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Original Article

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

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KEYWORDS
Burning mouth syndrome;
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Background/Purpose: Normocytosis is defined as having the mean corpuscular volume (MCV) between 80 fL and 99.9 fL. This study evaluated whether 770 burning mouth syndrome (BMS) patients with normocytosis (so-called normocytosis/BMS patients) had significantly higher frequencies of anemia, hematinic deficiencies, hyperhomocysteinemia, and serum gastric

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Vitamin B12 deficiency;
Hyperhomocysteinemia;
Normocytosis

parietal cell antibody (GPCA) positivity than 442 healthy control subjects or 884 BMS patients.
Materials and methods: Complete blood count, serum iron, vitamin B12, folic acid, homocysteine, and GPCA levels in 884 BMS patients (including 770 normocytosis/BMS patients) and 442 healthy control subjects were measured and compared.
Results: We found that 12.3%, 13.2%, 2.2%, 2.3%, 17.3%, and 10.5% of 770 normocytosis/BMS patients had blood hemoglobin (Hb), iron, vitamin B12, and folic acid deficiencies, hyperhomocysteinemia, and serum GPCA positivity, respectively. Furthermore, 770 normocytosis/BMS patients had significantly higher frequencies of blood Hb, iron, vitamin B12, and folic acid deficiencies, hyperhomocysteinemia, and serum GPCA positivity than 442 healthy control subjects (all *P*-values < 0.005). On the contrary, 770 normocytosis/BMS patients had significantly lower frequencies of blood Hb and vitamin B12 deficiencies than overall 884 BMS patients (both *P*-values < 0.01).
Conclusion: We conclude that there are significantly higher frequencies of anemia, serum iron, vitamin B12, and folic acid deficiencies, hyperhomocysteinemia, and serum GPCA positivity in normocytosis/BMS patients than in healthy control subjects. On the contrary, normocytosis/BMS patients do have significantly lower frequencies of blood Hb and vitamin B12 deficiencies than overall BMS patients.

Introduction

Normocytosis of erythrocyte is defined as having the mean corpuscular volume (MCV) between 80 fL and 99.9 fL.[1–6] Patients with both normocytosis and some specific conditions or diseases may have normocytic anemia (NA). These specific conditions or diseases include a decreased production of normal-sized erythrocytes (e.g., anemia of chronic disease or inflammation, aplastic anemia), an increased destruction or loss of red blood cells (e.g., hemolysis, hypersplenism, posthemorrhagic anemia), an uncompensated increase in plasma volume (e.g., pregnancy, fluid overload), and a mixture of conditions producing microcytic and macrocytic anemias.[1–3] Our previous studies showed that 95 (54.3%) of 175 anemic burning mouth syndrome (BMS) patients, 117 (57.9%) of 202 anemic atrophic glossitis patients, 32 (65.3%) of 49 anemic erosive oral lichen planus patients, and 56 (52.3%) of 107 anemic recurrent aphthous stomatitis patients had NA, suggesting that NA is the most common type of anemia in these four types of oral mucosal disease patients. Moreover, some of these oral mucosal disease patients with NA may have simultaneous iron, vitamin B12, and/or folic acid deficiencies.[4–7] It is also well known that severe iron deficiency can cause microcytic anemia[8,9] and severe vitamin B12/folic acid deficiency can lead to macrocytic anemia.[10–14] Therefore, concomitant deficiencies of iron plus vitamin B12 and/or folic acid may result in NA.[11]

Our previous study found blood hemoglobin (Hb), iron, vitamin B12, and folic acid deficiencies, hyperhomocysteinemia, and serum gastric parietal cell antibody (GPCA) positivity in 19.8%, 16.2%, 4.8%, 2.3%, 19.2%, and 12.3% of 884 BMS patients, respectively.[4] In this study, 770 BMS patients with normocytosis (so-called normocytosis/BMS patients), 884 BMS patients, and 442 healthy control subjects were retrieved from our previous study.[4] Complete blood count, serum iron, vitamin B12, folic acid, homocysteine, and serum GPCA levels in these 770 normocytosis/BMS patients, 884 BMS patients, and 442 healthy control subjects were measured and compared. We tried to find out whether normocytosis/BMS patients had significantly higher frequencies of anemia, serum iron, vitamin B12, and folic acid deficiencies, hyperhomocysteinemia, and serum GPCA positivity than 442 healthy control subjects or 884 BMS patients.

Materials and methods

Subjects

This study included 770 normocytosis/BMS patients (186 men and 584 women, age range 18—90 years, mean age 56.1 ± 14.1 years) retrieved from our previously-reported 884 BMS patients.[4] Furthermore, the blood examination data of 884 BMS patients (212 men and 672 women, age range 18—90 years, mean 56.1 ± 14.5 years) and 442 healthy control subjects (106 men and 336 women, age range 18—90 years, mean 57.5 ± 13.5 years) were also retrieved from the same previous study for comparison.[4] 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 (such as dry mouth, numbness of oral mucosa, and dysfunction of taste) but no apparent clinical oral mucosal abnormality was found.[4] The detailed inclusion and exclusion criteria for our BMS patients and healthy control subjects have been described previously.[4,15–22] 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 BMS patients and 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 consent forms before entering the study. This study was reviewed and approved by the Institutional Review Board at the NTUH (201212066RIND).

Determination of blood hemoglobin, 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, NTUH.[4,15–23]

Determination of serum gastric parietal cell antibody level

The serum GPCA level was detected by the indirect immunofluorescence technique with rat stomach as a substrate as described previously.[4,15–22] Sera were scored as positive when they produced fluorescence at a dilution of 10-fold or more.

Statistical analysis

Comparisons of the MCV and mean blood concentrations of Hb, iron, vitamin B12, folic acid, and homocysteine between 770 normocytosis/BMS patients and 442 healthy control subjects or 884 BMS patients were performed by Student's *t*-test. The differences in frequencies of blood Hb, iron, vitamin B12, and folic acid deficiencies, hyperhomocysteinemia, and serum GPCA positivity between 770 normocytosis/BMS patients and 442 healthy control subjects or 884 BMS patients were compared by chi-square test. Moreover, comparisons of frequencies of patients with low, moderate, or high serum levels of iron, vitamin B12, and folic acid between 95 BMS patients with NA and 675 BMS patients without NA were performed by chi-square test. The result was considered to be significant if the *P*-value was less than 0.05.

Results

The MCV, mean blood concentrations of Hb, iron, vitamin B12, folic acid, and homocysteine in 770 normocytosis/BMS patients, 884 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 that 770 normocytosis/BMS patients had significantly lower mean blood Hb (for men and women) and serum iron (for 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). In addition, 770 normocytosis/BMS patients had a significantly higher MCV and a significantly higher mean blood Hb level (for women only) than 884 BMS patients (both *P*-values < 0.01, Table 1).

According to the World Health Organization (WHO) criteria, normocytosis of erythrocyte was defined as having an MCV between 80 fL and 99.9 fL,[1–7] and men with Hb < 13 g/dL and women with Hb < 12 g/dL were defined as having Hb deficiency or anemia.[24] Furthermore, patients with the serum iron level <60 μg/dL,[25] the serum vitamin B12 level <200 pg/mL,[26] or the folic acid level <4 ng/mL,[27] were defined as having serum iron, vitamin B12 or folic acid deficiency, respectively. In addition, 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. By the above-mentioned definitions, 12.3%, 13.2%, 2.2%, 2.3%, 17.3%, and 10.5% of 770 normocytosis/BMS patients were diagnosed as having blood Hb, iron, vitamin B12, and folic acid deficiencies, hyperhomocysteinemia, and serum GPCA positivity, respectively. Furthermore, 770 normocytosis/BMS patients had significantly higher frequencies of blood Hb, iron, vitamin B12, and folic acid deficiencies, hyperhomocysteinemia, and serum GPCA positivity than 442 healthy control subjects (all *P*-values < 0.005, Table 2). On the contrary, 770 normocytosis/BMS patients had significantly lower frequencies of blood Hb and serum vitamin B12 deficiencies than overall 884 BMS patients (all *P*-values < 0.01, Table 2).

In this study, BMS patients with NA were defined as having anemia and normocytosis (MCV between 80 fL and 99.9 fL).[1–7] By this definition, 770 normocytosis/BMS patients could be divided into 95 (12.3%) with NA and 675 (87.7%) without NA.[4] Distribution of patients with low, moderate, or high serum levels of iron, vitamin B12, and folic acid in 95 BMS patients with NA and in 675 BMS patients without NA is shown in Table 3. We found that 95 BMS patients with NA had significantly higher frequencies of serum iron and folic acid deficiencies and significantly lower frequencies of patients with serum iron ≥ 100 μg/dL and of patients with vitamin B12 level ≥ 800 pg/mL than 675 BMS patients without NA (Table 3).

Discussion

The major findings of this study were that 95 (12.3%), 102 (13.2%), 17 (2.2%), 18 (2.3%), 133 (17.3%), and 81 (10.5%) of 770 normocytosis/BMS patients were diagnosed as having anemia, serum iron, vitamin B12, and folic acid deficiencies, hyperhomocysteinemia, and serum GPCA positivity, respectively. Moreover, 770 normocytosis/BMS patients had significantly higher frequencies of anemia and serum iron, vitamin B12, and folic acid deficiencies, hyperhomocysteinemia, and serum GPCA positivity than 442 healthy control subjects (Table 2).

Of 95 BMS patients with NA, 42 (44.2%) had iron deficiency, 4 (4.2%) had vitamin B12 deficiency, and 7 (7.4%) had folic acid deficiency (Table 3). Thus, from the nutritional point of view, the iron deficiency was the major contributing factor and the folic acid/vitamin B12 deficiency was the very minor contributing factor causing anemia in these 95 BMS patients with NA. A more detailed analysis showed that of the 95 BMS patient with NA, two

Table 1 Comparisons of mean corpuscular volume (MCV), mean blood concentrations of hemoglobin (Hb), iron, vitamin B12, folic acid, and homocysteine between 770 burning mouth syndrome (BMS) patients with normocytosis (MCV between 80 fL and 99.9 fL) and 442 healthy control subjects or 884 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			
BMS patients with normocytosis (n = 770)	90.4 ± 3.7	14.8 ± 1.4 (n = 186)	13.3 ± 1.0 (n = 584)	93.4 ± 25.4 (n = 186)	91.5 ± 30.5 (n = 584)	653.6 ± 259.9	14.5 ± 7.5	9.0 ± 3.5
[a]*P*-value	>0.999	0.044	0.001	<0.001	0.002	0.006	0.627	<0.001
[b]*P*-value	0.006	0.172	0.002	0.704	0.213	0.283	0.785	0.123
[c]BMS patients (n = 884)	89.6 ± 7.3	14.6 ± 1.5 (n = 212)	13.1 ± 1.2 (n = 672)	92.4 ± 26.8 (n = 212)	89.3 ± 31.8 (n = 672)	639.6 ± 268.1	14.4 ± 7.4	9.3 ± 4.3
[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 770 BMS patients with normocytosis and 442 healthy control subjects by Student's *t*-test.
[b] Comparisons of means of parameters between 770 BMS patients with normocytosis and 884 BMS patients by Student's *t*-test.
[c] The blood examination data of 884 BMS patients and 442 healthy control subjects were retrieved from our previous study.[4]

Table 2 Comparisons of frequencies of blood hemoglobin, iron, vitamin B12, and folic acid deficiencies, hyperhomocysteinemia, and gastric parietal cell antibody (GPCA) positivity between 770 burning mouth syndrome (BMS) patients with normocytosis (MCV between 80 fL and 99.9 fL) and 442 healthy control subjects or 884 BMS patients.

Group	Patient number (%)					
	Hemoglobin 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
BMS patients with normocytosis (n = 770)	95 (12.3)	102 (13.2)	17 (2.2)	18 (2.3)	133 (17.3)	81 (10.5)
[a]*P*-value	<0.001	<0.001	0.004	0.003	<0.001	<0.001
[b]*P*-value	<0.001	0.117	0.008	0.950	0.335	0.282
[c]BMS patients (n = 884)	175 (19.8)	143 (16.2)	42 (4.8)	20 (2.3)	170 (19.2)	109 (12.3)
[c]Healthy control subjects (n = 442)	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 770 BMS patients with normocytosis and 442 healthy control subjects by chi-square test.
[b] Comparisons of frequencies of parameters between 770 BMS patients with normocytosis and 884 BMS patients by chi-square test.
[c] The blood examination data of 884 BMS patients and 442 healthy control subjects were retrieved from our previous study.[4]

had concomitant deficiencies of iron and vitamin B12, two had concomitant deficiencies of iron and folic acid, 38 had iron deficiency only, two had vitamin B12 deficiency only, and 5 had folic acid deficiency only.[4] These findings indicate concomitant deficiencies of iron plus either vitamin B12 or folic acid are rarely found in our 95 BMS patients with NA. Similar results were also discovered in our atrophic glossitis, erosive oral lichen planus, and recurrent aphthous stomatitis patients with NA, in which 40 (34.2%) of 117 atrophic glossitis patients with NA, 13 (40.6%) of 32 erosive oral lichen planus patients with NA, and 30 (53.6%) of 56 recurrent aphthous stomatitis patients with NA had serum iron deficiency, suggesting serum iron deficiency play a significant role in causing NA in these four types of oral mucosal disease patients.[4–7]

The pathogenesis of NA is multifactorial and is associated with disorders such as chronic diseases, inflammatory diseases, infections, bone marrow hypoplasia, decreased production of erythropoietin or a poor response to erythropoietin, hemolytic blood diseases, mild but persistent blood loss from gastrointestinal tract, and cytokine-induced suppression of erythropoiesis.[1–3] Therefore, further studies are needed to explore the exact mechanisms that result in formation of NA in our BMS patients with NA, especially for those 46 NA/BMS patients without deficiencies of iron, vitamin B12, and folic acid.

Table 3 Distribution of patients with low, moderate, or high serum levels of iron, vitamin B12, and folic acid in 95 burning mouth syndrome (BMS) patients with normocytic anemia (NA) and 675 BMS patients without NA.

Parameter	Patient number (%)		*P*-value (Chi-square test)
	BMS patients with NA (n = 95)	BMS patients without NA (n = 675)	
Serum iron level (μg/dL)			
<60	42 (44.2)	60 (8.9)	<0.001
Between 60 and 100	37 (39.0)	327 (48.4)	0.104
≥100	16 (16.8)	288 (42.7)	<0.001
Serum vitamin B12 level (pg/mL)			
<200	4 (4.2)	13 (1.9)	0.296
Between 200 and 800	68 (71.6)	414 (61.3)	0.069
≥800	23 (24.2)	248 (36.8)	0.023
Serum folic acid level (ng/mL)			
<4	7 (7.4)	11 (1.6)	0.002
Between 4 and 15	54 (56.8)	364 (53.9)	0.671
≥15	34 (35.8)	300 (44.5)	0.138

This study demonstrated that 102 (13.2%) normocytosis/BMS patients had serum iron deficiency. Iron deficiency can be attributed to chronic blood loss associated with excessive menstrual flow or gastrointestinal diseases (such as peptic ulcer, diverticulosis or malignancies), hypochlorhydria, a decrease intake of iron during old-age stage, and a reduced absorption of iron in patients with gastrectomy or celiac sprue.[8] Of 102 normocytosis/BMS patients with serum iron deficiency, 61 (59.8%) were older than 50 years of age and 8 (7.8%) had GPCA positivity. Patients with GPCA positivity may have destruction of gastric parietal cells, resulting in hypochlorhydria and poor absorption of iron.[28] Some of our normocytosis/BMS patients may also have atrophic gastritis or *Helicobacter pylori* infection or take antacids or drugs that decrease gastric acid production (such as H_2-receptor antagonists) or transport (such as proton pump inhibitors). The atrophic gastritis, *H. pylori* infection, or administration of antacids or drugs that decrease gastric acid production or transport all can lead to malabsorption of iron and subsequently resulting in iron deficiency.[29] However, other underlying causes that lead to iron deficiency in our normocytosis/BMS patients may need further studies.[8,24]

Furthermore, 17 (2.2%) of 770 normocytosis/BMS patients had vitamin B12 deficiency. Of the 17 normocytosis/BMS patients with vitamin B12 deficiency, only one (5.9%) had serum GPCA positivity, suggesting that in only 5.9% of patients, the vitamin B12 deficiency can be attributed to the GPCA-induced lack of intrinsic factors.[30–33] Thus, the other 16 normocytosis/BMS patients with vitamin B12 deficiency might be due to insufficient intake of vitamin B12, food-bound vitamin B12 malabsorption, ileal malabsorption of vitamin B12, and biologic competition (including bacterial overgrowth and tapeworm infestation) or defective transport of vitamin B12 (such as transcobalamin II deficiency).[12]

The present study also found that 18 (2.3%) of 770 normocytosis/BMS patients had serum folic acid deficiency. Folic acid deficiency is reported to be associated with poor nutritional intake, malabsorption, hepatobiliary dysfunction, increased folate catabolism, and medication (e.g., methotrexate, 5-flurouracil, and phenytoin).[10] Our previous study of 131 oral precancer patients discovered significantly lower mean serum folic acid levels in 87 cigarette smokers than in 44 non-smokers ($P = 0.002$) and in 26 heavy smokers (consuming >20 cigarettes per day) than in 61 light smokers (consuming ≤20 cigarettes per day) ($P = 0.024$), indicating that smoking or heavy tobacco consumption can decrease the serum folic acid level.[34] The folic acid deficiency in oral precancer patients with smoking habit may be due to consumption of a relatively large amount of folic acid for repair of damaged DNAs caused by the carcinogens in the smoke in oral epithelial cells.[34] However, further investigations are needed to understand the real etiologies for folic acid deficiency in our normocytosis/BMS patients.

In this study, 133 (17.3%) of 770 normocytosis/BMS patients had hyperhomocysteinemia. Of the 133 normocytosis/BMS patients with hyperhomocysteinemia, 11 had vitamin B12 deficiency, 13 had folic acid deficiency, and 15 (one of them also had vitamin B12 deficiency) had GPCA positivity. Therefore, in only 24 normocytosis/BMS patients (18.0%), the hyperhomocysteinemia could be resulted from vitamin B12 or folic acid deficiency.[4] Deficiencies of vitamin B6, folic acid, and vitamin B12 can lead to high serum homocysteine levels (hyperhomocysteinemia).[35] However, in our hospital routine blood examination does not include the measurement of serum vitamin B6 level; thus, we did not know whether our patients had vitamin B6 deficiency or not. Furthermore, chronic consumption of alcohol may also result in increased serum homocysteine levels.[36,37] In addition, our previous study also discovered that of 131 oral precancer patients, the 87 cigarette smokers have significantly higher mean serum homocysteine level than the 44 non-smokers ($P = 0.034$), indicating that persistent tobacco

consumption may raise the serum homocysteine level.[34] Further survey of the oral habits in our normocytosis/BMS patients is necessary to understand whether the alcohol drinkers and smokers had significantly higher mean serum homocysteine levels than the non-drinkers and non-smokers, respectively. In addition, the main cause of hyperhomocysteinemia has been reported to be a dysfunction of enzymes and cofactors associated with the process of homocysteine biosynthesis. Other causes may include excessive methionine intake, certain diseases (chronic renal failure, hypothyroidism, pernicious or sickle cell anemia, and malignant tumors in the breast, ovary, and pancreas) and side effects of some drugs (cholestyramine, metformin, methotrexate, nicotinic acid, and fibric acid derivatives).[38] However, further studies are needed to explore the real mechanisms resulting in hyperhomocysteinemia in our normocytosis/BMS patients.

This study showed blood Hb and serum iron, vitamin B12, and folic acid deficiencies, hyperhomocysteinemia, and serum GPCA positivity in 12.3%, 13.2%, 2.2%, 2.3%, 17.3%, and 10.5% of 770 normocytosis/BMS patients, respectively. Furthermore, 770 normocytosis/BMS patients had significantly higher frequencies of blood Hb and serum iron, vitamin B12, and folic acid deficiencies, hyperhomocysteinemia, and serum GPCA positivity than 442 healthy control subjects. On the contrary, 770 normocytosis/BMS patients had significantly lower frequencies of anemia and serum vitamin B12 deficiency than overall 884 BMS patients. We conclude that there are significantly higher frequencies of anemia and serum iron, vitamin B12, and folic acid deficiencies, hyperhomocysteinemia, and serum GPCA positivity in normocytosis/BMS patients than in healthy control subjects. On the contrary, normocytosis/BMS patients have significantly lower frequencies of anemia and serum vitamin B12 deficiency than overall BMS patients.

Declaration of competing interest

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

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