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Original Article

Dentistry Section

Role of Serum ß-Carotene in the Diagnosis and Prevention of Oral Squamous Cell Carcinoma – A Case Control Study

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ABSTRACT

Context: Oral squamous cell carcinoma (OSCC) is the most common cancer of the head and neck. It accounts for more than 90% of all malignancies which occur in the head and neck region. The high incidence and mortality rate of oral cancer stimulates continuation of research on finding new diagnostic tools or markers for it.

Aim: To evaluate the possible role of serum β-carotene as a biochemical parameter in the diagnosis of oral squamous cell carcinoma.

Materials and Methods: The serum β-carotene levels in 40 patients with clinically diagnosed and histopathologically confirmed oral squamous cell carcinoma were estimated and the levels were compared with those of 40 healthy controls. The serum β-carotene levels were estimated by using a spectrophotometer. The data which was obtained was analyzed statistically by using unpaired t-test.

Results: Subjects with oral squamous cell carcinoma showed significantly lower levels of mean serum β-carotene (149.95 ± 61.29) as compared to those seen in controls (278.19 ± 90.12).

Conclusion: The results of the present study are encouraging and these suggest that the estimation of the low levels of β-carotene in the patients with oral squamous cell carcinoma may be a useful diagnostic tool for making the diagnosis of oral squamous Cell carcinoma and thereby improving the prognosis of this dreaded disease.

Keywords: Serum β-carotene levels, Biochemical marker, Oral squamous cell carcinoma

INTRODUCTION

Oral squamous cell carcinoma (OSCC) is the sixth most common malignancy in the world [1]. It is caused by a variety of factors, among which oxidants, the by-products of normal metabolism, rank high as a major culprit in the onset and development of the disease [2].

β-carotene, a potent antioxidant, has a potential role in prevention of oral cancer [3]. Studies have revealed that low levels of β-carotene may be one of the important causative factors of OSCC [4]. Therefore, its supplementation at a premalignant stage will perhaps prevent the transformation of such lesions into OSCC. Over the past several decades, enormous experience has been gained from various cancer studies, which have led to the recognition of the significant role of the β-carotene and its correlation with various cancers. However, studies which predict precise relevance of the serum β-carotene levels with OSCC are limited. Hence, this study was undertaken with an aim to evaluate the possible role of serum β-carotene as a biochemical parameter in the diagnosis of OSCC.

METHODOLOGY

This study included 80 subjects of either sex, who were in the age range of 30-80 years, after obtaining their informed consents. Ethical committee clearance was obtained. The study group comprised of 40 subjects with clinically diagnosed and histopathologically confirmed OSCC and the control group comprised of 40 healthy subjects. The patients in the study group were selected, based on the following criteria.

Inclusion Criteria

Patients with clinically and histopathologically diagnosed OSCC.

Exclusion Criteria

Patients suffering from any systemic diseases like diabetes mellitus, hypertension, cardiovascular system disease, renal dysfunction, liver disorders, etc or from any mental disorder. Patients with any other mucosal disease other than the primary lesion. After clinical examination, a blood investigation and a biopsy were performed. After histologic confirmation, the patients were recalled for collection of blood for β-carotene estimation. Whole blood samples were collected, they were allowed to clot and the serum was separated by centrifugation. Samples were refrigerated at 2-8°C until use. The serum β-carotene levels were estimated by Sobel's and Snow's method by using a spectrophotometer [5].

Preparation of the reagents

Ethanolic potassium hydroxide (KOH): It was prepared by adding 1 volume of 1 normal (56g/litre) to 10 volumes of absolute ethanol/ denatured ethanol (95% ethanol, 5% methanol). It must be prepared fresh. The stock solution of KOH remains stable for several months. **Stock β-carotene standard:** It was prepared by dissolving 2.5 mg of β-carotene powder in 250 mL of isooctane. **Working β-carotene standard:** It was prepared by diluting stock β-carotene standard with isooctane in the ratio of 1:10.

Procedure

It should be carried out in the absence of direct light, preferably

in dim light. Take the 3 centrifuge tubes and label them as blank, standard (As) and test (Ax). Place 1.5 mL distilled water in the tube which is labeled as blank, 1.5 mL working standard solution in the tube which is labeled as standard, and 1.5 mL sample serum in the tube which is labeled as test. Add ethanolic KOH solution to each of the centrifuge tubes and mix their contents.

Heat them at 60^0 for 20 minutes in a thermostat controlled water bath heater. Cool the tubes, add 4.5 mL isooctane and close with stopper lightly, and shake for 10 minutes. Centrifuge for 3 minutes at 2000 rpm.

For carotene estimation, transfer 1 mL supernatant to a Lowry-Bessey cuvette. Place 1 mL of working standard solution in a cuvette. Measure absorbances of the sample (Ax) and standard (As) against a blank of isooctane at 450 nm.

Calculation

Carotene (µg) / 100 ml = $\frac{AX}{AS} \times 1.0 \times \frac{4.5}{1.0} \times \frac{100}{1.5} = \frac{AX}{AS} \times 300$

STATISTICAL ANALYSIS

Results have been presented as Mean ± SD for quantitative data and as number and percentages for categorical data. Unpaired t-test was used for inter group comparisons. For all the tests, a p-value of 0.05 or less was considered for statistical significance. All statistical analyses were done by using SAS® V8.2 statistical software package (PC-SAS) (Cary, NC, USA).

RESULTS

[Table/Fig-1] provides the age and gender distributions in the study and the control groups. Mean age of OSCC patients was 53.4 ± 12.1 (Mean ± SD) years and that of controls was 51.6 ± 11.0 years. 50% of the subjects in both the groups were males. In the study group, all the patients had tobacco chewing habit and 16 out of 20 male patients were chronic smokers. In the control group, 16 males and 10 females had tobacco chewing habit and 14 males were smokers.

[Table/Fig-2] provides the comparison of serum β-carotene levels between the study and control groups. The mean serum β-carotene levels were 149.95 ± 61.29 and 278.19 ± 90.12 in the study and the control groups respectively. There was a decrease in the serum β-carotene levels in the study group as compared to those in the control group and the difference was highly significant, with a p-value of < 0.0005.

Groups	n	Males	Females	Age (in years)		p-value
				Mean ± SD	Range	
Study group	40	20	20	53.35± 12.054	30-80	0.625
Control group	40	20	20	51.55 ± 11.014	32-70	

[Table/Fig-1]: Gender and age distribution*

*Unpaired t-test was used to compare the age distribution between the study and the control groups. The difference in the mean age between the study and the control groups was not statistically significant (p=0.625)

	Group	N	Mean	Std. Deviation	Mean diff	p-value
Serum β-carotene levels (µg/100ml)	Study group	40	149.9565	61.29828	128.23	<0.0005
	Control group	40	278.1940	90.12225		

[Table/Fig-2]: Comparison of serum β-carotene levels between the study and the control groups *

*Unpaired t-test was used to compare the serum β-carotene levels between the study and the control groups. The difference in the serum β-carotene levels between the study and the control groups was highly significant (p<0.0005)

DISCUSSION

Oral cancer is the most life-threatening disease of oral tissues and it is currently the most frequent cause of cancer-related deaths [6]. Notably, the great morbidity and mortality rates which are associated with this devastating disease have not improved in decades [7]. Hence, an early recognition is imperative in attempting to improve oral cancer survival rates, preserving function and enhancing aesthetic and psychological outcomes.

During malignant transformation of cells, there may be either an up-regulation or down regulation of many biochemical substances. With the development of new and sensitive techniques for measuring very minute quantities of these biochemical substances, now, it is possible to identify early malignant transformation of the cells [8]. The determination of these biochemical substances contributes considerably to diagnosis of malignancy [9,10]. However, a majority of these are identified either in cell lines, or in biopsy specimens, thus making it challenging for large-scale screening. Measurement of these biochemical substances in serum could potentially aid in development of a useful practical screening tool [11]. One such biochemical marker is serum β-carotene. These blood-based tests are appealing from several points of view, including their ease, lesser invasiveness, lesser time consumption, ease of interpretation, in being economical yet quite confirmatory for diagnosis of OSCC and its prognosis [12].

β-carotene is the best known naturally occurring carotenoid with both provitamin A and antioxidant properties. It is primarily found in dark green, orange, or yellow vegetables such as carrots, sweet potatoes, and orange, and in orange fruits like apricots, papaya, mangoes, and cantaloupes. Availability of β-carotene as a nutrient is an added advantage, since it allows supplementation by dietary adjustments. It is capable of inhibiting carcinogenesis at both the early and late stages of cancer [1]. It produces salutary effects on cell-differentiation, immunologic function and interaction of cells with growth factors, e.g., epidermal growth factor (important in cancer inhibition activities) [1]. Therefore, its supplementation, either in the form of dietary adjustments or as β-carotene-containing supplements, at premalignant stages, will perhaps prevent the transformation of such lesions into OSCC. Decreased levels of serum β-carotene have been observed in patients with various malignancies [13]. However, literature search has revealed only a few studies which have correlated serum β-carotene and oral cancer.

The low β-carotene status among the study subjects in the present study probably explains the strong inverse relationship with the occurrence of OSCC. β-carotene, a potent antioxidant, scavenges the aggressive free radicals which induce chromosomal defects that may bring about cancer initiation and promotion, and thereby get depleted during this process. This could be the probable reason for lower levels of serum β-carotene which are seen in OSCC patients. Reddy et al., also observed significantly lower levels of serum β-carotene among oral cancer patients in comparison to those in control groups, in a study which was conducted on 70 subjects (50 oral cancer patients and 20 controls) [4]. Similar results were obtained by Torun et al., who observed that the mean levels of beta-carotene, vitamin E and vitamin C were significantly lower among the cases than the controls [14]. Radhakrishna Pillai found decreased levels of β-carotene in patients with oral cancer [15]. These results point in the direction of a causal association between β-carotene level and OSCC [16]. Low levels of β-carotene may be an important co-factor in the process of carcinogenesis [4]. Therefore, possibly, β-carotene evaluation can be incorporated in the array of tests which are done for diagnosis of oral cancer and as an adjunct to other tumour markers.

CONCLUSION

The results of the present study are encouraging and they substantiate the concept that low levels of β-carotene may be one of the important factors which is involved in the causation of the OSCC, in association with other contributing factors. However, since the sample size in the present study was small, further studies which are done on a larger sample size are recommended, for replication of these findings. Such studies can increase the likelihood that this association is real. Nonetheless, at present, our data strongly suggest the usefulness of β-carotene estimation as a diagnostic tool in the diagnosis of OSCC and they also encourages a higher intake of dietary β-carotene and β-carotene-containing supplements as a preventive measure against OSCC.

Future scope: Further research can be directed at assessing the variations in serum β-carotene levels in oral potentially malignant lesions and in different grades of OSCC, which may be used as a valuable tool in early diagnosis and prognosis of OSCC.

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