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Original Article

Anemia, hematinic deficiencies, hyperhomocysteinemia, and gastric parietal cell antibody positivity in burning mouth syndrome patients with vitamin B12 deficiency

Meng-Ling Chiang [a,b,c], Ying-Tai Jin [d,e], Chun-Pin Chiang [f,g,h,i], Yu-Hsueh Wu [f,g], Julia Yu-Fong Chang [g,h,i], Andy Sun [g,h*]

[a] Department of Pediatric Dentistry, Chang Gung Memorial Hospital, Taipei, Taiwan
[b] Department of Oral Pathology and Oral Diagnosis, Chang Gung Memorial Hospital, Taipei, Taiwan
[c] College of Medicine, Chang Gung University, Taoyuan, Taiwan
[d] Department of Pathology, Taiwan Adventist Hospital, Taipei, Taiwan
[e] Department of Pathology, National Cheng-Kung University Hospital, Tainan, Taiwan
[f] Department of Dentistry, Far Eastern Memorial Hospital, New Taipei City, Taiwan
[g] Graduate Institute of Clinical Dentistry, School of Dentistry, National Taiwan University, Taipei, Taiwan
[h] Department of Dentistry, National Taiwan University Hospital, College of Medicine, National Taiwan University, Taipei, Taiwan
[i] Graduate Institute of Oral Biology, School of Dentistry, National Taiwan University, Taipei, Taiwan

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Abstract *Background/purpose:* Our previous study found that 42 of 884 burning mouth syndrome (BMS) patients have vitamin B12 deficiency. This study assessed whether the vitamin B12-deficient BMS (B12D/BMS) patients had significantly higher frequencies of anemia, hematinic deficiencies, hyperhomocysteinemia, and serum gastric parietal cell antibody (GPCA) positivity than healthy control subjects and evaluated whether all B12D/BMS patients had pernicious anemia (PA).
Materials and methods: The blood hemoglobin (Hb) and serum iron, vitamin B12, folic acid, homocysteine, and GPCA levels in 42 B12D/BMS patients and 442 healthy control subjects were measured and compared.

* Corresponding author. Department of Dentistry, National Taiwan University Hospital, No. 1, Chang-Te Street, Taipei, 10048, Taiwan. Fax: +2 2389 3853.
E-mail address: andysun7702@yahoo.com.tw (A. Sun).

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Vitamin B12 deficiency

Results: We found that 42 B12D/BMS patients had significantly lower mean blood Hb and serum iron and vitamin B12 levels as well as significantly higher mean corpuscular volume (MCV) and mean serum homocysteine level than healthy control subjects (all *P*-values < 0.05). Moreover, 42 B12D/BMS patients had significantly higher frequencies of macrocytosis (52.4%), blood Hb (61.9%) and serum iron (26.2%) and vitamin B12 (100.0%) deficiencies, hyperhomocysteinemia (83.3%), and serum GPCA positivity (42.9%) than 442 healthy control subjects (all *P*-values < 0.001). Moreover, of 26 anemic B12D/BMS patients, 15 (57.7%) had PA, 5 (19.2%) had macrocytic anemia other than PA, 4 (15.4%) had normocytic anemia, and 2 (7.7%) had thalassemia trait-induced anemia.

Conclusion: B12D/BMS patients have significantly higher frequencies of macrocytosis, blood Hb and serum iron and vitamin B12 deficiencies, hyperhomocysteinemia, and serum GPCA positivity than healthy control subjects. Although PA is the most common type of anemia in our B12D/BMS patients, only 15 (35.7%) of 42 B12D/BMS patients have PA.

Introduction

Burning mouth syndrome (BMS) is characterized by the presence of burning sensation of the oral mucosa, but oral examination reveals none of clinically apparent oral mucosal alterations. BMS occurs more frequently in middle-aged and elderly women. 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.[1,2]

Our previous study found that 42 (4.8%) of 884 BMS patients have vitamin B12 deficiency.[2] The etiologies of vitamin B12 deficiency include inadequate intake, food-bound vitamin B12 malabsorption, the presence of gastric parietal cell antibody (GPCA) or intrinsic factor antibody or both in sera of patients, ileal malabsorption in patients with enteritis or ileal resection, biologic competition including bacterial overgrowth and tapeworm infestation, and defective transport such as transcobalamin II deficiency.[3] The GPCA can destroy gastric parietal cells, resulting in lack of intrinsic factors and hypochlorhydria.[4] Intrinsic factor deficiency may lead to malabsorption of vitamin B12 from terminal ileum and finally the vitamin B12 deficiency.[3–7] Vitamin B12 and/or folic acid deficiencies may lead to macrocytic anemia or hyperhomocysteinemia in BMS patients.[8–11] In addition, decreased gastric secretion of hydrochloric acid may cause iron malabsorption and subsequent iron deficiency.[12–14] Thus, it is interesting to know whether BMS patients with vitamin B12 deficiency (so-called B12D/BMS patients in this study) are prone to have significantly higher frequencies of anemia, serum iron vitamin B12, and folic acid deficiencies, hyperhomocysteinemia, and serum GPCA positivity than healthy control subjects.

In our oral mucosal disease clinic, patients with BMS, atrophic glossitis (AG), 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.[2,15–57] For patients with one of these seven specific diseases, complete blood count, 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.[2,15–57]

In this study, 42 B12D/BMS patients were retrieved from our previous study.[2] We tried to assess whether the 42 B12D/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 and evaluated whether all B12D/BMS patients had pernicious anemia (PA).

Materials and methods

Subjects

This study consisted of 42 (11 men and 31 women, age range 20–81 years, mean age 56.7 ± 15.5 years) B12D/BMS patients selected from 884 BMS patients in our previous study.[2] For two BMS patients, one age- ($\pm$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.[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.[2] The detailed including and excluding criteria for our BMS patients and healthy control subjects have been described previously.[2] 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 42 B12D/BMS patients and 442 healthy control subjects for the

measurement of complete blood count, serum iron, vitamin B12, folic acid, and homocysteine levels, and the serum GPCA positivity. All BMS patients and healthy control subjects signed the informed consents before entering the study. This study was reviewed and approved by the Institutional Review Board at the NTUH (201212066RIND).

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

The complete blood count and serum iron, vitamin B12, folic acid, and homocysteine levels were determined by the routine tests performed in the Department of Laboratory Medicine, NTUH.[2,15,17]

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.[2,15–17] 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 42 B12D/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),[12–14,52] macrocytosis (defined as MCV ≥ 100 fL),[49–51] blood Hb and serum iron, vitamin B12, and folic acid deficiencies, hyperhomocysteinemia, and serum GPCA positivity between 42 B12D/BMS patients and 442 healthy control subjects were compared by chi-square test. In addition, comparisons of frequencies of patients with low, moderate, or high serum levels of iron and folic acid between 26 anemic B12D/BMS patients and 16 non-anemic B12D/BMS patients were performed by chi-square or Fisher exact test, where appropriate. 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 42 B12D/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 mean blood Hb (for men and women) and serum iron (for men and women) and vitamin B12 levels as well as significantly higher MCV and mean serum homocysteine level in 42 B12D/BMS patients than in 442 healthy control subjects (all *P*-values < 0.05, Table 1).

According to the World Health Organization (WHO) criteria, microcytosis of erythrocyte was defined as having MCV < 80 fL,[12–14,52] macrocytosis of erythrocyte was defined as having MCV ≥ 100 fL,[49–51] and men with Hb < 13 g/dL and women with Hb < 12 g/dL were defined as having Hb deficiency or anemia.[58] Furthermore, patients with the serum iron level < 60 μg/dL,[59] the serum vitamin B12 level < 200 pg/mL,[60] or the serum folic acid level < 4 ng/mL[61] were defined as having 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, 7.1%, 52.4%, 61.9%, 26.2%, 100.0%, 0.0%, 83.3%, and 42.9% of 42 B12D/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 42 B12D/BMS patients had significantly higher frequencies of microcytosis, macrocytosis, blood Hb and serum iron and vitamin B12 deficiencies, hyperhomocysteinemia, and serum GPCA positivity than 442 healthy control subjects (all *P*-values < 0.001, Table 2).

We also found that 26 (61.9%) of 42 B12D/BMS patients had anemia (defined as having an Hb concentration < 13 g/dL for men and <12 g/dL for women).[58] Of the 26 anemic B12D/BMS patients, 15 had PA (defined as having anemia, an MCV ≥ 100 fL, a serum vitamin B12 level < 200 pg/mL, and the presence of serum GPCA positivity),[49–51] 5 had macrocytic anemia (defined as having anemia and an MCV ≥ 100 fL) other than PA,[49–51] 4 had normocytic anemia (defined as having

Table 1 Comparisons of mean corpuscular volume (MCV) and mean blood hemoglobin (Hb) and serum iron, vitamin B12, folic acid, and homocysteine levels between 42 burning mouth syndrome (BMS) patients with vitamin B12 deficiency (B12D/BMS patients) and 442 healthy control subjects.

Group	MCV (fL)	Hb (g/dL)		Iron (μg/dL)		Vitamin B12 (pg/mL)	Folic acid (ng/mL)	Homocysteine (μM)
		Men	Women	Men	Women			
B12D/BMS patients (n = 42)	96.7 ± 11.9	12.5 ± 1.1 (n = 11)	12.5 ± 1.1 (n = 31)	77.1 ± 20.8 (n = 11)	84.6 ± 28.4 (n = 31)	168.9 ± 31.4	15.2 ± 5.9	17.1 ± 9.7
[a]*P*-value	<0.001	<0.001	<0.001	0.002	0.010	<0.001	0.671	<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 42 B12D/BMS patients and 442 healthy control subjects 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) and serum iron, vitamin B12, and folic acid deficiencies, hyperhomocysteinemia, and gastric parietal cell antibody (GPCA) positivity between 42 burning mouth syndrome (BMS) patients with vitamin B12 deficiency (B12D/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
B12D/BMS patients (n = 42)	3 (7.1)	22 (52.4)	26 (61.9)	11 (26.2)	42 (100.0)	0 (0.0)	35 (83.3)	18 (42.9)
[a]*P*-value	<0.001	<0.001	<0.001	<0.001	<0.001	NA	<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)

NA = not assessed.
[a] Comparisons of frequencies of parameters between 42 B12D/BMS patients and 442 healthy control subjects by chi-square test.

Table 3 Anemia types, iron and folic acid deficiencies, hyperhomocysteinemia, and gastric parietal cell antibody (GPCA) positivity in 26 anemic burning mouth syndrome (BMS) patients with vitamin B12 deficiency (B12D/BMS patients).

Anemia type	Patient number (%)					
	Patient number (%)	Mean corpuscular volume (fL)	Iron deficiency (<60 μg/dL)	Folic acid deficiency (<4 ng/mL)	Hyperhomo-cysteinemia (>12.3 μM)	GPCA positivity
B12D/BMS patients (n = 42)						
Pernicious anemia	15 (57.7)	≥100	5 (33.3)	0 (0)	15 (100.0)	15 (100.0)
Other macrocytic anemia	5 (19.2)	≥100	1 (20.0)	0 (0)	5 (100.0)	0 (0)
Normocytic anemia	4 (15.4)	80.0–99.9	2 (50.0)	0 (0)	4 (100.0)	1 (25.0)
Thalassemia trait-induced anemia	2 (7.7)	<74	1 (50.0)	0 (0)	2 (100.0)	0 (0)
Total	26 (100.0)		9 (34.6)	0 (0)	26 (100.0)	16 (61.5)

anemia and an MCV between 80.0 fL and 99.9 fL),[62–65] two had thalassemia trait-induced anemia[53] (Table 3).

Distribution of patients with low, moderate, or high serum levels of iron and folic acid in 26 anemic B12D/BMS patients and in 16 non-anemic B12D/BMS patients is shown in Table 4. There were no significant differences in frequencies of patients with low, moderate, or high serum levels of iron and folic acid between 26 anemic B12D/BMS patients and 16 non-anemic B12D/BMS patients (Table 4).

Discussion

The major finding of this study was that 42 B12D/BMS patients had significantly higher frequencies of macrocytosis (52.4%), blood Hb (61.9%) and serum iron (26.2%) and vitamin B12 (100.0%) deficiencies, hyperhomocysteinemia (83.3%), and serum GPCA positivity (42.9%) than 442 healthy control subjects (all P-values < 0.001). Our previous study demonstrated that 56 vitamin B12-deficient AG (B12D/AG) patients had significantly higher frequencies of macrocytosis (53.6%), blood Hb (64.3%) and serum iron (26.8%) and folic acid (3.6%) deficiencies, hyperhomocysteinemia (89.3%), and serum GPCA positivity (55.4%) than 532 healthy control subjects (all P-values < 0.005).[20] Moreover, our previous study also found that 90 oral mucosal disease patients (including 34 with AG only, 21 with BMS only, 6 with oral lichen planus only, 15 with both oral lichen planus and AG, 9 with both recurrent aphthous stomatitis and AG, and 5 with both recurrent aphthous stomatitis and BMS) with vitamin B12 deficiency had significantly higher frequencies of macrocytosis (41.1%), blood Hb (38.9%) and serum iron (22.2%) deficiencies, hyperhomocysteinemia (72.2%), and serum GPCA positivity (47.8%) than 180 healthy control subjects (all P-values < 0.001).[50] These findings indicate that the frequencies of vitamin B12 deficiency-related macrocytosis, anemia, hyperhomocysteinemia, and serum GPCA positivity are slightly higher in AG patients than in BMS patients and are relatively higher in AG patients than in a mixed population of oral mucosal disease patients.[20,50]

Table 4 Distribution of patients with low, moderate, or high serum levels of iron and folic acid in 26 anemic burning mouth syndrome (BMS) patients with vitamin B12 deficiency (B12D/BMS patients) and in 16 non-anemic B12D/BMS patients.

Group	Patient number (%)		[a]P-value
	Anemic B12D/BMS patients (n = 26)	Non-anemic B12D/BMS patients (n = 16)	
Serum iron level (μg/dL)			
< 60	9 (34.6)	2 (12.5)	0.158
Between 60 and 100	15 (57.7)	9 (56.3)	0.819
≥ 100	2 (7.7)	5 (31.2)	0.085
Serum folic acid level (ng/mL)			
< 4	0 (0.0)	0 (0.0)	NA
Between 4 and 15	13 (50.0)	9 (56.3)	0.940
≥ 15	13 (50.0)	7 (43.7)	0.940

NA = not assessed.

[a] Comparisons of frequencies of patients with low, moderate, or high serum levels of iron and folic acid between 26 B12D/BMS patients and 16 non-anemic B12D/BMS patients by chi-square or Fisher exact test, where appropriate.

Severe vitamin B12 deficiency can result in macrocytosis of erythrocytes.[50,51,60] Macrocytosis was found in 22 (52.4%) of 42 B12D/BMS patients in the present study as well as in 30 (53.6%) of 56 B12D/AG patients[20] and in 37 (41.1%) of 90 vitamin B12-deficient oral mucosal disease patients in our previous studies.[50] Because both vitamin B12 and folic acid are involved in DNA synthesis, in patients with vitamin B12 or folic acid deficiency, decreased deoxynucleotide synthesis impairs S-phase progression of erythroblasts. Inhibition of erythroblast DNA replication can retard cell division, but it does not affect protein (Hb predominantly) synthesis rates, and therefore the relative sizes of the erythroblasts and their daughter cells increase.[64] This can explain why vitamin B12 deficiency results in macrocytosis of erythrocytes in oral mucosal disease patients (including BMS and AG patients) with vitamin B12 deficiency.

B12D/BMS patients are supposed to have macrocytic anemia or PA, because severe vitamin B12 deficiency can lead to macrocytosis or PA.[49–51] The present study found that 20 (47.6%) of 42 B12D/BMS patients have macrocytic anemia. Of these 20 BMS patients with macrocytic anemia, 15 had PA and 5 had macrocytic anemia other than PA. Our previous studies showed PA in 22 (39.3%) of 56 B12D/AG patients[20] and in 17 (18.9%) of 90 vitamin B12-deficient oral mucosal disease patients.[50] PA is also discovered in 10 (100.0%) of 10 vitamin B12-deficient BMS patients;[22] in 4 (12.5%) of 32 vitamin B12-deficient recurrent aphthous stomatitis patients;[33] in one (25%) of 4 vitamin B12-deficient Behcet's disease patients;[38] in 6 (24.0%) of 25 vitamin B12-deficient oral lichen planus patients;[23] in 3 (23.1%) of 13 vitamin B12-deficient erosive oral lichen planus patients;[26] in 13 (48.1%) of 27 vitamin B12-deficient erosive oral lichen planus patients with desquamative gingivitis.[27] Taken these findings together, for overall BMS, AG, recurrent aphthous stomatitis, Behcet's disease, and oral lichen planus patients, PA is detected in 12.5%—100% of these vitamin B12-deficient patients.[20,22,23,26,27,33,38,50]

Homocysteine is formed during methionine metabolism.[9] Both vitamin B12 and folic acid act as coenzymes for the conversion of homocysteine to methionine.[10] Moreover, vitamin B6 is a coenzyme for the conversion of homocysteine to cysteine.[10] Therefore, patients with vitamin B12, folic acid, and/or vitamin B6 deficiencies may have hyperhomocysteinemia.[9–11] This study demonstrated hyperhomocysteinemia in 35 (83.3%) of 42 B12D/BMS patients and folic acid deficiency in none (0%) of 35 hyperhomocysteinemia B12D/BMS patients. Our previous study also showed hyperhomocysteinemia in 50 (89.3%) of 56 B12D/AG patients and folic acid deficiency in 2 (4%) of 50 hyperhomocysteinemia B12D/AG patients.[20] Moreover, hyperhomocysteinemia and folic acid deficiency are found in 65 (72.2%) and none (0%) of 90 vitamin B12-deficient oral mucosal disease patients, respectively.[50] These findings suggest that vitamin B12 deficiency plays a major role in

causing hyperhomocysteinemia in 42 B12D/BMS patients, 56 B12D/AG patients, and 90 vitamin B12-deficient oral mucosal disease patients.[20,50]

GPCA can induce destruction of gastric parietal cells, resulting in failure of intrinsic factor production[4] and ileal malabsorption of vitamin B12 that finally leads to significantly higher frequencies of macrocytosis, anemia (including macrocytic, normocytic, and microcytic anemias), and vitamin B12 deficiency in our 42 B12D/BMS patients than in 442 healthy control subjects.[3–7] However, the serum GPCA positivity was found in only 18 (42.9%) of our 42 B12D/BMS patients, in only 31 (55.4%) of our 56 B12D/AG patients,[20] and in only 43 (47.8%) of 90 oral mucosal disease patients with vitamin B12 deficiency.[50] For 24 B12D/BMS patients, 25 B12D/AG patients, and 47 vitamin B12-deficient oral mucosal disease patients without serum GPCA positivity, the vitamin B12 deficiency may be due to other factors such as inadequate intake, vitamin B12 malabsorption, biologic competition including bacterial overgrowth and tapeworm infestation, and transcobalamin II deficiency.[3,50]

The serum iron deficiency was found in 11 (26.2%; 6 patients also had serum GPCA positivity) of 42 B12D/BMS patients. Our previous study discovered serum iron deficiency in 15 (26.8%; 12 patients also had serum GPCA positivity) of 56 B12D/AG patients.[20] These findings indicate that the GPCA-induced reduction of gastric hydrochloric acid secretion may play a major role in causing serum iron deficiency in our 42 B12D/BMS and 56 B12D/AG patients, although other factors such as decreased absorption (due to gastrectomy, duodenal bypass, *Helicobacter pylori* infection, celiac sprue, inflammatory bowel diseases, etc.), chronic blood loss (peptic ulcer, diverticulitis, angiodysplasia, hook worm infestation, ect.), and drug-related (glucocorticoids, nonsteroidal anti-inflammatory drugs, proton-pump inhibitors, etc.) iron deficiency may also play roles in causing iron deficiency in our 42 B12D/BMS and 56 B12D/AG patients.[12,13]

This study discovered that 42 B12D/BMS patients had significantly higher frequencies of macrocytosis (52.4%), blood Hb (61.9%) and serum iron (26.2%) and vitamin B12 (100.0%) deficiencies, hyperhomocysteinemia (83.3%), and serum GPCA positivity (42.9%) than 442 healthy control subjects. Of 26 anemic B12D/BMS patients, 15 (57.7%) had PA, 5 (19.2%) had macrocytic anemia other than PA, 4 (15.4%) had normocytic anemia, and two (7.7%) had thalassemia trait-induced anemia. We conclude that B12D/BMS patients have significantly higher frequencies of macrocytosis, blood Hb and serum iron and vitamin B12 deficiencies, hyperhomocysteinemia, and serum GPCA positivity than healthy control subjects. Although PA is the most common type of anemia in our B12D/BMS patients, only 15 (35.7%) of 42 B12D/BMS patients have PA, suggesting that not all B12D/BMS patients have PA.

Authorship statement

Conception and design of study: Chiang ML, Jin YT, Chiang CP, Sun A
Aacquisition of data: Chiang ML, Jin YT, Chiang CP, Chang JYF, Sun A
Analysis and/or interpretation of data: Chiang ML, Jin YT, Chiang CP, Sun A
Drafting the manuscript: Chiang ML, Jin YT, Chiang CP, Sun A
Revising the manuscript critically for important intellectual content: Chiang ML, Jin YT, Chiang CP, Chang JYF, Sun A
Approval of the version of the manuscript to be published (the names of all authors must be listed): Chiang ML, Jin YT, Chiang CP, Wu YH, Chang JYF, Sun A

Declaration of Competing Interest

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

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References

1. Sun A, Wu KM, Wang YP, Lin HP, Chen HM, Chiang CP. Burning mouth syndrome: a review and update. *J Oral Pathol Med* 2013;42:649–55.
2. Chiang CP, Wu YH, Wu YC, Chang JYF, Wang YP, Sun A. Anemia, hematinic deficiencies, hyperhomocysteinemia, and serum gastric parietal cell antibody positivity in 884 patients with burning mouth syndrome. *J Formos Med Assoc* 2019 (in press).
3. Oh RC, Brown DL. Vitamin B_{12} deficiency. *Am Fam Physician* 2003;67:979–86.
4. Taylor KB, Roitt IM, Doniach D, Coushman KG, Shapland C. Autoimmune phenomena in pernicious anemia: gastric antibodies. *BMJ* 1962;2:1347–52.
5. Snow CF. Laboratory diagnosis of vitamin B_{12} and folate deficiency. A guide for the primary care physician. *Arch Intern Med* 1999;159:1289–98.
6. Lahner E, Annibale B. Pernicious anemia: new insights from a gastroenterological point of view. *World J Gastroenterol* 2009;15:5121–8.
7. Green R. Vitamin B_{12} deficiency from the perspective of a practicing hematologist. *Blood* 2017;129:2603–11.
8. Aslinia F, Mazza JJ, Yale SH. Megaloblastic anemia and other causes of macrocytosis. *Clin Med Res* 2006;4:236–41.
9. Spence JD. Homocysteine-lowering therapy: a role in stroke prevention? *Lancet Neurol* 2007;6:830–8.
10. Chanarin I, Deacon R, Lumb M, Perry J. Cobalamin-folate interrelations. *Blood Rev* 1989;3:211–5.
11. Lonn E, Yusuf S, Arnold MJ, et al. Homocysteine lowering with folic acid and B vitamins in vascular disease. *N Engl J Med* 2006;354:1567–77.
12. Camaschella C. Iron-deficiency anemia. *N Engl J Med* 2015;372:1832–43.
13. Lopez A, Cacoub P, Macdougall IC, Peyrin-Biroulet L. Iron deficiency anaemia. *Lancet* 2016;387(10021):907–16.
14. Wu YC, Wang YP, Chang JYF, Cheng SJ, Chen HM, Sun A. Oral manifestations and blood profile in patients with iron deficiency anemia. *J Formos Med Assoc* 2014;113:83–7.
15. Chiang CP, Chang JYF, Wang YP, Wu YC, Wu YH, Sun A. Significantly higher frequencies of anemia, hematinic deficiencies, hyperhomocysteinemia, and serum gastric parietal cell antibody positivity in atrophic glossitis patients. *J Formos Med Assoc* 2018;117:1065–71.

16. Chiang CP, Chang JYF, Wang YP, Wu YH, Wu YC, Sun A. Gastric parietal cell and thyroid autoantibodies in patients with atrophic glossitis. *J Formos Med Assoc* 2019;118:973—8.
17. Chiang CP, Chang JYF, Wang YP, Wu YH, Wu YC, Sun A. Anemia, hematinic deficiencies, and hyperhomocysteinemia in gastric parietal cell antibody-positive and -negative atrophic glossitis patients. *J Formos Med Assoc* 2019;118:565—71.
18. Chiang CP, Chang JYF, Wang YP, Wu YH, Wu YC, Sun A. Hematinic deficiencies and hyperhomocysteinemia in gastric parietal cell antibody-positive or gastric and thyroid autoantibodies-negative atrophic glossitis patients. *J Formos Med Assoc* 2019;118:1114—21.
19. Kuo YS, Wu YH, Chang JYF, Wang YP, Wu YC, Sun A. Blood profile of atrophic glossitis patients with thyroglobulin antibody/thyroid microsomal antibody positivity but without gastric parietal cell antibody positivity. *J Formos Med Assoc* 2019;118:1218—24.
20. Wu YC, Wu YH, Chang JYF, Wang YP, Kuo YS, Sun A. Anemia, hematinic deficiencies, hyperhomocysteinemia, and serum gastric parietal cell antibody positivity in atrophic glossitis patients with vitamin B12 deficiency. *J Formos Med Assoc* 2019 (in press).
21. Sun A, Wang YP, Lin HP, Chen HM, Cheng SJ, Chiang CP. Significant reduction of homocysteine level with multiple B vitamins in atrophic glossitis patients. *Oral Dis* 2013;19:519—24.
22. Lin HP, Wang YP, Chen HM, Kuo YS, Lang MJ, Sun A. Significant association of hematinic deficiencies and high blood homocysteine levels with burning mouth syndrome. *J Formos Med Assoc* 2013;112:319—25.
23. Chen HM, Wang YP, Chang JYF, Wu YC, Cheng SJ, Sun A. Significant association of deficiencies of hemoglobin, iron, folic acid, and vitamin B12 and high homocysteine level with oral lichen planus. *J Formos Med Assoc* 2015;114:124—9.
24. Chang JYF, Chiang CP, Hsiao CK, Sun A. Significantly higher frequencies of presence of serum autoantibodies in Chinese patients with oral lichen planus. *J Oral Pathol Med* 2009;38:48—54.
25. Chang JYF, Chen IC, Wang YP, Wu YH, Chen HM, Sun A. Anemia and hematinic deficiencies in gastric parietal cell antibody-positive and antibody-negative erosive oral lichen planus patients with thyroid antibody positivity. *J Formos Med Assoc* 2016;115:1004—11.
26. Chang JYF, Wang YP, Wu YH, Su YX, Tu YK, Sun A. Hematinic deficiencies and anemia statuses in anti-gastric parietal cell antibody-positive or all autoantibodies-negative erosive oral lichen planus patients. *J Formos Med Assoc* 2018;117:227—34.
27. Chang JYF, Wang YP, Wu YC, Wu YH, Tseng CH, Sun A. Hematinic deficiencies and anemia statuses in antigastric parietal cell antibody-positive erosive oral lichen planus patients with desquamative gingivitis. *J Formos Med Assoc* 2016;115:860—6.
28. Chang JYF, Chiang CP, Wang YP, Wu YC, Chen HM, Sun A. Antigastric parietal cell and antithyroid autoantibodies in patients with desquamative gingivitis. *J Oral Pathol Med* 2017;46:307—12.
29. Sun A, Chang JYF, Chiang CP. Examination of circulating serum autoantibodies and hematinics is important for treatment of oral lichen planus. *J Formos Med Assoc* 2017;116:569—70.
30. Chiang CP, Chang JYF, Wang YP, Wu YH, Lu SY, Sun A. Oral lichen planus — differential diagnoses, serum autoantibodies, hematinic deficiencies, and management. *J Formos Med Assoc* 2018;117:756—65.
31. Sun A, Chen HM, Cheng SJ, et al. Significant association of deficiency of hemoglobin, iron, vitamin B12, and folic acid and high homocysteine level with recurrent aphthous stomatitis. *J Oral Pathol Med* 2015;44:300—5.
32. Wu YC, Wu YH, Wang YP, Chang JYF, Chen HM, Sun A. Antigastric parietal cell and antithyroid autoantibodies in patients with recurrent aphthous stomatitis. *J Formos Med Assoc* 2017;116:4—9.
33. Wu YC, Wu YH, Wang YP, Chang JYF, Chen HM, Sun A. Hematinic deficiencies and anemia statuses in recurrent aphthous stomatitis patients with or without atrophic glossitis. *J Formos Med Assoc* 2016;115:1061—8.
34. Wu YH, Chang JYF, Wang YP, Wu YC, Chen HM, Sun A. Anemia and hematinic deficiencies in anti-gastric parietal cell antibody-positive and —negative recurrent aphthous stomatitis patients with anti-thyroid antibody positivity. *J Formos Med Assoc* 2017;116:145—52.
35. Lin HP, Wu YH, Wang YP, Wu YC, Chang JYF, Sun A. Anemia and hematinic deficiencies in anti-gastric parietal cell antibody-positive or all autoantibodies-negative recurrent aphthous stomatitis patients. *J Formos Med Assoc* 2017;116:99—106.
36. Chiang CP, Chang JYF, Sun A. Examination of serum hematinics and autoantibodies is important for treatment of recurrent aphthous stomatitis. *J Formos Med Assoc* 2018;117:258—60.
37. Chiang CP, Chang JYF, Wang YP, Wu YH, Wu YC, Sun A. Recurrent aphthous stomatitis - Etiology, serum autoantibodies, anemia, hematinic deficiencies, and management. *J Formos Med Assoc* 2019;118:1279—89.
38. Kuo YS, Chang JYF, Wang YP, Wu YC, Wu YH, Sun A. Significantly higher frequencies of hemoglobin, iron, vitamin B12, and folic acid deficiencies and of hyperhomocysteinemia in patients with Behcet's disease. *J Formos Med Assoc* 2018;117:932—8.
39. Lin HP, Wu YH, Chang JYF, Wang YP, Chen HM, Sun A. Gastric parietal cell and thyroid autoantibodies in patients with Behcet's disease. *J Formos Med Assoc* 2018;117:505—11.
40. Wu YH, Chang JYF, Wang YP, Wu YC, Chen HM, Sun A. Gastric parietal cell and thyroid autoantibodies in Behcet's disease patients with or without atrophic glossitis. *J Formos Med Assoc* 2018;117:691—6.
41. Wu YH, Chang JYF, Wang YP, Wu YC, Chen HM, Sun A. Hemoglobin, iron, vitamin B12, and folic acid deficiencies and hyperhomocysteinemia in Behcet's disease patients with atrophic glossitis. *J Formos Med Assoc* 2018;117:559—65.
42. Chiang CP, Wu YH, Chang JYF, Wang YP, Wu YC, Sun A. Hematinic deficiencies and hyperhomocysteinemia in gastric parietal cell antibody-positive or gastric and thyroid autoantibodies-negative Behcet's disease patients. *J Formos Med Assoc* 2019;118:347—53.
43. Chiang CP, Wu YH, Chang JYF, Wang YP, Chen HM, Sun A. Serum thyroid autoantibodies are not associated with anemia, hematinic deficiencies, and hyperhomocysteinemia in patients with Behcet's disease. *J Dent Sci* 2018;13:256—62.
44. Chiang CP, Hsieh RP, Chen THH, et al. High incidence of autoantibodies in Taiwanese patients with oral submucous fibrosis. *J Oral Pathol Med* 2002;31:402—9.
45. Wang YP, Wu YC, Cheng SJ, Chen HM, Sun A, Chang JYF. High frequencies of vitamin B_{12} and folic acid deficiencies and gastric parietal cell antibody positivity in oral submucous fibrosis patients. *J Formos Med Assoc* 2015;114:813—9.
46. Chiang CP, Chang JYF, Wu YH, Sun A, Wang YP, Chen HM. Hematinic deficiencies and anemia in gastric parietal cell antibody-positive and -negative oral submucous fibrosis patients. *J Dent Sci* 2018;13:68—74.
47. Wu YH, Wu YC, Chu FY, Cheng SJ, Sun A, Chen HM. Significantly higher frequencies of hematinic deficiencies and hyperhomocysteinemia in oral precancer patients. *J Formos Med Assoc* 2019;118:1299—307.
48. Wu YH, Wu YC, Cheng SJ, Kuo YS, Sun A, Chen HM. Gastric parietal cell and thyroid autoantibodies in oral precancer patients. *J Formos Med Assoc* 2019;118:1393—400.
49. Sun A, Wang YP, Lin HP, Jia JS, Chiang CP. Do all the patients with gastric parietal cell antibodies have pernicious anemia? *Oral Dis* 2013;19:381—6.

50. Sun A, Chang JYF, Wang YP, Cheng SJ, Chen HM, Chiang CP. Do all the patients with vitamin B12 deficiency have pernicious anemia? *J Oral Pathol Med* 2016;45:23—7.
51. Chang JYF, Wang YP, Wu YC, Cheng SJ, Chen HM, Sun A. Hematinic deficiencies and pernicious anemia in oral mucosal disease patients with macrocytosis. *J Formos Med Assoc* 2015; 114:736—41.
52. Lin HP, Wu YH, Wang YP, Wu YC, Chang JYF, Sun A. Anemia and hematinic deficiencies in gastric parietal cell antibody-positive and —negative oral mucosal disease patients with microcytosis. *J Formos Med Assoc* 2017;116:613—9.
53. Wang YP, Chang JYF, Wu YC, Cheng SJ, Chen HM, Sun A. Oral manifestations and blood profile in patients with thalassemia trait. *J Formos Med Assoc* 2013;112:761—5.
54. Chang JYF, Wang YP, Wu YC, Cheng SJ, Chen HM, Sun A. Hematinic deficiencies and anemia statuses in oral mucosal disease patients with folic acid deficiency. *J Formos Med Assoc* 2015;114:806—12.
55. Chang JYF, Wang YP, Wu YC, Cheng SJ, Chen HM, Sun A. Blood profile of oral mucosal disease patients with both vitamin B12 and iron deficiencies. *J Formos Med Assoc* 2015;114:532—8.
56. Sun A, Chang JYF, Chiang CP. Blood examination is necessary for oral mucosal disease patients. *J Formos Med Assoc* 2016; 115:1—2.
57. Chiang CP, Chang JYF, Sun A. Majority of oral mucosal disease patients with thyroid autoantibodies have euthyroid with minority of them having either hypothyroidism or hyperthyroidism. *J Formos Med Assoc* 2019;118:1383—4.
58. WHO/UNICEF/UNU. *Iron deficiency anaemia assessment, prevention, and control: a guide for programme managers*. Geneva, Switzerland: World Health Organization, 2001.
59. Shine JW. Microcytic anemia. *Am Fam Physician* 1997;55: 2455—62.
60. Morris MS, Jacques PF, Rosenberg IH, Selhub J. Folate and vitamin B-12 status in relation to anemia, macrocytosis, and cognitive impairment in older Americans in the age of folic acid fortification. *Am J Clin Nutr* 2007;85:193—200.
61. de Benoist B. Conclusions of a WHO technical consultation on folate and vitamin B12 deficiencies. *Food Nutr Bull* 2008; 29(suppl):S238—44.
62. Brill JR, Baumgardner DJ. Normocytic anemia. *Am Fam Physician* 2000;62:2255—63.
63. Koury MJ. Abnormal erythropoiesis and the pathophysiology of chronic anemia. *Blood Rev* 2014;28:49—66.
64. Koury MJ, Rhodes M. How to approach chronic anemia. *ASH Educ Program B* 2012;2012:183—90.
65. Means Jr RT. Pathogenesis of the anemia of chronic disease: a cytokine-mediated anemia. *Stem Cells* 1995;13:32—7.