Blood profile of oral mucosal disease patients with both vitamin B₁₂ and iron deficiencies

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Received 7 February 2015; received in revised form 10 March 2015; accepted 17 March 2015

KEYWORDS
- gastric parietal cell antibody
- iron deficiency
- macrocytosis
- mean corpuscular volume
- microcytosis
- vitamin B₁₂ deficiency

Background/Purpose: Vitamin B₁₂ and iron deficiencies lead to macrocytosis [mean corpuscular volume (MCV) ≥ 100 fl] and microcytosis (MCV < 80 fl), respectively. This study evaluated anemic status, MCV, serum homocysteine level, and serum gastric parietal cell antibody (GPCA) level in oral mucosal disease patients with both vitamin B₁₂ and iron deficiencies.

Methods: The blood hemoglobin (Hb), iron, vitamin B₁₂, folic acid and homocysteine concentrations, MCV, and serum GPCA in 149 patients with both vitamin B₁₂ and iron deficiencies were measured and compared with the corresponding data in 149 age- and sex-matched healthy control subjects.

Results: We found that 54 (36.2%), 16 (10.7%), 44 (29.5%), and 36 (24.2%) patients with both vitamin B₁₂ and iron deficiencies had Hb deficiency (men < 13 g/dL, women < 12 g/dL), folic acid deficiency (< 6 mg/mL), abnormally high blood homocysteine level (> 12.6 μM), and serum GPCA positivity, respectively. Patients with both vitamin B₁₂ and iron deficiencies had a significantly higher frequency of Hb deficiency, abnormally elevated blood homocysteine level, and serum GPCA positivity than healthy control subjects (all p values < 0.001). Of 149 patients with both vitamin B₁₂ and iron deficiencies, 10 (6.7%) had high MCV (≥ 100 fl), 108 (72.5%) had normal MCV (between 80 fl and 99 fl), and 31 (20.8%) had low MCV (< 80 fl).

Conclusion: Approximately 73%, 30%, and 24% of patients with both vitamin B₁₂ and iron deficiencies are found to have normal MCV, abnormally high blood homocysteine level, and serum GPCA positivity, respectively.

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

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Introduction

Vitamin B₁₂ plays an important role in hemoglobin (Hb), DNA synthesis, and cell division. Patients with vitamin B₁₂ deficiency may have Hb deficiency and macrocytosis (mean corpuscular volume [MCV] ≥ 100 fL) that eventually leads to pernicious anemia. Vitamin B₁₂ deficiency may be due to an inadequate intake of vitamin B₁₂-containing foods, food-bound vitamin B₁₂ malabsorption, lack of intrinsic factor or parietal cells, ileal malabsorption in patients with enteritis or ileal resection, biologic competition including bacterial overgrowth and tapeworm infestation, and defective transport such as transcobalamin II deficiency. The intrinsic factor, which is produced by the parietal cells of the stomach lining, can tightly bind to dietary vitamin B₁₂. The vitamin B₁₂–intrinsic factor complex is carried to the terminal ileum, where it is absorbed after binding to intrinsic factor receptors on the luminal membranes of ileal cells. Vitamin B₁₂ deficiency may lead to pernicious anemia. In pernicious anemia patients, there are two mechanisms that cause the intrinsic factor deficiency or inactivation. Firstly, about 85% of pernicious anemia patients possess gastric parietal cell antibodies (GPCA), which induce destruction of parietal cells and in turn result in the failure of intrinsic factor production. Secondly, 40–80% of pernicious anemia patients have intrinsic factor antibodies that bind to the vitamin B₁₂–binding site of the intrinsic factor and subsequently inactivate the vitamin B₁₂ absorption-aiding function of the intrinsic factor. Iron is an essential element for Hb synthesis. Lack of iron in the body may cause iron deficiency anemia which is a kind of microcytic anemia (MCV < 80 fL). The causes of iron deficiency include chronic blood loss associated with excessive menstrual flow or gastrointestinal diseases such as peptic ulcer, diverticulosis, or malignancies, consumption of iron-poor foods, or a reduced absorption of iron in patients with total gastrectomy or celiac sprue. In our oral mucosal disease clinic, there are many patients with both vitamin B₁₂ and iron deficiencies were sometimes discovered in our oral mucosal disease clinic. Because vitamin B₁₂ deficiency may result in macrocytic anemia and iron deficiency may cause microcytic anemia, it was interesting to investigate whether patients with both vitamin B₁₂ and iron deficiencies may have macrocytic, normocytic or microcytic anemia. In this study, data from 149 patients with both vitamin B₁₂ and iron deficiencies were retrospectively collected from our oral mucosal disease clinic. We tried to find what percentages of this specific group of patients might have macrocytic or microcytic anemia, high, normal, or low MCV, high serum homocysteine level, serum GPCA positivity, and specific oral manifestations.

Materials and methods

Patients

In this study, oral mucosal disease patients with vitamin B₁₂ deficiencies were defined as those having a serum vitamin B₁₂ level ≤ 450 pg/mL and patients with iron deficiency were defined as those having a serum iron level ≤ 70 μg/dL for men, and ≤ 65 μg/dL for women. These vitamin B₁₂ and iron concentrations were chosen because they were the cut-off point concentrations for giving vitamin B₁₂ or iron supplement treatment to patients with either vitamin B₁₂ or iron deficiency. Based on the above selected concentrations for vitamin B₁₂ and iron deficiencies, 149 patients (44 men and 105 women, age range 18–90 years, mean 54.3 ± 16.6 years) with both vitamin B₁₂ (≤ 450 pg/mL) and iron deficiencies (serum iron level ≤ 70 μg/dL for men, and ≤ 65 μg/dL for women) were collected. For each patient, one age- (±2 years of each patient’s age) and sex-matched healthy control subject was selected. Thus, the normal control group consisted of 149 healthy control patients (44 men and 105 women, age range 19–89 years, mean 55.7 ± 13.9 years). All patients and healthy control subjects were seen consecutively, diagnosed, treated, and selected in the oral mucosal disease clinic or dental clinic of National Taiwan University Hospital (NTUH) from July 2007 to June 2014. The 149 patients with both vitamin B₁₂ and iron deficiencies had one or two oral mucosal diseases including BMS, AG, RAU, and OLP. BMS was diagnosed when patients had a burning sensation of the oral mucosa in the absence of clinically apparent mucosal alterations. Patients were diagnosed as having partial or complete AG when their dorsal tongues showed partial or complete absence or flattening of filiform papillae. RAU was diagnosed when patients had at least one episode of oral ulcers per month during the preceding years. OLP was diagnosed according to the following criteria: (1) a typical clinical presentation of radiating grayish-white Wickham striae or papules (nonerosive OLP) combined with erosion or ulceration on the bilateral buccal or vestibular mucosa (erosive OLP), and (2) biopsy specimens characteristic of OLP, that is, hyperkeratosis or parakeratosis, a slightly acanthotic epithelium with liquefaction degeneration of the basal epithelial cells, a pronounced band-like lymphocytic infiltrate in the lamina propria, and the absence of epithelial dysplasia. However, all patients with areca quid chewing habit, autoimmune diseases (such as systemic lupus erythematosus, rheumatoid arthritis, Sjögren’s syndrome, pemphigus vulgaris, and cicatricial pemphigoid), inflammatory diseases, malignancy, or recent surgery were excluded. In addition, all patients with serum creatinine concentrations indicative of renal dysfunction (i.e., men > 131 μM; women > 115 μM), and who reported a history of stroke, heavy alcohol use, or diseases of the liver, kidney, or coronary arteries were also excluded. Healthy control subjects had either dental caries or mild periodontal diseases but did not have any oral mucosal or systemic diseases. None of our patients with both vitamin B₁₂ and iron deficiencies had taken any prescription medication for BMS, AG, RAU, or OLP at least 3 months before entering the study.
According to the aforementioned diagnostic criteria, the 149 patients with both vitamin B₁₂ and iron deficiencies included 59 with BMS only, 46 with AG only, 14 with OLP only, 13 with both OLP and AG, nine with both RAU and BMS, and eight with both RAU and AG. For all patients with both vitamin B₁₂ and iron deficiencies and healthy control subjects, oral manifestations including a burning sensation and numbness of the oral mucosa, dry mouth, taste dysfunction, AG, RAU, and OLP were inquired, examined, and recorded. Blood samples were drawn from all patients and healthy control subjects for measurement of complete blood count, blood iron, vitamin B₁₂, folic acid, and homocysteine concentrations. In addition, the presence of GPCA in sera of patients and healthy control subjects was also checked. All 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 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 by routine tests performed in the Department of Laboratory Medicine at NTUH.

Determination of serum gastric parietal cell antibody level

Serum GPCA levels were detected using an indirect immunofluorescence technique with rat stomach as a substrate as described previously. In brief, 5 μm cryostat sections of substrate tissues on slides were reacted with serially diluted patients’ and control subjects’ sera in a moist chamber at room temperature for 30 minutes. The initial dilution of the patients’ and control subjects’ sera was 1:20 with phosphate-buffered saline (PBS). After washing, the sections were incubated with fluorescein isothiocyanate (FITC)-labeled goat anti-human IgG antiserum (Boehringer Mannheim Biochemicals, Indianapolis, IN, USA) which had been prediluted and kept in a dropper vial by the manufacturer and was ready to use, for another 30 minutes. The sections were washed again, mounted with buffered glycerine, and examined with an Olympus fluorescence microscope (Olympus, Tokyo, Japan). Sera were scored as positive when they produced fluorescence at a dilution of 20-fold or more.

Statistical analysis

Comparisons of the MCV and mean blood levels of Hb, iron, vitamin B₁₂, folic acid, and homocysteine between 149 patients with both vitamin B₁₂ and iron deficiencies and 149 age- and sex-matched healthy control subjects or between any two subgroups were performed using the Student t test. The differences in the frequency of Hb, vitamin B₁₂, or folic acid deficiency, of abnormally high blood homocysteine level, or of serum GPCA positivity between 149 patients with both vitamin B₁₂ and iron deficiencies and 149 age- and sex-matched healthy control subjects or between any two subgroups were compared using the Chi-square test. The result was considered to be significant if the p value was less than 0.05.

Results

The MCV and mean blood concentrations of Hb, iron, vitamin B₁₂, folic acid, and homocysteine in 149 patients with both vitamin B₁₂ and iron deficiencies and in 149 age- and sex-matched healthy control subjects are shown in Table 1. Because men usually had higher blood levels of Hb and iron than women, these two mean levels were calculated separately for men and women. We found that patients with both vitamin B₁₂ and iron deficiencies had a significantly lower mean Hb level (for both men and women, both p values < 0.001), a significantly lower mean MCV (p < 0.001), a significantly lower mean folic acid level (p < 0.05), and a significantly higher mean blood homocysteine level (p < 0.001) than healthy control subjects (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. For serum iron, vitamin B₁₂, and folic acid levels, this study chose serum iron levels ≤ 70 μg/dL for men and ≤ 65 μg/dL for women as the iron deficiency level, the serum vitamin B₁₂ level ≤ 450 pg/mL (148 pm) as the vitamin B₁₂ deficiency level, and the folic acid level ≤ 6 ng/mL (10 nM) as the folic acid deficiency level. In addition, patients with a blood homocysteine level >12.6 μM (which was the mean blood homocysteine level of healthy control subjects plus two standard deviations) were defined as having abnormally high homocysteine level. By the aforementioned definitions, 54 (36.2%) and 16 (10.7%) of the 149 patients with both vitamin B₁₂ and iron deficiencies had Hb and folic acid deficiencies, respectively. Moreover, 44 (29.5%) and 36 (24.2%) patients with both vitamin B₁₂ and iron deficiencies had abnormally high blood homocysteine level and serum GPCA positivity, respectively (Table 2). However, only 22 (14.8%), 33 (22.1%), 9 (6.0%), 4 (2.7%), and 3 (2.0%) normal control subjects had iron, vitamin B₁₂, and folic acid deficiencies, abnormally high blood homocysteine level, and serum GPCA positivity by the aforementioned criteria, respectively. Furthermore, patients with both vitamin B₁₂ and iron deficiencies had a significantly higher frequency of Hb deficiency, of abnormally elevated blood homocysteine level, and of serum GPCA positivity than healthy control subjects (all p values < 0.001) (Table 2).

When 149 patients with both vitamin B₁₂ and iron deficiencies were further divided into Group 1 (10 patients with MCV ≥ 100 fl), Group 2 (108 patients with MCV between 80 fl and 99 fl), and Group 3 (31 patients with MCV < 80 fl), we found that Group 1 patients had significantly higher MCV (p < 0.001), lower mean serum vitamin B₁₂ level (p < 0.001), higher mean serum homocysteine level (p < 0.001), and higher frequency of serum GPCA positivity (p = 0.025) than Group 2 patients (Table 3). Moreover, Group 3 patients had significantly lower mean blood Hb level (p < 0.001 for both men and women), lower MCV (p = 0.001), and lower mean serum...
Comparisons of MCV and blood concentrations of Hb, iron, vitamin B12, folic acid, and homocysteine in 149 patients with both high homocysteine levels were defined as patients with homocysteine levels greater than the mean serum homocysteine level of any of the aforementioned oral manifestations or diseases. Of the 149 patients with both vitamin B12 and iron deficiencies, 135 (90.6%) had a burning sensation of the oral mucosa, 110 (73.8%) had a dry mouth, 67 (45.0%) had AG, 52 (34.9%) had numbness of the oral mucosa, 27 (18.1%) had OLP, 25 (16.8%) had taste dysfunction, and 17 (11.4%) had RAU. However, none of healthy control subjects had RAU. However, none of healthy control subjects had any of the aforementioned oral manifestations or diseases.

Discussion

This study found that 54 (36.2%) of the 149 oral mucosal disease patients with both vitamin B12 (serum vitamin B12 level ≤ 450 pg/mL) and iron (serum iron level ≤ 70 µg/dL for men, and ≤ 65 µg/dL for women) deficiencies had anemia (men with Hb < 13 g/dL, and women with Hb < 12 g/dL). Moreover, only 36 (24.2%) of the 149 patients with both vitamin B12 and iron deficiencies were serum GPCA-positive. Our previous study discovered that 35 (38.9%) and 43 (47.8%) out of 90 patients with vitamin B12 deficiency (serum vitamin B12 level < 200 pg/mL) had anemia and serum GPCA-positivity, respectively (unpublished data). The significantly higher frequency of patients with serum GPCA-positivity in our previous study was due to the use of stricter criteria for patient selection in our previous study (serum vitamin B12 level < 200 pg/mL) than in this study (serum vitamin B12 level ≤ 450 pg/mL). The reason we used a serum vitamin B12 level ≤ 450 pg/mL as a

### Table 1

<table>
<thead>
<tr>
<th>Factor</th>
<th>Mean ± SD</th>
<th>Range</th>
<th>Healthy control subjects (n = 149)</th>
<th>p Student t test</th>
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<tbody>
<tr>
<td>Hb (g/dL)</td>
<td>13.6 ± 1.7 (n = 44)</td>
<td>7.4–16.4</td>
<td>15.1 ± 0.6 (n = 44)</td>
<td>&lt;0.001&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Men</td>
<td></td>
<td></td>
<td>13.8–16.3</td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>12.0 ± 1.5 (n = 105)</td>
<td>7.1–14.7</td>
<td>13.6 ± 0.7 (n = 105)</td>
<td>&lt;0.001&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>86.8 ± 9.5</td>
<td>55.8–117.4</td>
<td>90.9 ± 3.4</td>
<td>&lt;0.001&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Iron (µg/dL)</td>
<td>53.0 ± 14.8 (n = 44)</td>
<td>11.0–69.0</td>
<td>98.3 ± 23.9 (n = 44)</td>
<td>&lt;0.001&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Men</td>
<td>46.5 ± 16.6 (n = 105)</td>
<td>10.0–64.0</td>
<td>97.0 ± 27.3 (n = 105)</td>
<td>&lt;0.001&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Women</td>
<td>316.9 ± 93.4</td>
<td>150.0–449.0</td>
<td>651.9 ± 221.2</td>
<td>&lt;0.001&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vitamin B12 (pg/mL)</td>
<td>12.0 ± 5.8</td>
<td>2.2–24.0</td>
<td>13.6 ± 5.7</td>
<td>0.017&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Folic acid (ng/mL)</td>
<td>13.8 ± 18.3</td>
<td>3.6–174.0</td>
<td>8.6 ± 2.0</td>
<td>&lt;0.001&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Homocysteine (µM)</td>
<td>174.0 ± 44.0</td>
<td>7.6–151.0</td>
<td>174.0 ± 44.0</td>
<td>0.007</td>
</tr>
</tbody>
</table>

<sup>a</sup>Collections of MCV and blood concentrations of Hb, iron, vitamin B12, folic acid and homocysteine between 149 patients with both B12 and iron deficiencies, and 149 age- and sex-matched healthy control subjects by Student t test with p < 0.05.

### Table 2

<table>
<thead>
<tr>
<th>Factor</th>
<th>Subject number (%)</th>
<th>p Chi-square test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb deficiency (men &lt; 13 g/dL, women &lt; 12 g/dL)</td>
<td>54 (36.2)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Iron deficiency (men &lt; 70 µg/dL, women &lt; 65 µg/dL)</td>
<td>149 (100.0)</td>
<td>22 (14.8)</td>
</tr>
<tr>
<td>Vitamin B12 deficiency (≤ 450 pg/mL)</td>
<td>149 (100.0)</td>
<td>33 (22.1)</td>
</tr>
<tr>
<td>Folic acid deficiency (≤ 6 ng/mL)</td>
<td>16 (10.7)</td>
<td>9 (6.0)</td>
</tr>
<tr>
<td>High homocysteine level (&gt; 12.6 µM)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>44 (29.5)</td>
<td>4 (2.7)</td>
</tr>
<tr>
<td>GPCA positivity</td>
<td>36 (24.2)</td>
<td>3 (2.0)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Comparison of the frequency of Hb, iron, vitamin B12 or folic acid deficiency, of abnormally high blood homocysteine level, or of serum GPCA positivity between 149 patients with both B12 and iron deficiencies, and 149 age- and sex-matched healthy control subjects using the Chi-square test with p < 0.001.

<sup>b</sup>High homocysteine levels were defined as patients with homocysteine levels greater than the mean serum homocysteine level of healthy control subjects plus two standard deviations.

### Table 1

The mean corpuscular volume (MCV) and mean blood concentrations of hemoglobin (Hb), iron, vitamin B12, folic acid, and homocysteine in 149 patients with both B12 (≤ 450 pg/mL) and iron (≤ 70 µg/dL for men or ≤ 65 µg/dL for women) deficiencies, and in 149 age- and sex-matched healthy control subjects.
Comparisons of MCV and mean blood concentrations of Hb, iron, vitamin B12, folic acid, and homocysteine between Group 1 and Group 5.

Table 3

<table>
<thead>
<tr>
<th>Patients with B12 and iron deficiency (n = 149)</th>
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<tbody>
<tr>
<td>Group 1 MCV ≥ 100 fL (n = 10)</td>
</tr>
<tr>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Men: 12.7 ± 1.9 (n = 5)</td>
</tr>
<tr>
<td>Women: 12.0 ± 1.2 (n = 5)</td>
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<tr>
<td>Group 2 MCV 80–99 fL (n = 108)</td>
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<tr>
<td>Mean ± SD</td>
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<tr>
<td>Men: 10.3 ± 5.2 (n = 7)</td>
</tr>
<tr>
<td>Women: 12.0 ± 4.0 (n = 7)</td>
</tr>
<tr>
<td>Group 3 MCV &lt; 80 fL (n = 31)</td>
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<tr>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Men: 6.2 ± 2.9 (n = 26)</td>
</tr>
<tr>
<td>Women: 5.5 ± 3.0 (n = 26)</td>
</tr>
</tbody>
</table>

Comparisons of MCV and mean blood concentrations of Hb, iron, vitamin B12, folic acid, and homocysteine between Group 1 and Group 2, between Group 2 and Group 3, and between Group 1 and Group 3 using the Student t test or Chi-square test, where appropriate.

In our 36 GPCA-positive patients, the vitamin B12 deficiency could be attributed to the presence of GPCA, because the intestinal absorption of vitamin B12 is GPCA-dependent. Therefore, the vitamin B12 deficiency in the other 113 GPCA-negative 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.

This study divided our 149 patients with both vitamin B12 and iron deficiencies into three groups according to the patients’ MCV level. We found that 10 patients (Group 1) had macrocytosis (MCV ≥ 100 fL), 108 patients (Group 2) had a relatively normal MCV (between 80 fL and 99 fL), and 31 patients (Group 3) had microcytosis (MCV < 80 fL). We also discovered that compared with Group 2 (normal MCV) patients, Group 1 (macrocytosis) patients tended to have severe vitamin B12 deficiency, high serum homocysteine level, and a high frequency of GPCA positivity but tended not to have severe anemia and severe iron deficiency, and Group 3 (microcytosis) patients were prone to having severe anemia and severe iron deficiency but not to having a high serum homocysteine level and a high frequency of GPCA positivity. These findings suggest that GPCA positivity is an important factor leading to severe vitamin B12 deficiency which in turn causes high serum homocysteine levels as seen in Group 1 patients. Furthermore, severe iron deficiency is responsible for the severe anemic status seen in Group 3 patients. Because vitamin B12 deficiency can result in the generation of macrocytic RBCs, the macrocytic RBCs in our 10 macrocytosis patients are probably due to severe vitamin B12 deficiency. Moreover, iron deficiency can lead to the production of microcytic RBCs and thus the microcytic RBCs in our 31 microcytosis patients are probably due to severe iron deficiency. The MCV of the resting 108 patients with moderate vitamin B12 and iron deficiencies was within normal limits, because this specific group of patients may produce relatively normal-sized RBCs or generate nearly equal numbers of macrocytic or microcytic RBCs. In this study, approximately 90% of our patients had a normal or higher blood level of folic acid that might also reduce the vitamin B12-deficient effect on causing macrocytosis and further explain why 72.5% of our 149 patients had an MCV that fell into the normal limits.

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. This was the reason why we studied the blood homocysteine levels in our oral mucosal disease patients with both vitamin B12 and iron deficiencies. Homocysteine is a sulfur-containing amino acid which is formed during methionine metabolism. Both vitamin B12 and folic acid act as coenzymes for the conversion of homocysteine to methionine.
acid deficiency can lead to high homocysteine levels in their sera.\textsuperscript{21,22} Moreover, the high blood homocysteine levels in patients was found to be due to deficiencies in folic acid, vitamin B\textsubscript{12}, and vitamin B\textsubscript{6}, because a supplement therapy with folic acid, vitamin B\textsubscript{12}, and vitamin B\textsubscript{6} can reduce blood homocysteine levels in patients.\textsuperscript{15,16,22} In this study, an abnormally higher blood homocysteine level was detected in 44 (29.5\%) of the 149 patients with both vitamin B\textsubscript{12} and iron deficiencies. The high blood homocysteine level in this particular group of 44 patients was probably due to severe vitamin B\textsubscript{12} deficiency and the presence of GPCA in their sera.\textsuperscript{1,2,22} Moreover, only 10.7\% of our 149 patients with both vitamin B\textsubscript{12} and iron deficiencies had folic acid deficiency. Sufficient blood levels of folic acid in the majority of our patients may partially explain why the other 105 patients do not have abnormally high homocysteine levels in their sera.\textsuperscript{15,16,22}

For oral manifestations in our 149 patients with both vitamin B\textsubscript{12} and iron deficiencies, 135 (90.6\%) had a burning sensation of the oral mucosa, 110 (73.8\%) had a dry mouth, 67 (45.0\%) had AG, 52 (34.9\%) had numbness of the oral mucosa, 27 (18.1\%) had OLP, 25 (16.8\%) had numbness of the oral mucosa, dry mouth, numbness of the oral mucosa, and taste dysfunction in 100\%, 75.7\%, 43.9\%, and 19.8\% of 399 BMS patients, respectively,\textsuperscript{1} and in 100\%, 79.0\%, 57.4\%, and 27.8\% of 176 AG patients, respectively.\textsuperscript{10} Because 45.6\%, 45.0\%, 18.1\%, and 11.4\% of our 149 patients with both vitamin B\textsubscript{12} and iron deficiencies had concomitant BMS, AG, OLP, and RAU. Our previous studies found burning sensation of the oral mucosa, dry mouth, numbness of the oral mucosa, and taste dysfunction in 100\%, 75.7\%, 43.9\%, and 19.8\% of 399 BMS patients, respectively,\textsuperscript{1} and in 100\%, 79.0\%, 57.4\%, and 27.8\% of 176 AG patients, respectively.\textsuperscript{10} Because 45.6\%, 45.0\%, 18.1\%, and 11.4\% of our 149 patients with both vitamin B\textsubscript{12} and iron deficiencies had concomitant BMS, AG, OLP, and RAU, respectively, this could explain why our 149 patients with both vitamin B\textsubscript{12} and iron deficiencies had high or relatively high frequencies of burning sensation of the oral mucosa, dry mouth, and numbness of the oral mucosa. In addition, 36.2\% of our 149 patients with both vitamin B\textsubscript{12} and iron deficiencies had anemia according to the WHO criteria.\textsuperscript{6} Anemic patients have reduced Hb which carry insufficient oxygen to oral mucosa and finally result in atrophy of oral mucosa.\textsuperscript{12} Moreover, all of our 149 patients had both iron and vitamin B\textsubscript{12} deficiencies. Iron is essential to the normal functioning of oral epithelial cells\textsuperscript{12} and vitamin B\textsubscript{12} plays an important role in DNA synthesis and cell division.\textsuperscript{1,17–20} Oral epithelial cells have a high turnover rate. Therefore, deficiencies of both iron and vitamin B\textsubscript{12} may result in oral epithelial atrophy. Furthermore, high blood homocysteine level may result in an elevated frequency of thrombosis in feeding arterioles that supply the nutrients to the oral epithelial cells.\textsuperscript{21–23} This in turn leads to oral epithelial atrophy. Atrophic tongue dorsal or other oral mucosa in some of our patients could partially explain why a significant number of our patients had a burning sensation and numbness of the oral mucosa and taste dysfunction.\textsuperscript{10} The reasons why BMS and AG patients may have a burning sensation and numbness of the oral mucosa, dry mouth, and taste dysfunction compared with healthy control subjects have been explained in detail in our previous papers.\textsuperscript{8,10}

Our results demonstrated that 36.2\% and 10.7\% of our 149 patients with both vitamin B\textsubscript{12} and iron deficiencies had deficiencies of Hb and folic acid, respectively. Moreover, 29.5\% and 24.2\% of our 149 patients with both vitamin B\textsubscript{12} and iron deficiencies had abnormally high blood homocysteine levels and serum GPCA positivity, respectively. We also found significantly higher frequencies of Hb deficiency, of abnormally elevated blood homocysteine levels, and of GPCA positivity in our 149 patients with both vitamin B\textsubscript{12} and iron deficiencies than in the 149 healthy control subjects. Because 72.5\% of our 149 patients with both vitamin B\textsubscript{12} and iron deficiencies had an MCV that fell within normal limits, the MCV level was not a good indicator to assess the anemic status of patients with both vitamin B\textsubscript{12} and iron deficiencies.

References


