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Original Article

Higher frequencies of anemia, hematinic deficiencies, and gastric parietal cell antibody positivity in vitamin B12-deficient Taiwanese male oral submucous fibrosis patients

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KEYWORDS
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Abstract *Background/purpose:* Oral submucous fibrosis (OSF) is a progressive fibrotic oral mucosal disease associated with betel quid chewing. This study evaluated whether Taiwanese male OSF patients with vitamin B12 deficiency (the serum vitamin B12 level $\leq$ 450 pg/mL, B12-deficient OSF patients) had high frequencies of blood hemoglobin (Hb) and serum iron and folic acid deficiencies, and serum gastric parietal cell antibody (GPCA) positivity.
Materials and methods: The blood Hb and serum iron, vitamin B12, folic acid, and GPCA concentrations in 66 Taiwanese male B12-deficient OSF patients were measured and compared with the corresponding data in 132 age-matched healthy male control subjects.

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Vitamin B12 deficiency

Results: We found that 6 (9.1%), 19 (28.8%), 35 (53.0%), and 9 (13.6%) of the 66 B12-deficient OSF patients had blood Hb (<13 g/dL) and serum iron (≤70 μg/dL), and folic acid (≤6 ng/mL) deficiencies, and serum GPCA positivity, respectively. Furthermore, 66 OSF patients had significantly higher frequencies of blood Hb and serum iron and folic acid deficiencies, and serum GPCA positivity than 132 healthy control subjects (all *P*-values < 0.05). Of the 6 anemic B12-deficient OSF patients, one had macrocytic anemia, two normocytic anemia, and three thalassemia trait-induced anemia.
Conclusion: Taiwanese male B12-deficient OSF patients have high frequencies of blood Hb, serum iron and folic acid deficiencies, and serum GPCA positivity. The anemia and hematinic deficiencies in B12-deficient OSF patients are likely due to OSF symptoms and signs-caused insufficient intake, poor chewing, and malabsorption of hematinic elements from ingested food stuffs rather than the GPCA positivity.

Introduction

Oral submucous fibrosis (OSF) is a progressive fibrotic oral mucosal disease characterized by juxtaepithelial inflammatory cell infiltration followed by a gradual deposition of collagen in the subepithelial connective tissues of the oral mucosa and superficial muscle layer. The alkaloids, flavonoids, and copper in the areca nut are the main etiological factors causing OSF.[1,2] Alkaloids, mainly arecoline and arecaidine, can cause fibroblast proliferation and elevated collagen synthesis. Moreover, arecoline can augment collagen synthesis by upregulation of fibrogenic growth factors and profibrogenic cytokines, which subsequently result in fibrosis. Flavonoids (tannins and catechins) can inhibit collagenase, stabilize the collagen fibrils, and in turn render collagen fibrils resistant to degradation by collagenase. Furthermore, arecoline can reduce the matrix metalloproteinase (MMP)-2 secretion and increase the tissue inhibitor of MMP (TIMP) level, finally resulting in elevated deposition of collagen in the extracellular matrix. The high concentration of copper in the areca nut can stimulate lysyl oxidase activity that subsequently increases the cross-linking of collagen fibers.[1,2] In addition, arecoline can also cause a dose-dependent inhibition of collagen phagocytosis by OSF fibroblasts, resulting in insufficient collagen degradation. Therefore, areca nut ingredients can cause increased collagen synthesis and reduced collagen degradation in the oral tissues, finally leading to the formation of OSF.[1,2]

The TGF-β secreted by activated T-cells and macrophages in the subepithelial connective tissue of the OSF oral mucosa plays a pivotal role in controlling collagen production and degradation.[1,2] Through activation of the related genes, TGF-β can increase the procollagen production, elevate the procollagen proteinase level that augments the conversion of procollagens into collagen fibrils, and upregulate the lysyl oxidase activity that in turn increases the production of insoluble form of collagen. Furthermore, TGF-β can also raise the production of TIMPs that inhibit the activated collagenase and reduce the collagen degradation, and can elevate the synthesis of plasminogen activator inhibitor (PAI) that inhibits the conversion of plasminogen into plasmin and further block the conversion of procollagenase into active collagenases.[1,2] Thus, TGF-β can not only increase collagen production but also decrease collagen degradation, finally resulting in the development of OSF.

Our previous studies demonstrated low serum vitamin B12 and folic acid levels as well as high frequencies of vitamin B12 and folic acid deficiencies and gastric parietal cell antibody (GPCA) positivity in Taiwanese male OSF patients.[3,4] In this study, 66 Taiwanese male OSF patients with vitamin B12 deficiency (the serum vitamin B12 level ≤ 450 pg/mL, so-called B12-deficient OSF patients in the following text) were retrospectively collected from our oral mucosal disease clinic. We mainly wanted to know whether the Taiwanese male B12-deficient OSF patients also had high frequencies of blood hemoglobin (Hb) and serum iron and folic acid deficiencies, and serum gastric parietal cell antibody (GPCA) positivity, tried to explore the reasons why our Taiwanese male B12-deficient OSF patients had a lower mean serum vitamin B12 level and higher frequencies of anemia and hematinic deficiencies than healthy male control subjects, and assessed what were the major factors causing the anemia in our Taiwanese male B12-deficient OSF patients.

Materials and methods

Study and control subjects

The study group consisted of 66 male B12-deficient OSF patients (mean age 40.1 ± 13.5 years, range 21—80 years). For each B12-deficient OSF patient, two age-matched (±2 years of each OSF patient's age) healthy male control subjects were selected. Therefore, the normal control group included 132 healthy male control subjects (mean age 41.0 ± 12.2 years, range 20—80 years). All the B12-deficient OSF patients were collected from the Department of Dentistry, National Taiwan University Hospital, Taipei, Taiwan from 2007 to 2017 or the Department of Dentistry, Far Eastern Memorial Hospital, New Taipei City, Taiwan from 2019 to 2020. 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, bands of fibrous tissues in the buccal mucosa, and progressive mouth-opening limitation.[3,4] Because the clinical symptoms and signs were characteristic enough to make a precise clinical diagnosis, incisional biopsy of buccal mucosa was taken from only 10 of 66 B12-deficient OSF patients to further provide the histologic evidence of OSF, which has been described previously.[3,4] The oral mucosal sites of involvement (SOI, including soft palate, retromolar area, buccal mucosa, labial mucosa, floor of the mouth, and tongue) and the maximum mouth opening (MMO, the distance from the cutting edge of maxillary central incisor to the cutting edge of the mandibular central incisor) of B12-deficient OSF patients were recorded. The severity of OSF was determined according to the SOI and/or MMO; the more the SOI and the less the MMO, the more severe the OSF.[3,4] This study did not include mild OSF patients, because all our OSF patients had at least three SOI. Moreover, the exclusion criteria for OSF patients and the inclusion and exclusion criteria for healthy control subjects have also been described previously.[3,4]

Patients' oral habits including betel quid chewing, cigarette smoking, and alcohol drinking were recorded. In this study, all the 66 B12-deficient OSF patients were betel quid chewers, 60 (90.9%) of the 66 B12-deficient OSF patients were cigarette smokers, and 32 (48.5%) of the 66 B12-deficient OSF patients were alcohol drinkers according to the definitions described in our previous studies.[3,4]

The blood samples were drawn from all 66 B12-deficient OSF patients and 132 healthy control subjects for measurement of complete blood count as well as serum iron, vitamin B12, folic acid, and GPCA concentrations according to the methods described in our previous studies.[3–18] All B12-deficient 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 National Taiwan University Hospital (201212066RIND and 201512032RINB) and that at the Far Eastern Memorial Hospital (FEMH No.: 107116-E).

Statistical analysis

Comparisons of the mean corpuscular volume (MCV) as well as mean blood Hb and serum iron, vitamin B12, and folic acid concentrations between 66 B12-deficient OSF and 132 healthy control subjects or between two different groups of B12-deficient OSF patients were performed by Student's *t*-test. The differences in frequencies of macrocytosis, microcytosis, blood Hb and serum iron, vitamin B12, and folic acid deficiencies, and GPCA positivity between 66 B12-deficient OSF and 132 healthy control subjects or between two different groups of B12-deficient OSF patients were compared by chi-square test or Fisher exact test, where appropriate. The result was considered to be significant if the *P*-value was less than 0.05.

Results

The mean MMO of the 66 B12-deficient OSF patients was 29.9 ± 7.5 mm. Of the 66 B12-deficient OSF patients, 34 had MMO ≤ 30 mm and the other 32 had MMO > 30 mm (between 31 mm and 42 mm). The soft palate, retromolar area, and buccal mucosa were the three sites where were involved by OSF in every B12-deficient OSF patient, with extra involvement of the labial mucosa in 52 patients (78.8%), of the floor of the mouth in 35 patients (53.0%), and of the tongue in 15 patients (22.7%). Therefore, of the 66 B12-deficient OSF patients, 31 had SOI ≤ 4 sites and 35 had SOI > 4 sites.

The MCV, mean blood Hb level, and mean serum iron, vitamin B12, and folic acid levels in the 66 B12-deficient OSF patients and the 132 healthy control subjects are shown in Table 1. We found that the 66 B12-deficient OSF patients had significantly lower mean serum vitamin B12 level ($P < 0.001$) and lower mean serum folic acid level ($P < 0.001$) than the 132 healthy control subjects (Table 1).

We also discovered that the 34 B12-deficient OSF patients with MMO ≤ 30 mm had significantly lower mean serum folic acid level ($P < 0.001$) than the 32 B12-deficient OSF patients with MMO > 30 mm (Table 2). In addition, the 35 B12-deficient OSF patients with SOI > 4 sites had significantly lower MCV ($P = 0.025$) and lower mean serum folic acid level ($P < 0.001$) than the 31 B12-deficient OSF patients with SOI ≤ 4 sites (Table 2).

In this study, the MCV ≥ 100 fL and MCV < 80 fL was defined as having macrocytosis[5] and microcytosis,[19,20] respectively. The men with Hb < 13 g/dL were defined as having Hb deficiency or anemia.[19,20] Moreover, the serum iron level ≤ 70 μg/dL for men, vitamin B12 level ≤ 450 pg/mL, and folic acid level ≤ 6 ng/mL were defined as having serum iron, vitamin B12, and folic acid deficiencies as described previously.[21,22] By the above-mentioned definitions, 2 (3.0%), 7 (10.6%), 6 (9.1%), 19 (28.8%), 66 (100.0%), 35 (53.0%), and 9 (13.6%) of the 66 B12-deficient OSF patients as well as none (0%), none (0%), none (0%), 20 (15.2%), 27 (20.5%), 8 (6.1%), and 2 (1.5%) of the 132 healthy control subjects had macrocytosis, microcytosis, blood Hb and serum iron, vitamin B12, and folic acid deficiencies, and serum GPCA positivity by the

Table 1 Comparisons of the mean corpuscular volume (MCV), mean blood hemoglobin (Hb), and mean serum iron, vitamin B12, and folic acid levels between 66 vitamin B12-deficient (B12-deficient) oral submucous fibrosis (OSF) patients and 132 healthy control subjects.

Group	MCV (fL)	Hb (g/dL)	Iron (μg/dL)	Vitamin B12 (pg/mL)	Folic acid (ng/mL)
B12-deficient OSF patients (n = 66)	89.8 ± 9.3	15.3 ± 1.6	111.9 ± 55.5	322.7 ± 85.9	6.5 ± 2.8
[a]*P*-value	0.590	0.542	0.559	<0.001	<0.001
Healthy control subjects (n = 132)	90.3 ± 3.7	15.2 ± 0.7	115.3 ± 26.3	653.8 ± 201.6	13.8 ± 5.6

[a] Comparisons of means of parameters between 66 B12-deficient OSF patients and 132 healthy control subjects by Student's *t*-test.

Table 2 Comparisons of the mean corpuscular volume (MCV), mean blood hemoglobin (Hb), and mean serum iron, vitamin B12, and folic acid levels between the 34 vitamin B12-deficient (B12-deficient) oral submucous fibrosis (OSF) patients with maximum mouth opening (MMO) $\leq$ 30 mm and the 32 B12-deficient OSF patients with MMO $>$ 30 mm as well as between the 31 B12-deficient OSF patients with site of involvement (SOI) $\leq$ 4 sites and the 35 B12-deficient OSF patients with SOI $>$ 4 sites.

B12-deficient OSF patients	MCV (fL)	Hb (g/dL)	Iron (μg/dL)	Vitamin B12 (pg/mL)	Folic acid (ng/mL)
MMO $\leq$ 30 mm (n = 34)	87.8 ± 10.9	15.1 ± 1.8	112.5 ± 47.1	331.9 ± 89.9	5.1 ± 2.4
MMO $>$ 30 mm (n = 32)	91.9 ± 6.8	15.4 ± 1.3	111.4 ± 63.9	333.5 ± 82.8	8.0 ± 2.6
[a]*P*-value	0.073	0.443	0.937	0.940	$<$0.001
SOI $\leq$ 4 sites (n = 31)	92.5 ± 5.6	15.6 ± 1.0	114.6 ± 62.3	314.6 ± 87.7	7.7 ± 2.8
SOI $>$ 4 sites (n = 35)	87.4 ± 11.2	15.0 ± 1.9	109.5 ± 49.5	348.7 ± 82.2	5.4 ± 2.4
[b]*P*-value	0.025	0.120	0.713	0.108	$<$0.001

[a] Comparisons of means of parameters between the 34 B12-deficient OSF patients with MMO $\leq$ 30 mm and the 32 B12-deficient OSF patients with MMO $>$ 30 mm by Student's *t*-test.
[b] Comparisons of means of parameters between the 31 B12-deficient OSF patients with SOI $\leq$ 4 sites and the 35 B12-deficient OSF patients with SOI $>$ 4 sites by Student's *t*-test.

Table 3 Comparisons of frequencies of macrocytosis, microcytosis, blood hemoglobin (Hb), serum iron, vitamin B12, and folic acid deficiencies, and gastric parietal cell antibody (GPCA) positivity between the 66 vitamin B12-deficient (B12-deficient) oral submucous fibrosis (OSF) patients and the 132 healthy control subjects.

Group	Patient number (%)						
	Macrocytosis (MCV $\geq$ 100 fL)	Microcytosis (MCV $<$ 80 fL)	Hb deficiency ($<$13 g/dL)	Iron deficiency ($\leq$70 μg/dL)	Vitamin B12 deficiency ($\leq$450 pg/mL)	Folic acid deficiency ($\leq$6 ng/mL)	GPCA positivity
B12-deficient OSF patients (n = 66)	2 (3.0)	7 (10.6)	6 (9.1)	19 (28.8)	66 (100.0)	35 (53.0)	9 (13.6)
[a]*P*-value	0.209	$<$0.001	0.002	0.037	$<$0.001	$<$0.001	0.001
Healthy control subjects (n = 132)	0 (0.0)	0 (0.0)	0 (0.0)	20 (15.2)	27 (20.5)	8 (6.1)	2 (1.5)

[a] Comparisons of frequencies of parameters between the 66 B12-deficient OSF patients and the 132 healthy control subjects by chi-square test.

aforementioned criteria, respectively. Moreover, the 66 B12-deficient OSF patients had significantly higher frequencies of microcytosis ($P < 0.001$), blood Hb ($P = 0.002$), serum iron ($P = 0.037$), vitamin B12 ($P < 0.001$), and folic acid ($P < 0.001$) deficiencies, and serum GPCA positivity ($P = 0.001$) than the 132 healthy control subjects (Table 3).

Furthermore, we also found that 34 B12-deficient OSF patients with the MMO $\leq$ 30 mm had a significantly higher frequency of serum folic acid deficiency ($P < 0.001$) than the 32 B12-deficient OSF patients with MMO $>$ 30 mm (Table 4). In addition, the 35 B12-deficient OSF patients with SOI $>$ 4 sites had a significantly higher frequency of serum folic acid deficiency ($P = 0.015$) than the 31 B12-deficient OSF patients with SOI $\leq$ 4 sites (Table 4).

In this study, anemia (Hb $<$ 13 g/dL) was found in 6 male B12-deficient OSF patients (Table 5). Of these 6 male B12-deficient OSF patients with anemia, one had macrocytic anemia (defined as having Hb $<$ 13 g/dL for men and the MCV $\geq$ 100 fL), two had normocytic anemia (defined as having Hb $<$ 13 g/dL for men and the MCV between 80 and 99.9 fL), and 3 had thalassemia trait-induced anemia (defined as having Hb $<$ 13 g/dL for men, RBC count $>$ 5.0×10^{12}/L, MCV $<$ 74 fL, and Mentzer index (MCV/RBC) $<$ 13) (Table 5).[23]

Discussion

The major findings of this study were that 6 (9.1%), 19 (28.8%), 35 (53.0%), and 9 (13.6%) of the 66 B12-deficient OSF patients had blood Hb, serum iron, and serum folic acid deficiencies, and serum GPCA positivity, respectively. Furthermore, the 66 OSF patients had significantly higher frequencies of blood Hb, serum iron, and serum folic acid deficiencies, and serum GPCA positivity than the 132 healthy control subjects. Moreover, the 34 B12-deficient OSF patients with MMO $\leq$ 30 mm and the 35 B12-deficient OSF patients with SOI $>$ 4 sites also had significantly lower serum folic acid level (both *P*-values $<$ 0.001) and significantly higher frequencies of serum folic acid deficiency ($P < 0.001$ and $P = 0.015$, respectively) than the 32 B12-deficient OSF patients with MMO $>$ 30 mm and the 31 B12-deficient OSF patients with SOI $\leq$ 4 sites, respectively. These results suggest that the serum folic acid deficiency in B12-deficient OSF patients are significantly associated with the severity of OSF; i.e., the B12-deficient OSF patients with severe mouth-opening limitation and more oral mucosal sites involved by OSF both have significantly lower serum folic acid level and significantly higher frequencies of serum folic acid deficiency.

Table 4 Comparisons of frequencies of macrocytosis, microcytosis, blood hemoglobin (Hb), serum iron, vitamin B12, and folic acid deficiencies, and gastric parietal cell antibody (GPCA) positivity between the 34 vitamin B12-deficient (B12-deficient) oral submucous fibrosis (OSF) patients with maximum mouth opening (MMO) $\leq$ 30 mm and the 32 B12-deficient OSF patients with MMO $>$ 30 mm as well as between the 31 B12-deficient OSF patients with site of involvement (SOI) $\leq$ 4 sites and the 35 B12-deficient OSF patients with SOI $>$ 4 sites.

B12-deficient OSF patients	Patient number (%)						
	Macrocytosis (MCV $\geq$ 100 fL)	Microcytosis (MCV $<$ 80 fL)	Hb deficiency ($<$13 g/dL)	Iron deficiency ($\leq$70 μg/dL)	Vitamin B12 deficiency ($\leq$450 pg/mL)	Folic acid deficiency ($\leq$6 ng/mL)	GPCA positivity
MMO $\leq$ 30 mm (n = 34)	1 (2.9)	5 (14.7)	3 (8.8)	6 (17.6)	34 (100.0)	27 (79.4)	6 (17.6)
MMO $>$ 30 mm (n = 32)	1 (3.1)	2 (6.3)	3 (9.4)	13 (40.6)	32 (100.0)	8 (25.0)	3 (9.4)
[a]*P*-value	$>$0.999	0.428	$>$0.999	0.074	ND	$<$0.001	0.477
SOI $\leq$ 4 sites (n = 31)	1 (3.2)	1 (3.2)	1 (3.2)	10 (32.3)	31 (100.0)	11 (35.5)	6 (19.4)
SOI $>$ 4 sites (n = 35)	1 (2.9)	6 (17.1)	5 (14.3)	9 (25.7)	35 (100.0)	24 (68.6)	3 (8.6)
[b]*P*-value	$>$0.999	0.110	0.202	0.754	ND	0.015	0.287

ND = not done

[a] Comparisons of frequencies of parameters between the 34 B12-deficient OSF patients with MMO $\leq$ 30 mm and the 32 B12-deficient OSF patients with MMO $>$ 30 mm by chi-square test or Fisher exact test, where appropriate.

[b] Comparisons of frequencies of parameters between the 31 B12-deficient OSF patients with SOI $\leq$ 4 sites and the 35 B12-deficient OSF patients with SOI $>$ 4 sites by chi-square test or Fisher exact test, where appropriate.

Table 5 Anemia types, hemoglobin (Hb), mean corpuscular volume (MCV), red blood cell (RBC) number, serum iron, vitamin B12, folic acid levels, and gastric parietal cell antibody (GPCA) positivity in the 6 male vitamin B12-deficient oral submucous fibrosis (OSF) patients with anemia (Hb $<$ 13 g/dL).

Anemia type	Patient number (%)							
	Hb (g/dL)	MCV (fL)	RBC ($\times 10^{12}$/L)	Mentzer index (MCV/RBC)	Iron (μg/dL)	Vitamin B12 (pg/mL)	Folic acid (ng/mL)	GPCA positivity
Macrocytic anemia	10.0	114.3	2.59	44.1	37	228	4.8	–
Normocytic anemia	12.9	89.4	4.16	21.5	70	448	7	–
Normocytic anemia	12.0	95	4.02	23.6	67	343	12	–
Thalassemia trait-induced anemia	9.1	53.6	5.99	8.9	13	450	4.22	–
Thalassemia trait-induced anemia	12.4	70.2	5.57	12.6	130	449	6.0	–
Thalassemia trait-induced anemia	12.7	70	6.04	11.6	56	254	9	–

First, we discussed why the B12-deficient OSF patients were prone to have the significantly higher frequencies of serum iron and folic acid deficiencies than the 132 healthy control subjects. The GPCA can destroy gastric parietal cells, resulting in lack of intrinsic factors, reduced absorption of vitamin B12 from the terminal ileum, and finally the vitamin B12 deficiency.[24,25] In this study, only 9 of the 66 B12-deficient OSF patients had serum GPCA positivity. Thus, the vitamin B12 deficiency in our 66 B12-deficient OSF patients were not predominantly due to the presence of GPCA in the sera of these 66 B12-deficient OSF patients. Other etiological factors might be involved to cause the vitamin B12 deficiency; these include inadequate intake or malabsorption of vitamin B12, the presence of anti-intrinsic factor antibodies, or transcobalamin II deficiency.[24,25] The serum anti-intrinsic factor antibodies and transcobalamin II levels were not examined in this study. Therefore, it needs further studies to assess whether our B12-deficient OSF patients have serum anti-intrinsic factor antibody positivity and serum transcobalamin II deficiency. We suggest that the vitamin B12 deficiency in our B12-deficient OSF patients is more likely due to OSF symptoms and signs-caused insufficient intake, poor chewing, and malabsorption of vitamin B12-containing food stuffs.

The folic acid deficiency may result from poor nutritional intake, malabsorption, hepatobiliary dysfunction, increased folate catabolism, and medication (e.g., methotrexate, 5-fluoro-uracil, phenytoin).[26] The medication was not the factor causing the folic acid deficiency in our OSF patients, because all our OSF patients did not take any drugs described above. As mentioned before, our results suggest that the serum folic acid deficiency is significantly related to the severity of OSF. In this study, all our B12-deficient OSF patients had moderate or severe OSF with the mean MMO being nearly 30 mm and the mean SOI being 4.5 oral mucosal sites. Moreover, 52 (78.8%) of the 66 B12-deficient OSF patients had 4 to 6 oral mucosal sites involved by the OSF. In addition, our OSF patients frequently have stiffness of the oral mucosa, bands of fibrous tissues in the buccal mucosa, mouth-opening limitation, hypersensitivity to spicy foods, burning sensation of the oral mucosa, dry mouth, impaired tongue mobility, and severe attrition of the teeth.[3,4] These OSF-specific symptoms and signs may interfere with eating and chewing functions of OSF patients

and lead to insufficient food intake and difficulty in digestion and absorption of nutritional elements from the ingested food stuffs, finally resulting in hematinic deficiencies, including iron, vitamin B12, and folic acid deficiencies, in our OSF patients.[3,4]

Moreover, in this study, all the 66 B12-deficient OSF patients were betel quid chewers and 60 (90.9%) of the 66 B12-deficient OSF patients were cigarette smokers. Thus, the frequent betel quid chewing and smoking may cause DNA damage in oral epithelial cells due to the presence of carcinogenic substances in the betel quid and tobacco. In addition, the coarse fibers of betel nuts may also cause multiple microtraumas of the OSF oral mucosa during the betel quid chewing process. The repair of DNA damage in oral epithelial cells and microtraumas in OSF oral mucosa can result in a frequent cell proliferation and DNA replication, which in turn consume a great amount of vitamin B12 and folic acid and lead to a significantly lower serum vitamin B12 and folic acid levels in our OSF patients than in healthy control subjects.[27–30] Actually, a higher epithelial cell proliferation or turnover has been demonstrated in oral epithelial cells of OSF patients in our previous study that reported a higher labelling index of proliferating cell nuclear antigen (PCNA) in the OSF epithelial cells than in the normal oral mucosal epithelial cells.[31] Thus, the hematinic deficiencies including the iron, vitamin B12, and folic acid deficiencies are more likely attributed to OSF symptoms and signs-caused insufficient intake, poor chewing, and malabsorption of hematinic substances contained in the OSF patients' ingested food stuffs. Patil and Joshi[32] also reported a significantly lower mean serum vitamin B12 level in 40 OSF patients than in 25 healthy controls. Moreover, Abidullah et al.[33] also demonstrated a significantly lower mean serum vitamin B12 level in 50 OSF patients than in 50 healthy controls. In addition, Ramanathan[34] found folic acid deficiency in 6 of the 6 OSF patients.

Second, we wanted to know what might be the major factors causing anemia in our Taiwanese male B12-deficient OSF patients. In this study, 6 B12-deficient OSF patients were diagnosed as having anemia, including one with macrocytic anemia, two with normocytic anemia, and 3 with thalassemia trait-induced anemia. The only one B12-deficient OSF patient with macrocytic anemia also had iron and folic acid deficiencies. Both B12-deficient OSF patients with normocytic anemia also had iron deficiency. Of the three B12-deficient OSF patients with thalassemia trait-induced anemia, one had concomitant serum iron and folic acid deficiencies, another had simultaneous serum folic acid deficiency, and the other had concomitant iron deficiency. These 6 anemic B12-deficient OSF patients were all serum GPCA-negative (Table 5). Thus, the vitamin B12 deficiency in these 6 anemic B12-deficient OSF patients was not due to GPCA-positivity-induced malabsorption of vitamin B12.[24,25] We suggest that the iron, vitamin B12, and folic acid deficiencies are the major etiologic factors causing anemia in these 6 anemic B12-deficient OSF patients and the hematinic deficiencies in these 6 anemic B12-deficient OSF patients are more likely due to OSF symptoms and signs-caused insufficient intake, poor chewing, and malabsorption of hematinic substances from ingested food stuffs. However, because only one of the 6 B12-deficient OSF patients had macrocytic anemia, the vitamin B12 deficiency was not the major factor causing anemia in these 6 anemic B12-deficient OSF patients, although the vitamin B12 deficiency may also reduce bone marrow hematopoiesis and DNA synthesis of predecessors of the erythrocytes.[24,25] In addition, the α or β globin chain gene mutation-induced blood Hb deficiency is also an important contributing factor causing anemia in the three B12-deficient OSF patients with thalassemia trait-induced anemia.

We conclude that Taiwanese male B12-deficient OSF patients have significantly higher frequencies of blood Hb, serum iron, and serum folic acid deficiencies, and serum GPCA positivity than healthy male control subjects. The anemia and hematinic deficiencies in the B12-deficient OSF patients are likely due to OSF symptoms and signs-caused insufficient intake, poor chewing, and malabsorption of hematinic substances from ingested food stuffs rather than GPCA positivity in these B12-deficient OSF patients.

Declaration of Competing Interest

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

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