Journal of the Formosan Medical Association (2016) 115, 860–866

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.jfma-online.com

ORIGINAL ARTICLE

Hematinic deficiencies and anemia statuses in antigastric parietal cell antibody-positive erosive oral lichen planus patients with desquamative gingivitis

Julia Yu-Fong Chang [a,b,c], Yi-Ping Wang [a,b,c], Yang-Che Wu [a,b], Yu-Hsueh Wu [a,b], Chih-Huang Tseng [a,b], Andy Sun [a,b,*]

[a] *Graduate Institute of Clinical Dentistry, School of Dentistry, National Taiwan University, Taipei, Taiwan*
[b] *Department of Dentistry, National Taiwan University Hospital, College of Medicine, National Taiwan University, Taipei, Taiwan*
[c] *Graduate Institute of Oral Biology, School of Dentistry, National Taiwan University, Taipei, Taiwan*

Received 20 June 2016; accepted 5 July 2016

KEYWORDS
antigastric parietal cell antibody;
desquamative gingivitis;
macrocytosis;
oral lichen planus;
pernicious anemia;
vitamin B12 deficiency

Background/purpose: Erosive oral lichen planus (EOLP) patients with desquamative gingivitis (DG) are sometimes encountered in our oral mucosal disease clinic. This study assessed hematinic deficiencies and anemia statuses in antigastric parietal cell antibody (GPCA)-positive EOLP patients with DG ($GPCA^+/DG^+$/EOLP patients).

Methods: The blood hemoglobin, iron, vitamin B12, folic acid, and homocysteine concentrations and serum GPCA levels in 92 $GPCA^+/DG^+$/EOLP patients and 184 age- and sex-matched healthy controls were measured and compared between the two groups.

Results: We found that 27 (29.3%), 16 (17.4%), and 27 (29.3%) of 92 $GPCA^+/DG^+$/EOLP patients had hemoglobin (men $<$ 13 g/dL and women $<$ 12 g/dL), iron ($<$ 60 μg/dL), and vitamin B12 ($<$ 200 pg/mL) deficiencies, respectively. Moreover, 37 (40.2%) of 92 $GPCA^+/DG^+$/EOLP patients had an abnormally high blood homocysteine level ($>$ 12.1μM). $GPCA^+/DG^+$/EOLP patients had a significantly higher frequency of hemoglobin, iron, or vitamin B12 deficiency and an abnormally high blood homocysteine level than healthy control individuals (all $p < 0.001$). Of 27 anemic $GPCA^+/DG^+$/EOLP patients, 13 (48.2%) had pernicious anemia, five (18.5%) had iron deficiency anemia, one (3.7%) had thalassemia trait, and the remaining eight (29.6%) had normocytic anemia. Moreover, of the 92 $GPCA^+/DG^+$/EOLP patients, 24 had macrocytosis, and only 13 (54.2%) of these 24 patients had pernicious anemia.

Conclusion: We conclude that $GPCA^+/DG^+$/EOLP patients may have vitamin B12 deficiency, iron deficiency, and an abnormally high blood homocysteine level. In addition to pernicious

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

* Corresponding author. Department of Dentistry, National Taiwan University Hospital, Number 1, Chang-Te Street, Taipei 10048, Taiwan.
E-mail address: andysun7702@yahoo.com.tw (A. Sun).

http://dx.doi.org/10.1016/j.jfma.2016.07.006

anemia, GPCA$^+$/DG$^+$/EOLP patients may sometimes have normocytic anemia or iron deficiency anemia.

Introduction

Desquamative gingivitis (DG) presents mainly as painful erosions or ulcerations of the attached and free gingivae. It is now recognized to be a manifestation of several diseases, principally mucocutaneous autoimmune disorders such as oral lichen planus (OLP), mucous membrane pemphigoid, and pemphigus vulgaris.[1–3] Our recent study found that 455 (91%), 40 (8%), and five (1%) of 500 DG patients were associated with erosive oral lichen planus (EOLP), pemphigus vulgaris, and mucous membrane pemphigoid, respectively.[4]

Our previous study found that 84 (26.3%) of 320 OLP patients were antigastric parietal cell antibody (anti-GPCA) positive.[5] Our recent study also showed a GPCA-positive rate of 39.6% in 455 EOLP patients with DG (DG$^+$/EOLP patients).[4] The GPCA may induce destruction of gastric parietal cells and, in turn, result in failure of intrinsic factor production.[6,7] A lack of intrinsic factor may lead to vitamin B12 deficiency. Vitamin B12 plays an important role in hemoglobin (Hb) and DNA synthesis and cell division. Patients with vitamin B12 deficiency may have Hb deficiency and macrocytosis [mean corpuscular volume or (MCV) $\geq$ 100 fL] that finally causes pernicious anemia.[8,9] Vitamin B12 deficiency may also be due to an inadequate intake of vitamin B12-containing foods, vitamin B12 malabsorption, biologic competition including bacterial overgrowth and tapeworm infestation, and transcobalamin II deficiency.[9] As multiple causes are involved in vitamin B12 deficiency and macrocytosis, it is interesting to know the frequencies of vitamin B12 deficiency, macrocytosis, and macrocytic anemia in GPCA-positive DG$^+$/EOLP patients (GPCA$^+$/DG$^+$/EOLP patients).

Our previous studies demonstrated that some patients with oral mucosal diseases such as OLP, burning mouth syndrome, and atrophic glossitis may have autoantibodies in their sera. Thus, we frequently examined the presence of different types of autoantibodies such as GPCA, antithyroglobulin antibody (anti-TGA), and antithyroid microsomal antibody (anti-TMA, also known as antithyroid peroxidase antibody) in the sera of oral mucosal disease patients, especially the DG$^+$/EOLP patients.[4,5,10–16] In this study, we recruited 92 GPCA$^+$/DG$^+$/EOLP patients from the oral mucosal disease clinic of National Taiwan University Hospital (NTUH). For these GPCA$^+$/DG$^+$/EOLP patients, complete blood count, serum iron, vitamin B12, folic acid, homocysteine, TGA, and TMA levels were checked to assess whether these patients had microcytic, normocytic, or macrocytic anemia; thalassemia; deficiencies of hematinics; and serum TGA or TMA positivity. These data were further compared with the corresponding data of 184 age- and sex-matched healthy control individuals without oral mucosal and systemic diseases to evaluate whether GPCA$^+$/DG$^+$/EOLP patients had higher frequencies of anemia, vitamin B12 deficiency, macrocytosis (MCV $\geq$ 100 fL), abnormally high blood homocysteine level, and TGA or TMA positivity than with healthy control individuals.

Materials and methods

Patients

In this study, 92 (14 men and 78 women, age range 29–87 years, mean age 58 $\pm$ 12 years) GPCA$^+$/DG$^+$/EOLP patients were enrolled. For each GPCA$^+$/DG$^+$/EOLP patient, two age- ($\pm$2 years of each patient's age) and sex-matched healthy control individuals were selected. Thus, the normal control group consisted of 184 healthy individuals (28 men and 156 women, age range 29–87 years, mean age 57 $\pm$ 11 years). All the patients and healthy control individuals who were seen consecutively, diagnosed, and treated in the oral mucosal disease clinic of NTUH from July 2007 to May 2015 were selected for the study. DG was diagnosed when patients had painful erythematous lesions, erosions, or ulcerations on at least one-quarter of the total maxillary and mandibular gingivae.[1,2] OLP was diagnosed according to the criteria described previously.[5,16] However, all GPCA$^+$/DG$^+$/EOLP patients with areca quid chewing habit, autoimmune diseases (such as systemic lupus erythematosus, rheumatoid arthritis, Sjogren's syndrome, pemphigus vulgaris, and cicatricial pemphigoid), inflammatory diseases, recurrent aphthous ulcerations, malignancy, or recent surgery were excluded. In addition, all GPCA$^+$/DG$^+$/EOLP patients with serum creatinine concentrations indicative of renal dysfunction (men $>$ 131μM, women $>$ 115μM) and those who reported a history of stroke, heavy alcohol use, or diseases of the liver, kidney, or coronary arteries were also excluded.[17] Healthy control individuals had dental caries, pulpal disease, malocclusion, or missing of teeth, but did not have any oral mucosal or systemic diseases. None of our GPCA$^+$/DG$^+$/EOLP patients had taken any prescription medication for malignancies, epilepsy, diabetes mellitus, infection, inflammation, DG, or EOLP at least 3 months before entering the study.

The blood samples were drawn from all GPCA$^+$/DG$^+$/EOLP patients and healthy control individuals for measurement of complete blood count, blood iron, vitamin B12, folic acid, and homocysteine concentrations, as well as serum GPCA, TGA, and TMA levels. All the patients and healthy control individuals signed the informed consent forms before entering the study. This study was reviewed and approved by the Institutional Review Board of NTUH.

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

Complete blood count and blood iron, vitamin B12, folic acid, and homocysteine concentrations were determined with routine tests performed in the Department of Laboratory Medicine of NTUH as described previously.[10–16]

Determination of serum anti-GPCA level

The serum GPCA level was detected using the indirect immunofluorescence technique with rat stomach as a substrate, as described previously.[5,10–13] Sera were scored as positive when they produced fluorescence at a dilution of 10-fold or more.

Determination of serum anti-TGA or anti-TMA level

TGA and TMA titers were measured by a semiquantitative microtiter particle agglutination test using Serodia-AMC kits (Fujirebio Inc., Tokyo, Japan), as described previously.[5,12] Serum TGA or TMA titers equal to or greater than 1:40 were considered positive.

Statistical analysis

Comparisons of the MCV and the mean blood levels of Hb, iron, vitamin B12, folic acid, and homocysteine between 92 $GPCA^+/DG^+$/EOLP patients and 184 age- and sex-matched healthy control individuals were performed using the Student t test. The differences in the frequency of Hb, iron, vitamin B12, or folic acid deficiency; abnormally high blood homocysteine level; or serum TGA or TMA positivity between 92 $GPCA^+/DG^+$/EOLP patients and 184 age- and sex-matched healthy control individuals were compared using the Chi-square test. Comparisons of the MCV; mean blood concentrations of Hb, iron, vitamin B12, folic acid, and homocysteine; and serum TGA or TMA positivity between any two of the three groups of patients with macrocytic, normocytic, or microcytic red blood cells (RBCs) were performed using Student t test or Chi-square test where appropriate. The result was considered to be significant when $p < 0.05$.

Results

The MCV and mean blood concentrations of Hb, iron, vitamin B12, folic acid, and homocysteine in 92 $GPCA^+/DG^+$/EOLP patients and 184 age- and sex-matched healthy control individuals are shown in Table 1. As men usually have 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 Hb (for both men and women; $p < 0.001$ for both), iron (for women only; $p < 0.001$), and vitamin B12 ($p < 0.001$) levels as, well as a significantly higher mean blood homocysteine level ($p < 0.001$) in $GPCA^+/DG^+$/EOLP patients than in healthy control individuals (Table 1).

According to the World Health Organization (WHO) criteria, men with Hb < 13 g/dL and women with Hb < 12 g/dL were defined as having Hb deficiency or anemia.[18,19] Furthermore, patients with a serum iron level of < 60 μg/dL,[20] a vitamin B12 level of < 200 pg/mL,[17] or a folic acid level of < 4 ng/mL[21] were defined as having iron, vitamin B12, or folic acid deficiency, respectively. Moreover, patients with a blood homocysteine level of > 12.1μM (which was the mean blood homocysteine level of healthy control individuals plus two standard deviations) were defined as having an abnormally high homocysteine level. According to the abovementioned definitions, 27 (29.3%), 16 (17.4%), 27 (29.3%), and 0 (0%) of 92 $GPCA^+/DG^+$/EOLP patients had Hb, iron, vitamin B12, and folic acid deficiencies, respectively. Moreover, 37 (40.2%), 26 (28.3%), and 35 (38.0%) of

Table 1 Mean corpuscular volume and the mean blood concentrations of hemoglobin (Hb), iron, vitamin B12, folic acid, and homocysteine in 92 antigastric parietal cell antibody$^+$/desquamative gingivitis$^+$/erosive oral lichen planus ($GPCA^+/DG^+$/EOLP) patients and 184 age- and sex-matched healthy control individuals.

	$GPCA^+/DG^+$/EOLP patients ($n = 92$)		Healthy controls ($n = 184$)		p (Student t test)
	Mean ± SD	Range	Mean ± SD	Range	
Hb (g/dL)					
Men	14.4 ± 1.3 ($n = 14$)	12.7–16.0	15.4 ± 0.5 ($n = 28$)	14.3–16.4	<0.001[a]
Women	12.6 ± 1.2 ($n = 78$)	8.9–15.2	13.6 ± 0.7 ($n = 156$)	12.2–15.4	<0.001[a]
MCV (fL)	92.3 ± 8.9	66.7–117.2	91.2 ± 3.4	80.5–98.6	0.141
Iron (μg/dL)					
Men	108.6 ± 37.0 ($n = 14$)	50.0–196.0	103.2 ± 26.0 ($n = 28$)	63.0–153.0	0.586
Women	82.8 ± 34.0 ($n = 78$)	25.0–210.0	101.3 ± 27.3 ($n = 156$)	60.0–204.0	<0.001[a]
Vitamin B12 (pg/mL)	529.4 ± 318.3	150.0–1000.0	708.1 ± 225.8	267.0–1000.0	<0.001[a]
Folic acid (ng/mL)	15.0 ± 5.9	5.7–24.0	15.0 ± 6.2	4.1–24.0	>0.99
Homocysteine (μM)	10.7 ± 5.4	3.1–41.5	8.3 ± 1.9	4.3–13.5	<0.001[a]

SD = standard deviation.

[a] Comparisons of mean corpuscular volume, blood concentrations of hemoglobin, iron, vitamin B12, folic acid, and homocysteine between 92 antigastric parietal cell antibody$^+$/desquamative gingivitis$^+$/erosive oral lichen planus patients and 184 age- and sex-matched healthy controls by Student t test, with $p < 0.05$.

Table 2 Number and percentage of individuals with hemoglobin (Hb), iron, vitamin B12, or folic acid deficiency; abnormally high blood homocysteine level; or antithyroglobulin antibody (TGA) or antithyroid microsomal antibody (TMA) positivity in 92 antigastric parietal cell antibody$^+$/desquamative gingivitis$^+$/erosive oral lichen planus (GPCA$^+$/DG$^+$/EOLP) patients and 184 age- and sex-matched healthy control individuals.

Factor	GPCA$^+$/DG$^+$/EOLP patients (n = 92)	Healthy controls (n = 184)	p
Hb deficiency (men < 13 g/dL, women < 12 g/dL)	27 (29.3%)	0 (0%)	<0.001[a]
Iron deficiency (< 60 μg/dL)	16 (17.4%)	0 (0%)	<0.001[a]
Vitamin B12 deficiency (< 200 pg/mL)	27 (29.3%)	0 (0%)	<0.001[a]
Folic acid deficiency (< 4 ng/mL)	0 (0%)	0 (0%)	ND
High homocysteine level[b] (> 12.1μM)	37 (40.2%)	8 (4.3%)	<0.001[a]
TGA positivity	26 (28.3%)	3 (1.6%)	<0.001[a]
TMA positivity	35 (38.0%)	3 (1.6%)	<0.001[a]

DG = desquamative gingivitis; ND = not done.

[a] Comparison of frequency of hemoglobin, iron, vitamin B12, or folic acid deficiency; abnormally high blood homocysteine level; or antithyroglobulin antibody or antithyroid microsomal antibody positivity between 92 antigastric parietal cell antibody$^+$/desquamative gingivitis$^+$/erosive oral lichen planus patients and 184 age- and sex-matched healthy control individuals by Chi-square test, with $p < 0.05$.

[b] A homocysteine level was defined to be high when the homocysteine level of patients was greater than the mean serum homocysteine level of healthy control individuals plus two standard deviations.

our GPCA$^+$/DG$^+$/EOLP patients had an abnormally high blood homocysteine level (> 12.1μM), serum TGA positivity, and serum TMA positivity, respectively (Table 2). However, none of the normal control individuals was diagnosed as having Hb, iron, vitamin B12, and folic acid deficiencies according to the aforementioned strict WHO criteria. However, a high blood homocysteine level (> 12.1μM), serum TGA positivity, and serum TMA positivity were found in eight (4.3%), three (1.6%), and three (1.6%) normal control individuals, respectively. GPCA$^+$/DG$^+$/EOLP patients had a significantly higher frequency of Hb, iron, or vitamin B12 deficiency; abnormally high blood homocysteine level; and serum TGA or TMA positivity than healthy control individuals (all $p < 0.001$; Table 2).

In this study, 27 (29.3%) GPCA$^+$/DG$^+$/EOLP patients were diagnosed as having anemia according to the WHO criteria.[18] Considering the diagnostic criteria for pernicious anemia as having MCV ≥ 100 fL, vitamin B12 < 200 pg/mL, and serum GPCA positivity;[13] iron deficiency anemia as having MCV < 80 fL and iron < 60 μg/dL;[19,20] and thalassemia trait as having MCV < 74 fL, RBC count > 5.0 × 10^{12}/L, and Mentzer index (MCV/RBC) < 13,[22] we found that of 27 anemic GPCA$^+$/DG$^+$/EOLP patients, 13 had pernicious anemia, five had iron deficiency anemia, one had thalassemia trait, and the remaining eight had normocytic anemia (Table 3).

When 92 GPCA$^+$/DG$^+$/EOLP patients were further divided into three groups, Group 1 (24 patients with MCV ≥ 100 fL), Group 2 (61 patients with MCV between 80 fL and 99.9 fL), and Group 3 (seven patients with MCV < 80 fL), we found that Group 1 patients had significantly higher MCV ($p < 0.001$), a lower mean Hb level (for both men and women; men, $p = 0.001$; women, $p = 0.003$), a lower mean serum iron level (for men only; $p = 0.012$), a lower mean serum vitamin B12 level ($p < 0.001$), and a higher mean serum homocysteine level ($p < 0.001$) than Group 2 patients (Table 4). Moreover, Group 3 patients had a significantly lower mean Hb level (for women only; $p < 0.001$), lower MCV ($p < 0.001$), a lower mean serum iron level (for women only; $p = 0.003$), and a lower mean vitamin B12 level ($p = 0.035$) than Group 2 patients (Table 4). In addition, Group 1 patients had significantly higher MCV ($p < 0.001$), a higher mean Hb level (for women only; $p = 0.029$), a higher mean serum iron level (for women only; $p = 0.001$), and a lower mean serum vitamin B12 level ($p < 0.001$) than Group 3 patients (Table 4).

Discussion

This study found that of the 92 GPCA$^+$/DG$^+$/EOLP patients, 24 produced macrocytic RBCs, 61 normocytic RBCs, and seven microcytic RBCs. Moreover, 27 (29.3%) of the 92

Table 3 Anemia types of 27 antigastric parietal cell antibody$^+$/desquamative gingivitis$^+$/erosive oral lichen planus patients.

Anemia type	Patient no. (%)			
	Patient no. (%)	MCV (fL)	Vitamin B12 deficiency (< 200 pg/mL)	Iron deficiency (< 60 μg/dL)
Pernicious anemia	13 (48.2)	≥100	13 (100.0)	3 (23.1)
Normocytic anemia	8 (29.6)	80–99.9	0 (0.0)	3 (37.5)
Iron deficiency anemia	5 (18.5)	<80	3 (60.0)	5 (100.0)
Thalassemia trait	1 (3.7)	<74	0 (0.0)	0 (0.0)
Total	27 (100.0)		16 (59.3)	11 (40.7)

MCV = mean corpuscular volume.

Table 4 Mean corpuscular volume (MCV); mean blood concentrations of hemoglobin (Hb), iron, vitamin B12, folic acid, and homocysteine; and antithyroglobulin antibody (TGA) or antithyroid microsomal antibody (TMA) positivity in 92 antigastric parietal cell antibody$^+$/desquamative gingivitis$^+$/erosive oral lichen planus (GPCA$^+$/DG$^+$/EOLP) patients, including 24 with MCV $\geq$100 fL (Group 1), 61 with MCV between 80 fL and 99.9 fL (Group 2), and seven with MCV $<$ 80 fL (Group 3).

	GPCA-positive EOLP patients with DG (n = 92)					
	Group 1 MCV $\geq$ 100 fL (n = 24)	p[a] (Group 1 vs. Group 2)	Group 2 MCV 80—99.9 fL (n = 61)	p[a] (Group 2 vs. Group 3)	Group 3 MCV $<$ 80 fL (n = 7)	p[a] (Group 1 vs. Group 3)
	Mean ± SD		Mean ± SD		Mean ± SD	
Hb (g/dL)						
Men	12.8 ± 0.1 (n = 4)	0.001[a]	15.0 ± 1.0 (n = 10)	ND	(n = 0)	ND
Women	12.2 ± 1.2 (n = 20)	0.003[a]	13.0 ± 0.9 (n = 51)	<0.001[a]	11.0 ± 1.1 (n = 7)	0.029[a]
MCV (fL)	103.5 ± 3.5	<0.001[a]	90.1 ± 3.9	<0.001[a]	73.1 ± 4.8	<0.001[a]
Iron (μg/dL)						
Men	72.0 ± 14.9 (n = 4)	0.012[a]	123.2 ± 32.8 (n = 10)	ND	(n = 0)	ND
Women	82.2 ± 21.7 (n = 20)	0.417	89.1 ± 35.2 (n = 51)	0.003[a]	45.4 ± 26.9 (n = 7)	0.001[a]
Vitamin B12 (pg/mL)	172.5 ± 19.5	<0.001[a]	679.0 ± 262.8	0.035[a]	448.7 ± 316.1	<0.001[a]
Folic acid (ng/mL)	12.2 ± 6.1	0.109	14.5 ± 5.8	0.865	14.9 ± 6.5	0.318
Homocysteine (μM)	15.9 ± 6.7	<0.001[a]	8.6 ± 3.2	0.076	10.9 ± 3.2	0.068
TGA positivity	7 (29.2%)	0.997	16 (26.2%)	0.628	3 (42.9%)	0.824
TMA positivity	9 (37.5%)	0.988	21 (34.4%)	0.134	5 (71.4%)	0.248

ND = not done; SD = standard deviation.
[a] Comparisons of mean corpuscular volume; mean blood concentrations of hemoglobin, iron, vitamin B12, folic acid, and homocysteine; and serum thyroglobulin antibody or thyroid microsomal antibody positivity between Groups 1 and 2, between Groups 2 and 3, and between Groups 1 and 3 by Student t test or Chi-square test, where appropriate.

GPCA$^+$/DG$^+$/EOLP patients had anemia.[18,19] Of the 27 anemic GPCA$^+$/DG$^+$/EOLP patients, 13 had macrocytic anemia (MCV $\geq$ 100 fL; all had pernicious anemia including three having concomitant iron deficiency),[13,14] six had microcytic anemia (MCV $<$ 80 fL; five had iron deficiency anemia and one had thalassemia trait),[19,22] and eight had normocytic anemia (MCV between 80 fL and 99.9 fL; including three having concomitant iron deficiency). As GPCA may induce destruction of gastric parietal cells and further result in failure of intrinsic factor production, GPCA$^+$/DG$^+$/EOLP patients are supposed to have vitamin B12 deficiency, which in turn results in the generation of macrocytic RBCs and pernicious anemia.[13,14] However, we showed that only 27 (29.3%), 24 (26.1%), and 13 (14.1%) of 92 GPCA$^+$/DG$^+$/EOLP patients had vitamin B12 deficiency, macrocytosis, and pernicious anemia, respectively. We also found that 13 (48.1%) of our 27 vitamin B12 deficiency patients and 13 (54.2%) of our 24 macrocytosis patients had pernicious anemia. Our previous studies demonstrated that 16 (12.9%) of 124 GPCA-positive patients, 17 (18.9%) of 90 vitamin B12-deficient patients, and 10 (16.7%) of 60 macrocytosis patients with oral mucosal diseases (including atrophic glossitis, burning mouth syndrome, OLP, and recurrent aphthous ulcerations) had pernicious anemia.[13—15] Statistical analyses of the aforementioned data indicate that GPCA$^+$/DG$^+$/EOLP patients with vitamin B12 deficiency or macrocytosis had a significantly high frequency to contract pernicious anemia than oral mucosal disease patients with vitamin B12 deficiency (p = 0.005) or macrocytosis (p = 0.001), respectively.

This study found microcytosis in seven GPCA$^+$/DG$^+$/EOLP patients. Of these seven microcytosis patients, five had iron deficiency anemia including three having concomitant vitamin B12 deficiency and an abnormally high serum homocysteine level, one had thalassemia trait, and the remaining one had normal Hb, vitamin B12, folic acid, and iron levels. The five anemic microcytosis patients had severe iron deficiency (serum iron level $<$ 35 μg/dL). The severe iron deficiency may play an important role in the generation of microcytic RBCs in these five anemic microcytosis patients. In addition, vitamin B12 deficiency may be the predominant factor that results in an abnormally high serum homocysteine level in patients,[14] and both iron and vitamin B12 deficiencies may be the factors causing anemia in these five anemic microcytosis patients.[14,19]

In the present study, 61 of 92 GPCA$^+$/DG$^+$/EOLP patients had normocytosis. Of these 61 normocytosis patients, eight had normocytic anemia with three having concomitant iron deficiency, seven had iron deficiency (six with serum iron levels between 48 μg/dL and 59 μg/dL; one with a serum iron level of 33 μg/dL), and none had vitamin B12 and folic acid deficiencies. The normal vitamin B12 and folic acid

levels and only a small percentage (11.5%) of our patients with mild iron deficiency may be the major factors resulting in normocytosis in our patients. $GPCA^+/DG^+/EOLP$ patients may tend to have intrinsic factor deficiency that results in malabsorption of vitamin B12 from the terminal ileum and finally in vitamin B12 deficiency.[13–15] Therefore, it is interesting to know why our $GPCA^+/DG^+/EOLP$ patients do not have vitamin B12 deficiency. It has been reported that supplementation with oral vitamin B12 is a safe and effective treatment for the vitamin B12 deficiency state. Even when intrinsic factor is not present to aid the absorption of vitamin B12 or in other diseases that affect the usual absorption sites in the terminal ileum, oral therapy with vitamin B12 remains effective.[9] Therefore, although $GPCA^+/DG^+/EOLP$ patients have GPCA in their sera, enough dietary supply of vitamin B12 may prevent the occurrence of vitamin B12 deficiency in these patients. In addition, those GPCA-positive patients require a sufficiently long period of time to develop autoimmune gastritis, which finally results in complete failure of intrinsic factor production.[8,23] Therefore, the residual gastric parietal cells of patients with lower serum titer of GPCA, or those with medium or higher serum titer of GPCA of a shorter duration may still have some ability to produce intrinsic factors that help absorb vitamin B12 from the small intestine.

Carrozzo et al[24] discovered the presence of serum GPCA in three (6%) and antithyroid antibodies in five (10%) of 50 OLP patients. Our previous study demonstrated that 84 (26.3%), 68 (21.3%), and 78 (24.4%) of 320 OLP patients had GPCA, TGA, and TMA positivities, respectively.[5] Moreover, 34 (40.5%) of 84 GPCA-positive OLP patients also had serum TGA/TMA positivity and 34 (35.1%) of 97 TGA/TMA-positive OLP patients had concomitant GPCA positivity.[5] Our recent study found a GPCA, TGA, or TMA positive rate of 39.6% (180 patients), 46.4% (211 patients), or 45.1% (205 patients) in 455 $DG^+/EOLP$ patients.[4] Furthermore, 108 (60.0%) of 180 $GPCA^+/DG^+/EOLP$ patients also had serum TGA/TMA positivity and 108 (38.7%) of 279 TGA/TMA-positive $DG^+/EOLP$ patients had concomitant GPCA positivity.[4] This study showed a TGA or TMA positive rate of 28.3% or 38.0%, respectively, in 92 $GPCA^+/DG^+/EOLP$ patients. These findings suggest a high frequency of possessing both antigastric and antithyroid autoantibodies in EOLP and $DG^+/EOLP$ patients. In addition, the high GPCA, TGA, and TMA positive rates in EOLP and $DG^+/EOLP$ patients indicate an intimate association of EOLP and $DG^+/EOLP$ patients with autoimmune gastric and thyroid diseases.[4,5,24] Further studies are necessary to confirm this intimate association.

Homocysteine is a sulfur-containing amino acid that is formed during methionine metabolism.[25] Both vitamin B12 and folic acid act as coenzymes for the conversion of homocysteine to methionine.[26] Higher blood homocysteine levels can cause oxidative stress, damage endothelium, and enhance thrombogenicity in experimental studies, and thus are associated with increased rates of coronary heart disease and stroke.[25,27–30] Moreover, measurement of both serum methylmalonic acid and homocysteine levels has been discovered to be a more sensitive method of screening for vitamin B12 deficiency, with the sensitivity being 99.8%, and thus can be used for detecting subclinical vitamin B12 deficiency.[9,31] In this study, an abnormally higher blood homocysteine level was detected in 37 (40.2%) of the 92 $GPCA^+/DG^+/EOLP$ patients, but could be found in only eight (4.3%) of the 184 healthy control individuals. Previous studies demonstrated that the high blood homocysteine level found in patients is due to deficiencies in folic acid, vitamin B12, and vitamin B6, because a supplement therapy with folic acid, vitamin B12, and vitamin B6 can reduce their blood homocysteine levels.[28,32,33] In this study, of 37 patients with abnormally higher blood homocysteine levels, 24 (64.9%) had vitamin B12 deficiency according to the WHO definition (vitamin B12 level $<$ 200 pg/mL)[17] and 32 (86.5%) had relative vitamin B12 deficiency according to our previous definition (vitamin B12 level $\leq$ 450 pg/mL).[32,33] Furthermore, of our 37 patients with abnormally higher blood homocysteine levels, none had folic acid deficiency according to the WHO definition (folic acid level $<$ 4 ng/mL)[21] and only five (13.5%) had relative folic acid deficiency according to our previous definition (folic acid level $\leq$ 6 ng/mL).[32,33] Moreover, our 92 $GPCA^+/DG^+/EOLP$ patients also had a significantly lower mean serum vitamin B12 level than healthy control individuals. Therefore, the high blood homocysteine level in our 92 $GPCA^+/DG^+/EOLP$ patients may predominantly be due to vitamin B12 deficiency rather than folic acid deficiency.

Our results demonstrated that, according to WHO definitions, 27 (29.3%), 16 (17.4%), and 27 (29.3%) of 92 $GPCA^+/DG^+/EOLP$ patients had Hb, iron, and vitamin B12 deficiencies, respectively. Moreover, 37 (40.2%), 26 (28.3%), and 35 (38.0%) of our 92 $GPCA^+/DG^+/EOLP$ patients had an abnormally high blood homocysteine level, serum TGA positivity, and serum TMA positivity, respectively. $GPCA^+/DG^+/EOLP$ patients had a significantly higher frequency of Hb, iron, or vitamin B12 deficiency; abnormally high blood homocysteine level; and serum TGA or TMA positivity than healthy control individuals. Of 27 anemic $GPCA^+/DG^+/EOLP$ patients, 13 had pernicious anemia, five had iron deficiency anemia, one had thalassemia trait, and the remaining eight had normocytic anemia. Moreover, of the 92 $GPCA^+/DG^+/EOLP$ patients, 24 had macrocytosis, and only 13 of these 24 macrocytosis patients had pernicious anemia. We conclude that $GPCA^+/DG^+/EOLP$ patients have a significantly higher frequency of iron and vitamin B12 deficiencies, abnormally high blood homocysteine level, and serum TGA or TMA positivity than healthy control individuals. In addition to pernicious anemia (14.1%), $GPCA^+/DG^+/EOLP$ patients may have normocytic anemia (8.7%) or microcytic anemia (6.5%).

Acknowledgments

This study was supported by research grants of 102-2314-B-002-125-MY3 and 105-2314-B-002-075-MY2 from the Ministry of Science and Technology, Republic of China.

References

1. Alessi SS, Nico MM, Fernandes JD, Lourenço SV. Reflectance confocal microscopy as a new tool in the *in vivo* evaluation of desquamative gingivitis: patterns in mucous membrane pemphigoid, pemphigus vulgaris and oral lichen planus. *Br J Dermatol* 2013;**168**:257–64.

2. Gagari E, Damoulis PD. Desquamative gingivitis as a manifestation of chronic mucocutaneous disease. *J Dtsch Dermatol Ges* 2011;**9**:184—8.
3. Lo Russo L, Fierro G, Guiglia R, Compilato D, Testa NF, Lo Muzio L, et al. Epidemiology of desquamative gingivitis: evaluation of 125 patients and review of the literature. *Int J Dermatol* 2009;**48**:1049—52.
4. Chang JYF, Chiang CP, Wang YP, Wu YC, Chen HM, Sun A. Antigastric parietal cell and anti-thyroid autoantibodies in patients with desquamative gingivitis. *J Oral Pathol Med* 2016 (in press).
5. 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.
6. Taylor KB, Roitt IM, Doniach D, Coushman KG, Shapland C. Autoimmune phenomena in pernicious anemia: gastric antibodies. *BMJ* 1962;**2**:1347—52.
7. 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.
8. Lahner E, Annibale B. Pernicious anemia: new insights from a gastroenterological point of view. *World J Gastroenterol* 2009;**15**:5121—8.
9. Oh RC, Brown DL. Vitamin B_{12} deficiency. *Am Fam Phys* 2003;**67**:979—86.
10. 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.
11. Sun A, Lin HP, Wang YP, Chiang CP. Significant association of deficiency of hemoglobin, iron and vitamin B12, high homocysteine level, and gastric parietal cell antibody positivity with atrophic glossitis. *J Oral Pathol Med* 2012;**41**:500—4.
12. Wang YP, Lin HP, Chen HM, Kuo YS, Lang MJ, Sun A. Hemoglobin, iron, and vitamin B12 deficiencies and high blood homocysteine levels in patients with anti-thyroid autoantibodies. *J Formos Med Assoc* 2014;**113**:155—60.
13. 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.
14. 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.
15. 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.
16. Chen HM, Wang YP, Chang JYF, Wu YC, Cheng SJ, Sun A. Significant association of deficiency of hemoglobin, iron and vitamin B12 and high homocysteine level with oral lichen planus. *J Formos Med Assoc* 2015;**114**:124—9.
17. 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.
18. WHO/UNICEF/UNU. *Iron deficiency anaemia assessment, prevention, and control: a guide for programme managers*. Geneva, Switzerland: World Health Organization; 2001.
19. 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.
20. Shine JW. Microcytic anemia. *Am Fam Physician* 1997;**55**:2455—62.
21. de Benoist B. Conclusions of a WHO technical consultation on folate and vitamin B12 deficiencies. *Food Nutr Bull* 2008;**29**(Suppl.):S238—44.
22. 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.
23. Toh BH, van Driel IR, Gleeson PA. Pernicious anemia. *N Engl J Med* 1997;**337**:1441—8.
24. Carrozzo M, Gandolfo S, Lodi G, Carbone M, Garzino-Demo P, Carbonero C, et al. Oral lichen planus in patients infected or noninfected with hepatitis C virus: the role of autoimmunity. *J Oral Pathol Med* 1999;**28**:16—9.
25. Spence JD. Homocysteine-lowering therapy: a role in stroke prevention? *Lancet Neurol* 2007;**6**:830—8.
26. Chanarin I, Deacon R, Lumb M, Perry J. Cobalamin—folate interrelations. *Blood Rev* 1989;**3**:211—5.
27. Lonn E, Yusuf S, Arnold MJ, Sheridan P, Pogue J, Micks M, et al. Homocysteine lowering with folic acid and B vitamins in vascular disease. *N Engl J Med* 2006;**354**:1567—77.
28. Welch GN, Loscalzo J. Homocysteine and atherothrombosis. *N Engl J Med* 1998;**338**:1042—50.
29. Harker LA, Harlan JM, Ross R. Effect of sulfinpyrazone on homocysteine induced endothelial injury and arteriosclerosis in baboons. *Circ Res* 1983;**53**:731—9.
30. Harker LA, Slichter SJ, Scott CR, Ross R. Homocysteinemia: vascular injury and arterial thrombosis. *N Engl J Med* 1974;**291**:537—43.
31. Savage DG, Lindenbaum J, Stabler SP, Allen RH. Sensitivity of serum methylmalonic acid and total homocysteine determinations for diagnosing cobalamin and folate deficiencies. *Am J Med* 1994;**96**:239—46.
32. Sun A, Lin HP, Wang YP, Chen HM, Cheng SJ, Chiang CP. Significant reduction of serum homocysteine level and oral symptoms after different vitamin supplement treatments in patients with burning mouth syndrome. *J Oral Pathol Med* 2013;**42**:474—9.
33. 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.