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Original Article

# Anemia, hematinic deficiencies, and hyperhomocysteinemia in burning mouth syndrome patients with thyroglobulin antibody/thyroid microsomal antibody positivity but without gastric parietal cell antibody positivity

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**KEYWORDS**
Burning mouth syndrome;
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Thyroid microsomal antibody

**Abstract** *Background/purpose:* Our previous study found that 222 of 884 burning mouth syndrome (BMS) patients have thyroglobulin antibody (TGA) positivity and/or thyroid microsomal antibody (TMA) positivity but without gastric parietal cell antibody positivity ($GPCA^-TGA^+/TMA^+$BMS patients). This study mainly assessed whether the serum TGA/TMA positivity was significantly associated with anemia, hematinic deficiencies, and hyperhomocysteinemia in $GPCA^-TGA^+/TMA^+$BMS patients.
*Materials and methods:* The complete blood count, iron, vitamin B12, folic acid, and homocysteine levels were measured and compared between 222 $GPCA^-TGA^+/TMA^+$BMS patients and 553 GPCA-negative, TGA-negative, and TMA-negative BMS patients ($GPCA^-TGA^-TMA^-$BMS patients) or 442 healthy control subjects.
*Results:* We found that 222 $GPCA^-TGA^+/TMA^+$BMS patients had significantly lower mean corpuscular volume (MCV) and lower blood Hb and serum iron levels than 442 healthy control subjects and significantly lower MCV and lower serum homocysteine levels than 553 $GPCA^-TGA^-TMA^-$BMS patients. Moreover, 222 $GPCA^-TGA^+/TMA^+$BMS patients had significantly greater frequencies of microcytosis, macrocytosis, blood Hb and serum iron deficiencies, and hyperhomocysteinemia than 442 healthy control subjects and significantly higher frequency of microcytosis but significantly lower frequency of hyperhomocysteinemia than 553 $GPCA^-TGA^-TMA^-$BMS patients. However, no significant differences in the frequencies of macrocytosis, blood Hb, serum iron, vitamin B12, and folic acid deficiencies were discovered between 222 $GPCA^-TGA^+/TMA^+$BMS patients and 553 $GPCA^-TGA^-TMA^-$BMS patients.
*Conclusion:* We conclude that the disease of BMS itself does play a significant role in causing macrocytosis, anemia, hematinic deficiencies, and hyperhomocysteinemia in $GPCA^-TGA^+/TMA^+$BMS patients. However, the serum TGA/TMA-positivity is not significantly associated with anemia and serum iron, vitamin B12, and folic acid deficiencies in $GPCA^-TGA^+/TMA^+$BMS patients.


## Introduction

Burning mouth syndrome (BMS) is characterized by burning sensation of the oral mucosa in the absence of clinically apparent oral mucosal alterations.[1] Our previous study evaluated the symptoms of 884 BMS patients and found that 425 (48.1%) had dry mouth, 271 (30.7%) had numbness of oral mucosa, and 148 (16.7%) had dysfunction of taste.[1] 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]

Our previous study showed that 12.3%, 21.6%, and 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] Moreover, we also demonstrated that 19.8%, 16.2%, 4.8%, 2.3%, and 19.2% of 884 BMS patients have blood hemoglobin (Hb), iron, vitamin B12, and folic acid deficiencies and hyperhomocysteinemia, respectively.[1] Because GPCA can induce destruction of gastric parietal cells, resulting in failure of intrinsic factor and hydrochloric acid (HCl) production,[3,4] which in turn may lead to vitamin B12 deficiency, pernicious anemia, hyperhomocysteinemia, and iron deficiency in some GPCA-positive patients.[3—8] Thus, we also found 30.3%, 16.5%, 16.5%, 1.8%, and 29.4% of 109 GPCA-positive BMS patients have blood Hb, iron, vitamin B12, and folic acid deficiencies and hyperhomocysteinemia, respectively.[9] However, we have not yet known whether the serum TGA positivity and/or TMA positivity (TGA/TMA positivity) plays a significant role in causing anemia, hematinic deficiencies, and hyperhomocysteinemia in the 222 GPCA-negative TGA/TMA-positive BMS ($GPCA^-TGA^+/TMA^+$BMS) patients.

To assess the role of serum TGA/TMA positivity in the development of anemia, hematinic deficiencies, and hyperhomocysteinemia in BMS patients, 222 $GPCA^-TGA^+/TMA^+$BMS patients, 553 GPCA-negative, TGA-negative, and TMA-negative BMS patients ($GPCA^-TGA^-TMA^-$BMS patients), and 442 age- and sex-matched healthy control subjects were retrieved from our previous studies and included in this study.[1,2,9,10] The mean blood Hb, iron, vitamin B12, folic acid, and homocysteine levels in these 222 $GPCA^-TGA^+/TMA^+$BMS patients, 553 $GPCA^-TGA^-TMA^-$BMS patients, and 442 healthy control subjects were measured and compared one another to assess whether the serum TGA/TMA positivity was a significant factor causing anemia, hematinic deficiencies, and hyperhomocysteinemia in 222 $GPCA^-TGA^+/TMA^+$BMS patients.

## Materials and methods

### Subjects

This study included 222 (20 men and 202 women, age range 18–87 years, mean age 55.7 ± 13.2 years) GPCA$^-$TGA$^+$/TMA$^+$ BMS patients.[2] For evaluation of the role of serum TGA/TMA positivity in causing anemia, hematinic deficiencies, and hyperhomocysteinemia in BMS patients, 553 (166 men and 387 women, age range 18–90 years, mean age 56.0 ± 15.2 years) GPCA$^-$TGA$^-$TMA$^-$BMS patients and 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 retrieved from our previous studies and included in this study.[1,2,9,10] 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.[1,2,9–14] The detailed inclusion and exclusion criteria for our BMS patients and healthy control subjects have been described previously.[1,2,9–14] 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.[1,14,] 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] 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.[1,9–14]

### 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.[1,2] 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 222 GPCA$^-$TGA$^+$/TMA$^+$BMS patients and 553 GPCA$^-$TGA$^-$TMA$^-$BMS patients or 442 healthy control subjects 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 222 GPCA$^-$TGA$^+$/TMA$^+$BMS patients and 553 GPCA$^-$TGA$^-$TMA$^-$BMS patients or 442 healthy control subjects 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 222 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 significantly lower MCV and lower mean blood Hb and iron levels in 222 GPCA$^-$TGA$^+$/TMA$^+$BMS patients than in 442 healthy control subjects (all *P*-values $<$ 0.05, Table 1). However, there were no significant differences in the mean serum vitamin B12, folic acid, and homocysteine levels between 224 GPCA$^-$TGA$^+$/TMA$^+$BMS patients and 442 healthy control subjects. The 222 GPCA$^-$TGA$^+$/TMA$^+$BMS patients had significantly lower MCV and lower serum homocysteine level than 553 GPCA$^-$TGA$^-$TMA$^-$BMS patients (both *P*-values $<$ 0.001, Table 1). However, no significant differences in the mean blood Hb, iron, vitamin B12, and folic acid leve1s were discovered between 222 GPCA$^-$TGA$^+$/TMA$^+$BMS patients and 553 GPCA$^-$TGA$^-$TMA$^-$BMS patients, suggesting that the serum TGA/TMA-positivity does not play a significant role in causing the anemia and hematinic deficiencies in 222 GPCA$^-$TGA$^+$/TMA$^+$BMS patients (Table 1).

We also found significantly greater frequencies of microcytosis, macrocytosis, blood Hb and serum iron deficiencies, and hyperhomocysteinemia in 222 GPCA$^-$TGA$^+$/TMA$^+$BMS patients than in 442 healthy control subjects (all *P*-values $<$ 0.05, Table 2). The 222 GPCA$^-$TGA$^+$/TMA$^+$BMS patients also had greater frequencies of serum vitamin B12 and folic acid deficiencies than 442 healthy control subjects (both *P*-values = 0.066, marginal significance, Table 2). Moreover, 222 GPCA$^-$TGA$^+$/TMA$^+$BMS patients had significantly greater frequencies of microcytosis but significantly lower frequencies of hyperhomocysteinemia than 553

**Table 1** Comparisons of mean corpuscular volume (MCV) and mean blood hemoglobin (Hb), iron, vitamin B12, folic acid, and homocysteine levels between 222 thyroglobulin antibody (TGA)-positive and/or thyroid microsomal antibody (TMA)-positive burning mouth syndrome ($TGA^+/TMA^+$BMS) patients without serum GPCA positivity ($GPCA^-TGA^+/TMA^+$BMS patients) and 442 healthy control subjects or 553 GPCA-negative, TGA-negative, and TMA-negative BMS ($GPCA^-TGA^-TMA^-$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 = 222) | 87.9 ± 7.8 | 14.2 ± 1.7 (n = 20) | 13.1 ± 1.1 (n = 202) | 88.6 ± 38.4 (n = 20) | 88.5 ± 30.8 (n = 202) | 660.2 ± 252.2 | 14.6 ± 7.6 | 8.4 ± 3.2 |
| [a]*P*-value | <0.001 | <0.001 | <0.001 | 0.024 | <0.001 | 0.075 | 0.849 | 0.622 |
| [b]*P*-value | <0.001 | 0.079 | >0.999 | 0.473 | 0.546 | 0.449 | 0.403 | <0.001 |
| [c]$GPCA^-TGA^-TMA^-$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 |
| [c]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 222 $GPCA^-TGA^+/TMA^+$BMS patients and 442 healthy control subjects by Student's *t*-test.
[b] Comparisons of means of parameters between 222 $GPCA^-TGA^+/TMA^+$BMS patients and 553 $GPCA^-TGA^-TMA^-$BMS patients by Student's *t*-test.
[c] The blood examination data of 553 $GPCA^-TGA^-TMA^-$BMS patients and 442 healthy control subjects were retrieved from our previous studies.[1,2,10].

**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 222 thyroglobulin antibody (TGA)-positive and/or thyroid microsomal antibody (TMA)-positive burning mouth syndrome ($TGA^+/TMA^+$BMS) patients without serum gastric parietal cell antibody (GPCA) positivity ($GPCA^-TGA^+/TMA^+$BMS patients) and 442 healthy control subjects or 553 GPCA-negative, TGA-negative, and TMA-negative BMS ($GPCA^-TGA^-TMA^-$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 = 222) | 27 (12.2) | 4 (1.8) | 44 (19.8) | 37 (16.7) | 3 (1.4) | 3 (1.4) | 27 (12.2) |
| [a]*P*-value | <0.001 | 0.022 | <0.001 | <0.001 | 0.066 | 0.066 | <0.001 |
| [b]*P*-value | 0.008 | 0.231 | 0.562 | 0.881 | 0.122 | 0.382 | 0.012 |
| [c]$GPCA^-TGA^-TMA^-$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) |
| [c]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 222 $GPCA^-TGA^+/TMA^+$BMS patients and 442 healthy control subjects by chi-square test.
[b] Comparisons of frequencies of parameters between 222 $GPCA^-TGA^+/TMA^+$BMS patients and 553 $GPCA^-TGA^-TMA^-$BMS patients by chi-square test.
[c] The blood examination data of 553 $GPCA^-TGA^-TMA^-$BMS patients and 442 healthy control subjects were retrieved from our previous studies.[1,2,10].

$GPCA^-TGA^-TMA^-$BMS patients (both *P*-values < 0.05, Table 2). However, there were no significant differences in the frequencies of macrocytosis and blood Hb, iron, vitamin B12, and folic acid deficiencies between 222 $GPCA^-TGA^+/TMA^+$BMS patients and 553 $GPCA^-TGA^-TMA^-$BMS patients (Table 2). These findings also suggest that the serum TGA/TMA-positivity is not significantly associated with the anemia and serum iron, vitamin B12, and folic acid deficiencies in 222 $GPCA^-TGA^+/TMA^+$BMS patients, but the disease of BMS itself does play a significant role in causing the anemia,

hematinic deficiencies, and hyperhomocysteinemia in 222 GPCA$^-$TGA$^+$/TMA$^+$BMS patients.

In this study, 44 (19.8%) of 222 GPCA$^-$TGA$^+$/TMA$^+$ BMS patients were diagnosed as having anemia according to the WHO criteria.[15] In addition to having anemia (men with Hb $<$ 13 g/dL and women with Hb $<$ 12 g/dL), macrocytic anemia was diagnosed as having MCV $\geq$ 100 fL,[18–20] normocytic anemia as having MCV between 80 and 99.9 fL,[1,9,10] iron deficiency anemia (IDA) as having MCV $<$ 80 fL and iron $<$ 60 μg/dL,[7,8,13] and 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] By these definitions, of 44 anemic GPCA$^-$TGA$^+$/TMA$^+$BMS patients, two had macrocytic anemia rather than pernicious anemia, 25 had normocytic anemia, 7 had IDA, and 10 had thalassemia trait-induced anemia (Table 3).

## Discussion

This study predominantly assessed whether the serum TGA/TMA positivity was a significant factor causing anemia, hematinic deficiencies, and hyperhomocysteinemia in the GPCA$^-$TGA$^+$/TMA$^+$BMS patients. The rationale for the study design was that if the GPCA$^-$TGA$^+$/TMA$^+$BMS patients had significantly greater frequencies of anemia, hematinic deficiencies, and hyperhomocysteinemia than the GPCA$^-$TGA$^-$TMA$^-$BMS patients, then the serum TGA/TMA-positivity could be a significant factor causing anemia, hematinic deficiencies, and hyperhomocysteinemia in the GPCA$^-$TGA$^+$/TMA$^+$BMS patients. Our results found no significant differences in the mean blood Hb, serum iron, vitamin B12, and folic acid leve1s as well as no significant differences in the frequencies of blood Hb and serum iron, vitamin B12, and folic acid deficiencies between 222 GPCA$^-$TGA$^+$/TMA$^+$BMS patients and 553 GPCA$^-$TGA$^-$TMA$^-$BMS patients. In addition, the 222 GPCA$^-$TGA$^+$/TMA$^+$BMS patients had a significantly lower mean serum homocysteine level and a significantly lower frquency of hyperhomocysteinemia than the 553 GPCA$^-$TGA$^-$TMA$^-$BMS patients. These findings indicate that the serum TGA/TMA-positivity is not a significant factor causing anemia and serum iron, vitamin B12, and folic acid deficiencies in GPCA$^-$TGA$^+$/TMA$^+$BMS patients. Moreover, significantly lower mean blood Hb and serum iron levels, significantly greater frequencies of blood Hb and serum iron deficiencies and hyperhomocysteinemia, and marginally significantly greater frequencies of serum vitamin B12 and folic acid deficiencies were demonstrated in 222 GPCA$^-$TGA$^+$/TMA$^+$BMS patients than in 442 healthy control subjects, suggesting that the disease of BMS itself does play a significant role in causing anemia, hematinic deficiencies and hyperhomocysteinemia in 222 GPCA$^-$TGA$^+$/TMA$^+$BMS patients. Our previous study also discovered that 553 GPCA$^-$TGA$^-$TMA$^-$BMS patients had significantly lower mean blood Hb and serum iron and vitamin B12 levels, significantly higher mean serum homocysteine level, and significantly greater frequencies of blood Hb and serum iron, vitamin B12, and folic acid deficiencies and hyperhomocysteinemia than 442 healthy control subjects.[10] These findings also confirm that the disease of BMS itself is a significant factor causing anemia, hematinic deficiencies and hyperhomocysteinemia in 553 GPCA$^-$TGA$^-$TMA$^-$BMS patients.[10]

Our previous studies and this study found anemia in 175 (19.8%) of 884 BMS,[1] 33 (30.3%) of 109 GPCA$^+$BMS,[9] 142 (18.3%) of 775 GPCA$^-$BMS,[9] 20 (28.6%) of 70 GPCA$^+$TGA$^-$TMA$^-$BMS,[10] 98 (17.7%) of 553 GPCA$^-$TGA$^-$TMA$^-$BMS,[10] and 44 (19.8%) of 222 GPCA$^-$TGA$^+$/TMA$^+$BMS patients. These findings indicate that in the subgroups of BMS patients, GPCA-positive BMS patients with or without TGA/TMA positivity tend to have the higher frequencies of anemia (28.6—30.3%) and GPCA$^-$TGA$^-$TMA$^-$BMS patients have the lowest frequency of anemia (17.7%).[1,9,10]

For the iron deficiency in the subgroups of BMS patients, the iron deficiency was noted in 143 (16.2%) of 884 BMS,[1] 18 (16.5%) of 109 GPCA$^+$BMS,[9] 125 (16.1%) of 775 GPCA$^-$BMS,[9] 14 (20.0%) of 70 GPCA$^+$TGA$^-$TMA$^-$BMS,[10] 88 (15.9%) of 553 GPCA$^-$TGA$^-$TMA$^-$BMS,[10] and 37 (16.7%) of 222 GPCA$^-$TGA$^+$/TMA$^+$BMS patients. These findings indicate that in the subgroups of BMS patients, GPCA-positive AG patients with or without TGA/TMA positivity are prone to have the higher frequencies of iron deficiency (16.5—20.0%) and GPCA$^-$TGA$^-$TMA$^-$BMS patients have the lowest frequency of iron deficiency (15.9%).[1,9,10] The above findings also confirm the influence of GPCA positivity on the reduced absorption of

**Table 3** Anemia types of 44 anemic thyroglobulin antibody (TGA)-positive and/or thyroid microsomal antibody (TMA)-positive burning mouth syndrome (TGA$^+$/TMA$^+$BMS) patients without serum gastric parietal cell antibody (GPCA) positivity (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 = 222) | | | | | |
| Macrocytic anemia | 2 (4.6) | $\geq$100 | 1 (50.0) | 1 (50.0) | 0 (0.0) |
| Normocytic anemia | 25 (56.8) | 80—99.9 | 10 (40.0) | 0 (0.0) | 0 (0.0) |
| Iron deficiency anemia | 7 (15.9) | $<$80 | 7 (100.0) | 0 (0.0) | 0 (0.0) |
| Thalassemia trait-induced anemia | 10 (22.7) | $<$74 | 2 (20.0) | 0 (0.0) | 0 (0.0) |
| Total | 44 (100.0) | | 20 (45.5) | 1 (2.3) | 0 (0.0) |

iron from the stomach and duodenum and the subsequent iron deficiency.[1,9,10]

For the vitamin B12 deficiency in the subgroups of BMS patients, vitamin B12 deficiency was noted in 42 (4.8%) of 884 BMS,[1] 18 (16.5%) of 109 $GPCA^+$BMS,[9] 24 (3.1%) of 775 $GPCA^-$BMS,[9] 8 (11.4%) of 70 $GPCA^+TGA^-TMA^-$BMS,[10] 21 (3.8%) of 553 $GPCA^-TGA^-TMA^-$BMS,[10] and 3 (1.4%) of 222 $GPCA^-TGA^+/TMA^+$BMS patients. These findings indicate that in the subgroups of BMS patients, GPCA-positive BMS patients with or without TGA/TMA positivity do have the higher frequencies of vitamin B12 deficiency (11.4—16.5%) and $GPCA^-TGA^+/TMA^+$BMS patients have the lowest frequency of vitamin B12 deficiency (1.4%).[1,9,10] Moreover, the above findings also confirm a significant influence of GPCA positivity on the decreased absorption of vitamin B12 from the terminal ileum and the subsequent vitamin B12 deficiency.[1,9,10]

For the folic acid deficiency in the subgroups of BMS patients, the folic acid deficiency was noted in 20 (2.3%) of 884 BMS,[1] 2 (1.8%) of 109 $GPCA^+$BMS,[9] 18 (2.3%) of 775 $GPCA^-$BMS,[9] 2 (2.9%) of 70 $GPCA^+TGA^-TMA^-$BMS,[10] 15 (2.7%) of 553 $GPCA^-TGA^-TMA^-$BMS,[10] and 3 (1.4%) of 222 $GPCA^-TGA^+/TMA^+$BMS patients. These findings suggest that in the subgroups of BMS patients, $GPCA^+TGA^-TMA^-$BMS patients have the highest frequency of folic acid deficiency (2.9%) and $GPCA^-TGA^+/TMA^+$BMS patients have the lowest frequency of folic acid deficiency (1.4%).[1,9,10] Moreover, the above findings also suggest that GPCA positivity does not have a significant interference on the folic acid absorption from the jejunum.[1,9,10]

For the hyperhomocysteinemia in the subgroups of BMS patients, the hyperhomocysteinemia was noted in 170 (19.2%) of 884 BMS,[1] 32 (29.4%) of 109 $GPCA^+$BMS,[9] 138 (17.8%) of 775 $GPCA^-$BMS,[9] 18 (25.7%) of 70 $GPCA^+TGA^-TMA^-$BMS,[10] 111 (20.1%) of 553 $GPCA^-TGA^-TMA^-$BMS,[10] and 27 (12.2%) of 222 $GPCA^-TGA^+/TMA^+$BMS patients. GPCA-positive BMS patients with or without TGA/TMA positivity do have the higher frequencies of hyperhomocysteinemia (25.7—29.4%) and $GPCA^-TGA^+/TMA^+$BMS patients have the lowest frequency of hyperhomocysteinemia (12.2%).[1,9,10] Moreover, the above findings also provide evidence that GPCA positivity does have a significant influence on the absorption of vitamin B12 from the terminal ileum, resulting in the subsequent vitamin B12 deficiency and hyperhomocysteinemia.[1,9,10]

After analyses of the frequencies of anemia, hematinic deficiencies, and hyperhomocysteinemia in BMS patients and in different subgroups of BMS patients, we further conclude that the GPCA positivity plays a significant role in causing anemia, iron and vitamin B12 deficiencies, and hyperhomocysteinemia in BMS patients. The serum GPCA positivity does not have a significant influence on folic acid deficiency in BMS patients.[1,9,10] Moreover, the serum TGA/TMA-positivity is not significantly associated with anemia and hematinic deficiencies in $GPCA^-TGA^+/TMA^+$AG patients, but the disease of BMS itself does play a significant role in causing anemia, hematinic deficiencies, and hyperhomocysteinemia in BMS patients.

## Declaration of competing interest

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

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