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Original Article

Significantly higher frequencies of macrocytosis, anemia, serum vitamin B12 and folic acid deficiencies, and hyperhomocysteinemia in male than in female atrophic glossitis patients

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Abstract *Background/purpose:* Our previous study found that 19.0%, 16.9%, 5.3%, 2.3%, 11.9%, and 26.7% of 1064 atrophic glossitis (AG) patients have anemia, serum iron, vitamin B12, and folic acid deficiencies, hyperhomocysteinemia, and serum gastric parietal cell antibody (GPCA) positivity, respectively. This study evaluated the anemia, hematinic deficiencies, and hyperhomocysteinemia in 150 male and 914 female AG patients.
Materials and methods: The blood hemoglobin (Hb) and serum iron, vitamin B12, folic acid, homocysteine, GPCA levels in 150 male and 914 female AG patients were measured and compared with the corresponding levels in 75 male and 457 female healthy control subjects (HCSs), respectively.

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Results: We found that 150 male AG patients had significantly lower mean blood Hb, serum iron, vitamin B12, and folic acid levels, and significantly higher mean serum homocysteine levels than 75 male HCSs. Moreover, 914 female AG patients had significantly lower mean blood Hb and serum iron levels and significantly higher mean serum homocysteine level than 457 female HCSs. In addition, 150 male AG patients had significantly higher mean blood Hb and serum homocysteine levels, significantly lower mean serum vitamin B12 and folic acid levels, and significantly higher frequencies of Hb, vitamin B12, and folic acid deficiency and hyperhomocysteinemia than 914 female AG patients.
Conclusion: The male AG patients do have significantly higher mean blood Hb and serum homocysteine levels, significantly lower mean serum vitamin B12 and folic acid levels, and significantly higher frequencies of Hb, vitamin B12, and folic acid deficiencies and hyperhomocysteinemia than the female AG patients.

Introduction

The tongue is the mirror of general health or disease. Atrophic glossitis (AG) represents partial or complete loss of predominantly filiform papillae and minorly fungiform papillae on the dorsal surface of the tongue. The filiform papillae contain a relatively thick layer of keratinized stratified squamous epithelium that can protect the underlying connective and nerve cells from chemical, mechanical and physical stimuli. Moreover, fungiform papillae consist of plenty of taste cells that are responsible for mainly sweet and salty taste sensations. AG patients lacks protective function from filiform papillae and taste function from loss of taste cells. Therefore, our previous studies found burning sensation of the tongue, dry mouth, numbness of the tongue, and dysfunction of taste in 100.0%, 79.0%, 57.4%, and 27.8% of 176 AG patients and 98.5%, 70.1%, 50.7%, and 23.5% of 1064 AG patients, respectively.[1–3]

In our oral mucosal disease clinic, AG patients are relatively frequently encountered.[1–3] Our previous studies discovered that 19.0%, 16.9%, 5.3%, 2.3%, 11.9%, and 26.7% of 1064 AG patients and 22.2%, 26.7%, 7.4%, 1.7%, 21.6%, and 26.7% of 176 AG patients have anemia, serum iron, vitamin B12, and folic acid deficiencies, hyperhomocysteinemia, and serum gastric parietal cell antibody (GPCA) positivity, respectively.[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 male and female AG patients. Therefore, in this study, we divided the 1064 AG patients into 150 male and 914 female AG patients. We mainly evaluated whether 150 male AG patients had significantly higher frequencies of blood hemoglobin (Hb) and serum iron, vitamin B12, and folic acid deficiencies, hyperhomocysteinemia, and serum GPCA positivity than 914 female AG 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 150 male and 914 female AG patients than in 75 male and 457 female healthy control subjects, respectively.

Materials and methods

Participants

This study included 150 male AG patients (age range 26—90 years, mean 63.6 ± 15.2 years) and 914 female AG patients (age range 20—90 years, mean 62.5 ± 12.6 years).[1] For two AG patients, one age- (±2 years of each patient's age) and sex-matched healthy control subject was selected. Thus, 75 male (age range 28—89 years, mean 62.0 ± 14.5 years) and 457 female (age range 20—88 years, mean 61.6 ± 13.7 years) age- and sex-matched healthy control subjects were selected and included in this study.[1] All the patients and control subjects 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 1064 AG patients and 532 healthy control subjects have been described previously.[1] In addition, none of the AG patients had taken any prescription medication for AG at least 3 months before entering the study.

The blood samples were drawn from 1064 AG patients and 532 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 the AG 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.[1–21]

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.[1–19] 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 150 male or 914 female AG patients and 75 male or 457 female healthy control subjects, respectively, as well as between 150 male and 914 female AG patients were performed by Student's *t*-test. The differences in frequencies of microcytosis (MCV $<$80 fL),[22,23] macrocytosis (MCV $\geq$100 fL),[24–26] blood Hb and serum iron, vitamin B12, and folic acid deficiencies, hyperhomocysteinemia, and serum GPCA positivity between 150 male or 914 female AG patients and 75 male or 457 female healthy control subjects, respectively, as well as between 150 male and 914 female AG patients were compared by chi-square test. In addition, the differences in frequencies of 6 different types of anemia between 150 male and 914 female AG 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 150 male and 914 female AG patients and in 75 male and 457 female healthy control subjects are shown in Table 1. We found that 150 male AG patients had significantly lower mean blood Hb, serum iron, vitamin B12, and folic acid levels and a significantly higher mean serum homocysteine level than 75 male healthy control subjects (all *P*-values $<$ 0.05, Table 1). Moreover, 914 female AG patients had significantly lower mean blood Hb and serum iron levels, and a significantly higher mean serum homocysteine level than 457 female healthy control subjects (all *P*-values $<$ 0.05, Table 1). In addition, 150 male AG patients had significantly lower mean serum vitamin B12 and folic acid levels and significantly higher mean blood Hb and serum homocysteine levels than 914 female AG patients (all *P*-values $<$ 0.001, Table 1). However, no significant difference in the MCV and mean serum iron level was found between 150 male and 914 female AG patients (Table 1).

According to the World Health Organization (WHO) criteria, microcytosis of erythrocyte was defined as having MCV $<$80 fL,[22,23] macrocytosis of erythrocyte was defined as having MCV $\geq$100 fL,[24–26] and men with Hb $<$ 13 g/dL and women with Hb $<$ 12 g/dL were defined as having Hb deficiency or anemia.[27] Furthermore, patients with the serum iron level $<$60 μg/dL,[28] the serum vitamin B12 level $<$200 pg/mL,[29] or the folic acid level $<$4 ng/mL[30] were defined as having serum iron, vitamin B12 or folic acid deficiency, respectively. In addition, patients with the blood homocysteine level $>$12.1 μM (which was the mean serum homocysteine level of healthy control subjects plus two standard deviations) were defined as having hyperhomocysteinemia.[1] By the above-mentioned definitions, 10.7%, 10.0%, 26.0%, 15.3%, 12.0%, 6.7%, 27.3%, and 34.0% of 150 male AG patients and 6.9%, 2.8%, 17.8%, 17.2%, 4.2%, 1.5%, 9.4%, and 25.5% of 914 female AG 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, 150 male AG patients had significantly higher frequencies of microcytosis, macrocytosis, blood Hb and serum iron, and vitamin B12 deficiencies, hyperhomocysteinemia, and serum GPCA positivity than 75 male healthy control subjects (all *P*-values $<$ 0.05, Table 2). Furthermore, 914 female AG 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 457 female healthy control subjects (all *P*-values $<$ 0.05, Table 2). In addition, 150 male AG patients had significantly higher frequencies of macrocytosis, blood Hb, serum vitamin B12 and folic acid deficiencies, and hyperhomocysteinemis, and serum GPCA

Table 1 Comparisons of mean corpuscular volume (MCV) and mean blood hemoglobin (Hb) and serum iron, vitamin B12, folic acid, and homocysteine levels between 150 male or 914 female atrophic glossitis (AG) patients and 75 male or 457 female healthy control subjects (HCSs), respectively, as well as between 150 male and 914 female AG patients.

Group	MCV (fL)	Hb (g/dL)	Iron (μg/dL)	Vitamin B12 (pg/mL)	Folic acid (ng/mL)	Homocysteine (μM)
Male AG patients (n = 150)	90.5 ± 9.8	13.9 ± 1.9	91.9 ± 33.1	561.5 ± 294.0	12.6 ± 6.9	13.9 ± 18.6
[a]*P*-value	0.865	<0.001	0.006	0.002	0.129	0.042
[b]*P*-value	0.392	<0.001	0.149	<0.001	<0.001	<0.001
Female AG patients (n = 914)	89.9 ± 7.6	12.9 ± 1.3	87.9 ± 31.2	707.0 ± 269.7	15.5 ± 6.4	8.7 ± 7.1
[a]*P*-value	0.181	<0.001	<0.001	0.689	0.261	0.018
Male HCSs (n = 75)	90.7 ± 3.7	15.1 ± 0.8	104.3 ± 28.0	678.6 ± 197.3	14.0 ± 5.6	9.5 ± 1.8
Female HCSs (n = 457)	90.4 ± 3.5	13.6 ± 0.7	98.5 ± 27.6	701.1 ± 230.6	15.1 ± 5.8	7.9 ± 2.0

[a] Comparisons of means of parameters between 150 male or 914 female AG patients and 75 male or 457 female HCSs by Student's *t*-test, respectively.
[b] Comparisons of means of parameters between 150 male and 914 female AG 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, hyperhomocysteinemia, and gastric parietal cell antibody (GPCA) positivity between 150 male or 914 female atrophic glossitis (AG) patients and 75 male or 457 female healthy control subjects (HCSs), respectively, as well as between 150 male and 914 female AG 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 (>12.1 μM)	GPCA positivity
Male AG patients (n = 150)	16 (10.7)	15 (10.0)	39 (26.0)	23 (15.3)	18 (12.0)	10 (6.7)	41 (27.3)	51 (34.0)
[a]*P*-value	0.008	0.011	<0.001	<0.001	0.004	0.052	<0.001	<0.001
[b]*P*-value	0.143	<0.001	0.024	0.659	<0.001	<0.001	<0.001	0.037
Female AG patients (n = 914)	63 (6.9)	26 (2.8)	163 (17.8)	157 (17.2)	38 (4.2)	14 (1.5)	86 (9.4)	233 (25.5)
[a]*P*-value	<0.001	<0.001	<0.001	<0.001	<0.001	0.018	<0.001	<0.001
Male HCSs (n = 75)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	5 (6.7)	0 (0.0)
Female HCSs (n = 457)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	15 (3.3)	12 (2.6)

[a] Comparisons of frequencies of parameters between 150 male or 914 female AG patients and 75 male or 457 female HCSs by chi-square test, respectively.
[b] Comparisons of frequencies of parameters between 150 male and 914 female AG patients by chi-square test.

positivity than 914 female AG patients (all *P*-values < 0.05, Table 2).

Thirty-nine male and 163 female AG patients had anemia (defined as having an Hb concentration <13 g/dL for men and <12 g/dL for women).[27] Of the 39 anemic male AG patients, 9 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),[24–26] 4 had macrocytic anemia (defined as having anemia and an MCV ≥100 fL) other than PA,[24–26] 18 had normocytic anemia (defined as having anemia and an MCV between 80 fL and 99.9 fL),[31–34] 5 had iron deficiency anemia (IDA, defined as having anemia, an MCV <80 fL, and a serum iron level <60 μg/dL),[23,27,28] 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),[35] and one had microcytic anemia (defined as having anemia and an MCV <80 fL)[22,28] other than IDA and thalassemia trait-induced anemia. Thus, by strict WHO criteria the normocytic anemia (46.2%, 18/39) was the most common type of anemia in our 39 anemic male AG patients (Table 3).

Of the 163 anemic female AG patients, 13 had PA,[24–26] 4 had macrocytic anemia other than PA,[24–26] 99 had normocytic anemia,[31–34] 25 had IDA,[23,27,28] 19 had thalassemia trait-induced anemia,[35] and three had microcytic anemia[22,28] other than IDA and thalassemia trait-induced anemia. Therefore, by strict WHO criteria the normocytic anemia (60.7%, 99/163), IDA (15.3%, 25/163), and thalassemia trait-induced anemia (11.7%, 19/163) were the three most common types of anemia in our 163 anemic female AG patients (Table 3). In addition, 150 male AG patients had significantly higher frequency of PA ($P < 0.001$) and other macrocytic anemia ($P = 0.016$) than 914 female AG patients (Table 3).

Discussion

The major findings of this study were that the male AG patients had significantly higher mean blood Hb and serum

Table 3 Comparison of frequencies of 6 different types of anemia between 150 male and 914 female atrophic glossitis (AG) patients.

Anemia type	Patient number (%)		[a]*P*-value
	Male AG patients (n = 150)	Female AG patients (n = 914)	
Pernicious anemia	9 (6.0)	13 (1.4)	<0.001
Other macrocytic anemia	4 (2.7)	4 (0.4)	0.016
Normocytic anemia	18 (12.0)	99 (10.8)	0.777
Iron deficiency anemia	5 (3.3)	25 (2.7)	0.885
Thalassemia trait-induced anemia	2 (1.3)	19 (2.1)	0.771
Other microcytic anemia	1 (0.7)	3 (0.3)	0.927
Total	39 (26.0)	163 (17.8)	0.024

[a] Comparison of frequencies of 6 different types of anemia between 150 male and 914 female AG patients by chi-square test.

homocysteine levels, significantly lower mean serum vitamin B12 and folic acid levels, and significantly higher frequencies of Hb, vitamin B12, and folic acid deficiencies and hyperhomocysteinemia than the female AG patients. The male AG patients also had a higher mean serum iron level and a lower frequency of serum iron deficiency than the female AG patients, but the differences were not significant between the male and female AG patients.

Sex hormones can have a significant influence on the red blood cell (RBC), blood Hb, and serum iron levels.[36–42] Androgens can stimulate erythropoiesis and increase levels of RBCs, hemoglobin, and hematocrit. Androgens stimulate hematopoietic system by various mechanisms, including stimulation of erythropoietin release, increase in bone marrow activity, and augmentation of iron incorporation into the RBCs.[36,37] Erythropoietin is a kidney-produced hormone that stimulates the production and maintenance of RBCs.[38] However, in subjects with chronic mountain sickness (Monge's disease), estrogens have a striking negative effect on the erythropoietic response and may result in a significant decrease in RBC production.[39] In menopause women, estrogen decreases because of the cessation of ovarian functions and iron increases as a result of decreasing menstrual periods. Nevertheless, estrogen deficiency up-regulates hepcidin, which inhibits intestinal iron absorption, leading to lower serum iron levels.[40] Therefore, the difference in the sex hormone levels between men and women can partially explain why men usually have higher blood Hb and serum iron levels.

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. A man must absorb about 1 mg of iron to maintain equilibrium. During childbearing years, an adult female loses an average of 2 mg of iron daily and must absorb a similar quantity of iron in order to maintain equilibrium.[41] 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.[42] This difference is significantly greater during the childbearing years due to pregnancies and menses.[42] Because our male AG patients had a mean age of 63.6 years and the female AG patients had a mean age of 62.5 years that was far beyond the childbearing years, this could partially explain why the male AG patients had significantly higher blood Hb level but significantly higher frequency of anemia as well as non-significantly higher mean serum iron level and non-significantly lower frequency of serum iron deficiency than the female AG patients.

We further explained why male AG patients had the lower mean serum vitamin B12 and folic acid levels and higher frequencies of vitamin B12 and folic acid deficiencies than female AG 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.[43] Pivathilake et al.[44] also demonstrated lower buccal mucosal cell folate and vitamin Bl2 concentrations in 39 current smokers than in 60 noncurrent smokers.[44] 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 ≤20 cigarettes per day.[45] The mean serum folic acid level was also lower in 52 betel quid chewers than in 79 non-chewers.[45] 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.[43–45] We suggest that the mechanisms of vitamin B12 and folic acid deficiencies may arise from elevated vitamin B12 and folic acid consumption in response to rapid tissue proliferation or repair caused by the irritation or damage of oral mucosal cells by the carcinogens in tobacco or betel quid.[46,47] The present study found that male AG patients had significantly lower mean serum vitamin B12 and folic acid levels and significantly higher frequencies of vitamin B12 and folic acid deficiencies than female AG patients. In this study, we did not assess the frequencies of cigarette smoking and betel quid chewing habits in our 150 male and 914 female AG patients. However, in the Taiwan population, the males ≥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 ≥18 years of age.[48] Because there is a significantly higher prevalence of smoking or betel quid chewing habit in men than in women in the Taiwan population, we strongly suggest that the smoking or betel quid chewing habit may be the major causes that result in the lower mean serum vitamin B12 and folic acid levels and higher frequencies of vitamin B12 and folic acid deficiencies in male AG patients than in female AG patients.

In this study, the normocytic anemia (46.2%, 18/39) was the most common type of anemia in our 39 anemic male AG patients, followed by the PA (23.1%, 9/39), IDA (12.8%, 5/39), and other macrocytic anemia (10.3%, 4/39) (Table 3). Furthermore, the normocytic anemia (60.7%, 99/163), IDA (15.3%, 25/163), and thalassemia trait-induced anemia (11.7%, 19/163) were the three most common types of anemia in our 163 anemic female AG patients (Table 3). From the above-mentioned findings, we discovered that regardless of the normocytic anemia, the male AG patients tended to have macrocytic anemia (PA and other macrocytic anemia) and the female AG patients were prone to have microcytic anemia (IDA and thalassemia trait-induced anemia). As stated before, male AG patients had the significantly lower mean serum vitamin B12 and folic acid levels and significantly higher frequencies of vitamin B12 and folic acid deficiencies than female AG patients in the present study. Moreover, female AG patients had the lower mean blood Hb and serum iron levels and a higher frequency of serum iron deficiency than male AG patients. These results could easy explain why the male AG patients tended to have macrocytic anemia and the female AG patients were prone to have microcytic anemia in the present study.

Homocysteine is formed during methionine metabolism.[49] Both vitamin B12 and folic acid function as coenzymes for the conversion of homocysteine to methionine.[50] 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.[51] 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 AG or burning mouth syndrome.[20,21] In this study, significantly lower mean serum vitamin B12 and folic acid levels and significantly higher frequencies of serum vitamin B12 and folic acid deficiencies in male AG patients than in female AG patients could explain why our male AG patients had a significantly higher mean serum homocysteine level and a significantly higher frequency of hyperhomocysteinemia than our female AG patients.

The results of this study conclude that male AG patients do have significantly higher mean blood Hb and serum homocysteine levels, significantly lower mean serum vitamin B12 and folic acid levels, and significantly higher frequencies of Hb, vitamin B12, and folic acid deficiencies and hyperhomocysteinemia than the female AG patients.

Declaration of competing interest

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

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