Dental, Oral and Craniofacial Research

Research Article ISSN: 2058-5314

Serum levels of folate and vitamin B_{12} in oral epithelial dysplasia

Mohamed A Jaber*

Dubai College of Dental Medicine, MBR University of Medical and Health Sciences, United Arab Emirates

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 erythroplakia, 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.

Correspondence to: Mohamed A Jaber, Department of Oral Surgery, Dubai College of Dental Medicine, MBR University of Medical and Health Sciences, Dubai, P O Box-505097, United Arab Emirates, Tel: +971-044-248630, 971- 050-5178052; **E-mail:** mjaber4@hotmail.com, Mohamed.jaber@dcdm.ac.ae

Key words: *alcohol, folate, oral epithelial dysplasia, vitamin B12, tobacco*

Received: December 01, 2014; **Accepted:** January 29, 2015; **Published:** February 04, 2015

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 haematinic 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 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 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 B_{12}, iron, ferritin and total iron binding capacity (TIBC). 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 16·0, 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.

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.

	Cases		Control	
Variables	**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
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		
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	Serumm Folate µg/l Mean ± SD	RBC folate µg/l Mean ± SD	B_{12} 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

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. 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

Significance (t-test):

Serum Ferritin	Iron	TIBC
A *vs* B *P*=0.4	A *vs* B *P*=0.4	A *vs* B *P*=0.9
A *vs* C *P*=0.9	A *vs* C *P*=0.9	A *vs* C *P*=0.7
A *vs* D *P*=0.03	A *vs* D *P*=0.6	A *vs* D *P*=0.9
A *vs* E *P*= 0.8	A *vs* E *P*=0.8	A *vs* E *P*=0.8

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.

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)	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)	117.77
20-29	30 (33.7)	13 (22.0)	<0.001
>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)	

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

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.

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 erythropioeisis. 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 TIBC level may indicate anaemia of chronic disease, whereas low serum iron values and an elevated TIBC 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 depletion 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, B_{12}, 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 B_{12}, 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.

Acknowledgment

I would like to acknowledge the help and contribution of the other doctors and assisting staff.

References

1. Hazarey V, Daftary D, Kale A, Warnakulasuriya S (2007) Proceedings of the panel discussion on 'standardized reporting of oral epithelial dysplasia'. *J Oral Maxillofac Pathol* 11: 86-88.

2. Jaber MA, Porter SR, Speight P, Eveson JW, Scully C (2003) Oral epithelial dysplasia: clinical characteristics of western European residents. *Oral Oncol* 39: 589-596. [Crossref]

3. Gregg TA, Cowan CG, Kee F (1992) Trends in the relative frequency of histologically diagnosed epithelial dysplasia and intra-oral carcinoma in Northern Ireland, 1975-1989. *Br Dent J* 173: 234-236. [Crossref]

4. Crissman JD, Zarbo RJ (1989) Dysplasia, in situ carcinoma, and progression to invasive squamous cell carcinoma of the upper aerodigestive tract. *Am J Surg Pathol* 13 Suppl 1: 5-16. [Crossref]

5. Silverman S Jr, Gorsky M, Lozada F (1984) Oral leukoplakia and malignant transformation. A follow-up study of 257 patients. *Cancer* 53: 563-568. [Crossref]

6. Lumerman H, Freedman P, Kerpel S (1995) Oral epithelial dysplasia and the development of invasive squamous cell carcinoma. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 79: 321-329. [Crossref]

7. Maserejian NN, Joshipura KJ, Rosner BA, Giovannucci E, Zavras AI (2006) Prospective study of alcohol consumption and risk of oral premalignant lesions in men. *Cancer Epidemiol Biomarkers Prev* 15: 774-781. [Crossref]

8. Macigo FG, Mwaniki DL, Guthua SW (1995) The association between oral leukoplakia and use of tobacco, alcohol and khat based on relative risks assessment in Kenya. *Eur J Oral Sci* 103: 268-273. [Crossref]

9. Jaber MA, Porter SR, Scully C, Gilthorpe MS, Bedi R (1998) The role of alcohol in non-smokers and tobacco in non-drinkers in the aetiology of oral epithelial dysplasia. *Int J Cancer* 77: 333-336. [Crossref]

10. Jaber MA, Porter SR, Gilthorpe MS, Bedi R, Scully C (1999) Risk factors for oral epithelial dysplasia--the role of smoking and alcohol. *Oral Oncol* 35: 151-156. [Crossref]

11. Kulasegaram R, Downer MC, Jullien JA, Zakrzewska JM, Speight PM (1995) Case-control study of oral dysplasia and risk habits among patients of a dental hospital. *Eur J Cancer B Oral Oncol* 31B: 227-231. [Crossref]

12. Morse DE, Katz RV, Pendrys DG, Holford TR, Krutchkoff DJ, et al. (1996) Smoking and drinking in relation to oral epithelial dysplasia. *Cancer Epidemiol Biomarkers Prev* 5: 769-777. [Crossref]

13. Piyathilake CJ, Macaluso M, Hine RJ, Richards EW, Krumdieck CL (1994) Local and systemic effects of cigarette smoking on folate and vitamin B-12. *Am J Clin Nutr* 60: 559-566. [Crossref]

14. Zain RB (2001) Cultural and dietary risk factors of oral cancer and precancer--a brief overview. *Oral Oncol* 37: 205-210. [Crossref]

15. Petti S (2009) Lifestyle risk factors for oral cancer. *Oral Oncol* 45: 340-350. [Crossref]

16. Morse DE, Pendrys DG, Katz RV, Holford TR, Krutchkoff DJ, et al. (2000) Food group intake and the risk of oral epithelial dysplasia in a United States population. *Cancer Causes Control* 11: 713-720. [Crossref]

17. Stich HF, Mathew B, Sankaranarayanan R, Nair MK (1991) Remission of precancerous lesions in the oral cavity of tobacco chewers and maintenance of the protective effect of beta-carotene or vitamin A. *Am J Clin Nutr* 53: 298S-304S. [Crossref]

18. Benner SE, Winn RJ, Lippman SM, Poland J, Hansen KS, et al. (1993) Regression of oral leukoplakia with alpha-tocopherol: a community clinical oncology program chemoprevention study. *J Natl Cancer Inst* 85: 44-47. [Crossref]

19. Jaskiewicz K, Marasas WF, Lazarus C, Beyers AD, Van Helden PD (1988) Association of esophageal cytological abnormalities with vitamin and lipotrope deficiencies in populations at risk for esophageal cancer. *Anticancer Res* 8: 711-715. [Crossref]

20. Heimburger DC, Alexander CB, Birch R, Butterworth CE Jr, Bailey WC, et al. (1988) Improvement in bronchial squamous metaplasia in smokers treated with folate and vitamin B12. Report of a preliminary randomized, double-blind intervention trial. *JAMA* 259: 1525-1530. [Crossref]

21. Butterworth CE (1991) Folate deficiency and cancer. In: A. Bendich, C. E. Butterworth. Editors: Micronutrients in health and disease. New York: Marcel Decker 165-183.

22. Almadori G, Bussu F, Galli J, Cadoni G, Zappacosta B, et al. (2002) Serum folate and homocysteine levels in head and neck squamous cell carcinoma. *Cancer* 94: 1006-1011. [Crossref]

23. Hsieh LL, Wainfan E, Hoshina S, Dizik M, Weinstein IB (1989) Altered expression of retrovirus-like sequences and cellular oncogenes in mice fed methyl-deficient diets. *Cancer Res* 49: 3795-3799. [Crossref]

24. Sankaranarayanan R, Duffy SW, Day NE, Nair MK, Padmakumary G (1989) A case-control investigation of cancer of the oral tongue and the floor of the mouth in southern India. *Int J Cancer* 44: 617-621. [Crossref]

25. Ramaswamy G, Rao VR, Kumaraswamy SV, Anantha N (1996) Serum vitamins' status in oral leucoplakias--a preliminary study. *Eur J Cancer B Oral Oncol* 32B: 120-122. [Crossref]

26. MacDonald DG (1975) Premalignant lesions of oral mucosa. In: A. E. Dolby. edit. Oral mucosa in health and disease. Oxford: Blackwell Scientific publications 335-369.

27. Rennie JS, MacDonald DG, Dagg JH (1984) Iron and the oral epithelium: a review. *J R Soc Med* 77: 602-607. [Crossref]

28. Richie JP Jr, Kleinman W, Marina P, Abraham P, Wynder EL, et al. (2008) Blood iron, glutathione, and micronutrient levels and the risk of oral cancer. *Nutr Cancer* 60: 474-482. [Crossref]

29. Heimburger DC (1992) Localized deficiencies of folic acid in aerodigestive tissues. *Ann N Y Acad Sci* 669: 87-95. [Crossref]

30. Theaker JM1, Porter SR, Fleming KA (1989) Oral epithelial dysplasia in vitamin B12 deficiency. *Oral Surg Oral Med Oral Pathol* 67: 81-83. [Crossref]

31. Abu Khaled M, Watkins CL, Krumdieck CL (1986) Inactivation of B12 and folate coenzymes by butyl nitrite as observed by NMR: implications on one-carbon transfer mechanism. *Biochem Biophys Res Commun* 135: 201-207. [Crossref]

32. Hecht SS (1999) Tobacco smoke carcinogens and lung cancer. *J Natl Cancer Inst* 91: 1194-1210. [Crossref]

33. Longo DL, Herbert V (1976) Radioassay for serum and red cell folate. *J Lab Clin Med* 87: 138-151. [Crossref]

34. Waxman S, Schreiber C, Herbert V (1971) Radioisotopic assay for measurement of serum folate levels. *Blood* 38: 219-228. [Crossref]

35. Piyathilake CJ, Hine RJ, Dasanayake AP, Richards EW, Freeberg LE, et al. (1992) Effect of smoking on folate levels in buccal mucosal cells. *Int J Cancer* 52: 566-569. [Crossref]

36. Almadori G1, Bussu F, Galli J, Cadoni G, Zappacosta B, et al. (2005) Serum levels of folate, homocysteine, and vitamin B12 in head and neck squamous cell carcinoma and in laryngeal leukoplakia. *Cancer* 103: 284-292. [Crossref]

37. Eto I, Krumdieck CL (1986) Role of vitamin B12 and folate deficiencies in carcinogenesis. *Adv Exp Med Biol* 206: 313-330. [Crossref]

38. Khaled MA, Krumdieck CL (1985) Association of folate molecules as determined by proton NMR: implications on enzyme binding. *Biochem Biophys Res Commun* 130: 1273-1280. [Crossref]

39. Witter FR, Blake DA, Baumgardner R, Mellits ED, Niebyl JR (1982) Folate, carotene, and smoking. *Am J Obstet Gynecol* 144: 857. [Crossref]

40. Nakazawa Y, Chiba K, Imatoh N, Kotorii T, Sakamoto T, et al. (1983) Serum folic acid levels and antipyrine clearance rates in smokers and non-smokers. *Drug Alcohol Depend* 11: 201-207. [Crossref]

41. Dastur DK, Quadros EV, Wadia NH, Desai MM, Bharucha EP (1972) Effect of vegetarianism and smoking on vitamin B12, thiocyanate, and folate levels in the blood of normal subjects. *Br Med J* 3: 260-263. [Crossref]

42. Butterworth CE Jr, Hatch KD, Macaluso M, Cole P, Sauberlich HE, et al. (1992) Folate deficiency and cervical dysplasia. *JAMA* 267: 528-533. [Crossref]

43. Mason JB, Levesque T (1996) Folate: effects on carcinogenesis and the potential for cancer chemoprevention. *Oncology* (Williston Park) 10: 1727-1736, 1742-3. [Crossref]

44. Buckley DI, McPherson RS, North CQ, Becker TM (1992) Dietary micronutrients and cervical dysplasia in southwestern American Indian women. *Nutr Cancer* 17: 179-185. [Crossref]

45. Correa P (1992) Vitamins and cancer prevention. *Cancer Epidemiol Biomarkers Prev* 1: 241-243. [Crossref]

46. Piyathilake CJ, Macaluso M, Hine RJ, Vinter DW, Richards EW, et al. (1995) Cigarette smoking, intracellular vitamin deficiency, and occurrence of micronuclei in epithelial cells of the buccal mucosa. *Cancer Epidemiol Biomarkers Prev* 4:751–758.

47. Saito M, Kato H, Tsuchida T, Konaka C (1994) Chemoprevention effects on bronchial squamous metaplasia by folate and vitamin B12 in heavy smokers. *Chest* 106: 496-499. [Crossref]

48. Weinberg ED (1974) Iron and susceptibility to infectious disease. *Science* 184: 952-956. [Crossref]

49. Rennie JS, MacDonald DG, Dagg JH (1982) Quantitative analysis of human buccal epithelium in iron deficiency anaemia. *J Oral Pathol* 11: 39-46. [Crossref]

50. Khanna SS, Karjodkar FR (2006) Circulating immune complexes and trace elements (Copper, Iron and Selenium) as markers in oral precancer and cancer: a randomised, controlled clinical trial. *Head Face Med* 2: 33. [Crossref]

51. Ramanathan K (1981) Oral submucous fibrosis--an alternative hypothesis as to its causes. *Med J Malaysia* 36: 243-245. [Crossref]

52. Morse DE1, Pendrys DG, Katz RV, Holford TR, Krutchkoff DJ, et al. (2000) Food group intake and the risk of oral epithelial dysplasia in a United States population. *Cancer Causes Control* 11: 713-720. [Crossref]

53. Mitchell K, Ferguson MM, Lucie NP, MacDonald DG (1986) Epithelial dysplasia in the oral mucosa associated with pernicious anaemia. *Br Dent J* 161: 259-260. [Crossref]

54. Warnakulasuryia KA, Prabhu SR (1992) Anaemia in the Tropics. In: Prabhu S.R, Wilson D.F, Daftary D.K, Johnson N.W, editors. Oral diseases in the Tropics. Oxford Medical Publications: 325-36.