Journal of Dental Sciences 17 (2022) 1487–1493

Available online at www.sciencedirect.com

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journal homepage: www.e-jds.com

Original Article

Significantly lower mean serum vitamin B12 and folic acid levels and a significantly higher frequency of serum iron deficiency in younger than in older atrophic glossitis patients

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Received 25 May 2022
Available online 11 June 2022

KEYWORDS
Atrophic glossitis;
Hemoglobin;
Iron;
Vitamin B12;
Folic acid;
Younger or older patients

Abstract *Background/purpose:* : Our previous study found that 19.0%, 16.9%, 5.3%, 2.3%, and 11.9% of 1064 atrophic glossitis (AG) 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 224 younger (≤50 years old) and 840 older (>50 years old) AG patients.
Materials and methods: The blood hemoglobin (Hb) and serum iron, vitamin B12, folic acid, and homocysteine levels in 224 younger and 840 older AG patients were measured and compared with the corresponding levels in 112 younger (≤50 years old) and 420 older (>50

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https://doi.org/10.1016/j.jds.2022.05.023

years old) healthy control subjects (HCSs), respectively.
Results: We found that 224 younger AG patients had significantly lower mean blood Hb and serum iron levels than 112 younger HCSs. Moreover, 840 older AG patients had significantly lower mean blood Hb and serum iron levels and a significantly higher mean serum homocysteine level than 420 older HCSs. In addition, 224 younger AG patients had significantly lower mean serum vitamin B12 and folic acid levels, a lower mean serum homocysteine level (marginal significance, $P = 0.056$), a significantly higher frequency of serum iron deficiency, and a significantly lower frequency of hyperhomocysteinemia than 840 older AG patients.
Conclusion: The younger AG patients do have significantly lower mean serum vitamin B12 and folic acid levels, a significantly higher frequency of serum iron deficiency, and a significantly lower frequency of hyperhomocysteinemia than the older AG patients.

Introduction

A Chinese proverb says that the tongue is the mirror of the whole body's health and 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 lack 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 the previous studies compared the complete blood count data, serum iron, vitamin B12, folic acid, homocysteine, and GPCA levels between a large group of younger (≤50 years old) and older (>50 years old) AG patients. Moreover, the younger people tend to have better body adjustment ability than the older people. However, if the younger people present AG, they may have poorer physical condition than the older people. Therefore, in this study, we divided the 1064 AG patients into 224 younger and 840 older AG patients. We mainly evaluated whether the 224 younger AG 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 840 older 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 224 younger or 840 older AG patients than in 112 younger (≤50 years old) or 420 older (>50 years old) healthy control subjects (HCSs), respectively.

Materials and methods

Study subjects

This study included 224 younger AG patients (42 men and 182 women; age range 20—50 years, mean 44.0 ± 7.5 years) and 840 older AG patients (108 men and 732 women; age range 51—90 years, mean 67.6 ± 9.0 years).[1] For two AG patients, one age- (±2 years of each patient's age) and sex-matched HCS was selected. Thus, 112 age- and sex-matched younger HCSs (21 men and 91 women; age range 20—50 years, mean 42.3 ± 7.4 years) and 420 age- and sex-matched older HCSs (54 men and 366 women; age range 51—89 years, mean 66.9 ± 9.4 years) were selected and included in this study.[1] All the AG 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 1064 AG patients and 532 HCSs 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 HCSs for the measurement of complete blood count, serum iron, vitamin B12, folic acid, and homocysteine concentrations, and the serum GPCA positivity. All AG 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.[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 224 younger or 840 older AG patients and 112 younger or 420 older HCSs, respectively, as well as between 224 younger and 840 older 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 224 younger or 840 older AG patients and 112 younger or 420 older HCSs, respectively, as well as between 224 younger and 840 older AG patients were compared by chi-square test. In addition, the differences in frequencies of 6 different types of anemia between 224 younger and 840 older 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 224 younger and 840 older AG patients and in 112 younger and 420 older HCSs are shown in Table 1. We found that 224 younger AG patients had significantly lower MCV, mean blood Hb and serum iron levels than 112 younger HCSs (all *P*-values $<$ 0.005, Table 1). Although the 224 younger AG patients also had a higher mean serum homocysteine level than 112 younger HCSs, the difference was not significant ($P = 0.171$) (Table 1). Moreover, 840 older AG patients had significantly lower mean blood Hb and serum iron levels, and a significantly higher mean serum homocysteine level than 420 older HCSs (all *P*-values $<$ 0.01, Table 1). In addition, 224 younger AG patients had significantly lower MCV and mean serum vitamin B12 and folic acid levels than 840 older AG patients (all *P*-values $<$ 0.001, Table 1). The 224 younger AG patients also had a lower mean serum homocysteine level (marginal significance, $P = 0.056$) than 840 older AG patients. However, no significant differences in the mean blood Hb and serum iron levels were found between 224 younger and 840 older 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, 13.4%, 1.8%, 18.8%, 28.1%, 4.0%, 3.6%, 7.1%, and 21.0% of 224 younger AG patients and 5.8%, 4.4%, 19.0%, 13.9%, 5.6%, 1.9%, 13.2%, and 28.2% of 840 older 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, 224 younger AG patients had significantly higher frequencies of microcytosis, blood Hb and serum iron deficiencies, hyperhomocysteinemia, and serum GPCA positivity than 112 younger HCSs (all *P*-values $<$ 0.05, Table 2). Furthermore, 840 older AG patients had significantly higher frequencies of microcytosis, macrocytosis, blood Hb and serum iron, vitamin B12, and folic acid deficiencies, hyperhomocysteinemia, and serum

Table 1 Comparisons of mean corpuscular volume (MCV) and mean blood hemoglobin (Hb) and serum iron, vitamin B12, folic acid, and homocysteine levels between 224 younger ($\leq$ 50 years old) or 840 older ($>$ 50 years old) atrophic glossitis (AG) patients and 112 younger ($\leq$ 50 years old) or 420 older ($>$ 50 years old) healthy control subjects (HCSs), respectively, as well as between 224 younger and 840 older AG patients.

Group	MCV (fL)	Hb (g/dL)	Iron (μg/dL)	Vitamin B12 (pg/mL)	Folic acid (ng/mL)	Homocysteine (μM)
Younger AG patients (n = 224)	87.3 ± 9.0	13.1 ± 1.9	85.0 ± 39.8	628.4 ± 258.3	13.2 ± 6.3	8.3 ± 7.6
[a]*P*-value	0.003	<0.001	<0.001	0.964	0.886	0.171
[b]*P*-value	<0.001	>0.999	0.070	<0.001	<0.001	0.056
Older AG patients (n = 840)	90.7 ± 7.5	13.1 ± 1.3	89.3 ± 28.9	702.0 ± 280.9	15.6 ± 6.6	9.7 ± 10.2
[a]*P*-value	0.796	<0.001	<0.001	0.348	0.598	0.005
Younger HCSs (n = 112)	89.9 ± 3.4	13.8 ± 0.9	104.0 ± 31.3	627.1 ± 217.7	13.1 ± 5.5	7.3 ± 1.8
Older HCSs (n = 420)	90.6 ± 3.6	13.8 ± 0.9	98.1 ± 26.6	716.8 ± 224.8	15.4 ± 5.8	8.3 ± 2.1

[a] Comparisons of means of parameters between 224 younger or 840 older AG patients and 112 younger or 420 older HCSs by Student's *t*-test, respectively.
[b] Comparisons of means of parameters between 224 younger and 840 older 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 224 younger (≤50 years old) or 840 older (>50 years old) atrophic glossitis (AG) patients and 112 younger (≤50 years old) or 420 older (>50 years old) healthy control subjects (HCSs), respectively, as well as between 224 younger and 840 older 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
Younger AG patients (n = 224)	30 (13.4)	4 (1.8)	42 (18.8)	63 (28.1)	9 (4.0)	8 (3.6)	16 (7.1)	47 (21.0)
[a]P-value	<0.001	0.374	<0.001	<0.001	0.073	0.100	0.028	<0.001
[b]P-value	<0.001	0.107	0.996	<0.001	0.441	0.215	0.018	0.037
Older AG patients (n = 840)	49 (5.8)	37 (4.4)	160 (19.0)	117 (13.9)	47 (5.6)	16 (1.9)	111 (13.2)	237 (28.2)
[a]P-value	<0.001	<0.001	<0.001	<0.001	<0.001	0.010	<0.001	<0.001
Younger HCSs (n = 112)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.9)	0 (0.0)
Older HCSs (n = 420)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	19 (4.5)	12 (2.9)

[a] Comparisons of frequencies of parameters between 224 younger or 840 older AG patients and 112 younger or 420 older HCSs by chi-square test, respectively.
[b] Comparisons of frequencies of parameters between 224 younger and 840 older AG patients by chi-square test.

GPCA positivity than 420 older HCSs (all *P*-values < 0.05, Table 2). In addition, 224 younger AG patients had significantly higher frequencies of microcytosis and serum iron deficiency, and significantly lower frequencies of hyperhomocysteinemia and serum GPCA positivity than 840 older AG patients (all *P*-values < 0.05, Table 2).

Forty-two younger and 160 older AG patients had anemia (defined as having an Hb concentration < 13 g/dL for men and <12 g/dL for women).[27] Of the 42 anemic younger AG 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),[24–26] one had macrocytic anemia (defined as having anemia and an MCV ≥ 100 fL) other than PA[24–26], 15 had normocytic anemia (defined as having anemia and an MCV between 80 fL and 99.9 fL)[31–34], 22 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 none 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 IDA (52.4%, 22/42,) and normocytic anemia (35.7%, 15/42) were the two most common types of anemia in our 42 anemic younger AG patients (Table 3).

Of the 160 anemic older AG patients, 20 had PA[24–26], 7 had macrocytic anemia other than PA[24–26], 102 had normocytic anemia[31–34], 8 had IDA[23,27,28], 19 had thalassemia trait-induced anemia,[35] and 4 had microcytic anemia[22,28] other than IDA and thalassemia trait-induced anemia. Therefore, by strict WHO criteria the normocytic anemia (63.8%, 102/160), PA (12.5%, 20/160), and thalassemia trait-induced anemia (11.9%, 19/160) were the three most common types of anemia in our 160 anemic older AG patients (Table 3). In addition, 224 younger AG patients had significantly higher frequency of IDA ($P < 0.001$) and significantly lower frequency of normocytic anemia ($P = 0.028$) than 840 older AG patients (Table 3).

Discussion

This study found that the younger AG patients had significantly lower mean blood vitamin B12 and folic acid levels, a lower mean serum homocysteine level (marginal significance, $P = 0.056$), a significantly higher frequency of serum iron deficiency, a significantly lower frequencies of hyperhomocysteinemia and serum GPCA positivity than the older AG patients. To explain why we had these findings, first, we had to understand the composition of our two groups of AG patients. The younger (≤ 50 years old) AG patients consisted of 42 men and 182 women, with a male to female ratio of approximately 1:4.3 and a mean age of 44.0 years. Thus, the majority of our male, younger AG patients might have sufficient total body androgen levels, and the majority of our female, younger AG patients might still have menstrual cycles and enough total body estrogen levels. The older (> 50 years old) AG patients was composed of 108 men and 732 women, with a male to female ratio of approximately 1:6.8 and a mean age of 67.6 years. Thus, our male, older AG patients might have slightly

Table 3 Comparison of frequencies of 6 different types of anemia between 224 younger (≤50 years old) and 840 older (>50 years old) atrophic glossitis (AG) patients.

Anemia type	Patient number (%)		[a]*P*-value
	Younger AG patients (n = 224)	Older AG patients (n = 840)	
Pernicious anemia	2 (0.9)	20 (2.4)	0.260
Other macrocytic anemia	1 (0.4)	7 (0.8)	0.873
Normocytic anemia	15 (6.7)	102 (12.1)	0.028
Iron deficiency anemia	22 (9.8)	8 (1.0)	<0.001
Thalassemia trait-induced anemia	2 (0.9)	19 (2.3)	0.299
Other microcytic anemia	0 (0.0)	4 (0.5)	0.674
Total	42 (18.8)	160 (19.0)	0.996

[a] Comparison of frequencies of 6 different types of anemia between 224 younger and 840 older AG patients by chi-square test.

decreased total body androgen level and nearly all the female, older AG 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 (RBCs) and Hb through the mechanisms of stimulation of erythropoietin release, increase in bone marrow activity, and augmentation of iron incorporation into the RBCs.[36–38] 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).[39] 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 upregulates hepcidin, which inhibits intestinal iron absorption, leading to lower serum iron levels.[40] 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.[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] In this study, men constituted approximately one-fifth of younger AG patients and approximately one-eighth of older AG patients, suggesting that the androgen factor may play a more important role in the group of our younger AG patients than in the group of our older AG patients. On the contrary, menopausal women constituted seven-eighths of our older AG patients, indicating that the menopause is a relevant factor influencing the blood Hb and serum iron levels in the group of our older AG patients. Moreover, younger women consisted of four-fifths of our younger AG patients, indicating pregnancies and menses are two important factors influencing the blood Hb and serum iron levels in the group of our younger AG patients. Taken the above-mentioned evidences together, for the younger AG 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 AG 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 AG patients had lower mean serum iron level and significantly higher frequency of serum iron deficiency than the older AG patients.[36–42]

We further explained why the younger AG patients had the significantly lower mean serum vitamin B12 and folic acid levels and a non-significantly higher frequency of folic acid deficiency than the older 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 folic acid levels in oral precancer patients also found significantly lower mean serum folic acid levels in cigarette smokers or heavy smokers than in non-smokers, and in betel quid chewers than in 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. 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.[46,47] In this study, we did not assess the frequencies of cigarette smoking and betel quid chewing habits in our 224 younger and 840 older 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 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 AG patients than in the older AG patients.[43–48] 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 AG patients should have more severe deficiencies of vitamin B12 and folic acid to express the symptoms of AG. 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 AG patients than in the older AG patients.

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, although significantly lower mean serum vitamin B12 and folic acid levels and a higher frequency of serum folic acid deficiency in the younger AG patients than in the older AG patients were found, there were a marginally significant lower mean serum homocysteine level and a significantly lower frequency of hyperhomocysteinemia in the younger AG patients than in the older AG patients. We suggest that these results may be also due to the relatively minor deviations of the mean serum vitamin B12 and folic acid levels of the younger or older AG patients from those of the younger or older HCSs, respectively (Table 1).

In this study, the younger AG patients had a significantly higher frequency of IDA (9.8%) than the older AG patients (1.0%, $P < 0.001$). This could be due to the finding that the younger AG patients had a higher frequency of serum iron deficiency (28.1%) than the older AG patients (13.9%, $P < 0.001$). On the contrary, the older AG patients had a significantly higher frequency of normocytic anemia (12.1%) than the younger AG patients (6.7%, $P = 0.028$). We suggest that the significantly higher frequency of normocytic anemia in the older AG patients than in the younger AG patients may result from the relatively higher frequencies chronic or inflammatory diseases in the older AG patients than in the younger AG patients.[31–34]

The results of this study conclude that the younger AG patients do have significantly lower mean serum vitamin B12 and folic acid levels, a significantly higher frequency of serum iron deficiency, and a significantly lower frequency of hyperhomocysteinemia than the older AG 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 (Nos. 102-2314-B-002-125-MY3 and 105-2314-B-002-075-MY2) of Ministry of Science and Technology, Taiwan.

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