



Original Article

Anemia, hematinic deficiencies, hyperhomocysteinemia, and gastric parietal cell antibody positivity in burning mouth syndrome patients with iron deficiency



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Received 7 November 2019

Available online 9 December 2019

KEYWORDS

Burning mouth syndrome;
Gastric parietal cell antibody;
Hyperhomocysteinemia;
Iron deficiency;
Normocytic anemia;
Iron deficiency anemia

Background/purpose: Our previous study found that 143 of 884 burning mouth syndrome (BMS) patients have iron deficiency (ID). This study assessed whether all BMS patients with ID (so-called ID/BMS patients) had iron deficiency anemia (IDA) and evaluated whether the ID/BMS patients had significantly higher frequencies of anemia, hematinic deficiencies, hyperhomocysteinemia, and serum gastric parietal cell antibody (GPCA) positivity than healthy control subjects.

Materials and methods: The blood hemoglobin (Hb) and serum iron, vitamin B12, folic acid, homocysteine, and GPCA levels in 143 ID/BMS patients and 442 healthy control subjects were measured and compared.

Results: We found that 143 ID/BMS patients had significantly lower mean blood Hb and serum iron, vitamin B12, folic acid levels as well as significantly higher mean serum homocysteine level than healthy control subjects (all *P*-values < 0.01). Moreover, 143 ID/BMS patients had

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significantly higher frequencies of blood Hb (55.9%) and serum iron (100.0%), vitamin B12 (7.7%), and folic acid (2.1%) deficiencies, hyperhomocysteinemia (27.3%), and serum GPCA positivity (12.6%) than 442 healthy control subjects (all P -values < 0.001). Furthermore, of 80 anemic ID/BMS patients, 5 (6.3%) had pernicious anemia, 5 (6.3%) had macrocytic anemia other than pernicious anemia, 42 (52.5%) had normocytic anemia, 21 (26.3%) had IDA, and 7 (8.8%) had thalassemia trait-induced anemia.

Conclusion: ID/BMS patients had significantly higher frequencies of blood Hb and serum iron, vitamin B12, and folic acid deficiencies, hyperhomocysteinemia, and serum GPCA positivity than healthy control subjects. Normocytic anemia is the most common type of anemia in ID/BMS patients.

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Introduction

Patients with burning mouth syndrome (BMS) have burning sensation of the oral mucosa, but oral examination reveals none of clinically apparent oral mucosal alterations. BMS occurs more commonly in middle-aged and elderly women. Clinically, BMS can be classified into the primary and secondary forms. The primary BMS is essential or idiopathic, in which none of organic local/systemic causes can be identified, and peripheral and central neuropathies are the possible etiologies. The secondary BMS is caused by local, systemic, and/or psychological factors.^{1–3}

Our previous study found that 143 (16.2%) and 109 (12.3%) of 884 BMS patients have iron deficiency (ID) and gastric parietal cell antibody (GPCA) positivity, respectively.³ The etiologies of ID may include a reduced intake of iron during old-age stage, a decreased absorption of iron in patients who have celiac disease, gastrectomy, atrophic gastritis, or *Helicobacter pylori* infection or who take antacids, H₂-receptor antagonists, or proton pump inhibitors, and chronic blood loss related to excessive menstrual flow, hematuria, epistaxis, hemoptysis, hemodialysis, or gastrointestinal diseases (such as gastric or colonic carcinoma, inflammatory bowel disease, ulcers, angiodysplasia, or intestinal worm colonization).^{4–6} Moreover, the serum GPCA can destroy gastric parietal cells, resulting in lack of secretion of intrinsic factors and hydrochloric acid.⁷ Intrinsic factor deficiency may lead to malabsorption of vitamin B12 from terminal ileum and finally the vitamin B12 deficiency.^{7–10} Furthermore, decreased gastric secretion of hydrochloric acid may cause iron malabsorption and subsequent iron deficiency.^{4–6} Therefore, it is interesting to know whether all BMS patients with ID (so-called ID/BMS patients in this study) have ID anemia (IDA) and whether ID/BMS patients are prone to have significantly higher frequencies of anemia, hematinic deficiencies, hyperhomocysteinemia, and serum GPCA positivity than healthy control subjects.

In our oral mucosal disease clinic, patients with BMS, atrophic glossitis, oral lichen planus, recurrent aphthous stomatitis, oral submucous fibrosis, or oral precancerous lesions are frequently encountered and patients with Behcet's disease are less commonly seen.^{3,11–30,31–53} For patients with one of these seven specific diseases, complete

blood count and serum iron, vitamin B12, folic acid, homocysteine, GPCA, thyroglobulin antibody, and thyroid microsomal antibody levels are frequently examined to assess whether these patients have anemia, hematinic deficiencies, hyperhomocysteinemia, and serum GPCA, thyroglobulin antibody, and thyroid microsomal antibody positivities.^{3,11–30,31–53}

In this study, 143 ID/BMS patients were retrieved from 884 BMS patients reported in our previous study.³ We tried to find out whether all ID/BMS patients had IDA and to assess whether the ID/BMS patients had significantly higher frequencies of anemia, serum iron, vitamin B12, and folic acid deficiencies, hyperhomocysteinemia, and serum GPCA positivity than healthy control subjects.

Materials and methods

Subjects

This study consisted of 143 (28 men and 115 women, age range 24–90 years, mean age 53.3 ± 15.9 years) ID/BMS patients retrieved from 884 BMS patients reported in our previous study.³ 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.³ 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.³ The detailed including and excluding criteria for our BMS patients and healthy control subjects have been described previously.³ 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 143 ID/BMS patients and 442 healthy control subjects for the measurement of complete blood count, serum iron, vitamin B12, folic acid, and homocysteine concentrations, 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.^{3,11–13}

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.^{3,11–13} 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 143 ID/BMS patients and 442 healthy control subjects were performed by Student's *t*-test. The differences in frequencies of microcytosis (defined as $MCV < 80$ fL),^{4–6,54,55} macrocytosis (defined as $MCV \geq 100$ fL),^{45–47} blood Hb and serum iron, vitamin B12, and folic acid deficiencies, hyperhomocysteinemia, and serum GPCA positivity between 143 ID/BMS patients and 442 healthy control subjects were compared by chi-square test. Moreover, comparisons of frequencies of patients with low, moderate, or high serum levels of vitamin B12 and folic acid between 80 anemic ID/BMS patients and 63 non-anemic ID/BMS 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 143 ID/BMS patients 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 MCV and lower mean blood Hb (for men and women) and serum iron (for men and women), vitamin B12, and folic acid levels as well as significantly higher mean serum homocysteine level in 143 ID/BMS patients than in 442 healthy control subjects (all *P*-values < 0.01 , Table 1).

According to the World Health Organization (WHO) criteria, microcytosis of erythrocyte was defined as having $MCV < 80$ fL,^{4–6,54,55} macrocytosis of erythrocyte was defined as having $MCV \geq 100$ fL,^{45–47} and men with

Table 1 Comparisons of mean corpuscular volume (MCV) and mean blood levels of hemoglobin (Hb), iron, vitamin B12, folic acid, and homocysteine between 143 burning mouth syndrome (BMS) patients with iron deficiency (ID/BMS patients) and 442 healthy control subjects.

Group	MCV (fL)	Hb (g/dL)		Iron (μ g/dL)		Vitamin B12 (pg/mL)	Folic acid (ng/mL)	Homocysteine (μ M)
		Men	Women	Men	Women			
ID/BMS patients (n = 143)	87.6 \pm 9.2	13.2 \pm 1.5 (n = 28)	12.2 \pm 1.4 (n = 115)	46.5 \pm 9.7 (n = 28)	43.8 \pm 13.9 (n = 115)	604.7 \pm 271.6	13.2 \pm 6.3	9.8 \pm 6.3
^a <i>P</i> -value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.008	< 0.001
Healthy control subjects (n = 442)	90.4 \pm 3.6	15.1 \pm 0.8 (n = 106)	13.5 \pm 0.7 (n = 336)	105.2 \pm 28.0 (n = 106)	97.8 \pm 27.2 (n = 336)	694.2 \pm 220.2	14.7 \pm 5.7	8.3 \pm 2.0

^a Comparisons of means of parameters between 143 ID/BMS patients and 442 healthy control subjects by Student's *t*-test.

Hb < 13 g/dL and women with Hb < 12 g/dL were defined as having Hb deficiency or anemia.⁵⁴ Furthermore, patients with the serum iron level < 60 µg/dL,⁵⁵ the serum vitamin B12 level < 200 pg/mL,⁵⁶ or the serum folic acid level < 4 ng/mL⁵⁷ 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, 21.0%, 7.7%, 55.9%, 100.0%, 7.7%, 2.1%, 27.3% and 12.6% of 143 ID/BMS patients were diagnosed as having microcytosis, macrocytosis, blood Hb and serum iron, vitamin B12, and folic acid deficiencies, hyperhomocysteinemia, and serum GPCA positivity, respectively. We found that 143 ID/BMS patients had significantly higher frequencies of microcytosis, macrocytosis, blood Hb and serum iron, vitamin B12, and folic acid deficiencies, hyperhomocysteinemia, and serum GPCA positivity than 442 healthy control subjects (all *P*-values < 0.001, Table 2)

We also found that 80 (55.9%) of 143 ID/BMS patients had anemia (defined as having an Hb concentration < 13 g/dL for men and <12 g/dL for women).⁵⁴ Of the 80 anemic ID/BMS patients, 5 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),⁴⁵⁻⁴⁷ 5 had macrocytic anemia (defined as having anemia and an MCV ≥ 100 fL) other than PA, 42 had normocytic anemia (NA, defined as having anemia and an MCV between 80.0 fL and 99.9 fL),⁵⁸⁻⁶¹ 21 had IDA (defined as having anemia, an MCV < 80 fL, and a serum iron level < 60 µg/dL),^{4-6,54,55} and 7 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).⁴⁹

Distribution of patients with low, moderate, or high serum levels of vitamin B12, and folic acid in 80 anemic ID/BMS patients and in 63 non-anemic ID/BMS patients is shown in Table 4. We found that 80 anemic ID/BMS patients had significantly lower frequencies of serum vitamin B12 level ≥ 800 pg/mL than 63 non-anemic ID/BMS patients (*P* = 0.027, Table 4).

Discussion

There were three major findings in this study. First, we found that 143 ID/BMS patients had significantly lower MCV, mean blood Hb and serum iron, vitamin B12, and folic acid levels as well as significantly higher mean serum homocysteine level than 442 healthy control subjects (all *P*-values < 0.01). Second, 143 ID/BMS patients had significantly higher frequencies of blood Hb (55.9%) and serum iron (100.0%), vitamin B12 (7.7%), and folic acid (2.1%) deficiencies, hyperhomocysteinemia (27.3%), and serum GPCA positivity (12.6%) than 442 healthy control subjects (all *P*-values < 0.001). Third, it was also interesting to know that not all ID/BMS patients had IDA and IDA (26.3%) was not the most common type of anemia in ID/BMS patients. On the contrary, NA (52.5%) was the most common type of anemia in ID/BMS patients, and IDA was only the second common type of anemia in ID/BMS patients.

Table 2 Comparisons of frequencies of microcytosis (mean corpuscular volume or MCV < 80 fL), macrocytosis (MCV ≥ 100 fL), blood hemoglobin (Hb) and serum iron, vitamin B12, and folic acid deficiencies, hyperhomocysteinemia, and gastric parietal cell antibody (GPCA) positivity between 143 burning mouth syndrome (BMS) patients with iron deficiency (ID/BMS patients) 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)	Hyperhomocysteinemia (>12.3 µM)	GPCA positivity
ID/BMS patients (n = 143)	30 (21.0)	11 (7.7)	80 (55.9)	143 (100.0)	11 (7.7)	3 (2.1)	39 (27.3)	18 (12.6)
^a <i>P</i> -value	<0.001	<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)	8 (1.8)

^a Comparisons of frequencies of parameters between 143 ID/BMS patients and 442 healthy control subjects by chi-square test.

Table 3 Anemia types, vitamin B12 and folic acid deficiencies, hyperhomocysteinemia, and gastric parietal cell antibody (GPCA) positivity in 80 anemic burning mouth syndrome (BMS) patients with iron deficiency (ID/BMS patients).

Anemia type	Patient number (%)					
	Patient number (%)	Mean corpuscular volume (fL)	Vitamin B12 deficiency (<200 pg/mL)	Folic acid deficiency (<4 ng/mL)	Hyperhomocysteinemia (>12.3 μM)	GPCA positivity
ID/BMS patients (n = 143)						
Pernicious anemia	5 (6.3)	≥100	5 (100.0)	0 (0.0)	5 (100.0)	5 (100.0)
Other macrocytic anemia	5 (6.3)	≥100	1 (20.0)	0 (0.0)	2 (40.0)	2 (40.0)
Normocytic anemia	42 (52.5)	80.0–99.9	2 (4.8)	2 (4.8)	24 (57.1)	2 (4.8)
Iron deficiency anemia	21 (26.3)	<80	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.8)
Thalassemia trait-induced anemia	7 (8.8)	<74	1 (14.3)	0 (0.0)	2 (28.6)	1 (14.3)
Total	80 (100.0)		9 (11.3)	2 (2.5)	33 (41.3)	11 (13.8)

NA was discovered in 42 (52.5%) of 80 anemic ID/BMS patients in this study. Of these 42 NA/ID/BMS patients, all of them had ID, two had vitamin B12 deficiency, two had folic acid deficiency, and two had serum GPCA positivity. These findings suggest that ID may be the major factor causing NA in these 42 NA/ID/BMS patients. Whereas the vitamin B12/folic acid deficiency was a very minor factor resulting in NA in these 42 NA/ID/BMS patients. The GPCA-induced hypochlorhydria and subsequent poor iron absorption could explain the ID in 2 GPCA-positive NA/ID/BMS patients. In addition to ID, NA could also be caused by 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.^{58–61}

Because iron is a structural element of heme (heme is composed of protoporphyrin IX and iron), in patients with ID, the heme synthesis is reduced. When intracellular heme is decreased in the erythroblast, heme-regulated inhibitor kinase (HRI) is active and its phosphorylation of the α -subunit of eukaryotic translational initiation factor 2 (eIF2 α) inhibits protein (Hb predominantly) synthesis, resulting in production of hypochromic and microcytic red blood cells.⁶⁰ Therefore, ID/BMS patients are supposed to

have microcytic anemia.^{4–6,55} However, 5 of our 143 ID/BMS patients were found to have macrocytosis, vitamin B12 deficiency, and serum GPCA positivity simultaneously. These 5 ID/BMS patients were diagnosed as having PA by the definition of PA.^{45–47} The serum GPCA can destroy gastric parietal cells, resulting in lack of intrinsic factors, malabsorption of vitamin B12, and finally vitamin B12 deficiency.⁷ Therefore, we suggest the GPCA-induced vitamin B12 deficiency plays an important role in causing macrocytosis and PA in our 5 ID/BMS patients with PA.

This study found IDA in 21 of 143 ID/BMS patients. Of the 21 ID/BMS patients with IDA, none had vitamin B12 and folic acid deficiencies, and only one had serum GPCA positivity. Thus, the GPCA-induced hypochlorhydria and subsequent iron malabsorption could explain the ID in only one of 21 ID/BMS patients with IDA.^{4–6} The ID in the rest of 20 ID/BMS patients with IDA could be attributed to the iron deficiency causes described in the second paragraph of the introduction section in this study.^{4–6}

This study discovered anemia was in 80 (55.9%) of 143 ID/BMS patients. The remaining 63 ID/BMS patients had no anemia. Our diagnosis of ID was based on the finding of low iron levels (<60 μg/dL) in the sera of BMS patients. The serum iron is not the only source of iron that can be used for making Hb in the body, because iron can be stored in serum

Table 4 Distribution of patients with low, moderate, or high serum levels of vitamin B12 and folic acid in 80 anemic burning mouth syndrome (BMS) patients with iron deficiency (ID/BMS patients) and in 63 non-anemic ID/BMS patients.

Group	Patient number (%)		*P-value
	Anemic ID/BMS patients (n = 80)	Non-anemic ID/BMS patients (n = 63)	
Serum vitamin B12 level (pg/mL)			
<200	9 (11.3)	2 (3.2)	0.138
Between 200 and 800	54 (67.5)	36 (57.1)	0.272
≥800	17 (21.2)	25 (39.7)	0.027
Serum folic acid level (ng/mL)			
<4	2 (2.5)	1 (1.6)	0.834
Between 4 and 15	54 (67.5)	38 (60.3)	0.475
≥15	24 (30.0)	24 (38.1)	0.401

*Comparisons of frequencies of patients with low, moderate, or high serum levels of vitamin B12 and folic acid between 80 anemic ID/BMS patients and 63 non-anemic ID/BMS patients by chi-square test.

ferritin (which reflects total body iron stores), bone marrow, and hepatocytes (appear to be a long-term reservoir for iron).^{4,5} In addition, iron is an essential component of Hb in red blood cells and of myoglobin in muscles, which contain around 60% of total body iron. Iron in erythrocytes can be recycled and further used for making Hb.⁵ The low serum iron level does not necessarily mean that the total body iron stores are deficient. Therefore, when the serum iron level is low (<60 µg/dL), if the patients still have enough body iron stores, some of these body-stored iron can still be available for construction of Hb for a period of several weeks or months.^{4,5} This can partially explain why those ID/BMS patients have serum ID but do not have anemia. We have to bear in mind that the low serum ferritin level (<30 µg/L) is the most sensitive and specific indicator of ID.^{4,5} However, serum ferritin concentrations are increased independently of iron status in acute and chronic inflammatory disorders, malignant disease, and liver disease - a serious diagnostic limitation. In these situations, patients with a ferritin concentration of 50 µg/L or higher could still be iron-deficient. In patients with chronic kidney disease, the serum ferritin level of 100 µg/L has been suggested as a cutoff point for the diagnosis of ID, and the serum ferritin level of 200 µg/L has been suggested as a cutoff point for the diagnosis of ID in hemodialysis patients.^{4,5} In addition, measurement of serum ferritin, transferrin saturation (i.e., the ratio of serum iron to total iron-binding capacity; transferrin saturation < 16% means IDA), serum soluble transferrin receptors (in case of ID, synthesis of transferrin receptors is increased, leading to a corresponding increase in soluble transferrin receptors), and the serum soluble transferrin receptors-ferritin index (when the index is low, anemia is probably caused by chronic disease; when it is high, ID is probably the major cause of anemia) are more accurate than classic red cell indices in the diagnosis of IDA.^{4,5}

This study found hyperhomocysteinemia in 39 (27.3%) of 143 ID/BMS patients. Of these 39 ID/BMS patients, 10 had serum GPCA positivity, 10 had vitamin B12 deficiency, and two had folic acid deficiency. The two folic acid-deficient ID/BMS patients did not have concomitant vitamin B12 deficiency. Therefore, we suggest that the folic acid and vitamin B12 deficiencies can explain the hyperhomocysteinemia in 12 of 39 ID/BMS patients with hyperhomocysteinemia. The hyperhomocysteinemia in the remaining 27 ID/BMS patients may be due to other reasons such as chronic consumption of alcohol or tobacco, a dysfunction of enzymes and cofactors associated with the process of homocysteine biosynthesis, 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).⁶²

In this study, we found that 143 ID/BMS patients had significantly higher frequencies of blood Hb (55.9%) and serum iron (100.0%), vitamin B12 (7.7%), and folic acid (2.1%) deficiencies, hyperhomocysteinemia (27.3%), and serum GPCA positivity (12.6%) than 442 healthy control subjects. In addition, NA (52.5%) is the most common type of anemia in 143 ID/BMS patients, followed by IDA (26.3%), thalassemia trait-induced anemia (8.8%), PA (6.3%), and macrocytic anemia other than PA (6.3%). We conclude that

ID/BMS patients had significantly higher frequencies of blood Hb and serum iron, vitamin B12, and folic acid deficiencies, hyperhomocysteinemia, and serum GPCA positivity than healthy control subjects. Not all ID/BMS patients have anemia. Moreover, NA but not IDA is the most common type of anemia in our ID/BMS patients. ID/BMS patients are also possible to have PA, if they have concomitant serum GPCA positivity and vitamin B12 deficiency.

Declaration of Competing Interest

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

Acknowledgements

This study was supported by the grants (No. 102-2314-B-002-125-MY3 and No. 105-2314-B-002-075-MY2) of Ministry of Science and Technology, Taiwan.

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