Serum levels of folate and vitamin B$_{12}$ in oral epithelial dysplasia

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Abstract

Oral epithelial dysplasia (OED) is a histopathologic diagnosis associated with an increased risk of cancer. Deficiency of vitamin B$_{12}$ and folate is associated with causation of certain precancerous and cancerous lesions. The aims of this study were: to evaluate the circulating levels of vitamin B$_{12}$, folate status and iron, in patients with OED and to compare these levels with the values obtained in normal control subjects with and without tobacco smoking and alcohol drinking. To evaluate the circulating level of vitamin B$_{12}$, serum folate, red blood cells folate and iron among OED patients. Data were collected from 120 patients with OED and 120 healthy control Subjects matched for age and gender, selected from patients with oral diseases not caused by tobacco or alcohol or related to knowing haematinic deficiency. Measurement of serum folate and vitamin B$_{12}$ were carried out using radioassay. The majority of OED were graded either mild (46.7%) or moderate (40.0%) lesions and most of patients with OED were current smokers of more than 20 cigarettes per day for more than 20 years compared with normal healthy control. A significant decrease in the serum levels of folate, red blood cell folate was found in OED compared to normal tobacco smokers ($p<0.05$). No significant differences in vitamin B$_{12}$ was found between OED cases and normal control subjects. Likewise significant differences in serum ferritin level were found between OED cases and normal drinkers of alcoholic beverages ($p<0.05$). And no significant differences in the TIBC level in OED compared with control subjects. These findings support the notion that OED may develop in persons who expose to tobacco smoking and have low folate level. A possible inverse association between iron concentrations and the risk of OED needs further study.

Introduction

Oral epithelial dysplasia (OED) is defined as a lesion in which part of the thickness of the epithelium replaced by cells showing varying degrees of cellular atypia and maturational disturbances [1]. OED may occur in clinically identifiable lesions including erythroleukoplakia, leukoplakia and erythroleukoplakia [2]. These clinically defined lesions have been stated to harbour an increased risk compared to normal mucosa for transformation into squamous cell carcinoma [3,4]. Studies reported transformation rates ranging from 6.6 to 36.4% after mean follow-up periods of 1.5 to 8.5 years [1,5,6].

Tobacco and alcohol use is accepted as the most important risk factors for oral potentially malignant lesions [7,8] and OED [9-12]. Exposure to cigarette smoke may result in folate deficiency via chemical inactivation and thus render the epithelium more susceptible to neoplastic transformation by the carcinogenic hydrocarbons of tobacco smoke [13].

Some aspects of diet is considered to be associated with the risk of cancer, precancer and OED [14-16] and intake of certain food products such as beta-carotene, vitamin E and vitamin A, or its analogues may cause regression of oral leukoplakia, thus preventing its progression to malignancy [17,18]. There is evidence that folate deficiency may be involved in the aetiology of carcinoma of oesophagus [19], bronchi [20], cervix [21], and oral cavity [22], as well as in certain experimental models of carcinogenesis [23]. Several studies have reported an association between low systemic levels of folate and/or vitamin B$_{12}$ and an increased risk of cancer and precancer in epithelial tissues [24,25]. Mucosal atrophy is a common feature of various conditions considered to increase the liability to oral cancer and precancer [26]. In experimental animals, iron deficiency lead to changes in the cell kinetics [27] and mild iron deficiency levels which are associated with increased oxidative stress, increase the risk of oral cavity cancer [28].

Epidemiological and clinical evidence suggest that folate deficiency in certain epithelial tissue, regardless of systemic folate status, may be a factor that predispose to the development of neoplasms arising from these tissues [29]. Folate supplementation thought to have resulted in correction of cellular abnormalities associated with diminished folate status [20], and profound vitamin B$_{12}$ deficiency can cause moderate-to-severe oral mucosal dysplasia that resolves after correction of the vitamin B$_{12}$ deficiency [30].

Several studies have reported alterations in circulating levels of vitamin B$_{12}$ and folate in humans due to the habit of tobacco smoking or chewing [31,32], but there is paucity of information about the role of folate and vitamin B$_{12}$ in OED, thus, the aim of this study was: to establish the circulating levels of the vitamin B$_{12}$, serum folate, red blood cells folate, ferritin, iron, and total iron binding capacity (TIBC) in patients with OED and to compare these levels with the values obtained in normal control subjects with and without tobacco smoking.

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Materials and methods

Study population

The study group comprised a total of 120 patients with histologically confirmed OED (64 males, 56 females, median age 54 years, range 29-80) attending the Oral Surgery Oral Medicine Department of the College of Dentistry, Ajman University of Science and Technology, United Arab Emirates between 2002 and June 2012 were selected for the study after obtaining their informed consent. Control subjects were selected from those attending the college dental clinics with oral diseases not caused by smoking or drinking or related to known haematonic deficiency. Control participants were from same geographic area as the patients. Patients and control subjects were matched for gender, and date of birth (within 5 years). A total of 120 patients with OED and 120 control subjects were included in the study. Case and control subjects were interviewed in person and relevant data was collected in a standard, structured questionnaire. Information on prior use of tobacco and alcohol, type, site, duration of the dysplastic lesions, Dysplastic lesions have been classified microscopically according to degree of cytologic atypia and changes in architectural patterns, by a single pathologist into mild, moderate and severe dysplasia [1], treatment, lifelong occupational history, past medical history, and family history of OED and cancer were also collected. A current smoker was defined as someone who had smoked within the year preceding diagnosis, and previous smoker as some one who had smoked but had stopped more than one year prior to diagnosis. Questions regarding the major parameters of tobacco use included: type of tobacco used (filter cigarettes, cigars, pipe, roll-up and chewing tobacco/taking snuff, chew betel quid); duration of smoking in years; average number of cigarette smoked per day. Data on alcohol consumption included: type of alcoholic beverage used, amount of alcohol consumed per day (glass/daily) and total duration of drinking in years. Because most of the patients with OED (74.2%) were smokers, and because cigarette smoking can determine alterations in vitamin B12, and folate status and may be a confounding factor, the results from patients with OED was compared with the results from an age-matched and gender-matched control group of 59 smokers and with the results from another age-matched and gender-matched control group of 61 nonsmokers. All participants in the smoker group were current smokers, and all participants in the non-smoker group did not ever smoke.

Because heavy alcohol drinking can alter folate absorption and metabolism and also considered as a risk factor for head and neck carcinoma and OED, this may have a confounding effect in the study, thus any known heavy drinkers were excluded from the study. No participants who were included in the study had received folate or vitamin B12 supplements in the last 6 months before the study. No participants who were included in the study had received folate or vitamin B12 supplements in the last 6 months before the study. Any participant with clinically evident nutritional deficiencies was excluded from the study. All subjects gave written informed consent to participate in the study. The study protocol was approved by the institutional review boards at the College of Dentistry of Ajman University of Science and Technology.

Haematological assessment

A venous blood sample was drawn from each patient and control subject and divided for determination of serum folate, red blood cell folate, vitamin B12, iron, total iron binding capacity and ferritin among cases and controls was carried out using Student’s t-test. Significance was accepted when the p-value was less than 0.05.

Results

Demographic details

Age and gender distribution of the study subjects is detailed in Table 1. Most cases were male (53.3%), and the median age at diagnosis of the patients was 54 years (range 29 to 80). The majority of OED were graded as mild (46.7%) or moderate (40.0%) epithelial dysplasia.

Serum folate, red blood cell folate and vitamin B12 among cases and control subjects

Mean serum levels of vitamin B12, folate, and red blood cells folate in normal non-smokers and smokers control subjects compared with OED are detailed in Table 2. A significant decrease in the serum levels of folate, red blood cell folate were found in OED compared to normal tobacco smokers (p<0.05). No significant differences in vitamin B12 was found between OED cases and normal control subjects.

Serum ferritin, iron and total iron binding capacity among cases and control subjects

Table 1. Demographic characteristics of patients with oral epithelial dysplasia and control subjects.

| Variables | Cases | % | Control | % |
|-----------|-------|---|---------|---|
| Age (years) | | | | |
| < 30 | 1 | 0.8 | 3 | 2.5 |
| 30-50 | 43 | 35.8 | 40 | 33.3 |
| 51-70 | 60 | 50.0 | 57 | 47.5 |
| > 70 | 16 | 13.3 | 20 | 16.7 |
| Total | 120 | 100.0 | 120 | 100.0 |
| Gender | | | | |
| Male | 64 | 53.3 | 64 | 53.3 |
| Female | 56 | 46.7 | 56 | 46.7 |
| Total | 120 | 100.0 | 120 | 100.0 |
| Marital status | | | | |
| Single | 10 | 8.3 | 15 | 12.5 |
| Married | 81 | 67.5 | 72 | 60.0 |
| Widow | 17 | 14.1 | 19 | 15.8 |
| Divorce | 12 | 10.0 | 14 | 11.7 |
| Total | 120 | 100.0 | 120 | 100.0 |
| Income Status | | | | |
| Low | 67 | 55.8 | 42 | 35.0 |
| Middle | 35 | 29.2 | 56 | 46.7 |
| High | 18 | 15.0 | 22 | 18.3 |
| Total | 120 | 100.0 | 120 | 100.0 |
| Oral epithelial dysplasia | | | | |
| Mild | 56 | 46.7 | 46.7 |
| Moderate | 48 | 40.0 | 40.0 |
| Severe | 16 | 13.3 | 13.3 |
| Total | 120 | 100.0 | 100.0 |
Estimation of serum ferritin, iron, total iron binding capacity among cases and control subjects revealed low mean serum ferritin and iron in the control subjects compared with OED cases and significant differences in serum ferritin level were found between OED cases and normal drinkers of alcoholic beverages (p<0.05), but this association could reflect disease-related inflammation or comorbidity. And no significant differences in the TIBC level in OED compared with control subjects (Table 3).

### Tobacco and alcohol habits of subjects

Tobacco and alcohol usage are detailed in Table 4. Significantly more of OED patients were current tobacco smokers of more than 20 cigarettes per day for more than 20 years compared with normal healthy control.

### Discussion

It is generally agreed that tobacco consumption is a major aetiological factor for OED and many studies have shown an over-representation of tobacco smokers amongst patients with OED [8-11]. In this study tobacco smoking was recorded in at least 74.2% of patients with OED compared with 49.0% in healthy controls thus confirm the significance of tobacco smoking and alcohol consumption as risk factors in the aetiology of OED.

One of the harmful effects of tobacco consumption is the alterations in the plasma/serum levels of micronutrients [13,25,32,35]. In this study a decrease in the plasma folate levels was observed in the patients consuming tobacco as compared to the non smokers, thus confirming recent observation by Almadori et al. from Italy [36] who found that serum folate levels were significantly lower in patients with head and neck carcinoma and in patients with laryngeal leukoplakia compared with serum folate levels in both the smoker and nonsmoker control group. Likewise, Ramaswamy et al. [25] have reported low

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Table 2. Serum and Red Blood cell folate and serum vitamin B<sub>12</sub> in patients with oral epithelial dysplasia and control subjects.

| Groups       | No | Serum Folate µg/l Mean ± SD | RBC folate µg/l Mean ± SD | B<sub>12</sub> ng/l Mean ± SD |
|--------------|----|-----------------------------|---------------------------|-------------------------------|
| (A) Oral dysplasia | 120 | 3.1 ± 2.9 | 228.8 ± 138.7 | 277.6 ± 201.3 |
| Controls     |    |                        |                          |                              |
| (B) Smokers  | 59 | 13.7 ± 2.6 | 587.0 ± 189.7 | 272.3 ± 161.3 |
| (C) Non-smokers | 61 | 10.1 ± 2.8 | 504.6 ± 197.2 | 326.9 ± 193.2 |
| (D) Drinkers | 55 | 3.4 ± 3.1 | 350.9 ± 167.3 | 308.7 ± 177.6 |
| (E) Non-drinkers | 65 | 3.7 ± 3.1 | 392.6 ± 186.2 | 317.0 ± 204.1 |

S.D.= Standard deviation

### Table 3. Serum ferritin, iron and total iron binding capacity in patients with oral epithelial dysplasia and control subjects.

| Groups       | No | Serum Ferritin mg/l Mean ± SD | Iron umol/l Mean ± SD | TIBC umol/l Mean ± SD |
|--------------|----|-----------------------------|----------------------|-----------------------|
| (A) Oral dysplasia | 120 | 118 ± 113.3 | 18.8 ± 7.2 | 58.2 ± 14.9 |
| Controls     |    |                        |                      |                        |
| (B) Smokers  | 59 | 107 ± 118 | 12.0 ± 6.7 | 58.6 ± 10.3 |
| (C) Non-smokers | 61 | 112 ± 147 | 14.1 ± 7.5 | 75.3 ± 10.2 |
| (D) Drinkers | 55 | 103 ± 125 | 12.3 ± 6.9 | 62.9 ± 12.1 |
| (E) Non-drinkers | 65 | 114 ± 147 | 13.5 ± 7.1 | 67.9 ± 14.1 |

S.D.≈ Standard deviation

TIBC=Total iron binding capacity

### Table 4. Frequency of tobacco and alcohol usage in 120 patients with oral epithelial dysplasia and healthy control subjects.

| Habits            | Cases No (%) | Controls No (%) | Chi-square test P value |
|-------------------|--------------|-----------------|-------------------------|
| Tobacco smoking   |              |                 |                         |
| Yes               | 89 (74.2)    | 59 (49.1)       | 15.86                   |
| No                | 31 (25.8)    | 60 (50.9)       | <0.001                  |
| Total             | 120 (100.0)  | 120 (100.0)     |                         |
| Years smoking     |              |                 |                         |
| 1-19              | 15 (16.8)    | 34 (57.6)       | 60.96                   |
| 20-39             | 41 (46.0)    | 18 (30.5)       | <0.001                  |
| >39 years         | 33 (37.2)    | 7 (11.8)        |                         |
| Total             | 89 (100.0)   | 59 (100.0)      |                         |
| Type of tobacco habit |          |                 |                         |
| Filter            | 61 (68.5)    | 41 (69.4)       | 0.15                    |
| Non filter        | 28 (31.5)    | 18 (30.5)       |                         |
| Total             | 89 (100.0)   | 59 (100.0)      |                         |
| Cigarette (per day) |           |                 |                         |
| 1-9               | 8 (9.0)      | 22 (37.2)       | 117.77                  |
| 10-19             | 19 (21.3)    | 15 (25.4)       | <0.001                  |
| 20-29             | 30 (33.7)    | 13 (22.0)       |                         |
| >29               | 32 (36.0)    | 9 (15.2)        |                         |
| Total             | 89 (100.0)   | 59 (100.0)      |                         |
| Alcohol consumption |             |                 |                         |
| Yes               | 18 (15.0)    | 55 (45.8)       | 26.95                   |
| No                | 102 (85.0)   | 65 (54.2)       | <0.001                  |
| Total             | 120 (100.0)  | 120 (100.0)     |                         |
levels of vitamin B₁₂ and folate in a group of Indian patients with oral leukoplakia, furthermore, several other investigators have suggested that deficiency of folate enhances development of preneoplastic and neoplastic lesions, which are suppressed by folate supplementation [37]. Low folate level probably does not have an independent role as an initiating factor. Instead, presumably, acts synergistically with other genetic and environmental factors, such as tobacco carcinogens, making cells more susceptible to mutagens and increasing the rate of tumor progression. Some of the carcinogenic substances present in tobacco smoke ‘primarily organic nitrates, cyanates, and isocyanates’, have been shown to interact with folate and vitamin B₁₂ coenzymes, transforming them into biologically inactive compounds [32,38]. These chemical interactions may have physiological significance is supported by reports of lower circulating folate [39,40] and B₁₂ [41] levels in smokers and the buccal mucosal cells of tobacco smokers were shown to have a decreased concentration of folate [35].

The rationale for folate’s possible protection against cancer is based on its roles in DNA synthesis and repairing damaged DNA [42,43]. Folate is involved in DNA methylation, through which it may influence gene stability and expression [43]. The benefits of folate [20,42] cobalamin [20] in reducing the risk of cancer or precancer in epithelial tissues have been reported in the literature.

Eto and Krumdieck [37] in a review of the role of vitamin B₁₂ and folate deficiencies in carcinogenesis, observed that neither deficiency is carcinogenic by itself but that each may increase susceptibility to the action of other carcinogens. A deficiency of folate has also been reported to enhance the expression of endogenous and exogenous oncogenes [23]. It is generally acknowledged that RBC folate levels provide a more accurate indication of long term nutritional status than plasma or serum folate level, which is influenced by recent ingestion of food. The findings of this study provide evidence that inadequate reserve of folate, as reflected in RBC folate contents may enhance the effect of tobacco smoking on OED risk. Furthermore low level of folate was found to be related to an increased risk of epithelial dysplasia or carcinoma-in-situ [44,45]. These nutrients are likely to take the active role in the risk reduction effect.

Vitamin B₁₂ deficiency reportedly has been associated with chromosomal damage to buccal mucosal cells in smokers [46] and vitamin B₁₂ and folate supplementation in the treatment of precancerous lesions like cervical dysplasia and bronchial metaplasia have been reported [42,47]. Nevertheless, in the current study focused on OED, differences in vitamin B₁₂ serum levels between OED patients and healthy control subjects lacked significance.

Serum iron assay alone are of little significance without relating these to total iron binding capacity. Both these values are subject to variability and serum iron levels is also subject to diurnal variation and merely indicate the efficacy of iron transportation within the body to sites of erythropoiesis. The diurnal variation is reported to exceed 50 percent [48]. For this reason, all blood samples were drawn in the morning to provide consistency in interpretation. Low serum iron and TTBC level may indicate anaemia of chronic disease, whereas low serum iron values and an elevated TTBC represent true iron deficiency. A more accurate assay is serum ferritin level, which reflects the level of total body iron stores. In this study serum iron, total iron binding capacity and ferritin levels were all within normal limits among OED cases and the normal healthy control subjects. The biochemical changes in iron deficient epithelium including decrease cytochrome C levels and enzyme depletions have been reported [49]. Occurrence of iron deficiency is known to present in oral cancer.

Haematological abnormalities in oral cancer and precancerous lesions were reported by Khanna and Karjodkar [50], and the abnormalities may be associated with the pathogenesis and progressions of oral cancer and potentially malignant lesions. It has been suggested that mucosal atrophy, increased mitotic activity, and diminished repair capacity are among the major common underlying predisposing factors in oral cancer and potentially malignant lesions [51].

It is recognised however, that in certain cases other associated deficiencies of essential nutrients and vitamins may arise and complicate the situation [52]. Nutritional factors are of great importance in maintaining the integrity of the oral mucosa [27,53] and thorough haematinic investigation is recommended in the management of potentially malignant oral lesions, particularly in patients in whom these deficiencies are prevalent [54].

These findings support the notion that OED may develop in persons who expose to tobacco smoking and have low folate level. This was a prospective investigation, allowing assessment of serum folate, B₁₂ and iron status. Clearly, however, more prospective studies are needed to supply the additional pieces of information that will eventually resolve the role, if any, of the vitamins B₁₂, folate and iron in the etiology of OED. Clinical trials to investigate the effectiveness of supplementation of this micronutrient in reducing the incidence of oral OED and its subsequent malignant transformation may be warranted.

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