Journal of Dental Sciences 17 (2022) 1144–1150

Available online at www.sciencedirect.com

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journal homepage: www.e-jds.com

Original Article

# Anemia, hematinic deficiencies, and hyperhomocysteinemia in younger and older burning mouth syndrome patients

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Received 4 February 2022
Available online 25 February 2022

**KEYWORDS**
Burning mouth syndrome;
Hemoglobin;
Iron;
Vitamin B12;
Folic acid;
Younger or older patients

*Abstract Background/purpose:* Our previous study found that 19.8%, 16.2%, 4.8%, 2.3%, and 19.2% of 884 burning mouth syndrome (BMS) patients have anemia, serum iron, vitamin B12, and folic acid deficiencies, and hyperhomocysteinemia, respectively. This study mainly evaluated the anemia, hematinic deficiencies, and hyperhomocysteinemia in 272 younger (≤50 years old) and 612 older (>50 years old) BMS patients.
*Materials and methods:* The blood hemoglobin (Hb) and serum iron, vitamin B12, folic acid, and homocysteine levels in 272 younger and 612 older BMS patients were measured and compared with the corresponding levels in 136 younger (≤50 years old) and 306 older (>50 years old) healthy control subjects (HCSs), respectively.

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https://doi.org/10.1016/j.jds.2022.02.005
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*Results:* We found that 272 younger BMS patients had significantly lower mean blood Hb and serum iron, vitamin B12, and folic acid levels than 136 younger HCSs. Moreover, 612 older BMS patients had significantly lower mean blood Hb, and serum iron and vitamin B12 levels and significantly higher mean serum homocysteine level than 306 older HCSs. In addition, 272 younger BMS patients had higher mean blood Hb level (marginal significance, $P = 0.056$), significantly lower mean serum vitamin B12 and folic acid levels, and significantly higher frequencies of iron and folic acid deficiencies than 612 older BMS patients.
*Conclusion:* The younger BMS patients do have higher mean blood Hb level, significantly lower mean serum vitamin B12 and folic acid levels, and significantly higher frequencies of serum iron and folic acid deficiencies than the older BMS patients.
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## Introduction

Burning mouth syndrome (BMS) is an oral disease with burning sensation of the oral mucosa but without clinically apparent mucosal alterations. It occurs more commonly in the middle-aged and elderly women and the prevalence of BMS increases with advancing age.[1,2] However, there are still some younger BMS patients clinically. Compared to the older people, younger people tend to have more active physiological function and better regeneration or tissue repair ability. Thus, they should be more resistant to become the BMS patients, but if these younger people have BMS, more severe underlying organic local/systemic causes or peripheral and central neurogenic defects may be present in these younger BMS patients.

In our oral mucosal disease clinic, BMS patients are relatively frequently encountered.[1–11] Our previous study of 884 BMS patients found burning sensation, dry mouth, numbness of oral mucosa, and dysfunction of taste in 100%, 48.1%, 30.7% and 16.7% of 884 BMS patients.[2] We also discovered that 19.8%, 16.2%, 4.8%, 2.3%, 19.2%, and 12.3% of 884 BMS patients have anemia, serum iron, vitamin B12, and folic acid deficiencies, hyperhomocysteinemia, and serum gastric parietal cell antibody (GPCA) positivity, respectively.[2] We also showed that treatment with vitamin BC capsules plus none, one, or two deficient hematinics (iron, vitamin B12, and folic acid) can result in complete remission of all oral symptoms in 177 (44.4%) of 399 BMS patients after treatment.[11] Because the BMS patients often have eating problem, the results of our previous studies suggest that single or multiple hematinic deficiencies can be one of the pivotal factors leading to the development of BMS.[1,2] To the best of our knowledge, none of previous studies compared the complete blood count data, serum iron, vitamin B12, folic acid, homocysteine, and GPCA levels between a large group of younger ($\leq$50 years old) and older ($>$50 years old) BMS patients. Therefore, in this study, we divided the 884 BMS patients into 272 younger and 612 older BMS patients. We mainly evaluated whether the 272 younger BMS patients had significantly lower mean blood hemoglobin (Hb) and serum iron, vitamin B12, and folic acid levels, significantly higher frequencies of blood Hb and serum iron, vitamin B12, and folic acid deficiencies, hyperhomocysteinemia, and serum GPCA positivity than the 612 older BMS patients. We also assessed whether there were significantly higher frequencies of blood Hb and serum iron, vitamin B12, and folic acid deficiencies, hyperhomocysteinemia, and serum GPCA positivity in 272 younger or 612 older BMS patients than in 136 younger ($\leq$50 years old) or 306 older ($>$50 years old) healthy control subjects (HCSs), respectively.

## Materials and methods

### Participants

This study included 272 younger BMS patients (90 men and 182 women; age range 18–50 years, mean 38.8 $\pm$ 8.6 years) and 612 older BMS patients (122 men and 490 women; age range 51–90 years, mean 63.8 $\pm$ 8.8 years).[2] For two BMS patients, one age- ($\pm$2 years of each patient's age) and sex-matched HCS was selected. Thus, 136 age- and sex-matched younger HCSs (45 men and 91 women; age range 18–50 years, mean 40.6 $\pm$ 8.5 years) and 306 age- and sex-matched older HCSs (61 men and 245 women; age range 51–90 years, mean 65.0 $\pm$ 8.3 years) were selected and included in this study.[2] All the BMS patients and HCSs were seen consecutively, diagnosed, and treated in the department of dentistry of national Taiwan university hospital (NTUH) from July 2007 to July 2017. The detailed inclusion and exclusion criteria for 884 BMS patients and 442 HCSs 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 884 BMS patients and 442 HCSs 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 HCSs 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.[2–11]

### 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.[2–10] 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 Hb and serum iron, vitamin B12, folic acid, and homocysteine levels between 272 younger or 612 older BMS patients and 136 younger or 306 older HCSs, respectively, as well as between 272 younger and 612 older BMS patients were performed by Student's *t*-test. The differences in frequencies of microcytosis (MCV $<$ 80 fL),[12,13] macrocytosis (MCV $\geq$ 100 fL),[14–16] blood Hb and serum iron, vitamin B12, and folic acid deficiencies, hyperhomocysteinemia, and serum GPCA positivity between 272 younger or 612 older BMS patients and 136 younger or 306 older HCSs, respectively, as well as between 272 younger and 612 older BMS patients were compared by chi-square test. In addition, the differences in frequencies of 6 different types of anemia between 272 younger and 612 older BMS patients were also compared 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 Hb and serum iron, vitamin B12, folic acid, and homocysteine levels in 272 younger and 612 older BMS patients and in 136 younger and 306 older HCSs are shown in Table 1. We found that 272 younger BMS patients had significantly lower MCV, mean blood Hb, and serum iron, vitamin B12, and folic acid levels than 136 younger HCSs (all *P*-values $<$ 0.05, Table 1). Although the 272 younger BMS patients also had higher mean serum homocysteine level than 136 younger HCSs, the difference was not significant ($P = 0.117$) (Table 1). Moreover, 612 older BMS patients had significantly lower mean blood Hb and serum iron and vitamin B12 levels, and significantly higher mean serum homocysteine level than 306 older HCSs (all *P*-values $<$ 0.01, Table 1). In addition, 272 younger BMS patients had significantly lower MCV and mean serum vitamin B12 and folic acid levels than 612 older BMS patients (all *P*-values $<$ 0.001, Table 1). The 272 younger BMS patients also had higher mean blood Hb level (marginal significance, $P = 0.056$) than 612 older BMS patients. However, no significant differences in the mean serum iron and homocysteine levels were found between 272 younger and 612 older BMS patients (Table 1).

According to the world health organization (WHO) criteria, microcytosis of erythrocyte was defined as having MCV $<$ 80 fL,[12,13] macrocytosis of erythrocyte was defined as having MCV $\geq$ 100 fL,[14–16] and men with Hb $<$ 13 g/dL and women with Hb $<$ 12 g/dL were defined as having Hb deficiency or anemia.[17] Furthermore, patients with the serum iron level $<$ 60 μg/dL,[18] the serum vitamin B12 level $<$ 200 pg/mL,[19] or the folic acid level $<$ 4 ng/mL[20] were defined as having serum 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 HCSs plus two standard deviations) were defined as having hyperhomocysteinemia.[2] By the above-mentioned definitions, 13.2%, 2.2%, 23.2%, 24.3%, 4.0%, 4.8%, 21.3%, and 9.2% of 272 younger BMS patients and 5.2%, 6.5%, 18.3%, 12.6%, 5.1%, 1.1%, 18.3%, and 13.7% of 61 2 older 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 (Table 2). Moreover, 272 younger BMS patients had significantly higher frequencies of microcytosis, blood Hb and serum iron, vitamin B12, and folic acid deficiencies, hyperhomocysteinemia, and serum GPCA positivity than 136 younger HCSs (all *P*-values $<$ 0.05, Table 2). Furthermore, 612 older BMS patients had significantly higher

**Table 1** Comparisons of mean corpuscular volume (MCV) and mean blood hemoglobin (Hb) and serum iron, vitamin B12, folic acid, and homocysteine levels between 272 younger ($\leq$50 years old) or 612 older ($>$50 years old) BMS patients and 136 younger ($\leq$50 years old) or 306 older ($>$50 years old) healthy control subjects (HCSs), respectively, as well as between 272 younger and 612 older BMS patients.

| Group | MC (fL) | Hb (g/dL) | Iron (μg/dL) | Vitamin B12 (pg/mL) | Folic acid (ng/mL) | Homocysteine (μM) |
|---|---|---|---|---|---|---|
| Younger BMS patients (n = 272) | 86.8 ± 7.0 | 13.6 ± 1.7 | 87.6 ± 35.7 | 559.9 ± 244.9 | 11.4 ± 5.9 | 9.2 ± 4.2 |
| [a]*P*-value | <0.001 | 0.002 | <0.001 | 0.005 | 0.047 | 0.117 |
| [b]*P*-value | <0.001 | 0.056 | 0.117 | <0.001 | <0.001 | >0.999 |
| Older BMS patients (n = 612) | 90.9 ± 7.1 | 13.4 ± 1.3 | 91.1 ± 28.1 | 675.0 ± 270.5 | 15.7 ± 7.6 | 9.2 ± 4.3 |
| [a]*P*-value | 0.642 | <0.001 | <0.001 | 0.007 | 0.838 | <0.001 |
| Younger HCSs (n = 136) | 89.6 ± 3.5 | 14.1 ± 1.1 | 103.8 ± 29.4 | 629.1 ± 205.3 | 12.6 ± 5.4 | 8.6 ± 2.1 |
| Older HCSs (n = 306) | 90.7 ± 3.5 | 13.8 ± 0.9 | 97.7 ± 26.6 | 723.2 ± 220.8 | 15.6 ± 5.6 | 8.2 ± 2.0 |

[a] Comparisons of means of parameters between 272 younger or 612 older BMS patients and 136 younger or 306 older HCSs by Student's *t*-test, respectively.
[b] Comparisons of means of parameters between 272 younger and 612 older BMS patients by Student's *t*-test.

**Table 2** Comparisons of frequencies of microcytosis (mean corpuscular volume or MCV < 80 fL), macrocytosis (MCV ≥ 100 fL), blood hemoglobin (Hb) and serum iron, vitamin B12, and folic acid deficiencies, and gastric parietal cell antibody (GPCA) positivity between 272 younger (≤50 years old) or 612 older (>50 years old) BMS patients and 136 younger (≤50 years old) or 306 older (>50 years old) healthy control subjects (HCSs), respectively, as well as between 272 younger and 612 older 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) | Hyperhomo -cysteinemia (>12.3 μM) | GPCA positivity |
| Younger BMS patients (n = 272) | 36 (13.2) | 6 (2.2) | 63 (23.2) | 66 (24.3) | 11 (4.0) | 13 (4.8) | 58 (21.3) | 25 (9.2) |
| [a]*P*-value | <0.001 | 0.191 | <0.001 | <0.001 | 0.040 | 0.003 | <0.001 | 0.002 |
| [b]*P*-value | <0.001 | 0.012 | 0.114 | <0.001 | 0.626 | 0.002 | 0.337 | 0.075 |
| Older BMS patients (n = 612) | 32 (5.2) | 40 (6.5) | 112 (18.3) | 77 (12.6) | 31 (5.1) | 7 (1.1) | 112 (18.3) | 84 (13.7) |
| [a]*P*-value | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | 0.140 | <0.001 | <0.001 |
| Younger HCSs (n = 136) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 5 (3.7) | 1 (0.7) |
| Older HCSs (n = 306) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 6 (2.0) | 7 (2.3) |

[a] Comparisons of frequencies of parameters between 272 younger or 612 older BMS patients and 136 younger or 306 older HCSs by chi-square test, respectively.
[b] Comparisons of frequencies of parameters between 272 younger and 612 older BMS patients by chi-square test.

frequencies of microcytosis, macrocytosis, blood Hb and serum iron and vitamin B12 deficiencies, hyperhomocysteinemia, and serum GPCA positivity than 306 older HCSs (all *P*-values < 0.001, Table 2). In addition, 272 younger BMS patients had significantly higher frequencies of microcytosis, serum iron and folic acid deficiencies and significantly lower frequencies of macrocytosis than 612 older BMS patients (all *P*-values < 0.05, Table 2).

Fifty-five younger and 120 older BMS patients had anemia (defined as having an Hb concentration < 13 g/dL for men and < 12 g/dL for women).[17] Of the 55 anemic younger BMS patients, two 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),[14–16] one had macrocytic anemia (defined as having anemia and an MCV ≥ 100 fL) other than PA,[14–16] 32 had normocytic anemia (defined as having anemia and an MCV between 80 fL and 99.9 fL),[21–24] 17 had iron deficiency anemia (IDA, defined as having anemia, an MCV < 80 fL, and a serum iron level < 60 μg/dL),[13,17,18] two had thalassemia trait-induced anemia (defined as having anemia, a red blood cell count > 5.0 M/μL, an MCV < 74 fL, and a Mentzer index (MCV/RBC) < 13),[25] and one had microcytic anemia (defined as having anemia and an MCV < 80 fL)[12,18] other than IDA and thalassemia trait-induced anemia. Thus, by strict WHO criteria the normocytic anemia (58.2%, 32/55) and IDA (17/55, 30.9%) were the two most common types of anemia in our 55 anemic younger BMS patients (Table 3).

Of the 120 anemic older BMS patients, 13 had PA,[14–16] 14 had macrocytic anemia other than PA,[14–16] 63 had normocytic anemia,[21–24] 4 had IDA,[13,17,18] 25 had thalassemia trait-induced anemia,[25] and one had microcytic anemia[12,18] other than IDA and thalassemia trait-induced anemia. Therefore, by strict WHO criteria the normocytic anemia (51.1%, 63/120), and thalassemia trait-induced anemia (20.8%, 25/125) were the two most common types of anemia in our 120 anemic older BMS patients (Table 3). In addition, 272 younger BMS patients

**Table 3** Comparison of frequencies of 6 different types of anemia between 272 younger (≤50 years old) and 612 older (>50 years old) burning mouth syndrome (BMS) patients.

| Anemia type | Patient number (%) | | [a]*P*-value |
|---|---|---|---|
| | Younger BMS patients (n = 272) | Older BMS patients (n = 612) | |
| Pernicious anemia | 2 (0.7) | 13 (2.1) | 0.233 |
| Other macrocytic anemia | 1 (0.4) | 14 (2.3) | 0.079 |
| Normocytic anemia | 32 (11.8) | 63 (10.3) | 0.593 |
| Iron deficiency anemia | 17 (6.3) | 4 (0.7) | <0.001 |
| Thalassemia trait-induced anemia | 2 (0.7) | 25 (4.1) | 0.014 |
| Other microcytic anemia | 1 (0.4) | 1 (0.2) | 0.860 |
| Total | 55 (20.2) | 120 (19.6) | 0.905 |

[a] Comparison of frequencies of 6 different types of anemia between 272 younger and 612 older BMS patients by chi-square test.

had significantly higher frequency of IDA and significantly lower frequency of thalassemia trait-induced anemia than 612 older BMS patients (both *P*-values $< 0.05$, Table 3).

## Discussion

This study found that the younger BMS patients had higher mean blood Hb level (marginal significance, $P = 0.056$), lower mean serum iron level, higher frequency of blood Hb deficiency, and significantly higher frequency of serum iron deficiency than the older BMS patients. To explain why we had these findings, first, we had to understand the composition of our two groups of BMS patients. The younger ($\leq$50 years old) BMS patients consisted of 90 men and 182 women, with a male to female ratio of approximately 1: 2 and a mean age of 38.8 years. Thus, the majority of our male, younger BMS patients might have sufficient total body androgen levels, and the majority of our female, younger BMS patients might still have menstrual cycles and enough total body estrogen levels. The older (>50 years old) BMS patients was composed of 122 men and 490 women, with a male to female ratio of approximately 1: 4 and a mean age of 63.8 years. Thus, our male, older BMS patients might have slightly decreased total body androgen level and nearly all the female, older BMS patients might be in the menopause status and had a reduced total body estrogen level. It is well known that androgens can stimulate erythropoiesis and increase levels of red blood cells and Hb through the mechanisms of stimulation of erythropoietin release, increase in bone marrow activity, and augmentation of iron incorporation into the red blood cells.[26–28] However, estrogens do not have this erythropoiesis-enhancement effect and even have a striking negative effect on the erythropoiesis, especially in patients with chronic mountain sickness (Monge's disease).[29] In menopause women, total body estrogen level decreases because of the cessation of ovarian functions and iron increases as a result of cease of menstrual blood loss. Nevertheless, estrogen deficiency up-regulates hepcidin, which inhibits intestinal iron absorption, leading to lower serum iron levels.[30] In general, each healthy pregnancy depletes the mother of approximately 500 mg of iron. Menstrual blood losses are highly variable, ranging from 10 to 250 mL (4—100 mg of iron) per period. During childbearing years, an adult female loses an average of 2 mg of iron daily.[31] However, in the postmenopausal women, iron deficiency is uncommon in the absence of menstrual bleeding. Furthermore, because women eat less food than men, they must be more than twice as efficient as men in the absorption of iron to avoid iron deficiency. Therefore, anemia is twice as prevalent in females as in males.[32] This difference is significantly greater during the childbearing years due to pregnancies and menses.[32] In this study, men constituted one-third of younger BMS patients and one-fifth of older BMS patients, suggesting that the androgen factor may play a more important role in the group of our younger BMS patients than in the group of our older BMS patients. On the contrary, menopausal women constituted four-fifths of our older BMS patients and two-thirds of our younger BMS patients, indicating that the menopause factor may play a more relevant role in the group of our older BMS patients than in the group of our younger BMS patients. Taken the above-mentioned evidences together, for the younger BMS patients, the active total body physiological function and relatively high total body androgen level are positive factors that increase the blood Hb and serum iron levels, but the repeated menstrual blood losses and one or more times of pregnancy are negative factors that decrease the blood Hb and serum iron levels. Moreover, for the older BMS patients, the menopause is the positive factor that enhances the blood Hb and serum iron levels, whereas the slightly decrease total body physiological function and relatively low total body androgen level are negative factors that reduce the blood Hb and serum iron levels. Therefore, the overall effects of these positive and negative factors could finally explain why the younger BMS patients had higher mean blood Hb level, lower mean serum iron level, higher frequency of blood Hb deficiency, and significantly higher frequency of serum iron deficiency than the older BMS patients.[26–32]

We further explained why the younger BMS patients had the significantly lower mean serum vitamin B12 and folic acid levels and a significantly higher frequency of folic acid deficiency than the older BMS patients. Previous studies discovered significantly lower mean folate levels in buccal mucosal cells and sera of 25 smokers than in those of 34 non-smokers.[33] Pivathilake et al.[34] also demonstrated lower buccal mucosal cell folate and vitamin Bl2 concentrations in 39 current smokers than in 60 noncurrent smokers.[34] Our previous study of serum vitamin B12 and folic acid levels in oral precancer patients also found significantly lower mean serum folic acid levels in 87 cigarette smokers than in 44 non-smokers and in 26 smokers consuming $>$ 20 cigarettes per day than in 61 smokers consuming $\leq$ 20 cigarettes per day.[35] The mean serum folic acid level was also lower in 52 betel quid chewers than in 79 non-chewers.[35] The findings of above-mentioned studies indicate the existence of vitamin B12 and folic acid deficiencies in the sera and oral mucosal cells of the smokers and betel quid chewers. We suggest that the mechanisms of vitamin B12 and folic acid deficiencies may result from elevated vitamin B12 and folic acid consumption in response to rapid cell proliferation or tissue repair caused by the irritation or damage of oral mucosal cells by the carcinogens in tobacco or betel quid.[36,37] In this study, we did not assess the frequencies of cigarette smoking and betel quid chewing habits in our 272 younger and 612 older BMS patients. However, in the Taiwan population, the males $\geq$ 18 years of age had a significantly higher prevalence of smoking habit (23.1% for men and 2.9% for women) or betel quid chewing habit (16.8% for men and 1.2% for women) than the females $\geq$ 18 years of age.[38] Because there is a significantly higher prevalence of smoking or betel quid chewing habit in men than in women in the Taiwan population as well as in younger people than in older people, we strongly suggest that the smoking or betel quid chewing habit may be the major factors that result in the lower mean serum vitamin B12 and folic acid levels and higher frequency of folic acid deficiency in the younger BMS patients than in the older BMS patients.[33—38] In addition, although the younger people tend to have more active physiological function including relatively higher intestinal absorption rate and better regeneration and tissue repair functions, these younger

BMS patients should have more severe deficiencies of vitamin B12 and folic acid to express the symptoms of BMS. Thus, it is not surprised to see the significantly lower mean serum vitamin B12 and folic acid levels and a significantly higher frequency of folic acid deficiency in the younger BMS patients than in the older BMS patients.

Homocysteine is formed during methionine metabolism.[39] Both vitamin B12 and folic acid function as coenzymes for the conversion of homocysteine to methionine.[40] Thus, patients with vitamin B12 and/or folic acid deficiencies may have hyperhomocysteinemia. A previous study has shown that a supplementation with folic acid and vitamins B12 and B6 can reduce blood homocysteine levels.[41] Our previous studies also demonstrated that supplementations with vitamin BC capsules plus corresponding deficient vitamin B12 and/or folic acid can reduce the abnormally high serum homocysteine level to significantly lower levels in patients with either BMS or atrophic glossitis.[11,42] In this study, although significantly lower mean serum vitamin B12 and folic acid levels and a significantly higher frequency of serum folic acid deficiency in the younger BMS patients than in the older BMS patients were found, there were no significant differences in the mean serum homocysteine level and in the frequency of hyperhomocysteinemia between the younger BMS patients and the older BMS patients. We suggest that these results may be due to the relatively minor deviations of the mean serum vitamin B12 and folic acid levels of the younger or older BMS patients from those of the younger or older HCSs, respectively (Table 1).

In this study, the younger BMS patients had a significantly higher frequency of IDA (6.3%) than the older BMS patients (0.7%, $P < 0.001$). This could be due to the finding that the younger BMS patients had a significantly higher frequency of serum iron deficiency (24.3%) than the older BMS patients (12.6%, $P < 0.001$). On the contrary, the older BMS patients had a significantly higher frequency of thalassemia trait-induced anemia (4.1%) than the younger BMS patients (0.7%, $P = 0.014$). Because thalassemia trait-induced anemia results from the inherent defects in the genes of the $\alpha$-globin or $\beta$-globin molecule, we do not know whether the significantly higher frequency of thalassemia trait-induced anemia in the older BMS patients (4.1%) than in the younger BMS patients (0.7%) is due to a coincidence only or other specific reasons.

The results of this study conclude that the younger BMS patients do have higher mean blood Hb level, significantly lower mean serum vitamin B12 and folic acid levels, and significantly higher frequencies of serum iron and folic acid deficiencies than the older BMS patients.

## Declaration of competing interest

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

## Acknowledgments

This study was partially 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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