Journal of Oral Science, Vol. 53, No. 4, 427-431 2011

Original

Estimation of serum beta carotene levels in patients with oral submucous fibrosis in India

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(Received 8 January and accepted 15 August 2011)

Abstract: Oral submucous fibrosis (OSMF) is a chronic, insidious and disabling condition affecting the oral cavity, being especially prevalent in India and South East Asia. However, considering its high prevalence and potential to undergo malignant transformation, OSMF has not been widely investigated with respect to levels of antioxidants, especially beta carotene. In the present study, an attempt was made to analyze serum levels of beta carotene in 45 patients with oral submucous fibrosis and 45 age- and sex-matched controls. The serum beta carotene level was estimated using the Bradley and Hornbeck method. The serum beta carotene level was significantly lower in the patients with oral submucous fibrosis than in the controls. When the values were compared between different disease stages, the maximum reduction of beta carotene was evident for Grade III OSMF, as compared with Grade I and II. From the present results, it is evident that beta carotene plays an important role in the pathogenesis of OSMF, and that its level decreases with disease progression. OSMF patients should be treated with a diet rich in beta carotene to reduce disease severity and progression towards malignancy. (J Oral Sci 53, 427-431, 2011)

Keywords: OSMF; beta carotene; antioxidant.

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Introduction

Oral submucous fibrosis (OSMF) is a chronic, insidious, disabling precancerous condition of the oral cavity seen predominantly in India and South East Asia (1,2). It is characterized by excessive production of collagen leading to inelasticity of the oral mucosa and atrophic changes of the epithelium (3). The chief complaint is a progressive inability to open the mouth owing to accumulation of inelastic fibrous tissue in the juxta-epithelial region of the oral mucosa. Also there is ulceration of the oral mucosa and a burning sensation when eating spicy foods (4). The fibrosis also leads to difficulty in mastication, speech and swallowing, pain in the throat and ears, and a relative loss of auditory acuity due to stenosis of the opening of the eustachian tube (5).

The pathogenesis of OSMF is not well established, but is believed to be multi-factorial. The chewing of betel quid (containing areca nut, tobacco and slaked lime) has been recognized as one of the most important risk factors for OSMF (6). It has been suggested that consumption of chillies, nutritional deficiency, genetic susceptibility, altered salivary constituents, autoimmunity and collagen disorders may be involved in the pathogenesis of this condition (4). Over the years, the incidence of OSMF has increased in various parts of the Indian subcontinent (7). Malignant transformation rates as high as 7.6% have been reported from India over a 17-year period (8).

The diagnosis and prognosis of OSMF can be established by biopsy, which is an invasive and time-consuming procedure (9). Biochemical investigations are of prime importance because of their advantages such as simplicity and minimal invasiveness, as well as being

useful for monitoring the response to therapy.

Epidemiological and experimental studies have shown that the process of carcinogenesis is triggered by generation of reactive oxygen species (ROS), which initiate lipid peroxidation (LPO). The extent of oxidative damage caused by ROS can be exacerbated by a decrease in the efficiency of the body's antioxidant defense mechanisms (10). Beta carotene is an essential precursor of vitamin A or retinol. It is an excellent antioxidant and radical trapping agent, especially for peroxyl and hydroxyl radicals, which have been implicated in the genesis of a number of cancers. Therefore adequate levels of beta-carotene need to be maintained in the blood (11).

In spite of its high prevalence in India and potential to undergo malignant transformation, OSMF has not been widely investigated with respect to antioxidants, especially beta carotene. Moreover, to our knowledge, literature pertaining to the serum levels of beta carotene in relation to the clinical grading of OSMF is not easily available or scanty. Therefore we conducted a case control study to estimate the serum levels of beta carotene in relation to the clinical grading of OSMF, and to determine the probable role of beta carotene in the pathogenesis of OSMF.

Materials and Methods

A total of 45 patients with OSMF who consecutively attended the Department of Oral Medicine and Radiology, KLE's V. K. Institute of Dental Sciences, Belgaum, Karnataka, were included in the study. Diagnosis of OSMF was made on the basis of history and characteristic clinical features including mucosal blanching, burning, hardening and presence of characteristic fibrous bands. Clinical diagnosis was confirmed histopathologically following incisional biopsy from the most affected area of the buccal mucosa. OSMF was divided clinically and functionally into three stages according to the criteria reported by Haider et al. (2), based on the presence of fibrous bands at various anatomical sites, and functional staging was based on the degree of mouth opening.

Clinical Staging:

Stage 1: Faucial bands only

Stage 2: Faucial and buccal bands

Stage 3: Faucial, buccal and labial bands

Functional Staging:

Stage 1: Mouth opening >20 mm

Stage 2: Mouth opening 11-19 mm

Stage 3: Mouth opening <10 mm

Patients with systemic diseases/conditions that may be associated with alterations in the serum level of beta carotene, i.e., diabetes, pregnancy, lactation, fever, liver disorders, and medication with anticonvulsive drugs, anticoagulants, broad-spectrum antibiotics and systemic steroids, were excluded from the study. An equal number of age- and sex-matched patients who consecutively presented at the same department for various other complaints, and had no adverse habits or oral lesions, were included as a control group. Informed consent was obtained from patients in both the case and control groups. A predetermined data sheet was used to record demographic data, history, clinical findings and details of betel chewing, tobacco smoking and alcohol intake. The study was done after obtaining approval from the institutional ethics committee. The subjects of the study were as follows:

Group 1 (Grade I OSMF): 15 Patients

Group 2 (Grade II OSMF): 15 Patients

Group 3 (Grade III OSMF): 15 Patients

Group 4 (Controls): 45 Patients

Table 1 Mean value for age among various groups

	Mean	Median	SD
Group 1	32.33	33	7.85
Group 2	32.27	33	7.76
Group 3	31.33	29	6.22
Group 4	31.98	31	7.16

SD: Standard deviation

Table 2 ANOVA test for ages among various groups

	Sum of Squares	df	Mean Square	F-ratio	Sig.	Inf.
Between Groups	9.38	3	3.1259	0.0596	0.9808	NS
Within Groups	4512.58	86	52.4718			
Total	4521.96	89				

df: Degree of freedom, Sig.: Significance value, Inf.: Inference, NS: Not significant

Table 3 Mean value of beta carotene among various groups

	Mean	Median	SD
Group 1	67	67	2.53
Group 2	56.40	55	3.77
Group 3	51.33	52	0.97
Group 4	93.02	94	8.67

SD: Standard deviation

Table 4 ANOVA test for beta carotene among various groups

	Sum of squares	df	Mean square	F-ratio	*P* value	Inf.
Between Groups	29130.98	3	9710.3296	230.8206	5.11E-41	S
Within Groups	3617.91	86	42.0687			
Total	32748.9	89			<0.0001	

df: Degree of freedom, Inf.: Inference, S: Statistically significant

Collection of blood samples

First, the details of each patient, including medical history, were taken. After a clinical diagnosis of OSMF had been made, the purpose and procedure was explained to each patient, who then provided informed consent to participate. The patient was then asked to attend the next morning after an overnight fast, to avoid any dietary influence on the serum beta carotene level. Upon returning, the patient was asked to sit comfortably in a dental chair in a reclining position. A tourniquet was then applied above the right cubital fossa, and the needle of a disposable 2-ml, 23-gauge syringe was inserted into the vein. About 2 ml of venous blood was withdrawn and then trnasferred to a plain 10-ml glass test tube. After the blood had coagulated, the test tube containing the blood was subjected to centrifugation for about 4-5 min at 2500 rpm. The test tube was then removed from the centrifuge, and the serum layer was pipetted into a vial, which was then stored in a refrigerator under protection from light. Beta carotene levels were estimated by the Bradley and Hornbeck method using a beta-carotene stock standard (Sigma-Aldrich Corp. St. Louis, MO, USA). The above procedure was repeated for all of the patients.

Statistical analysis

Serum beta carotene levels in patients with different grades of OSMF and the control group were analyzed statistically using the Statistical Package for the Social Sciences program (SPSS version 13.0). To assess the statistical significance of differences between the grades of OSMF and the control group, ANOVA test was carried out. Comparison of serum beta carotene levels in all three case groups with those in the control group was performed using Student's unpaired *t* test.

Results

A total of 90 patients were included in the study, of whom 78 were males and 12 were females. In the case group comprising 45 patients, Groups 1, 2 and 3 comprised 12, 14 and 13 males and 3, 1 and 2 females, respectively. The age of the patients varied between 20 and 45 yr. (Tables 1 and 2).

Group 4 comprised 45 age- and sex-matched healthy individuals without any contributory habits or premalignant lesions/conditions. All of the patients had been taking areca nut in one form or another. The mean beta carotene values were 67.00, 56.40 and 51.33 µg/dl in Groups 1, 2 and 3 (cases), and 93.02 µg/dl in Group 4 (controls) (Table 3).

Once inter-group and intra-group comparisons of beta carotene levels had been done using ANOVA, the p-value indicated non-homogeneity for the means of the four groups (Table 4).

For pairwise comparisons, a post-hoc Tukey test was conducted, and a table was obtained showing significant differences for all comparisons except between Groups 2 and 3 (Table 5).

Comparison of the mean serum beta carotene levels using unpaired *t* test demonstrated statistically significant differences between the cases (58.24 µg/dl) and controls (93.02 µg/dl). (Table 6).

Discussion

OSMF is a precancerous condition of the oral cavity and oropharynx that is widespread in India. OSMF is considered to be an oral health problem with a high degree of malignant potential, i.e., between 2.3% and 7.6% (12). Unfortunately, the pathophysiology of the OSMF is complex, and treatment modalities for relieving the symptoms have not been successful so far.

Table 5 Multiple comparison of serum beta carotene among various groups using post-hoc Ttukey's test

Group	Group	Mean difference	*P* value	Inf.
1	2	10.60	0.000134782	S
1	3	15.67	1.80054E-08	S
1	4	-26.02	5.54445E-13	S
2	3	5.07	0.1490	NS
2	4	-36.62	5.54445E-13	S
3	4	-41.69	5.54445E-13	S

Inf.: Inference, S: Statistically significant, NS: Not significant

Table 6 Comparison of serum beta carotene the three groups with the control group using unpaired *t*-test

	Mean	SD
Cases	58.24	7.10
Controls	93.02	8.67

SD: Standard deviation

In this study, an attempt was made to analyze serum beta-carotene levels in patients with OSMF in comparison with normal healthy individuals, thereby providing an indicator of oxidative stress. An attempt was also made to compare these levels among different stages of the disease.

The mean age of our study patients was 32.3 yr, which was similar to that in the study by Ranganathan et al. (13). Most of our patients were in the second or third decade of life. The higher prevalence of OSMF in younger patients is explained by the popularity of refined areca nut products, which are readily available, allowing the habit of betel nut chewing to begin at an early age.

Among our 45 OSMF patients, 39 were males and 6 were females, and the sex ratio was 6.5:1 (male:female). The sex ratio has varied among previous studies due to differences in habits in the studied populations (4,13).

All the patients in our study had been chewing areca nut in some form. Areca nut chewing has been identified as the most important etiological factor of OSMF. In our patients, the mean serum beta carotene level was 67 µg/dl, 56.40 µg/dl and 51.33 µg/dl in Groups I, II and III, respectively. However, it was 93.02 µg/dl in the control group. This may have been attributable to utilization of the antioxidants by affected tissues or in combating excessive oxidative stress in the general circulation.

The possible protective benefits of beta carotene are probably related to its role as an antioxidant in decreasing free-radical damage and its ability to quench singlet oxygen, which is a reactive unstable molecule. Beta carotene also has immunoregulatory properties, possibly retarding the development of cancer cells (13). Ingestion of beta carotene quickly increases the number of CD4 helper T-lymphocytes and significantly increases their response to mitogens. Therefore it seems that beta carotene supplementation might enhance the immunoresponse of patients who are deficient in beta carotene (15).

The present study clearly indicated a decreased level of beta carotene in Groups I, II, and III. Therefore, beta carotene supplementation in these patients might have a direct protective action against cancer. Our study findings were also significant in identifying persons who are vulnerable to cancer, and might have a higher risk of developing it. Such individuals should be given additional beta carotene supplementation to protect them against cancer.

Stahelin (16) demonstrated a dose-dependent effect of beta carotene by comparing the relative risk of developing cancer with serum beta carotene levels. Subjects with lower levels of beta carotene had a higher risk than those with average or higher levels. Similarly, in the present study, Group III appeared to have the highest risk of developing cancer, as this group had the minimum level of serum beta carotene, followed in order by Group II and Group I.

The present study has shown that beta carotene plays an important role in the pathogenesis of OSMF, and that its level decreases with disease progression. Our findings suggest that the degree of oxidative damage in OSMF can be assessed by estimation of serum beta carotene levels in affected patients, and that the underlying deficiency of antioxidants can be corrected by dietary supplementation of beta carotene. This, may be helpful for successful management of this condition, and for avoiding the consequences of malignancy. However, further detailed studies with a larger sample size, including OSMF patients with coexisting oral cancer, along with follow-up data, are needed to clarify the actual relationship between beta carotene and the initiation and promotion of carcinogenesis.

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