severe vitamin B12 deficiency (vitamin B12 $<$ 200 pg/mL) in 55 patients, the lower normal levels of vitatamin B12 in 38 patients, and the severe folic acid deficiency ($<$4 ng/mL) in two patients. Thus, the vitamin B12 deficiency was the major factor causing the hyperhomocysteinemia in our 124 hyperhomocysteinemia/OLP patients. For the other 29 hyperhomocysteinemia/OLP patients without the vitamin B12/folic acid deficiency and serum GPCA positivity, the hyperhomocysteinemia was probably due to chronic consumption of alcohol or tobacco, a dysfunction of enzymes and cofactors associated with the process of homocysteine biosynthesis (main cause), excessive methionine intake, certain diseases (chronic renal failure, hypothyroidism, anemia, and malignant tumors), and side effects of some drugs (cholestyramine, metformin, methotrexate, etc.).[58–60]

In this study, the serum iron deficiency was observed in 34 (27.4 %) of the 124 hyperhomocysteinemia/OLP patients. Moreover, 21 (61.8 %) of the 34 iron-deficient hyperhomocysteinemia/OLP patients were serum GPCA-positive. These findings indicate that the serum GPCA-induced reduction of gastric hydrochloric acid secretion play a major role in causing the serum iron deficiency in our 34 iron-deficient hyperhomocysteinemia/OLP patients.[8,13] The iron deficiency in the other 13 iron-deficient hyperhomocysteinemia/OLP patients might be due to other factors, such as chronic blood loss related to excessive menstrual flow or gastrointestinal diseases, a reduced intake of iron during old-age stage, a decreased absorption of iron in patients who take antacids, H2-receptor antagonists, or proton pump inhibitors, etc.[13]

This study also demonstrated a significantly higher frequency of anemia (46.8 %, 58 cases) in the 124 hyperhomocysteinemia/OLP patients. The macrocytic anemia (41.4 %, 24 cases including 17 with PA and 7 with macrocytic anemia other than PA), normocytic anemia (37.9 %, 22 cases), and IDA (17.2 %, 10 cases) were the three most common types of anemia in our 124 hyperhomocysteinemia/OLP patients. The serum vitamin B12 deficiency was discovered in 22 of the 24 macrocytic anemia hyperhomocysteinemia/OLP patients, suggesting that the serum vitamin B12 deficiency is the major factor causing the macrocytic anemia in these 24 macrocytic anemia hyperhomocysteinemia/OLP patients.[44–48] For the 22 normocytic anemia hyperhomocysteinemia/OLP patients, the iron deficiency was found in 11 patients, the vitamin B12 deficiency was detected in 10 patients. Moreover, 6 normocytic anemia hyperhomocysteinemia/OLP patients had both iron and vitamin B12 deficiency. Thus, these 6 of the 22 normocytic anemia hyperhomocysteinemia/OLP patients might be due to a mixed condition of producing both microcytic and macrocytic red

blood cells in the same normocytic anemia patient.[61] The other 16 normocytic anemia hyperhomocysteinemia/OLP patients might be attributed to other factors, such as chronic diseases, inflammatory diseases, infections, bone marrow hypoplasia, decreased production of erythropoietin or a poor response to erythropoietin, hemolytic disorders, mild but persistent blood loss from gastrointestinal tract, and cytokine-induced suppression of erythropoiesis.[53–56] However, further studies are needed to explore whether our normocytic anemia hyperhomocysteinemia/OLP patients have the above-mentioned conditions associated with normocytic anemia.

In conclusion, hyperhomocysteinemia/OLP patients had significantly higher frequencies of macrocytosis, anemia, serum iron and vitamin B12 deficiencies, and serum GPCA positivity than healthy control subjects or non-hyperhomocysteinemia/OLP patients.

Declaration of competing interest

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

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None.

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