



Original Article

Hematinic deficiencies and anemia in gastric parietal cell antibody-positive and -negative oral submucous fibrosis patients



Chun-Pin Chiang^{a,b,c,d}, Julia Yu-Fong Chang^{b,c,d},
Yu-Hsueh Wu^{a,b}, Andy Sun^{b,c}, Yi-Ping Wang^{b,c,d},
Hsin-Ming Chen^{b,c,d*}

^a Department of Dentistry, Far Eastern Memorial Hospital, Taipei, Taiwan

^b Graduate Institute of Clinical Dentistry, School of Dentistry, National Taiwan University, Taipei, Taiwan

^c Department of Dentistry, National Taiwan University Hospital, College of Medicine, National Taiwan University, Taipei, Taiwan

^d Graduate Institute of Oral Biology, School of Dentistry, National Taiwan University, Taipei, Taiwan

Received 6 February 2018

Available online 21 March 2018

KEYWORDS

Anemia;
Folic acid deficiency;
Hemoglobin;
Vitamin B12
deficiency;
Gastric parietal cell
antibody;
Oral submucous
fibrosis

Abstract *Background/purpose:* Previous studies showed that approximately 13–15% of male oral submucous fibrosis (OSF) patients are serum gastric parietal cell antibody (GPCA)-positive. This study assessed whether serum GPCA or OSF itself was a significant factor that caused hematinic deficiencies and anemia statuses in GPCA-positive or GPCA-negative OSF patients (GPCA⁺/OSF and GPCA⁻/OSF patients).

Materials and methods: The frequencies of macrocytosis (mean corpuscular volume or MCV \geq 100 fL) and blood hemoglobin (Hb), iron, vitamin B12 and folic acid deficiencies were determined and compared between any two of the four groups of 149 male OSF, 23 male GPCA⁺/OSF, and 126 male GPCA⁻/OSF patients and 149 age-matched male healthy control subjects.

Results: All three groups of OSF patients (including 149 OSF, 23 GPCA⁺/OSF, and 126 GPCA⁻/OSF patients) had a significantly higher frequency of Hb, vitamin B12, or folic acid deficiency and of macrocytosis than 149 healthy control subjects (all *P*-values < 0.05). The 23 GPCA⁺/OSF patients did have a significantly lower mean serum vitamin B12 level and a significantly higher MCV or frequency of vitamin B12 deficiency than the 126 GPCA⁻/OSF patients. Two of the 23 GPCA⁺/OSF patients had pernicious anemia. Of the 126 GPCA⁻/OSF patients, 6 had macrocytic anemia, 2 had iron deficiency anemia, and 4 had thalassemia trait-induced anemia.

Conclusion: We conclude that OSF itself does play a significant role in causing hematinic

* Corresponding author. Department of Dentistry, National Taiwan University Hospital, No. 1, Chang-Te Street, Taipei 10048, Taiwan. Fax: +886 02 2389 3853.

E-mail address: hmchen51@ntuh.gov.tw (H.-M. Chen).

deficiencies and anemia in OSF, GPCA⁺/OSF, and GPCA⁻/OSF patients. The serum GPCA is the major factor that causes vitamin B12 deficiency, macrocytosis, and pernicious anemia in GPCA⁺/OSF patients.

© 2018 Taiwan Association of Obstetrics & Gynecology. Publishing services by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Introduction

Oral submucous fibrosis (OSF) is a chronic progressive scarring oral mucosal disease characterized by juxtaepithelial inflammatory cell infiltration followed by a marked deposition of collagen in the lamina propria and submucosa of the oral mucosa and superficial muscle layer. The areca nut is the main etiological factor in OSF.^{1,2} Areca nut contains alkaloids, flavonoids, and copper. Alkaloids, mainly arecoline and arecaidine, are found to stimulate fibroblasts to produce collagen. Flavonoids (tannins and catechins) can inhibit collagenase, stabilize the collagen fibrils (through an increase in cross-linking in the collagen fibrils), and in turn render collagen fibrils resistant to degradation by collagenase. The high concentration of copper in areca nut has been reported to stimulate lysyl oxidase activity, an enzyme essential to the final cross-linking of collagen fibers.^{1,2} Therefore, areca nut ingredients can cause increased collagen deposition in the oral tissue, leading to OSF.

The localized mucosal inflammation caused by areca nut leads to the recruitment of activated T-cells and macrophages that can secrete transforming growth factor- β (TGF- β), a pivotal regulator controlling collagen production and degradation.^{1,2} TGF- β can increase the collagen production by activation of procollagen genes (resulting in enhanced production of procollagen), elevation of procollagen proteinase levels (leading to conversion of procollagens to collagen fibrils), and upregulation of lysyl oxidase activity (causing an increased production of insoluble form of collagen). Furthermore, TGF- β can inhibit collagen degradation by activating the tissue inhibitor of matrix metalloproteinase (TIMP) genes (resulting in an increased production of TIMPs that inhibit the activated collagenase) and plasminogen activator inhibitor (PAI) genes (leading to an elevated production of PAI that blocks the conversion of plasminogen to plasmin and in turn block the conversion of procollagenase to active collagenases).^{1,2} Thus, TGF- β can not only increase collagen production but also decrease collagen degradation, finally resulting in development of OSF.

In our oral mucosal disease clinic or dental clinic, patients with atrophic glossitis, burning mouth syndrome, oral lichen planus, and recurrent aphthous stomatitis are frequently encountered and patients with OSF or specific jaw bone lesions are sometimes seen.^{3–21} For these particular groups of patients, complete blood count as well as serum iron, vitamin B12, folic acid, gastric parietal cell antibody (GPCA), thyroglobulin antibody (TGA), and thyroid microsomal antibody (TMA, also known as thyroid peroxidase antibody, TPO) levels were frequently

examined to assess whether these patients have anemia, hematinic deficiencies, and serum GPCA, TGA, or TMA positivity.^{3–21} Because the majority of OSF patients can not tolerate spicy food, suffer from burning sensation of oral mucosa, and have a certain degree of mouth opening limitation, these functional impairments may affect normal food intake and lead to nutritional deficiencies.^{22,23} Our previous study demonstrated high frequencies of vitamin B12 and folic acid deficiencies and GPCA positivity in OSF patients.^{22,23} In this study, 149 male OSF patients were retrospectively collected from our oral mucosal disease clinic. The mean corpuscular volume (MCV) and blood hemoglobin (Hb), iron, vitamin B12, folic acid, and GPCA levels were examined and compared with the corresponding data in 149 age-matched male healthy control subjects. We tried to assess whether serum GPCA or OSF itself was a significant factor that caused hematinic deficiencies and anemia statuses in GPCA-positive or GPCA-negative OSF patients (GPCA⁺/OSF and GPCA⁻/OSF patients).

Materials and methods

Subjects

This study included 149 male OSF patients (mean age 39.2 ± 12.3 years, range 18–62 years) and 149 age-matched (± 2 years of each patient's age, mean age 40.1 ± 12.6 years, range 19–63 years) male healthy control subjects. All the OSF patients and healthy control subjects were seen consecutively, diagnosed, treated, and selected in oral mucosal disease clinic and dental clinic of National Taiwan University Hospital (NTUH) from July 2007 to July 2016. Clinical diagnosis of OSF was made when patients showed characteristic features of OSF, including intolerance to spicy foods, blanching and stiffness of the oral mucosa, fibrous bands in the buccal mucosa, and progressive inability to open the mouth.^{22,23} Some OSF patients might also have burning sensation of the oral mucosa, xerostomia, the presence of vesicles or ulcers on the oral mucosa, depapillation of the tongue, and impaired tongue mobility.^{22,23} Incisional biopsy of buccal mucosa was taken from 43 of 149 OSF patients. Histological diagnosis was made based on the examination of hematoxylin and eosin-stained tissue sections. The histological criteria for a diagnosis of OSF were: (1) atrophic epithelium with either parakeratosis or hyperkeratosis, and (2) moderate to marked fibrosis or hyalinization in the lamina propria, submucosa, and superficial muscle layer.^{22,23} The oral mucosal sites (soft palate, retromolar area, buccal mucosa,

labial mucosa, floor of the mouth, and tongue) of involvement and the maximum mouth opening (MMO) of OSF patients were recorded. The severity of OSF was determined according to the sites of involvement or MMO; the more the sites involved and the less the MMO, the more severe the OSF. No mild OSF patient was included in the study, because all our OSF patients had at least three sites of involvement. OSF patients with severe systemic diseases, autoimmune diseases (such as systemic lupus erythematosus, rheumatoid arthritis, Sjogren's syndrome, pemphigus vulgaris, and cicatricial pemphigoid), inflammatory diseases, malignancy, or recent surgery were excluded. Healthy control subjects had dental caries, pulpal disease, malocclusion, or missing of teeth but did not have any oral mucosal or systemic diseases. None of our OSF patients had taken any prescription medication at least 3 months before entering the study.

All the 149 OSF patients had areca quid chewing habit; they chewed 1–150 (mean, 28) quids per day for 1–39 (mean, 14.2) years. One hundred and two (68.5%) OSF patients claimed that they swallowed some of the "juice" of areca quid during the chewing process. One hundred and thirty-five (90.6%) of the 149 OSF patients were smokers; they smoked 6–60 (mean, 23) cigarettes per day for 2–39 (mean, 18.3) years. Ninety-nine (66.4%) of the 149 OSF patients were drinkers; they drank 46–6528 (mean, 942) g of pure alcohol per week for 1–33 (mean, 13.6) years. Of the 149 healthy control subjects, none were areca quid chewers (one quid or more daily for at least 1 year), 39 (26.2%) were smokers (one cigarettes or more per day for at least 1 year), and 29 (19.5%) were drinkers (drinking more than 3 days a week).

The blood samples were drawn from all OSF patients and healthy control subjects for measurement of complete blood count as well as serum iron, vitamin B12, and folic acid concentrations. In addition, the presence of GPCA in sera of OSF patients and healthy control subjects was also checked. All OSF patients and healthy control subjects signed the informed consents before entering the study. This study was reviewed and approved by the Institutional Review Board at the NTUH.

Determination of complete blood count as well as serum iron, vitamin B12, and folic acid concentrations

The complete blood count as well as serum iron, vitamin B12, and folic acid concentrations were determined by the routine tests performed in the Department of Laboratory Medicine of NTUH.^{3–15,17,18}

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.^{6–8} Sera were scored as positive when they produced fluorescence at a dilution of 20-fold or more.^{6–8}

Statistical analysis

Comparisons of the MCV and mean blood levels of Hb, iron, vitamin B12, and folic acid between 149 OSF, 23 GPCA⁺/OSF, or 126 GPCA⁻/OSF patients and 149 age-matched healthy control subjects as well as between 23 GPCA⁺/OSF and 126 GPCA⁻/OSF patients were performed by Student's *t*-test. The differences in frequency of blood Hb, iron, vitamin B12 or folic acid deficiency or of macrocytosis (MCV \geq 100 fL) between any two of the four groups of 149 OSF, 23 GPCA⁺/OSF, and 126 GPCA⁻/OSF patients and 149 healthy control subjects were compared by chi-square test. The result was considered to be significant if the *P*-value was less than 0.05.

Results

The mean MMO of 149 OSF patients were 30.1 \pm 8.5 mm; 75 had MMO \leq 31 mm and 74 had MMO between 31 mm and 42 mm. The soft palate, retromolar area, and buccal mucosa were the three sites where were involved by OSF in

Table 1 Comparisons of the mean corpuscular volume (MCV) and mean blood concentrations of hemoglobin (Hb), iron, vitamin B12, and folic acid between any two of the four groups of 149 oral submucous fibrosis (OSF) patients, 23 gastric parietal cell antibody (GPCA)-positive OSF patients (GPCA⁺/OSF patients), 126 GPCA-negative OSF patients (GPCA⁻/OSF patients), and 149 healthy control subjects.

Group	Hb (g/dL)	MCV (fL)	Iron (μ g/dL)	Vitamin B12 (pg/mL)	Folic acid (ng/mL)
OSF patients (n = 149)	15.1 \pm 1.7	91.8 \pm 8.1	112.1 \pm 48.5	497.0 \pm 217.7	7.0 \pm 2.9
^a P-value	0.064	0.091	0.281	<0.001	<0.001
GPCA ⁺ /OSF patients (n = 23)	14.5 \pm 0.9	95.2 \pm 4.1	97.4 \pm 34.3	362.4 \pm 228.6	6.0 \pm 3.2
^a P-value	<0.001	<0.001	<0.001	<0.001	<0.001
^b P-value	0.056	0.029	0.114	0.001	0.067
GPCA ⁻ /OSF patients (n = 126)	15.2 \pm 1.7	91.2 \pm 8.5	114.8 \pm 50.3	521.5 \pm 207.3	7.2 \pm 2.8
^a P-value	0.227	0.422	0.652	<0.001	<0.001
Healthy control subjects (n = 149)	15.4 \pm 1.0	90.6 \pm 3.0	116.9 \pm 24.3	653.2 \pm 224.8	13.7 \pm 6.1

^a Comparisons of means of parameters between 149 OSF, 23 GPCA⁺/OSF or 126 GPCA⁻/OSF patients and 149 healthy control subjects by Student's *t*-test.

^b Comparisons of means of parameters between 23 GPCA⁺/OSF patients and 126 GPCA⁻/OSF patients by Student's *t*-test.

every patient, with extra involvement of labial mucosa in 95 patients (63.8%), of floor of the mouth in 74 patients (49.7%), and of tongue in 61 patients (40.9%). Of the 149 OSF patients, 32 had 3-site involvement, 40 had 4-site involvement, 41 had 5-site involvement, and 36 had 6-site involvement.

There were 23 GPCA⁺/OSF patients and 126 GPCA⁻/OSF patients in this study. Comparisons of the MCV and mean blood concentrations of Hb, iron, vitamin B12, and folic acid between any two of the four groups of 149 OSF, 23 GPCA⁺/OSF, and 126 GPCA⁻/OSF patients, and 149 healthy control subjects are shown in Table 1. We found that both 149 OSF and 126 GPCA⁻/OSF patients had a significantly lower mean serum vitamin B12 ($P < 0.001$) or folic acid level ($P < 0.001$) than healthy control subjects (Table 1). Moreover, the 23 GPCA⁺/OSF had a significantly lower mean blood Hb, iron, vitamin B12, or folic acid level and a significantly higher mean MCV level (all P -values < 0.001) than healthy control subjects. In addition, the 23 GPCA⁺/OSF also had a significantly lower mean serum vitamin B12 level ($P = 0.001$) and a significantly higher mean MCV level ($P = 0.029$) than healthy control subjects (Table 1).

According to the World Health Organization (WHO) criteria, men with Hb < 13 g/dL were defined as having Hb deficiency or anemia.²⁴ For serum iron, vitamin B12, and folic acid levels, this study chose the serum iron level ≤ 70 μ g/dL for men, vitamin B12 level ≤ 450 pg/mL, and folic acid level ≤ 6 ng/mL as having the iron, vitamin B12, and folic acid deficiencies, respectively.^{25,26} These iron, vitamin B12 and folic acid concentrations were chosen because they were the cut-off point concentrations for giving the iron, vitamin B12, and folic acid supplement treatment to oral mucosal disease patients with iron, vitamin B12 or folic acid deficiency.^{25,26} Moreover, macrocytosis was defined as having the MCV ≥ 100 fL.⁸ By the above-mentioned definitions, 14 (9.4%), 32 (21.5%), 76 (51.0%) and 61 (40.9%) of the 149 OSF patients had blood Hb, iron, vitamin B12, and folic acid deficiencies, respectively. Moreover, 12 (8.1%) OSF patients had macrocytosis

(Table 2). However, 0 (0%), 21 (14.1%), 31 (20.8%), 8 (5.4%) and 2 (1.3%) of normal control subjects had blood Hb, iron, vitamin B12, and folic acid deficiencies and macrocytosis by the aforementioned criteria, respectively. Furthermore, all three groups of OSF patients (including 149 OSF, 23 GPCA⁺/OSF, and 126 GPCA⁻/OSF patients) had a significantly higher frequency of macrocytosis or blood Hb, vitamin B12, or folic acid deficiency than healthy control subjects (all P -values < 0.05) (Table 2). In addition, the 23 GPCA⁺/OSF patients had a significantly higher frequency of vitamin B12 deficiency than the 126 GPCA⁻/OSF patients ($P = 0.031$, Table 2).

Of the 23 GPCA⁺/OSF patients, 2 had pernicious anemia (defined as having Hb < 13 g/dL for men, serum vitamin B12 level < 200 pg/mL, MCV ≥ 100 fL, and serum GPCA positivity) and 21 had no anemia (Table 3).^{6-8,27-30} Of the 126 GPCA⁻/OSF patients, 6 had macrocytic anemia (defined as having Hb < 13 g/dL for men and MCV ≥ 100 fL),⁸ 2 had iron deficiency anemia (defined as having Hb < 13 g/dL for men and serum iron level < 60 μ g/dL),^{24,31} 4 had thalassemia trait-induced anemia (defined as having Hb < 13 g/dL for men, RBC count $> 5.0 \times 10^{12}$ /L, MCV < 74 fL, and Mentzer index (MCV/RBC) < 13),³² and 114 had no anemia (Table 3).

Discussion

This study found that 14 (9.4%), 32 (21.5%), 76 (51.0%) and 61 (40.9%) of the 149 OSF patients had blood Hb, iron, vitamin B12, and folic acid deficiencies, respectively. Furthermore, both 149 OSF and 126 GPCA⁻/OSF patients had a significantly higher frequency of blood Hb, vitamin B12, or folic acid deficiency than healthy control subjects (all P -values < 0.05). In addition, single, double, and triple hematinic (iron, vitamin B12, and folic acid) deficiencies were found in 55 (36.9%), 42 (28.2%), and 10 (6.7%) of the 149 OSF patients, respectively. These findings suggest that hematinic deficiencies are frequently found in OSF patients and OSF disease itself is the etiology of single or multiple

Table 2 Comparisons of frequencies of macrocytosis as well as blood hemoglobin (Hb), iron, vitamin B12, and folic acid deficiencies between any two of the four groups of 149 oral submucous fibrosis (OSF) patients, 23 gastric parietal cell antibody (GPCA)-positive OSF patients (GPCA⁺/OSF patients), 126 GPCA-negative OSF patients (GPCA⁻/OSF patients), and 149 healthy control subjects.

Group	Patient number (%)				
	Hb deficiency (< 13 g/dL)	Macrocytosis (MCV ≥ 100 fL)	Iron deficiency (≤ 70 μ g/dL)	Vitamin B12 deficiency (≤ 450 pg/mL)	Folic acid deficiency (≤ 6 ng/mL)
OSF patients (n = 149)	14 (9.4)	12 (8.1)	32 (21.5)	76 (51.0)	61 (40.9)
^a P-value	< 0.001	0.014	0.130	< 0.001	< 0.001
GPCA ⁺ /OSF patients (n = 23)	2 (8.7)	3 (13.0)	6 (26.1)	17 (73.9)	14 (60.9)
^a P-value	0.010	0.015	0.245	< 0.001	< 0.001
^b P-value	0.792	0.589	0.757	0.031	0.060
GPCA ⁻ /OSF patients (n = 126)	12 (9.5)	9 (7.1)	26 (20.6)	59 (46.8)	47 (37.3)
^a P-value	< 0.001	0.033	0.202	< 0.001	< 0.001
Healthy control subjects (n = 149)	0 (0.0)	2 (1.3)	21 (14.1)	31 (20.8)	8 (5.4)

^a Comparisons of frequencies of parameters between 149 OSF patients, 23 GPCA⁺/OSF patients or 126 GPCA⁻/OSF patients and 149 healthy control subjects by chi-square test.

^b Comparisons of frequencies of parameters between 23 GPCA⁺/OSF patients and 126 GPCA⁻/OSF patients by chi-square test.

Table 3 Anemia types of 2 of 23 gastric parietal cell antibody (GPCA)-positive oral submucous fibrosis (OSF) patients (GPCA⁺/OSF patients) and of 12 of 126 GPCA-negative OSF patients (GPCA⁻/OSF patients).

Anemia type	Patient number (%)				
	Patient number (%)	MCV	Vitamin B12 deficiency (≤ 450 pg/mL)	Iron deficiency (≤ 70 μ g/dL)	Folic acid deficiency (≤ 6 ng/mL)
GPCA⁺/OSF patients (n = 23)					
Pernicious anemia	2 (100.0)	≥ 100 fL	2 (100.0)	0 (0.0)	2 (100.0)
Total	2 (100.0)		2 (100.0)	0 (0.0)	2 (100.0)
GPCA⁻/OSF patients (n = 126)					
Macrocytic anemia	6 (50.0)	≥ 100 fL	2 (33.3)	2 (33.3)	2 (33.3)
Iron deficiency anemia	2 (16.7)	< 80 fL	0 (0.0)	2 (100.0)	0 (0.0)
Thalassemia trait-induced anemia	4 (33.3)	< 74 fL	2 (50.0)	0 (0.0)	2 (50.0)
Total	12 (100.0)		4 (33.3)	4 (33.3)	4 (33.3)

hematinic deficiencies in OSF patients. Because all of our OSF patients had moderate or severe OSF with the mean MMO being 30.1 mm, it was possible that the blood Hb, iron, vitamin B12, and folic acid deficiencies were at least partially due to difficult and insufficient food intake secondary to the limited mouth-opening and intolerance to spicy food in OSF patients. Wahi et al.³³ also reported a significantly higher prevalence of malnutrition in 104 OSF patients than in 200 normal control subjects. In their study, poor nutritional status was evident on clinical examination in 28% of the 70 male and 35% of the 34 female OSF patients. In addition, of 104 OSF patients, 40 (38.5%) had multiple vitamin deficiencies, 20 (19.2%) had vitamin A deficiency, 56 (53.8%) had vitamin B deficiency, and 54 (51.9%) had vitamin C deficiency.³³

In this study, the 23 GPCA⁺/OSF patients did have a significantly lower mean serum vitamin B12 level and a significantly higher MCV or frequency of vitamin B12 deficiency than the 126 GPCA⁻/OSF patients. Because GPCA⁺/OSF patients may have partial or total destruction of their gastric parietal cells which can secrete intrinsic factors to help absorb vitamin B12 from the ileum,^{27,28} our findings indicate that the serum GPCA-positivity can explain why 23 GPCA⁺/OSF patients have a significantly lower mean serum vitamin B12 level and a significantly higher MCV or frequency of vitamin B12 deficiency than 126 GPCA⁻/OSF patients.

Regarding the serum iron level, Ramanathan³⁴ reported that 5 (63%) of the 8 OSF patients have a depressed serum iron level below the normal value. Rajendran et al.³⁵ found a significantly lower serum iron level in 50 OSF patients than in 50 age- and sex-matched control subjects. Anuradha and Devi³⁶ attributed the deficiencies of vitamin C and iron in 36 OSF patients to the increased utilization of these two substances in collagen synthesis. Tadakamadla et al.³⁷ also discovered a significantly lower mean serum iron level in 50 OSF patients than in 50 age- and sex-matched control subjects. In this study, there were no significant differences in the mean serum iron level and frequency of iron deficiency between 149 OSF or 126 GPCA⁻/OSF patients and 149 healthy control subjects. However, the 23 GPCA⁺/OSF patients did have a significantly lower mean serum iron level than the 149 healthy control subjects. Because GPCA⁺/OSF patients may have

partial or total destruction of their gastric parietal cells which can secrete HCl to help absorb iron from the small intestine,^{27,28} the lack of HCl secretion can explain why GPCA⁺/OSF patients have a significantly lower mean serum iron level than healthy control subjects.

Ramanathan³⁴ reported that 10 (77%) of the 13 OSF patients have iron deficiency anemia. The present study showed a significantly greater frequency of anemia in 149 OSF patients (9.4%) than in 149 healthy control subjects (0%). Wahi et al.³³ discovered that 6% of the 70 male OSF patients and 11% of the 34 female OSF patients had anemia, but the frequency of anemia did not differ significantly from that of the control subjects. A South African study also found no significant difference in the prevalence of iron deficiency anemia between OSF patients and the general population.³⁸

In a South African study, Seedat reported that serum vitamin B12 and folate levels of OSF patients are within normal limits.³⁸ However, Ramanathan³⁴ found folic acid deficiency in 6 of the 6 OSF patients. This study also discovered that 76 (51.0%) and 61 (40.9%) of OSF patients had vitamin B12 and folic acid deficiencies, respectively. Only 17 (22.4%) of the 76 vitamin B12-deficient OSF patients were GPCA-positive. In these 17 GPCA⁺/OSF patients, the vitamin B12 deficiency could be attributed to the presence of GPCA in sera, because GPCA can induce destruction of gastric parietal cells and in turn result in failure of intrinsic factor production.^{27,28} Lack of intrinsic factor finally leads to failure of vitamin B12 absorption.^{29,30} Furthermore, the vitamin B12 deficiency in the other 59 GPCA⁻/OSF patients may be due to other causes including inadequate intake or malabsorption of vitamin B12, the presence of anti-intrinsic factor antibodies, or transcobalamin II deficiency, etc.³⁰ Folic acid deficiency may be resulted from poor nutritional intake, malabsorption, hepatobiliary dysfunction, increased folate catabolism, and medication (e.g., methotrexate, 5-fluoro-uracil, phenytoin, etc.).^{28,30} In the present study, we suggest that the folic acid deficiency is probably due to poor or insufficient intake of folic acid-containing diet because OSF patients usually have functional impairment in food intake and chewing due to the limited mouth opening. Further studies are needed to explore the real causes resulting in vitamin B12 and folic acid deficiencies in OSF patients.

Our previous study demonstrated that 14.7% of 109 and 13.2% of 68 male OSF patients are GPCA-positive.^{22,23} Similar result was found in this study that showed serum GPCA-positivity in 23 (15.4%) of 149 male OSF patients. In this study, 2 (8.7%) of 23 GPCA-positive, 2 (2.6%) of 76 vitamin B12-deficient, and 2 (16.7%) of 12 macrocytosis male OSF patients had pernicious anemia by the strict WHO definitions.^{6–8} Our previous studies demonstrated that 12.9% of 124 GPCA-positive, 18.9% of 90 vitamin B12-deficient, and 16.7% of 60 macrocytosis male and female patients with oral mucosal diseases had pernicious anemia by the strict WHO definitions.^{6–8} The discrepancies in parts of the data may be due to the gender (male vs. both sexes), age (middle-aged OSF patients vs. elderly patients with other oral mucosal diseases), and disease (OSF vs. atrophic glossitis, burning mouth syndrome, oral lichen planus, or recurrent aphthous stomatitis) differences.^{6–8}

Our results demonstrated that 14 (9.4%), 32 (21.5%), 76 (51.0%), 61 (40.9%), 12 (8.1%), and 23 (15.4%) of the 149 OSF patients had blood Hb, iron, vitamin B12, and folic acid deficiencies, macrocytosis, and serum GPCA positivity, respectively. All three groups of OSF patients (including 149 OSF, 23 GPCA⁺/OSF, and 126 GPCA⁻/OSF patients) had a significantly higher frequency of macrocytosis or blood Hb, vitamin B12, or folic acid deficiency than healthy control subjects. Furthermore, the 23 GPCA⁺/OSF patients had a significantly higher frequency of vitamin B12 deficiency than the 126 GPCA⁻/OSF patients. We conclude that OSF itself does play a significant role in causing hematinic deficiencies and anemia in OSF, GPCA⁺/OSF, and GPCA⁻/OSF patients. The serum GPCA is the major factor that causes vitamin B12 deficiency, macrocytosis, and pernicious anemia in GPCA⁺/OSF patients.

Conflicts of interest

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

References

- Rajalalitha P, Vali S. Molecular pathogenesis of oral submucous fibrosis – a collagen metabolic disorder. *J Oral Pathol Med* 2005;34:321–8.
- Arakeri G, Brennan PA. Oral submucous fibrosis: an overview of the aetiology, pathogenesis, classification, and principles of management. *Br J Oral Maxillofac Surg* 2013;51:587–93.
- 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.
- 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.
- 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.
- 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.
- 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.
- 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.
- 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.
- Chang JYF, Wang YP, Wu YC, Wu YH, Tseng CH, Sun A. Hematinic deficiencies and anemia statuses in antigastric parietal cell antibody-positive erosive oral lichen planus patients with desquamative gingivitis. *J Formos Med Assoc* 2016;115:860–6.
- Chang JYF, Chiang CP, Wang YP, Wu YC, Chen HM, Sun A. Antigastric parietal cell and antithyroid autoantibodies in patients with desquamative gingivitis. *J Oral Pathol Med* 2017;46:307–12.
- Sun A, Chang JYF, Wang YP, Cheng SJ, Chen HM, Chiang CP. Effective vitamin B12 treatment can reduce serum anti-gastric parietal cell antibody titer in patients with oral mucosal disease. *J Formos Med Assoc* 2016;115:837–44.
- Chang JYF, Chen IC, Wang YP, Wu YH, Chen HM, Sun A. Anemia and hematinic deficiencies in gastric parietal cell antibody-positive and antibody-negative erosive oral lichen planus patients with thyroid antibody positivity. *J Formos Med Assoc* 2016;115:1004–11.
- Sun A, Chen HM, Cheng SJ, et al. Significant association of deficiency of hemoglobin, iron, vitamin B12, and folic acid and high homocysteine level with recurrent aphthous stomatitis. *J Oral Pathol Med* 2015;44:300–5.
- Wu YC, Wu YH, Wang YP, Chang JYF, Chen HM, Sun A. Antigastric parietal cell and antithyroid autoantibodies in patients with recurrent aphthous stomatitis. *J Formos Med Assoc* 2017;116:4–9.
- Liao PH, Yang HW, Huang YF. Genetic expression signatures of oral submucous fibrosis and oral cancer – a preliminary microarray report. *J Dent Sci* 2016;11:457–62.
- Wu YH, Chang JYF, Wang YP, Wu YC, Chen HM, Sun A. Anemia and hematinic deficiencies in anti-gastric parietal cell antibody-positive and -negative recurrent aphthous stomatitis patients with anti-thyroid antibody positivity. *J Formos Med Assoc* 2017;116:145–52.
- Lin HP, Wu YH, Wang YP, Wu YC, Chang JYF, Sun A. Anemia and hematinic deficiencies in anti-gastric parietal cell antibody-positive or all autoantibodies-negative recurrent aphthous stomatitis patients. *J Formos Med Assoc* 2017;116:99–106.
- Wu YH, Wang YP, Sun A, Chang JYF. Oral plexiform neurofibroma. *J Dent Sci* 2016;11:468–9.
- Kuo YS, Wu YH, Sun A, Chiang CP. Burkitt's lymphoma of the mandible. *J Dent Sci* 2017;12:421–3.
- Kuo YS, Wu YH, Sun A, Chiang CP. Eosinophilic granuloma of the mandible mimicking a periapical lesion. *J Dent Sci* 2017;12:424–5.
- Chiang CP, Hsieh RP, Chen THH, et al. High incidence of autoantibodies in Taiwanese patients with oral submucous. *J Oral Pathol Med* 2002;31:402–9.
- Wang YP, Wu YC, Cheng SJ, Chen HM, Sun A, Chang JYF. High frequencies of vitamin B₁₂ and folic acid deficiencies and gastric parietal cell antibody positivity in oral submucous fibrosis patients. *J Formos Med Assoc* 2015;114:813–9.
- WHO/UNICEF/UNU. *Iron Deficiency Anaemia Assessment, Prevention, and Control: A Guide for Programme Managers*. Geneva, Switzerland: World Health Organization, 2001.
- 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.
- 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.
27. Taylor KB, Roitt IM, Doniach D, Coughman KG, Shapland C. Autoimmune phenomena in pernicious anemia: gastric antibodies. *BMJ* 1962;2:1347–52.
 28. Snow CF. Laboratory diagnosis of vitamin B₁₂ and folate deficiency. A guide for the primary care physician. *Arch Intern Med* 1999;159:1289–98.
 29. Lahner E, Annibale B. Pernicious anemia: new insights from a gastroenterological point of view. *World J Gastroenterol* 2009;15:5121–8.
 30. Oh RC, Brown DL. Vitamin B₁₂ deficiency. *Am Fam Phys* 2003;67:979–86.
 31. 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.
 32. 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.
 33. Wahi PN, Kapur VL, Luthra UK, Srivastava MC. Submucous fibrosis of the oral cavity: 2. Studies on epidemiology. *Bull WHO* 1966;35:793–9.
 34. Ramanathan K. Oral submucous fibrosis – an alternative hypothesis as to its causes. *Med J Malaysia* 1981;36:243–5.
 35. Rajendran R, Vasudevan DM, Vijayakumar T. Serum levels of iron and proteins in oral submucous fibrosis (OSMF). *Annals Dent* 1990;49:23–5. 45.
 36. Anuradha CD, Devi CS. Serum protein, ascorbic acid & iron & tissue collagen in oral submucous fibrosis—a preliminary study. *Indian J Med Res* 1993;98:147–51.
 37. Tadakamadla J, Kumar S, GP M. Evaluation of serum copper and iron levels among oral submucous fibrosis patients. *Med Oral Patol Oral Cir Bucal* 2011;16:e870–3.
 38. Seedat HA. *Oral Submucous Fibrosis in Durban, Natal: A Study of its Epidemiology, Aetiology and Morphological Features*. Thesis. Stellenbosch, South Africa: University of Stellenbosch, 1985.