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Original Article

Anemia, hematinic deficiencies, and hyperhomocysteinemia in serum gastric parietal cell antibody-positive burning mouth syndrome patients without serum thyroid autoantibodies

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KEYWORDS

Abstract *Background/purpose:* Our previous study found that 70 of 884 burning mouth syndrome (BMS) patients have serum gastric parietal cell antibody (GPCA) positivity but without

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Gastric parietal cell antibody;
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Hyperhomocysteinemia

thyroglobulin antibody (TGA) and thyroid microsomal antibody (TMA) (so-called GPCA$^+$TGA$^-$TMA$^-$BMS patients). This study assessed whether these 70 GPCA$^+$TGA$^-$TMA$^-$BMS patients had significantly higher frequencies of macrocytosis, anemia, hematinic deficiencies, and hyperhomocysteinemia than 553 GPCA-negative, TGA-negative, and TMA-negative BMS (GPCA$^-$TGA$^-$TMA$^-$BMS) patients or 442 healthy control subjects.

Materials and methods: Complete blood count, serum iron, vitamin B12, folic acid, homocysteine, GPCA, TGA, and TMA levels in 70 GPCA$^+$TGA$^-$TMA$^-$BMS patients, 553 GPCA$^-$TGA$^-$TMA$^-$BMS patients, and 442 healthy control subjects were measured and compared.

Results: We found that 15.7%, 28.6%, 20.0%, 11.4%, 2.9%, and 25.7% of 70 GPCA$^+$TGA$^-$TMA$^-$BMS patients and 3.8%, 17.7%, 15.9%, 3.8%, 2.7%, and 20.1% of 553 GPCA$^-$TGA$^-$TMA$^-$BMS patients had macrocytosis, blood hemoglobin, iron, vitamin B12, and folic acid deficiencies, and hyperhomocysteinemia, respectively. Moreover, both 70 GPCA$^+$TGA$^-$TMA$^-$BMS patients and 553 GPCA$^-$TGA$^-$TMA$^-$BMS patients had significantly greater frequencies of macrocytosis, blood hemoglobin, serum iron, vitamin B12, and folic acid deficiencies, and hyperhomocysteinemia than 442 healthy control subjects (all P-values < 0.05). In addition, 70 GPCA$^+$TGA$^-$TMA$^-$BMS patients also had greater frequencies of macrocytosis, anemia, serum vitamin B12 deficiency, and hyperhomocysteinemia than 553 GPCA$^-$TGA$^-$TMA$^-$BMS patients (all P-values < 0.05).

Conclusion: The GPCA $^+$ TGA$^-$TMA$^-$BMS patients have significantly greater frequencies of macrocytosis, anemia, serum iron, vitamin B12, and folic acid deficiencies, and hyperhomocysteinemia than healthy control subjects and significantly greater frequencies of macrocytosis, anemia, serum vitamin B12 deficiency, and hyperhomocysteinemia than GPCA$^-$TGA$^-$TMA$^-$BMS patients.

Introduction

Burning mouth syndrome (BMS) is a disease characterized by burning sensation of the oral mucosa in the absence of clinically apparent oral mucosal alterations.[1,2] Our previous study has shown that 109 (12.3%), 191 (21.6%), and 201 (22.7%) of 884 BMS patients have serum gastric parietal cell antibody (GPCA), thyroglobulin antibody (TGA), and thyroid microsomal autoantibody (TMA, also known as anti-thyroid peroxidase antibody, anti-TPO antibody) positivities, respectively.[2] It is well known that GPCA can induce destruction of gastric parietal cells, resulting in failure of intrinsic factor and hydrochloric acid (HCl) production.[3,4] The intrinsic factor deficiency can cause vitamin B12 deficiency and finally lead to pernicious anemia (PA) in some of the vitamin B12-deficient patients.[5,6] The HCl deficiency can cause malabsorption of iron and finally result in iron deficiency.[7,8] The vitamin B12 deficiency may also lead to hyperhomocysteinemia in BMS patients.[9,10] Thus, GPCA positivity may have a significant influence on the red blood cell size and blood Hb, iron, vitamin B12, and homocysteine levels in GPCA-positive BMS patients.[1,9,10]

Moreover, we also demonstrated that 19.3%, 30.3%, 16.5%, 16.5%, 1.8%, and 29.4% of 109 GPCA-positive BMS patients have macrocytosis, blood hemoglobin (Hb), iron, vitamin B12, and folic acid deficiencies and hyperhomocysteinemia, respectively.[10] Of the 109 GPCA-positive BMS patients, 20 also have serum TGA and TMA positivities, 7 also have serum TGA positivity, 12 also have serum TMA positivity, and 70 have serum GPCA positivity only but without TGA and TMA positivities (so-called GPCA$^+$TGA$^-$TMA$^-$BMS patients).[2] Thus, these 70 GPCA$^+$TGA$^-$TMA$^-$BMS patients could be used to assess the relatively pure role of serum GPCA positivity in causing macrocytosis, blood hemoglobin (Hb), iron, vitamin B12, and folic acid deficiencies, and hyperhomocysteinemia in BMS patients. Furthermore, our previous study also discovered 553 BMS patients who were GPCA-negative, TGA-negative, and TMA-negative (so-called GPCA$^-$TGA$^-$TMA$^-$BMS patients).[2] These 553 GPCA$^-$TGA$^-$TMA$^-$BMS patients could be used to clarify the disease of BMS itself in the final development of macrocytosis, blood hemoglobin (Hb), iron, vitamin B12, and folic acid deficiencies, and hyperhomocysteinemia in BMS patients.

Therefore, the main purpose of this study was to evaluate whether 70 GPCA$^+$TGA$^-$TMA$^-$BMS patients had significantly higher frequencies of macrocytosis, anemia, hematinic deficiencies, and hyperhomocysteinemia than 553 GPCA$^-$TGA$^-$TMA$^-$BMS patients or 442 healthy control subjects. In addition, we also explored whether 553 GPCA$^-$TGA$^-$TMA$^-$BMS patients had significantly higher frequencies of macrocytosis, anemia, hematinic deficiencies, and hyperhomocysteinemia than 442 healthy control subjects.

Materials and methods

Subjects

In this study, 70 (19 men and 51 women, age range 21—85 years, mean age 56.7 $\pm$ 14.8 years) GPCA$^+$TGA$^-$TMA$^-$BMS patients and 553 (166 men and 387 women, age range 18—90 years, mean age 56.0 $\pm$ 15.2 years) GPCA$^-$TGA$^-$TMA$^-$BMS

patients were retrieved from our 884 BMS patients (212 men and 672 women, age range 18—90 years, mean 56.1 ± 14.5 years) whose anemia statuses, hematinic deficiencies, hyperhomocysteinemia, and frequencies of serum GPCA, TGA and TMA positivities were published before.[1,2] For comparisons of blood data, 442 age- (±2 years of each patient's age) and sex-matched healthy control subjects (106 men and 336 women, age range 18—90 years, mean 57.5 ± 13.5 years) were also retrieved from our previous study and included in this study.[1,2] 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.[1,2,10–14] The detailed inclusion and exclusion criteria for our BMS patients and healthy control subjects have been described previously.[1,2,10–14] 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 our BMS patients and healthy control subjects for measurement of complete blood count, serum iron, vitamin B12, folic acid, and homocysteine concentrations as well as serum GPCA, TGA, and TMA levels. All the BMS patients and healthy control subjects signed the informed consent forms before entering the study. This study was reviewed and approved by the Institutional Review Board at the NTUH (201212066RIND).

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

The complete blood count and serum iron, vitamin B12, folic acid, and homocysteine concentrations were determined by the routine tests performed in the Department of Laboratory Medicine of NTUH as described previously.[1,2,10–14] This study defined the Hb and hematinic deficiencies according to the World Health Organization (WHO) criteria. Thus, men with Hb $<$ 13 g/dL and women with Hb $<$ 12 g/dL were defined as having Hb deficiency or anemia.[15] Patients with serum iron level $<$60 μg/dL,[7,8,13] vitamin B12 level $<$200 pg/mL[16] or folic acid level $<$4 ng/mL[17] were defined as having iron, vitamin B12 or folic acid deficiency, respectively. Moreover, patients with the serum 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.[1,10–14]

Determination of serum gastric parietal cell antibody, thyroglobulin antibody, and thyroid microsomal antibody levels

GPCA, TGA, and TMA levels were measured by the routine tests performed in the Department of Laboratory Medicine, NTUH. Serum GPCA level was measured by indirect immunofluorescence assay. Sera were scored as positive for GPCA when they produced fluorescence at a serum dilution of 10-fold or more. Moreover, serum TGA and TMA levels were measured by chemiluminescent microparticle immunoassay. Sera were scored as positive for TGA or TMA when the serum TGA level was greater than 14.4 IU/mL or when the serum TMA level was greater than 5.6 IU/mL, respectively.[2]

Statistical analysis

Comparisons of the mean corpuscular volume (MCV), the mean blood levels of Hb, iron, vitamin B12, folic acid, and homocysteine between 70 $GPCA^+TGA^-TMA^-$BMS patients or 553 $GPCA^-TGA^-TMA^-$BMS patients and 442 healthy control subjects as well as between 70 $GPCA^+TGA^-TMA^-$BMS patients and 553 $GPCA^-TGA^-TMA^-$BMS patients were performed by Student's *t*-test. The differences in frequencies of microcytosis, macrocytosis, blood Hb, iron, vitamin B12, and folic acid deficiencies and hyperhomocysteinemia between 70 $GPCA^+TGA^-TMA^-$BMS patients or 553 $GPCA^-TGA^-TMA^-$BMS patients and 442 healthy control subjects as well as between 70 $GPCA^+TGA^-TMA^-$BMS patients and 553 $GPCA^-TGA^-TMA^-$BMS patients were compared by chi-square test. The result was considered to be significant if the *P*-value was less than 0.05.

Results

The MCV and mean blood concentrations of Hb, iron, vitamin B12, folic acid, and homocysteine in 70 $GPCA^+TGA^-TMA^-$BMS patients, 553 $GPCA^-TGA^-TMA^-$BMS patients, and 442 healthy control subjects are shown in Table 1. Because men and women usually had different normal blood Hb and iron levels, these two mean levels were calculated separately for men and women. We found that both 70 $GPCA^+TGA^-TMA^-$BMS and 553 $GPCA^-TGA^-TMA^-$BMS patients had significantly lower mean blood Hb (for both men and women) and serum iron (for both men and women) and vitamin B12 levels as well as a significantly higher mean serum homocysteine level than 442 healthy control subjects (all *P*-values $<$ 0.05, Table 1). Moreover, we also found a significantly lower mean serum vitamin B12 level (P = 0.030) and a higher mean serum homocysteine level (marginal significance, P = 0.067) in 70 $GPCA^+TGA^-TMA^-$BMS patients than in 553 $GPCA^-TGA^-TMA^-$BMS patients (Table 1).

We also found that 8.6%, 15.7%, 28.6%, 20.0%, 11.4%, 2.9%, and 25.7% of 70 $GPCA^+TGA^-TMA^-$BMS patients and 6.1%, 3.8%, 17.7%, 15.9%, 3.8%, 2.7%, and 20.1% of 553 $GPCA^-TGA^-TMA^-$BMS patients were diagnosed as having microcytosis, macrocytosis, blood Hb, iron, vitamin B12, and folic acid deficiencies, and hyperhomocysteinemia, respectively. Moreover, both 70 $GPCA^+TGA^-TMA^-$BMS patients and 553 $GPCA^-TGA^-TMA^-$BMS patients had significantly greater frequencies of microcytosis, macrocytosis, blood Hb, serum iron, vitamin B12, and folic acid deficiencies, and hyperhomocysteinemia than 442 healthy control subjects (all *P*-values $<$ 0.05). In addition, 70 $GPCA^+TGA^-TMA^-$BMS patients also had greater frequencies of macrocytosis, anemia, serum vitamin B12 deficiency, and hyperhomocysteinemia than 553 $GPCA^-TGA^-TMA^-$BMS patients (all *P*-values $<$ 0.05, Table 2).

In this study, 20 (28.6%) of 70 $GPCA^+TGA^-TMA^-$BMS patients and 98 (17.7%) of 553 $GPCA^-TGA^-TMA^-$BMS patients

Table 1 Comparisons of mean corpuscular volume (MCV) and mean blood concentrations of hemoglobin (Hb), iron, vitamin B12, folic acid, and homocysteine between 70 gastric parietal cell antibody (GPCA)-positive but thyroglobulin antibody (TGA)-negative and thyroid microsomal antibody (TMA)-negative burning mouth syndrome ($GPCA^+TGA^-TMA^-BMS$) patients or 553 GPCA-negative, TGA-negative, and TMA-negative BMS ($GPCA^-TGA^-TMA^-BMS$) patients and 442 healthy control subjects as well as between 70 $GPCA^+TGA^-TMA^-BMS$ patients and 553 $GPCA^-TGA^-TMA^-BMS$ patients.

Group	MCV (fL)	Hb (g/dL)		Iron (μg/dL)		Vitamin B12 (pg/mL)	Folic acid (ng/mL)	Homocysteine (μM)
		Men	Women	Men	Women			
$GPCA^+TGA^-TMA^-BMS$ patients (n = 70)	91.0 ± 8.7	14.3 ± 1.6 (n = 19)	12.9 ± 1.1 (n = 51)	90.6 ± 27.1 (n = 19)	85.5 ± 29.0 (n = 51)	570.4 ± 279.9	14.0 ± 6.2	10.3 ± 3.6
[a]*P*-value	0.314	0.001	<0.001	0.038	0.003	<0.001	0.346	<0.001
[b]*P*-value	0.213	0.148	0.259	0.675	0.336	0.030	0.915	0.067
$GPCA^-TGA^-TMA^-BMS$ patients (n = 553)	89.9 ± 6.7	14.8 ± 1.4 (n = 166)	13.1 ± 1.2 (n = 387)	93.2 ± 25.4 (n = 166)	90.2 ± 33.2 (n = 387)	644.4 ± 266.5	14.1 ± 7.5	9.4 ± 3.9
[a]*P*-value	0.158	0.046	<0.001	<0.001	<0.001	0.002	0.165	<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 70 $GPCA^+TGA^-TMA^-BMS$ patients or 553 $GPCA^-TGA^-TMA^-BMS$ patients and 442 healthy control subjects by Student's *t*-test.
[b] Comparisons of means of parameters between 70 $GPCA^+TGA^-TMA^-BMS$ patients and 553 $GPCA^-TGA^-TMA^-BMS$ 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), iron, vitamin B12, and folic acid deficiencies, and hyperhomocysteinemia between 70 serum gastric parietal cell antibody (GPCA)-positive but thyroglobulin antibody (TGA)-negative and thyroid microsomal antibody (TMA)-negative burning mouth syndrome ($GPCA^+TGA^-TMA^-BMS$) patients or 553 GPCA-negative, TGA-negative, and TMA-negative BMS ($GPCA^-TGA^-TMA^-BMS$) patients and 442 healthy control subjects as well as between 70 $GPCA^+TGA^-TMA^-BMS$ patients and 553 $GPCA^-TGA^-TMA^-BMS$ 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 (>12.3 μM)
$GPCA^+TGA^-TMA^-BMS$ patients (n = 70)	6 (8.6)	11 (15.7)	20 (28.6)	14 (20.0)	8 (11.4)	2 (2.9)	18 (25.7)
[a]*P*-value	<0.001	<0.001	<0.001	<0.001	<0.001	0.011	<0.001
[b]*P*-value	0.603	<0.001	0.043	0.484	0.011	0.749	<0.001
$GPCA^-TGA^-TMA^-BMS$ patients (n = 553)	34 (6.1)	21 (3.8)	98 (17.7)	88 (15.9)	21 (3.8)	15 (2.7)	111 (20.1)
[a]*P*-value	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	<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)	11 (2.5)

[a] Comparisons of frequencies of parameters between 70 $GPCA^+TGA^-TMA^-BMS$ patients or 553 $GPCA^-TGA^-TMA^-BMS$ patients and 442 healthy control subjects by chi-square test.
[b] Comparisons of frequencies of parameters between 70 $GPCA^+TGA^-TMA^-BMS$ patients and 553 $GPCA^-TGA^-TMA^-BMS$ patients by chi-square test.

were diagnosed as having anemia according to the WHO criteria.[15] In addition to having Hb deficiency (men with Hb < 13 g/dL and women with Hb < 12 g/dL), PA was diagnosed as having the MCV ≥ 100 fL, serum vitamin B12 < 200 pg/mL, and serum GPCA positivity,[3,5,18–20] macrocytic anemia as having the MCV ≥ 100 fL,[18–20] normocytic anemia as having the MCV between 80 and 99.9 fL,[1] iron deficiency anemia (IDA) as having the MCV < 80 fL and

serum iron $<$ 60 μg/dL,[7,8,13] thalassemia trait-induced anemia as having the red blood cell count $>$ 5.0 M/μL, the MCV $<$ 74 fL, and a Mentzer index (MCV/RBC) $<$ 13,[21] and microcytic anemia as having the MCV $<$ 80 fL.[8,14] By these definitions, of 20 anemic GPCA$^+$TGA$^-$TMA$^-$BMS patients, 8 had PA, three had macrocytic anemia other than PA, four had normocytic anemia, one had IDA, and four had thalassemia trait-induced anemia (Table 3). In addition, of the 98 anemic GPCA$^-$TGA$^-$TMA$^-$BMS patients, 9 had macrocytic anemia, 62 had normocytic anemia, 13 had IDA, 12 had thalassemia trait-induced anemia, and two had microcytic anemia rather than IDA and thalassemia trait-induced anemia (Table 3).

Discussion

This study found significantly greater frequencies of microcytosis, macrocytosis, blood Hb, iron, and vitamin B12 deficiencies, and hyperhomocysteinemia in 70 GPCA$^+$TGA$^-$TMA$^-$BMS patients than in 442 healthy control subjects. Moreover, 70 GPCA$^+$TGA$^-$TMA$^-$BMS patients did have significantly greater frequencies of macrocytosis, anemia, vitamin B12 deficiency, and hyperhomocysteinemia than 553 GPCA$^-$TGA$^-$TMA$^-$BMS patients. These two findings suggest that the significantly greater frequencies of microcytosis, macrocytosis, blood Hb, iron, and vitamin B12 deficiencies, and hyperhomocysteinemia in 70 GPCA$^+$TGA$^-$TMA$^-$BMS patients may be attributed to both serum GPCA positivity and the disease of BMS itself. Moreover, the significantly greater frequencies of macrocytosis, anemia, vitamin B12 deficiency, and hyperhomocysteinemia are mainly caused by the serum GPCA positivity and the significantly greater frequencies of microcytosis and serum iron and folic acid deficiencies in 70 GPCA$^+$TGA$^-$TMA$^-$BMS patients are predominantly caused by the disease of BMS itself.

In addition, this study also discovered that 553 GPCA$^-$TGA$^-$TMA$^-$BMS patients did have significantly greater frequencies of microcytosis, macrocytosis, blood Hb, iron, vitamin B12, and folic acid deficiencies, and hyperhomocysteinemia than 442 healthy control subjects. This finding indicates that the disease of BMS itself does play a significant role in causing microcytosis, macrocytosis, anemia, serum iron, vitamin B12, and folic acid deficiencies, and hyperhomocysteinemia in 553 GPCA$^-$TGA$^-$TMA$^-$BMS patients.

We further explained why GPCA might result in microcytosis, macrocytosis, blood Hb, iron, and vitamin B12 deficiencies, and hyperhomocysteinemia in 70 GPCA$^+$TGA$^-$TMA$^-$BMS patients. GPCA can induce destruction of gastric parietal cells, resulting in failure of intrinsic factor and HCl production.[3,4] The intrinsic factor deficiency can cause malabsorption of vitamin B12 from the terminal ileum and vitamin B12 deficiency.[5,6] The vitamin B12 deficiency in turn leads to macrocytosis, anemia, and hyperhomocysteinemia.[5,6,11,12,18–20] Moreover, the HCl deficiency can cause malabsorption of iron from the stomach and upper portion of the duodenum and iron deficiency.[7,13] The iron deficiency subsequently results in

Table 3 Anemia types of 20 anemic gastric parietal cell antibody (GPCA)-positive but thyroglobulin antibody (TGA)-negative and thyroid microsomal antibody (TMA)-negative burning mouth syndrome (GPCA$^+$TGA$^-$TMA$^-$BMS) patients and 98 anemic GPCA-negative, TGA-negative, and TMA-negative BMS (GPCA$^-$TGA$^-$TMA$^-$BMS) patients.

Anemia type	Patient number (%)				
	Patient number (%)	Mean corpuscular volume (fL)	Iron deficiency ($<$60 μg/dL)	Vitamin B12 deficiency ($<$200 pg/mL)	Folic acid deficiency ($<$4 ng/mL)
GPCA$^+$TGA$^-$TMA$^-$BMS patients (n = 70)					
Pernicious anemia	8 (40.0)	≥100	5 (62.5)	8 (100.0)	0 (0.0)
Macrocytic anemia	3 (15.0)	≥100	1 (33.3)	0 (0.0)	0 (0.0)
Normocytic anemia	4 (20.0)	80–99.9	2 (50.0)	0 (0.0)	0 (0.0)
Iron deficiency anemia	1 (5.0)	$<$80	1 (100.0)	0 (0.0)	0 (0.0)
Thalassemia trait-induced anemia	4 (20.0)	$<$74	1 (25.0)	0 (0.0)	1 (25.0)
Total	20 (100.0)		10 (50.0)	8 (40.0)	1 (5.0)
GPCA$^-$TGA$^-$TMA$^-$BMS patients (n = 553)					
Macrocytic anemia	9 (9.2)	≥100	2 (22.2)	4 (44.4)	0 (0.0)
Normocytic anemia	62 (63.3)	80–99.9	30 (48.4)	3 (4.8)	7 (11.3)
Iron deficiency anemia	13 (13.3)	$<$80	13 (100.0)	0 (0.0)	0 (0.0)
Thalassemia trait-induced anemia	12 (12.2)	$<$74	4 (33.3)	2 (16.7)	1 (8.3)
Other microcytic anemia	2 (2.0)	$<$80	0 (0.0)	0 (0.0)	0 (0.0)
Total	98 (100.0)		49 (50.0)	9 (9.2)	8 (8.2)

microcytosis and anemia.[7,13,14] The size of red blood cell is influenced by the serum levels of iron, vitamin B12 and folic acid.[4—8,13—20] If the vitamin B12 deficiency plays a more important role than iron deficiency in GPCA $^{+}$ TGA^{-}TMA^{-}BMS patients as seen in this study, then the MCV in our GPCA $^{+}$ TGA^{-}TMA^{-}BMS patients may be slightly larger than that in healthy control subjects.[13,14,20]

Our previous studies demonstrated that vitamin B12 and folic acid deficiencies can lead to high serum homocysteine level in oral mucosal disease patients.[1,22—26] Supplementation of multiple B vitamins especially vitamin B12 and folic acid can reduce the serum homocysteine levels in patients with atrophic glossitis or BMS.[9,22] In this study, GPCA^{+}TGA^{-}TMA^{-}BMS patients did not have a lower mean serum folic acid level and a higher frequency of folic acid deficiency than healthy control subjects or GPCA^{-}TGA^{-}TMA^{-}BMS patients, but had a significantly lower serum vitamin B12 level and a significantly higher frequency of vitamin B12 deficiency than healthy control subjects or GPCA^{-}TGA^{-}TMA^{-}AG patients. These findings suggest that the higher frequency of hyperhomocysteinemia in GPCA^{+}TGA^{-}TMA^{-}BMS patients than in healthy control subjects or in GPCA^{-}TGA^{-}TMABMS patients may be predominantly due to vitamin B12 deficiency in GPCA^{+}TGA^{-}TMA^{-}BMS patients.

In this study, 20 (28.6%) of 70 GPCA^{+}TGA^{-}TMA^{-}BMS patients and 98 (17.7%) of 553 GPCA^{-}TGA^{-}TMA^{-}BMS patients had anemia according to the strict WHO criteria.[15] Therefore, the frequency of anemia (28.6%) in 70 GPCA^{+}TGA^{-}TMA^{-}BMS patients was significantly higher than that (17.7%) in 553 GPCA^{-}TGA^{-}TMA^{-}BMS patients. PA was the most common type of anemia in 70 GPCA^{+}TGA^{-}TMA^{-}BMS patients (8 cases, 11.4%). Of the 8 GPCA^{+}TGA^{-}TMA^{-}BMS patients with PA, 5 had iron deficiency, 8 had vitamin B12 deficiency, and none had folic acid deficiency. Thus, the serum GPCA positivity and iron and vitamin B12 deficiencies were the major causes resulting in anemia in these 8 GPCA^{+}TGA^{-}TMA^{-}BMS patients with PA. Normocytic anemia was the second common type of anemia in 70 GPCA^{+}TGA^{-}TMA^{-}BMS patients (4 cases, 5.7%; two had iron deficiency and none had vitamin B12 or folic acid deficiency) and was the most common type of anemia in 553 GPCA^{-}TGA^{-}TMA^{-}BMS patients (62 cases, 11.2%; 30 had iron deficiency, 3 had vitamin B12 deficiency, and 7 had folic acid deficiency). Although the normocytic anemia was predominantly associated with 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,[27—29] the normocytic anemia in our GPCA $^{+}$ TGA^{-}TMA^{-}BMS and GPCA^{-}TGA^{-}TMA^{-}BMS patients was also partially attributed to the iron deficiency with occasional and concomitant vitamin B12 and/or folic acid deficiencies.

The present study revealed that only a small percentage (11.4%) of 70 GPCA^{+}TGA^{-}TMA^{-}BMS patients had PA. Our previous studies showed that 12.9% of 124 GPCA-positive oral mucosal disease patients (including 75 AG and 49 burning mouth syndrome patients) have PA,[18] 22 (7.7%) of 284 GPCA-positive atrophic glossitis patients have PA,[30] 7.3% of 41 GPCA-positive erosive oral lichen planus patients have PA,[31] 14.1% of 92 GPCA-positive erosive oral lichen planus patients with desquamative gingivitis have PA,[32] 13.3% of 15 GPCA-positive recurrent aphthous stomatitis patients with TGA/TMA positivity have PA,[33] and 9.7% of 31 recurrent aphthous stomatitis patients with GPCA positivity only (without TGA or TMA positivity) have PA.[34] These findings indicate that not all GPCA-positive oral mucosal disease patients have PA and only approximately 7.3%—14.1% of GPCA-positive oral mucosal disease patients have PA.

Our previous study found burning sensation of oral mucosa, dry mouth, numbness of oral mucosa, and dysfunction of taste in 100.0%, 48.1%, 30.7%, and 16.7% of 884 BMS patients, respectively. The oral mucosa-associated symptoms such as burning sensation, dry mouth, numbness, and dysfunction of taste all may interfere with the eating and swallowing function of BMS patients.[1] The eating and swallowing difficulties may result in reduced food intake that in turn leads to anemia, hematinic deficiencies, and hyperhomocysteinemia in a certain percentage of our BMS patients.[1]

We conclude that the GPCA is a major factor causing vitamin B12 deficiency, macrocytosis, and hyperhomocyteinemia in GPCA^{+}TGA^{-}TMA^{-}BMS patients. BMS itself does play a significant role in causing anemia, hematinic deficiencies, and hyperhomocysteinemia in both GPCA^{+}TGA^{-}TMA^{-}BMS and GPCA^{-}TGA^{-}TMA^{-}BMS patients. In addition, the GPCA^{+}TGA^{-}TMA^{-}BMS patients have significantly greater frequencies of macrocytosis, blood Hb, serum iron, vitamin B12, and folic acid deficiencies, and hyperhomocysteinemia than healthy control subjects and significantly greater frequencies of macrocytosis, anemia, serum vitamin B12 deficiency, and hyperhomocysteinemia than GPCA^{-}TGA^{-}TMA^{-}BMS patients.

Declaration of competing interest

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

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