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Original Article

Anemia, hematinic deficiencies, and gastric parietal cell antibody positivity in burning mouth syndrome patients with or without hyperhomocysteinemia

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KEYWORDS
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Abstract *Background/purpose:* Our previous study found that 170 of 884 burning mouth syndrome (BMS) patients have hyperhomocysteinemia. This study assessed whether these 170 BMS patients with hyperhomocysteinemia had significantly higher frequencies of anemia, hematinic deficiencies, and serum gastric parietal cell antibody (GPCA) positivity than 714 BMS patients without hyperhomocysteinemia or 442 healthy control subjects.
Materials and methods: The blood hemoglobin (Hb) and serum iron, vitamin B12, folic acid, homocysteine, and GPCA levels in 170 BMS patients with hyperhomocysteinemia, 714 BMS patients without hyperhomocysteinemia, and 442 healthy control subjects were measured and compared.
Results: We found that 170 BMS patients with hyperhomocysteinemia had significantly higher frequencies of macrocytosis, blood Hb and serum iron, vitamin B12, and folic acid deficiencies, and serum GPCA positivity than 442 healthy control subjects (all *P*-values < 0.001) or 714 BMS patients without hyperhomocysteinemia (all *P*-values < 0.05). Anemia was found in 77 of 170

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BMS patients with hyperhomocysteinemia and in 98 of 714 BMS patients without hyperhomocysteinemia. Normocytic anemia (47 cases) and pernicious anemia (15 cases) were the two most common types of anemia in 170 BMS patients with hyperhomocysteinemia. Moreover, normocytic anemia (48 cases), iron deficiency anemia (21 cases), and thalassemia trait-induced anemia (21 cases) were the three most common types of anemia in 714 BMS patients without hyperhomocysteinemia.

Conclusion: BMS patients with hyperhomocysteinemia had significantly higher frequencies of macrocytosis, anemia, serum iron, vitamin B12, and folic acid deficiencies, and serum GPCA positivity than healthy control subjects or BMS patients without hyperhomocysteinemia.

Introduction

Burning mouth syndrome (BMS) is characterized by the presence of burning sensation of the oral mucosa, but oral examination reveals none of clinically apparent oral mucosal alterations. BMS occurs more frequently in middle-aged and elderly women.[1] Our previous study found that 170 (19.2%) of 884 BMS 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.[13] Thus, it is interesting to know whether BMS patients with hyperhomocysteinemia are prone to have significantly higher frequencies of anemia, serum iron, vitamin B12, and folic acid deficiencies, and serum GPCA positivity than BMS patients without hyperhomocysteinemia or healthy control subjects.

In this study, we divided the 884 BMS patients into two groups: one group containing 170 BMS patients with hyperhomocysteinemia and the other group consisting of 714 BMS patients without hyperhomocysteinemia.[1] We tried to find out whether the 170 BMS patients with hyperhomocysteinemia had significantly higher frequencies of macrocytosis, anemia, serum iron, vitamin B12, and folic acid deficiencies, and serum GPCA positivity than 714 BMS patients without hyperhomocysteinemia or 442 healthy control subjects.

Materials and methods

Subjects

This study consisted of 170 (69 men and 101 women, age range 18—90 years, mean age 56.2 ± 17.6 years) BMS patients with hyperhomocysteinemia and 714 (143 men and 571 women, age range 18—87 years, mean age 56.1 ± 13.7 years) BMS patients without hyperhomocysteinemia.[1] For two BMS patients, one age- (±2 years of each patient's age) and sex-matched healthy control subject was selected. Thus, 442 age- and sex-matched healthy control subjects (106 men and 336 women, age range 18—90 years, mean 57.5 ± 13.5 years) were selected and included in this study.[1] These 170 BMS patients with hyperhomocysteinemia, 714 BMS patients without hyperhomocysteinemia, and 442 healthy control subjects were retrieved from our previous study.[1] All the BMS patients and healthy control subjects were seen consecutively, diagnosed, and treated in the Department of Dentistry, National Taiwan University Hospital (NTUH) from July 2007 to July 2017. Patients were diagnosed as having BMS when they complained of burning sensation and other symptoms of the oral mucosa but no apparent clinical oral mucosal abnormality was found.[1,14,15] The detailed inclusion and exclusion criteria for our BMS patients and healthy control subjects have been described previously.[1,14,15] In addition, none of the BMS patients had taken any prescription medication for BMS at least 3 months before entering the study.

The blood samples were drawn from 170 BMS patients with hyperhomocysteinemia, 714 BMS patients without hyperhomocysteinemia, and 442 healthy control subjects for the measurement of complete blood count, serum iron, vitamin B12, folic acid, and homocysteine levels, and the serum GPCA positivity. All BMS patients and healthy control subjects signed the informed consents before entering the study. This study was reviewed and approved by the Institutional Review Board at the NTUH (201212066RIND).

Determination of complete blood count and serum iron, vitamin B12, folic acid, and homocysteine levels

The complete blood count and serum iron, vitamin B12, folic acid, and homocysteine levels were determined by the routine tests performed in the Department of Laboratory Medicine, NTUH.[1,14,15]

Determination of serum gastric parietal cell antibody level

The serum GPCA level was detected by the indirect immunofluorescence assay with rat stomach as a substrate as described previously.[1] 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 hemoglobin (Hb) and serum iron, vitamin B12, folic acid, and homocysteine levels between 170 BMS patients with hyperhomocysteinemia or 714 BMS patients without hyperhomocysteinemia and 442 healthy control subjects as well as between 170 BMS patients with hyperhomocysteinemia and 714 BMS patients without hyperhomocysteinemia were performed by Student's *t*-test. The differences in frequencies of microcytosis (defined as MCV $<$ 80 fL),[13,16] macrocytosis (defined as MCV $\geq$ 100 fL),[17–19] blood Hb and serum iron, vitamin B12, and folic acid deficiencies, and serum GPCA positivity between 170 BMS patients with hyperhomocysteinemia or 714 BMS patients without hyperhomocysteinemia and 442 healthy control subjects as well as between 170 BMS patients with hyperhomocysteinemia and 714 BMS patients without hyperhomocysteinemia were compared by chi-square test. Comparisons of frequencies of macrocytic, normocytic or microcytic anemia between 170 BMS patients with hyperhomocysteinemia and 714 BMS patients without hyperhomocysteinemia were performed by chi-square test, too. In addition, comparisons of frequencies of patients with low, moderate, or high serum levels of iron, vitamin B12, and folic acid between 170 BMS patients with hyperhomocysteinemia and 714 BMS patients without hyperhomocysteinemia 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 170 BMS patients with hyperhomocysteinemia, 714 BMS patients without hyperhomocysteinemia, and 442 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 170 BMS patients with hyperhomocysteinemia than in 442 healthy control subjects (all *P*-values $<$ 0.01, Table 1). Moreover, we also found significantly lower mean blood Hb (for men and women) and serum iron (for men only), vitamin B12, and folic acid levels as well as significantly higher MCV and mean serum homocysteine level in 170 BMS patients with hyperhomocysteinemia than in 714 BMS patients without hyperhomocysteinemia (all *P*-values $<$ 0.001, Table 1). Furthermore, 714 BMS patients without hyperhomocysteinemia also had significantly lower MCV, mean

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 170 burning mouth syndrome (BMS) patients with hyperhomocysteinemia, 714 BMS patients without hyperhomocysteinemia, and 442 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			
BMS patients with hyperhomocysteinemia (n = 170)	91.7 ± 8.5	13.9 ± 1.7 (n = 69)	12.5 ± 1.3 (n = 101)	81.2 ± 23.4 (n = 69)	84.6 ± 30.0 (n = 101)	382.0 ± 233.2	11.8 ± 6.0	15.8 ± 5.4
[a]*P*-value	0.008	<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	0.109	<0.001	<0.001	<0.001
BMS patients without hyperhomocysteinemia (n = 714)	89.1 ± 6.9	14.9 ± 1.2 (n = 143)	13.2 ± 1.1 (n = 571)	97.8 ± 26.7 (n = 143)	90.1 ± 32.0 (n = 571)	700.9 ± 237.7	15.0 ± 7.6	7.7 ± 1.9
[a]*P*-value	<0.001	0.138	<0.001	0.035	<0.001	0.632	0.475	<0.001
Healthy control subjects (n = 442)	90.4 ± 3.6	15.1 ± 0.8 (n = 106)	13.5 ± 0.7 (n = 336)	105.2 ± 28.0 (n = 106)	97.8 ± 27.2 (n = 336)	694.2 ± 220.2	14.7 ± 5.7	8.3 ± 2.0

[a] Comparisons of means of parameters between 170 BMS patients with hyperhomocysteinemia or 714 BMS patients without hyperhomocysteinemia and 442 healthy control subjects by Student's *t*-test.

[b] Comparisons of means of parameters between 170 BMS patients with hyperhomocysteinemia and 714 BMS patients without hyperhomocysteinemia by Student's *t*-test.

blood Hb (for women only) and serum iron (for men and women) and homocysteine levels than healthy control subjects (all *P*-values < 0.05, Table 1).

According to the World Health Organization (WHO) criteria, microcytosis of erythrocyte was defined as having MCV < 80 fL,[13,16] macrocytosis of erythrocyte was defined as having MCV ≥ 100 fL,[17–19] and men with Hb < 13 g/dL and women with Hb < 12 g/dL were defined as having Hb deficiency or anemia.[20] Furthermore, patients with the serum iron level < 60 μg/dL,[21] the serum vitamin B12 level < 200 pg/mL,[22] or the serum folic acid level < 4 ng/mL[23] were defined as having iron, vitamin B12 or folic acid deficiency, respectively. In addition, patients with the blood homocysteine level >12.3 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, 5.3%, 16.5%, 45.3%, 22.9%, 20.6%, 8.2%, and 18.8% of 170 BMS patients with hyperhomocysteinemia and 8.3%, 2.5%, 13.7%, 14.6%, 1.0%, 0.8%, and 10.8% of 714 BMS patients without hyperhomocysteinemia 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 170 BMS patients with hyperhomocysteinemia had significantly higher frequencies of microcytosis, macrocytosis, blood Hb and serum iron, vitamin B12, and folic acid deficiencies, and serum GPCA positivity than 442 healthy control subjects (all *P*-values < 0.001) and significantly higher frequencies of macrocytosis, blood Hb and serum vitamin B12 and folic acid deficiencies, and serum GPCA positivity than 714 BMS patients without hyperhomocysteinemia (all *P*-values < 0.01). Moreover, 714 BMS patients without hyperhomocysteinemia had significantly higher frequencies of microcytosis, macrocytosis, blood Hb and serum iron deficiencies, and serum GPCA positivity than healthy control subjects (all *P*-values < 0.005, Table 2).

We also found that 77 (45.3%) of 170 BMS patients with hyperhomocysteinemia and 98 (13.7%) of 714 BMS patients without hyperhomocysteinemia had anemia (defined as having an Hb concentration < 13 g/dL for men and <12 g/dL for women).[20] Of the 77 anemic BMS patients with hyperhomocysteinemia, 15 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),[17–19] 9 had macrocytic anemia (defined as having anemia and an MCV ≥ 100 fL) other than PA,[17–19] 47 had normocytic anemia (defined as having anemia and an MCV between 80.0 fL and 99.9 fL),[24–27] and 6 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) (Table 3).[28] Of the 98 anemic BMS patients without hyperhomocysteinemia, 6 had macrocytic anemia other than PA, 48 had normocytic anemia, 21 had iron deficiency anemia (IDA), 21 had thalassemia trait-induced anemia, and two had microcytic anemia other than IDA and thalassemia trait-induced anemia (Table 3).

Comparisons of frequencies of macrocytic, normocytic or microcytic anemia between 170 BMS patients with hyperhomocysteinemia and 714 BMS patients without hyperhomocysteinemia are shown in Table 4. We found that 170 BMS patients with hyperhomocysteinemia had significantly higher frequencies of macrocytic anemia and normocytic anemia than 714 BMS patients without hyperhomocysteinemia (both *P*-values < 0.001, Table 4).

Distribution of patients with low, moderate, or high serum levels of iron, vitamin B12, and folic acid in 170 BMS patients with hyperhomocysteinemia and in 714 BMS patients without hyperhomocysteinemia is shown in Table 5. We found that 170 BMS patients with

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 170 burning mouth syndrome (BMS) patients with hyperhomocysteinemia, 714 BMS patients without hyperhomocysteinemia, and 442 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
BMS patients with hyperhomocysteinemia (n = 170)	9 (5.3)	28 (16.5)	77 (45.3)	39 (22.9)	35 (20.6)	14 (8.2)	32 (18.8)
[a]*P*-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
[b]*P*-value	0.252	<0.001	<0.001	0.011	<0.001	<0.001	0.006
BMS patients without hyperhomocysteinemia (n = 714)	59 (8.3)	18 (2.5)	98 (13.7)	104 (14.6)	7 (1.0)	6 (0.8)	77 (10.8)
[a]*P*-value	<0.001	0.002	<0.001	<0.001	0.090	0.131	<0.001
Healthy control subjects (n = 442)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	8 (1.8)

[a] Comparisons of frequencies of parameters between 170 BMS patients with hyperhomocysteinemia or 714 BMS patients without hyperhomocysteinemia and 442 healthy control subjects by chi-square test.
[b] Comparisons of frequencies of parameters between 170 BMS patients with hyperhomocysteinemia and 714 BMS patients without hyperhomocysteinemia by chi-square test.

Table 3 Anemia types and hematinic deficiencies in 77 anemic burning mouth syndrome (BMS) patients with hyperhomocysteinemia and in 98 anemic BMS patients without hyperhomocysteinemia.

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)
BMS patients with hyperhomocysteinemia (n = 170)					
Pernicious anemia	15 (19.5)	≥100	5 (33.3)	15 (100.0)	0 (0.0)
Other macrocytic anemia	9 (11.7)	≥100	2 (22.2)	5 (55.6)	0 (0.0)
Normocytic anemia	47 (61.0)	80—99.9	24 (51.1)	4 (8.5)	7 (14.9)
Thalassemia trait-induced anemia	6 (7.8)	<74	2 (33.3)	2 (33.3)	1 (16.7)
Total	77 (100.0)		33 (42.9)	26 (33.8)	8 (10.4)
BMS patients without hyperhomocysteinemia (n = 714)					
Other macrocytic anemia	6 (6.1)	≥100	3 (50.0)	0 (0.0)	0 (0.0)
Normocytic anemia	48 (49.0)	80—99.9	18 (37.5)	0 (0.0)	0 (0.0)
Iron deficiency anemia	21 (21.4)	<80	21 (100.0)	0 (0.0)	0 (0.0)
Thalassemia trait-induced anemia	21 (21.4)	<74	5 (23.8)	0 (0.0)	1 (4.8)
Other microcytic anemia	2 (2.1)	<80	0 (0.0)	0 (0.0)	0 (0.0)
Total	98 (100.0)		47 (48.0)	0 (0.0)	1 (1.0)

Table 4 Comparisons of frequencies of macrocytic, normocytic or microcytic anemia between 170 burning mouth syndrome (BMS) patients with hyperhomocysteinemia and 714 BMS patients without hyperhomocysteinemia.

Anemia type	Patient number (%)		[a]*P*-value
	BMS patients with hyperhomocysteinemia (n = 170)	BMS patients without hyperhomocysteinemia (n = 714)	
Macrocytic anemia	24 (14.1)	6 (0.8)	<0.001
Normocytic anemia	47 (27.7)	48 (6.7)	<0.001
Microcytic anemia	6 (3.5)	44 (6.2)	0.250
Total	77 (45.3)	98 (13.7)	<0.001

[a] Comparison of frequencies of macrocytic, normocytic or microcytic anemia between 170 BMS patients with hyperhomocysteinemia and 714 BMS patients without hyperhomocysteinemia by chi-square test.

hyperhomocysteinemia had significantly higher frequencies of serum iron, vitamin B12, and folic acid deficiencies as well as significantly higher frequencies of serum vitamin B12 between 200 and 800 pg/mL and folic acid between 4 and 15 ng/mL than 714 BMS patients without hyperhomocysteinemia (all *P*-values < 0.05, Table 5). However, 714 BMS patients without hyperhomocysteinemia had significantly higher frequencies of patients with serum iron level ≥100 μg/dL, vitamin B12 level ≥800 pg/mL or folic acid level ≥15 ng/mL than 170 BMS patients with hyperhomocysteinemia (Table 5).

Discussion

In this study, hyperhomocysteinemia was discovered in 170 (19.2%) of 884 BMS patients.[1] Our previous study also found hyperhomocysteinemia in 127 (11.9%) of 1064 atrophic glossitis patients,[29] in 52 (14.8%) of 352 oral lichen planus patients,[30] in 21 (7.7%) of 273 recurrent aphthous stomatitis patients,[31] in 9 (14.3%) of 63 Behcet's disease patients,[32] and in 29 (22.1%) of 131 oral precancer patients.[33] These findings suggest that among the five oral mucosal disease patients reported, the prevalence of hyperhomocysteinemia is highest in oral precancer patients and lowest in recurrent aphthous stomatitis patients.[29—33]

Our previous study found that 170 of 884 BMS patients have hyperhomocysteinemia.[1] Of 170 BMS patients with hyperhomocysteinemia, 9 (5.3%) had microcytosis, 28 (16.5%) macrocytosis, 77 (45.3%) anemia, 39 (22.9%) serum iron, 35 (20.6%) vitamin B12, and 14 (8.2%) folic acid deficiencies, and 32 (18.8%) serum GPCA positivity. Of the 884 BMS patients, 68 (7.7%) had microcytosis, 46 (5.2%) macrocytosis, 175 (19.8%) anemia, 143 (16.2%) serum iron,

Table 5 Distribution of patients with low, moderate, or high serum levels of iron, vitamin B12, and folic acid in 170 burning mouth syndrome (BMS) patients with hyperhomocysteinemia and in 714 BMS patients without hyperhomocysteinemia.

Group	Patient number (%)		[a]*P*-value
	BMS patients with hyperhomocysteinemia (n = 170)	BMS patients without hyperhomocysteinemia (n = 714)	
Serum iron level (μg/dL)			
< 60	39 (22.9)	104 (14.6)	0.011
Between 60 and 100	89 (52.4)	316 (44.2)	0.069
≥ 100	42 (24.7)	294 (41.2)	<0.001
Serum vitamin B12 level (pg/mL)			
< 200	35 (20.6)	7 (1.0)	<0.001
Between 200 and 800	118 (69.4)	421 (59.0)	0.015
≥ 800	17 (10.0)	286 (40.0)	<0.001
Serum folic acid level (ng/mL)			
< 4	14 (8.3)	6 (0.8)	<0.001
Between 4 and 15	108 (63.5)	384 (53.8)	0.027
≥ 15	48 (28.2)	324 (45.4)	<0.001

[a] Comparisons of frequencies of patients with low, moderate, or high serum levels of iron, vitamin B12, and folic acid between 170 BMS patients with hyperhomocysteinemia and 714 BMS patients without hyperhomocysteinemia by chi-square test.

42 (4.8%) vitamin B12, and 20 (2.3%) folic acid deficiencies, and 109 (12.3%) serum GPCA positivity.[1] Thus, when hyperhomocysteinemia is used as a biomarker, it can detect 13.2%, 60.9%, 44.0%, 27.3%, 83.3%, 70.0%, and 29.4% of BMS patients with microcytosis, macrocytosis, anemia, and serum iron, vitamin B12, and folic acid deficiencies, and serum GPCA positivity, respectively, indicating that hyperhomocysteinemia is a pretty good biomarker for screening the BMS patients with macrocytosis, anemia, serum vitamin B12 deficiency, or serum folic acid deficiency.[1] Moreover, when a physician wants to check the serum vitamin B12 and folic acid levels for a patient who is suspected to have serum vitamin B12 and/or folic acid deficiencies, concomitant examination of the serum homocysteine level is necessary. Furthermore, when the patients are discovered to have hyperhomocysteinemia, they are suggested to give vitamin B12 and/or folic acid supplement treatments even if they have a moderate serum vitamin B12 level (up to 600 pg/mL) or folic acid level (up to 14 ng/mL). Compared to WHO definitions for vitamin B12, folic acid and iron deficiencies,[21–23] our previous study used slightly higher cutoff points for serum vitamin B12 (≤450 pg/mL), folic acid (≤6 ng/mL) or iron (≤70 μg/dL for men and ≤65 μg/dL for women) level to start the relatively high doses of hematinic supplement treatments for 399 BMS patients regardless of having hyperhomocysteinemia or not.[15] 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.[15] 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.[15]

The GPCA is a gastric autoantibody that can cause destruction of gastric parietal cells, resulting in lack of intrinsic factor production[8] 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 170 BMS patients with hyperhomocysteinemia than in our 714 BMS patients without hyperhomocysteinemia.[9–12] However, the serum GPCA positivity was found in only 32 (18.8%) of our 170 BMS patients with hyperhomocysteinemia. Vitamin B12 and/or folic acid deficiencies also play important roles in causing hyperhomocysteinemia in patients. By WHO definitions, vitamin B12/folic acid deficiency was noted in 49 (28.8%) of our 170 BMS patients with hyperhomocysteinemia.[22,23] Moreover, serum GPCA positivity and/or vitamin B12/folic acid deficiency were found in 63 (37.1%) of our 170 BMS patients with hyperhomocysteinemia. These findings suggest that there may be other factors causing hyperhomocysteinemia in our 107 hyperhomocysteinemic BMS patients without serum GPCA positivity and/or vitamin B12/folic acid deficiency. Chronic consumption of alcohol or tobacco has been shown as the possible causes resulting in increased serum levels of homocysteine in patients.[33–35] In addition, other causes of hyperhomocysteinemia include 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, nicotinic acid, and fibric acid derivatives).[36]

In this study, we showed serum iron deficiency in 39 (22.9%; 10 patients also had serum GPCA positivity) of 170 BMS patients with hyperhomocysteinemia and in 104 (14.6%; 8 patients also had serum GPCA positivity) of 714 BMS patients without hyperhomocysteinemia. These findings indicate that the serum GPCA-induced reduction of

gastric hydrochloric acid secretion only play a minor role in causing serum iron deficiency in our 170 BMS patients with hyperhomocysteinemia and 714 BMS patients without hyperhomocysteinemia. Thus, there may be 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, and others) that result in serum iron deficiency in our BMS patients.[13]

This study also found folic acid deficiency in 14 (8.2%) of 170 BMS patients with hyperhomocysteinemia and in only 6 (0.8%) of 714 BMS patients without hyperhomocysteinemia. Folic acid deficiency can be due to several factors such as poor nutritional intake, malabsorption, hepatobiliary dysfunction, increased folate catabolism, and medication (e.g., methotrexate, 5-fluoro-uracil, phenytoin, etc.).[37] However, the exact etiologies resulting in folic acid deficiency in our 170 BMS patients with hyperhomocysteinemia and in our 714 BMS patients without hyperhomocysteinemia may need further studies.

This study also demonstrated a significantly higher frequency of anemia in 170 BMS patients with hyperhomocysteinemia (45.3%) than in 714 BMS patients without hyperhomocysteinemia (13.7%). The normocytic anemia (61.0%), PA (19.5%), and macrocytic anemia other than PA (11.7%) were the three most common types of anemia in our 170 BMS patients with hyperhomocysteinemia. Moreover, the normocytic anemia (49.0%), IDA (21.4%), and thalassemia trait-induced anemia (21.4%) were the three most common types of anemia in our 714 BMS patients without hyperhomocysteinemia. Further analyses demonstrated that macrocytic anemia and normocytic anemia occurred more commonly in 170 BMS patients with hyperhomocysteinemia than in 714 BMS patients without hyperhomocysteinemia. This result could be partially due to significantly higher frequencies of serum vitamin B12 and folic acid deficiencies and serum GPCA positivity in our 170 BMS patients with hyperhomocysteinemia than in our 714 BMS patients without hyperhomocysteinemia. The normocytic anemia in BMS patients could also be attributed to a mixture of conditions producing microcytic and macrocytic anemias, 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.[24–27] However, further studies are needed to explore whether our BMS patients have the above-mentioned conditions associated with normocytic anemia.

In conclusion, BMS patients with hyperhomocysteinemia had significantly higher frequencies of macrocytosis, anemia, serum iron, vitamin B12, and folic acid deficiencies, and serum GPCA positivity than healthy control subjects or BMS patients without hyperhomocysteinemia.

Declaration of Competing Interest

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

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