Mouth and Teeth

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Hematinic Deficiency and the risk of Oral Epithelial Dysplasia

Mohamed A Jaber*

Hamdan Bin Mohamed College of Dental Medicine, Mohammed Bin Rashid University of Medicine and Health Sciences, UAE

Abstract

Oral squamous cell carcinoma could be preceded by clinically evident oral potentially malignant disorders harboring oral epithelial dysplasia (OED. Hematinic deficiencies are associated with certain potentially malignant disorders and cancerous lesions. The aims of this study were: to evaluate the circulating levels of vitamin B_{12}, folate status in patients with OED and to assess the impact tobacco smoking on these levels. To evaluate the circulating level of vitamin B_{12}, serum folate, red blood cells folate 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 hematinic deficiency. 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. These findings support the notion that OED may develop in persons who expose to tobacco smoking and have low folate level.

Introduction

Epithelial dysplasia represents the sum of various disturbances of epithelial proliferation and differentiation as seen microscopically. Individual cellular features of dysplasia are called epithelial atypia. Dysplastic epithelium characterized by alteration in the part of the thickness of the epithelium, which replaced by cells showing varying degrees of cellular atypia and maturational disturbances [1]. OED may occur in clinically identifiable lesions including erythroplakia, leukoplakia and erythroleukoplakia [2], These clinically defined lesions have been stated to harbor 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 are 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 esophagus [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 foliate 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 in patients with OED and to compare these levels with the values obtained in normal control subjects with and without tobacco smoking.

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, United Arab Emirates between 2002 and June 2012 were selected for the study after obtaining their

Correspondence to: Mohamed A Jaber, Department of Oral Surgery, Hamdan Bin Mohamed College of Dental Medicine, Mohammed Bin Rashid University of Medicine and Health Sciences, Dubai P. O. Box 505097, United Arab Emirates. Tel: (+971) 044248630; E-mail : mjaber4@hotmail.com; mohamed.jaber@mbru.ac.ae

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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 hematinic 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]. A current smoker was defined as someone who had smoked within the year preceding diagnosis, and previous smoker as someone 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 B_{12}, 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 B_{12} supplements in the last 6 months before the study. In addition, nutritional status may be the primary determinant of folate and vitamin B_{12} levels so any participant with clinically evident nutritional deficiencies was excluded from the study. All subjects gave written informed consent to participate in the study. The institutional review board of the College of Dentistry, Ajman University, approved the study protocol.

A venous blood sample was drawn from each patient and control subject and divided for determination of serum folate, red blood cell folate, vitamin B_{12}. Blood samples were stabilized and frozen at -70°C until assayed. The complete blood count (CBC) included determination of haemoglobin, red blood cell, red blood cell indices, and white blood cells with differential using standard methods. All blood samples from patients and controls were drawn in the morning to provide consistency in interpretation of results. Serum folate, whole blood folate and RBC folates were measured in duplicate using standard technique [33,34]. None of the patients or controls was taking any medications at the time of testing.

Data analysis

Statistical procedures were carried out using the SPSS programme [version 21, SPSS Inc., Chicago, IL, USA for Windows]. Analysis of the differences between serum folate, red blood cell folate, vitamin B_{12}, 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 B_{12} among cases and control subjects

Mean serum levels of vitamin B_{12}, 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 B_{12} was found between OED cases and normal control subjects.

Tobacco and alcohol habits of subjects

Tobacco and alcohol usage are detailed in (Table 3). 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 alteration 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 nonsmokers, thus confirming the observation of Almadori et al. from Italy [36] who found that serum

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

Variables	Cases		Control	
	No	%	No	%
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
Oral epithelial dysplasia				
Mild	56	46.7		
Moderate	48	40.0		
Severe	16	13.3		
Total	120	100.0		

Table 2. Serum and Red Blood cell folate and serum vitamin B_{12} in patients with oral epithelial dysplasia and control subjects

Groups	No	Serum Folate µg/l		RBC folate g/l		B12 ng/l	
		Mean	± SD	Mean	± SD	Mean	± SD
(A) **Oral dysplasia**	120	3.1	2.9	228.8	38.7	277.6	20.3
Controls							
(B) Smokers	59	13.7	2.6	587.0	18.7	272.3	16.3
(C) Non-smokers	61	10.1	2.8	504.6	19.2	326.9	19.2
(D) Drinkers	55	3.4	3.1	350.9	16.3	308.7	17.6
(E) Non-drinkers	65	3.7	3.1	392.6	14.2	317.0	24.1

S.D.= Standard deviation

Significance (t-test):

Serum Folate	RBC folate	Vitamin B12
A vs B $P<0.05$	A vs B $P<0.05$	A vs B $P=0.9$
A vs C $P=0.07$	A vs C $P=0.09$	A *vs* C $P=0.5$
A vs D $P=0.9$	A vs D $P=0.7$	A vs D $P=0.8$
A vs E $P=0.9$	A vs E $P=0.6$	A vs E $P=0.7$

Table 3. 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)	61(50.9)	<0.001
Total	120(100.0	120(100.0)	
Years smoking			
1-19	15 (16.8)	34 (57.6)	
20-39	41(46.0)	18(30.5)	60.96
>39 years	33 (37.2)	7(11.8)	<0.001
Total	89(100.0)	59 (100.0)	
Type of tobacco habit			
Filter	61 (68.5)	41(69.4)	
Non filter	28 (31.5)	18(30.5)	0.15
Total	89 (100.0)	59(100.0)	>0.001
Cigarette (per day)			
1-9	8 (9.0)	22(37.2)	
10-19	19(21.3)	15(25.4)	
20-29	30(33.7)	13(22.0)	117.77
>29	32(36.0)	9(15.2)	<0.001
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)	

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 levels of vitamin B_{12} 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 nitrites, cyanates, and isocyanates', have been shown to interact with folate and vitamin B_{12} 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 B_{12} [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 B_{12} 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 B_{12} deficiency reportedly has been associated with chromosomal damage to buccal mucosal cells in smokers [46] and vitamin B_{12} 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 B_{12} serum levels between OED patients and healthy control subjects lacked significance.

Hematological abnormalities in oral cancer and precancerous lesions were reported by Khanna and Karjodkar [48], 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 [49].

It is recognized however, that in certain cases other associated deficiencies of essential nutrients and vitamins may arise and complicate the situation [50]. Nutritional factors are of great importance in maintaining the integrity of the oral mucosa [27,51] and thorough hematinic investigation is recommended in the management of potentially malignant oral lesions, particularly in patients in whom these deficiencies are prevalent [52].

These findings support the notion that OED may develop in persons who expose to tobacco smoking and have low folate level, however, 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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Conflict of interests

The author confirms that there are no any conflicts of interest to be declared.

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