RESEARCH COMMUNICATION

A Study of Various Sociodemographic Factors and Plasma Vitamin Levels in Oral and Pharyngeal Cancer in Gujarat, India

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Abstract

Present study examined various socio-demographic factors, dietary patterns, habit of tobacco consumption and plasma vitamin levels in 56 healthy individuals, 146 patients with oral precancerous conditions (OPC) and 132 untreated oral and pharyngeal cancer patients. The subjects were interviewed with a detailed health, habit and diet questionnaire. Plasma β-carotene, vitamin-A and vitamin-E levels were determined spectrophotometrically. An increased incidence of OPC was observed in the age group of <30 years which was associated with tobacco chewing. Whereas, incidence of cancer was in the age group of 30-60 years where habit of tobacco smoking was more prevalent. Majorities of the subjects were from rural area, poor, unaware about association of diet with cancer. The body mass index was lower (p=0.045) in patients with OPC and cancer patients as compared to the controls. Plasma β-carotene and vitamin-E levels were lower in patients with OPC (p=0.000 and 0.031, respectively) and untreated cancer patients (p=0.000 and 0.071, respectively) than the controls. ROC curve revealed that plasma vitamin levels have ability to discriminate between controls and cancer patients. Lower plasma β-carotene and vitamin-E levels were observed in tobacco consumers as compared to non-consumers. Odds ratio revealed that controls and patients with OPC having tobacco habit and lower plasma levels of β-carotene were at a higher risk (p<0.05) of developing cancer. Regression study and Analysis of Variance revealed that plasma β-carotene levels were inversely associated (r^2=0.14, p=0.001 and F=0.000, respectively) with increase in the stage of cancer. The data provide interesting clues of potential role of diet, tobacco habits, socio-demographic status and plasma vitamin levels in etiology of oral and pharyngeal cancer in Gujarat, where no such findings are reported.

Key Words: dietary factors - oral and pharyngeal cancer - plasma vitamins - oral precancerous conditions - sociodemographic factors - tobacco habits

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Introduction

The incidence of oral cavity and pharyngeal cancer has shown increasing trend world wide, as have the mortality rate for patients with these malignancies (Blot et al., 1994). Oral cavity cancer is one of the 10 most frequent cancers globally (Parkin et al., 1993). In India, approximately 30-40% of all cancer cases are from oral cavity in origin, which are much higher as compared to Western world (Jayant and Notani, 1991). As estimated by WHO, 90% of oral cancer cases among Indian men are attributable to tobacco consumption (Bull WHO, 1984). Oral cavity and pharyngeal cancer account for about 56.9% of tobacco related cancers at The Gujarat Cancer & Research Institute, Ahmedabad,

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India (Annual Report, 1995).

Oral carcinomas are commonly preceded by premalignant lesions like leukoplakia and submucous fibrosis (Paterson et al., 1996). Therefore, these oral precancerous conditions (OPC) can serve as a good model for investigating carcinogenesis of oral cavity. In India, tobacco and betel nut use is accepted as one of the few unequivocal risk factors for development of OPC and oral cavity cancer (Nandakumar et al., 1990). Dietary antioxidants prevent damage to the mucosa of oral cavity and pharynx due to oxidative stress resulting from tobacco consumption. Recent reports and monographs have also documented protective effects of antioxidant micronutrients (Steinmetz and Potter, 1991 [I]; World Cancer Fund, 1997; Steinmetz and Potter, 1991 [II]). The importance of vitamins in cancer biology, stems primarily from the fact that they act as antioxidants which if present in sufficient amount may prevent cancer (Stich et al., 1991; Benner et al., 1993; Knekt et al., 1988). As a group, cancer patients are more likely to be malnourished and with certain cancers such as upper aerodigestive tract, majorities of them are more so at the time of diagnosis (Nixon et al., 1980). Hence, in addition to tobacco use, factors such as dietary deficiency of particular vitamins such as β-carotene, vitamin-A, and Vitamin-E are thought to predispose individuals to oral cavity cancer (Palan et al., 1996; Zeigler, 1986). Previous reports have described the benefits of carotenes, vitamin-A and vitamin-E in reducing risk of cancer or precancerous lesions in epithelial tissues (Peto et al., 1981; Zeigler, 1989; Ibrahim et al., 1977). The relation between serum concentration of β-carotene, retinol and α-tocopherol with development of cancer is recognised during last two decades (Palan et al., 1996; Wald et al., 1980; Zheng et al., 1993).

If detected earlier, survival of oral cavity and pharyngeal cancer patients can be dramatically increased and hence can reduce mortality due to it. Although the analysis of diet through food is strongly recommended for public health purpose, the study could be more advantageous if compared with analysis of tobacco consumption, mainly through better understanding of mechanism of diseases. Many studies from the Western countries in the past have put emphasis on serum levels of micronutrients and tobacco consumption with subsequent risk of development of oral cavity cancer but not much is known about this among Indian population. Epidemiological data indicate that ethnic and racial groups differ significantly in terms of cancer incidence and mortality rate (Perera, 1997). These geographic differences relate to the incidence rates of these cancers, with demographic, socio-educational and occupational differences or food and tobacco consumption habits. As etiology of cancer in Indian population is different, information of type and duration of tobacco consumption, type of food can provide distinctive data to address association of plasma vitamin levels with oral carcinoma. In a large group, study focused on early onset of oral cavity cancer, we evaluated the influence of socio-demographic status, habit of tobacco as well as plasma β-carotene, vitamin-A and vitamin-E levels in oral and pharyngeal cancer.

Materials and Methods

Subjects

Cancer patients: The study enrolled 132 untreated patients with oral cavity and pharyngeal cancer from the out patients' department of The Gujarat Cancer and Research Institute, Ahmedabad. Clinical details of the patients were gathered from the case files. The diagnosis of the patients was based on their clinical examinations, radiological findings and histopathological reports. Staging of the disease was done according to UICC classification (UICC, 1987). Clinical details of cancer patients are shown in Table 1. Around 19% of the patients had early disease (stage I & II) while 77% of the patients had advanced disease (stage III & IV). Out of 132 untreated cancer patients, 77 (58%) had oral cavity cancer and 55 (42%) had pharyngeal cancer. The patients were also categorized according to tumour differentiation. 26% of the patients had well differentiated carcinoma, 55% had moderately differentiated carcinoma, 14% had poorly differentiated carcinoma and 2% had undifferentiated carcinoma. Histopathologically, 97% of the patients had squamous cell carcinoma. 52% of the patients were positive for nodal invasion.

Patients with OPC: To assess the parameters in premalignant diseases, 146 patients with OPC were included

Table 1. Clinical Details of Cancer Patients

Variable	Sub-class	Observations
Age (in years)	Median	50
	Range	20-80
Gender	Male	115 (87.1%)
	Female	17(12.9%)
Clinical Stage	I	8 (6.06%)
	II	17(12.87%)
	III	21(15.9%)
	IV	81(61.4%)
	Unknown	5(3.8%)
Clinical Classification	T1	14 (10.6%)
	T2	34(25.8%)
	T3	43(32.6%)
	T4	37(28.0%)
	Unknown	4(3.0%)
Tumor Differentiation	Well	34 (25.76%)
	Moderate	72(54.55 %)
	Poor	19 (14.39%)
	Undifferentiated	3(2.28%)
	Unknown	4(3.02%)
Histopathological Examination	Squamous Cell Ca.	128(96.97%)
	Others	4(3.03%)
Lymphnode Involvement	Yes	69(52.3%)
	No	34(25.7%)
	Unknown	29(22.0%)

from the Government Dental College & Hospital, Civil Hospital Campus, Ahmedabad. 69% of the patients had oral submucous fibrosis, 27% had oral leukoplakia and 4% represented with other disease like verrucous hyperplasia and dysplasia.

Controls: 56 first degree (father, mother, brother, sister etc.) relatives of the patients without history of any illness in recent past were included as controls. The controls were age and sex matched.

Health, Habit and Diet Questionnaire

All the subjects were interviewed face to face using a health, habit and diet questionnaire designed specially for this study. The questionnaire included socio-demographic details, medical information, occupational details, family history of cancer, habit of tobacco consumption and detailed dietary patterns (through food-frequency questionnaire including over fifty food items). Details regarding habit of tobacco consumption included type (chewing, smoking, or snuff), duration and frequency. The history on dietary habits included information regarding food (vegetarian, mixed, non-vegetarian), food type (boiled, oily), food taste (non-spicy, moderate, spicy), nutritional supplementation, amount of oil and ghee consumed and food-frequency details. The food-frequency details also included food items in terms of staple food (wheat, rice, maize, bajra, pulses, cereals, legumes, and sprouts), frequency of food rich or good sources of vitamins like green leafy vegetables (spinach, shepu, fenugreek etc.), cruciferous (radish, carrot, cabbage, cauliflower, colocasia, tomato), fruits and fruit juices (Indian gooseberry, lemon, orange, apple, mango, grapes, papaya, banana, guava and others), nuts (ground-nuts, cashew-nuts, almond, pista, grape-fruit, walnut), milk and milk products (curd, butter milk, cheese etc.) and non-vegetarian items (egg, meat, fish, chicken, pork and beef). Though, it was a time consuming task, which required many trained workers, the subjects were convinced, and the information were gathered skillfully.

Sample Collection

Fasting blood samples were collected into foiled vials containing EDTA as an anticoagulant. Prior consent was obtained from all the subjects. The blood samples were centrifuged, plasma were collected into foiled vials and stored at -80^{o}C until analysed. To avoid exposure to light, all the procedures including, collection, separation and storage of the samples were carried out under dark conditions. The samples were analysed in duplicates.

Table 2. Socio-Demographic Details of the Subjects

DETAILS	GROUPS	CONTROLS (n=56)	OPC (n=146)	CANCER PATIENTS (n=132)
AGE (in years)	16-30	25(44.6%)	78(53.4%)	06(4.5%)
	31-45	24(42.9%)	49(33.6%)	48(36.4%)
	46-60	06(10.7%)	16(10.9%)	62(46.9%)
	61-75	01(1.8%)	03(2.1%)	16(12.1%)
MEDIAN AGE	in years	32.5	30.0	50.0
GENDER	Male	40 (71.4%)	142 (97.3%)	115 (87.1%)
	Female	16 (28.6%)	4 (2.7%)	17(12.9%)
AREA	Urban	31 (55.4%)	122(83.6%)	46 (34.8%)
	Rural	25 (44.6%)	24 (16.4%)	86 (65.2%)
EDUCATION	Uneducated	4 (7.1%)	22 (15.1%)	41 (31.1%)
	Under graduates	33 (58.9%)	117 (80.0%)	86 (65.2%)
	Graduates	19 (33.9%)	7 (4.8%)	05 (3.7%)
MONTHLY INCOME (in Rupees)	<1000	28(50.0%)	63 (43.2%)	105 (79.5%)
	1000-2000	9 (16.1%)	40 (27.4%)	8 (6.1%)
	2000-5000	9 (16.1%)	32 (21.9%)	15 (11.4%)
	>5000	10 (17.8%)	11 (7.5%)	4 (3.0%)
EXPOSURE TO CHEMICALS	No	36 (64.3%)	105 (71.9%)	75 (56.8%)
	Yes	20 (35.7%)	41(28.1%)	57 (43.2%)
BMI (Kg/m^2)	<20	25 (44.6%)	75 (51.2%)	73 (55.3%)
	20-25	22 (39.3%)	53 (36.2%)	54 (40.9%)
	26-30	07 (12.5%)	14 (9.4%)	05 (3.8%)
	>30	02(3.6%)	04(3.1%)	-
MEAN BMI		21.18±0.811	20.76±0.342	19.79±0.327 (p=0.045)*

* As compared to the controls; OPC- patients with oral precancerous conditions; BMI- Body mass index

Table 3. Details Regarding Habit of Tobacco Consumption

DETAILS		CONTROLS (n=56)	OPC (n=146)	CANCER PATIENTS (n=132)
TOBACCO	NO	31 (55.4%)	3 (2.1%)	10 (7.6%)
HABIT	YES	25 (44.6%)	143(97.9%)	122 (92.4%)
TYPE OF	SMOKING	5 (8.9%)	21 (14.4%)	57 (43.2%)
TOBACCO	CHEWING	15 (26.8%)	81 (55.5%)	32 (24.2%)
HABIT	BOTH	4 (7.1%)	39 (26.7%)	26 (19.7%)
	SNUFF	1 (1.8%)	2 (1.4%)	7 (5.3%)
	<5	-	11 (18.3%)	5(6.0%)
SMOKING	5-9	2(22.2%)	7 (11.7%)	3(3.6%)
DURATION	10-19	1 (11.1%)	13 (21.7%)	14(16.9%)
(in years)	≥20	6 (66.7%)	29 (48.3%)	61 (73.5%)
	<5	3 (15.8 %)	43 (35.8%)	6 (10.3%)
CHEWING	5-9	6 (31.6%)	36 (30.0%)	12 (20.7%)
DURATION	10-19	7(36.8%)	28 (23.4%)	18 (31.0%)
(in years)	≥20	3 (15.8%)	13 (10.8%)	22(38.0%)
	<5	-	1 (50.0%)	1 (14.3%)
SNUFF	5-9	-	-	1 (14.3%)
DURATION	10-19	-	-	2 (28.6%)
(in years)	≥20	1 (100%)	1(50.0%)	3 (42.8%)

OPC - Oral precancerous condition, BOTH – Tobacco smoking and chewing both

Assays

β-Carotene and Vitamin-A: β-carotene and vitamin-A were estimated from plasma as described by Dugan et al. (1976). Briefly, proteins were precipitated out using ethanol. Retinol and carotenes were extracted in petroleum ether. Intensity of yellow colour was read at 450 nm which directly correlated to the amount of β-carotene. Petroleum ether was evaporated and the amount of blue colour produced after adding trifluoroacetic acid, was read at 620 nm (for vitamin-A) within 15 to 20 seconds. β-carotene (Merck, Germany) and retinol (Sigma chemicals, U.S.A.) were used as standards.

Vitamin-E: Vitamin-E was estimated spectrophotometrically as described by Baker and Frank (1968). Plasma proteins were precipitated using ethanol. Tocopherol and carotene were extracted in xylene and the absorbance was read at 460 nm to measure total carotenes levels. α'-bipyridyl and ferric chloride were added, the intensity of colour was read at 520 nm. Vitamin-E (α-tocopherol) purchased from Merck, India, was used as standard. Vitamin-E concentrations were calculated using following formula:

$$\frac{(\text{O.D. of sample at 520 nm - O. D. of sample at 460nm} * 0.29\ ) * \text{Standard Concentration}}{\text{O.D of standard at 520 nm}}$$

Statistical Methods

Statistical analysis was carried out using windows based SPSS (version 6.0). For statistical analysis $p<0.05$ was considered statistically significant. 't' test was performed for comparison of marker levels between two groups of the subjects. 95% Confidence Interval (CI) was calculated for range of observations. Odds ratio were determined to examine the disease risk among the subjects with habit of tobacco. Receiver's Operating Characteristic (ROC) curves were plotted to check discriminatory efficacy of the markers between controls and cancer patients. To evaluate the association of plasma vitamin levels with the extent of malignancy, regression analysis was carried out. ANOVA test was also performed to examine variations in the mean plasma vitamin levels with stage of malignant disease.

Results

Distribution of the controls, patients with OPC and untreated oral and pharyngeal cancer patients with respect to socio-demographic characteristics and other confounding variables obtained from health, habit and diet questionnaire are provided in Table 2. The subjects were distributed into four different age groups. 53.4% of the patients with OPC were from 16-30 years age group (younger age group) while majorities of the cancer patients were from age group 31-60 years. Study included more than 70% males in all groups of subjects. Out of 334 subjects, 59.6% were from urban area and 40.4% were from rural area. Most of the subjects received only primary education. 71% of the subjects were from low-income group (<Rs. 2000 per month). Body mass index (BMI, ratio of weight to height) was calculated for all the subjects and they were categorized into four different

groups. As shown, 51% of patients with OPC and 55% of cancer patients had BMI below normal value (BMI<20Kg/m^2). Mean BMI level was lower in patients with OPC and untreated cancer patients as compared to the controls. Difference in mean BMI levels between controls and cancer patients was statistically significant with p= 0.045.

Details regarding habit of tobacco consumption are shown in Table 3. Approximately 45% of the controls, 98% of the patients with OPC and 92% of the patients with oral and pharyngeal cancer were found to have habit of tobacco consumption in one or another form. 27% of the controls, 56% of the patients with OPC and 24% of the cancer patients were having habit of tobacco chewing, while 9% of the controls, 14% of the patients with OPC and 43% of the cancer patients were smokers. 20% of the patients with OPC and untreated cancer patients and 7% of controls were found to have habit of both tobacco chewing as well as smoking. 1.4% of subjects had habit of snuff, which was more common among females. The cancer patients were found to have habit of tobacco on an average for more than 10 years.

Figure 1 shows the mean plasma levels of β-carotene, vitamin-A and vitamin-E in controls, patients with OPC and untreated oral and pharyngeal cancer patients. A decrease in the plasma levels of β-carotene in patients with OPC was observed when compared to the controls. Further, there was a significant decrease in plasma β-carotene values in cancer patients as compared to the patients with OPC (p=0.000) as well as healthy individuals (p=0.000). Mean plasma vitamin-E levels were lower in patients with OPC and untreated oral and pharyngeal cancer patients as compared to the controls (p=0.031, p=0.071, respectively). However, mean plasma vitamin-E levels in patients with OPC and in untreated oral and pharyngeal cancer patients were comparable. Patients with OPC and untreated oral and pharyngeal cancer patients showed higher plasma vitamin-A levels than the controls. However, the levels were comparable between patients with OPC and untreated oral and pharyngeal cancer patients.

Table 4a shows comparison of plasma vitamin levels with 95% confidence intervals between tobacco consumers and

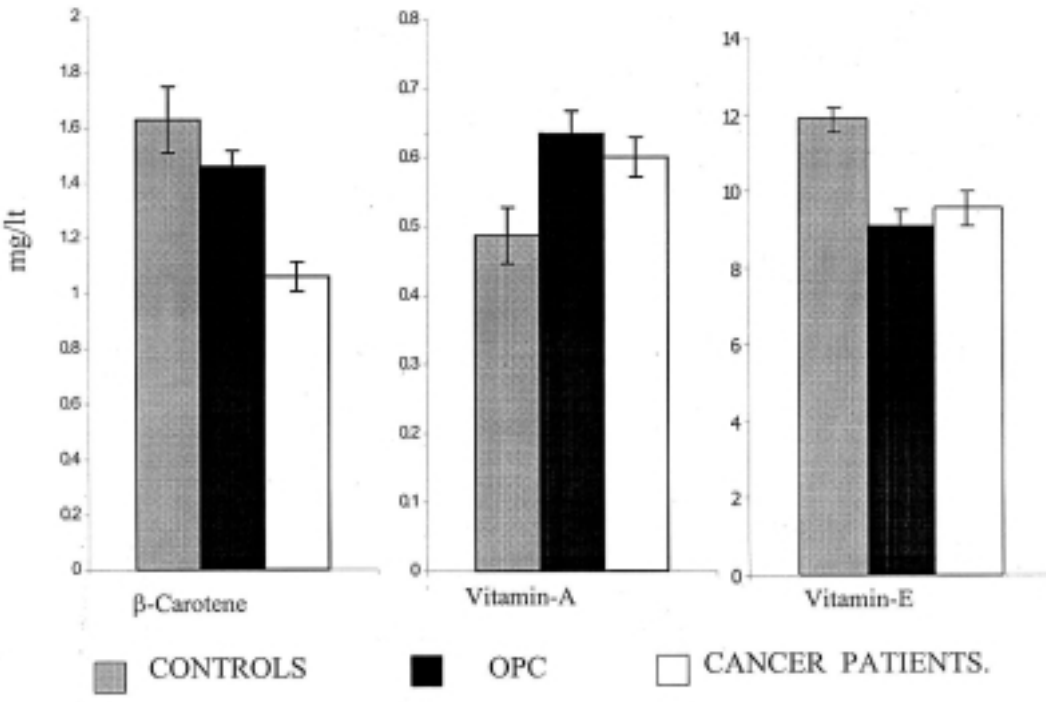

Figure 1. Bar Graph Showing Plasma Levels of β-Carotene, Vitamin-A and Vitamin–E in Controls and Patients

Statistical significance:

COMPARISON	β-CAROTENE		VITAMIN-A		VITAMIN-E	
	't' value	'p' value	't' value	'p' value	't'value	'p' value
Controls vs. OPC	1.29	0.198	1.95	0.054	2.18	0.031
Controls vs. Ca. Pts.	4.75	0.000	1.71	0.090	1.83	0.071
OPC vs. Ca. Pts.	5.08	0.000	0.078	0.438	0.73	0.466

OPC – Oral precancerous Conditions
Ca.Pts – Cancer patients

Table 4a. Habit of Tobacco and Vitamin Levels in Controls, Patients with OPC and Untreated Oral and Pharyngeal Cancer Patients

Groups		β-Carotene MEAN±S.E (95%C.I.)	Vitamin-A MEAN±S.E (95%C.I.)	Vitamin-E MEAN±S.E (95%C.I.)
Controls	NHT	1.73 ± 0.23 (1.29 – 2.17)	0.40 ± 0.09 (0.28 – 0.52)	12.17 ± 0.55 (11.08 – 13.26)
	WHT	1.55 ± 0.12 (1.31 – 1.79)	0.55 ±0.05 (0.45–0 .65)	11.53 ± 0.25 (11.03 – 12.04)
OPC	WHT	1.46 ± 0.05 (1.35 – 1.58)	0.64 ± 0.03 (0.57 – 0.70)	09.08 ± 0.43 (08.23 – 9.93)
Cancer PTS.	WHT	1.03 ± 0.05 (0.93 – 1.13)	0.63 ± 0.03 (0.55 – 0.68)	09.84 ± 0.46 (08.93 – 10.75)

Table 4b. Odds Ratio for Comparison of Vitamin Levels Between Tobacco Consumers in Controls and Patients

Comparisons	β-Carotene	Vitamin-A	Vitamin-E
WHT vs. NHT (Controls)	3.20	0.50	1.33
OPC vs Controls*	1.48	0.63	3.26
Cancer PTS. vs. Controls*	4.15**	0.60	2.70
Cancer PTS. vs. OPC*	2.80**	0.95	0.80

*OPC vs. Controls, Ca. Pts. vs.Controls, Ca. Pts. vs. OPC comparisons are between the tobbaco consumbers of respective group.
** P<0.05
OPC - Oral precancerous condition; NHT - No habit of tobacco
WHT - With habit of tobacco; C.I - Confidence interval
CANCER PTS. - Cancer patients; Values expressed as mg/lt.

nonconsumers. A decrease in the mean plasma levels of β-carotene and vitamin-E was found in healthy individuals having habit of tobacco consumption, patients with OPC as well as untreated oral and pharyngeal cancer patients when compared to the controls without habit of tobacco consumption. 95% confidence intervals are also provided in the table to indicate the lower and upper limits of the observations. The Odds ratio was determined to examine the disease risk in the subjects. It is clear from Table IVb that the controls and patients with OPC having habit of tobacco as well as lower plasma β-carotene levels were at a higher risk of developing cancer (OR = 4.15, 2.8, respectively, p<0.05).

Figure 2 shows ROC curves between controls and cancer patients. ROC curve is a more meaningful statistical method because it simultaneously considers both sensitivity and specificity of the parameters to discriminate between the groups under comparison. The ROC curves of β-carotene, vitamin-A and vitamin-E were within acceptable range with a good discriminatory efficacy between controls and cancer patients.

Figure 3 shows the regression curve for β-carotene with respect to the stage of disease. Plasma β-carotene levels showed inverse association with extent of malignant disease (r^2= 0.141, p= 0.001). The plasma vitamin-A and vitamin-E levels did not show any association with stage of the disease (The regression curves for vitamin-E and vitamin-A are not shown). Figure as well as Table 5a and Table 5b (ANOVA 'F'= 0.000), clearly indicate that a significant decrease in plasma β-carotene levels was observed with increase in the extent of disease i.e. from stage I to stage IV.

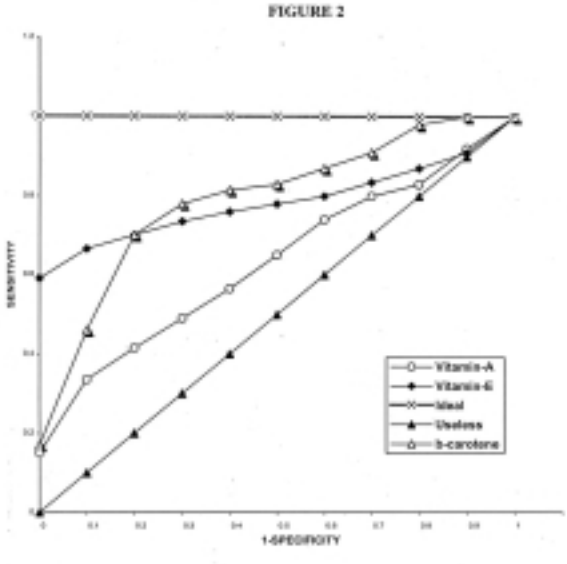

Figure 2. Receiver's Operating Characteristic (ROC) Curves: Controls Versus Cancer Patients

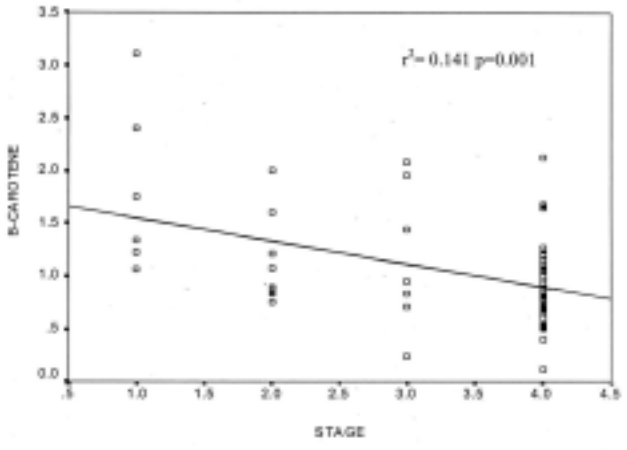

Figure 3. Association of Plasma Vitamin Levels with the Extent of Malignant Diseases

Table 5a. Statistical Significance of Regression Analysis

PARAMETER	r^2	'p' VALUE
β-CAROTENE	0.141	0.001
VITAMIN-A	0.022	0.225
VITAMIN-E	0.003	0.649

Table 5b. Vitamin Level Vetsus. Stage of the Disease: Anova Table

PARAMETER	'F'VALUE	SIGNIFICANCE OF 'F'
β-CAROTENE	7.238	0.000
VITAMIN-A	1.124	0.346
VITAMIN-E	1.874	0.139

Observations on food habits of the subjects (derived from Health, habit and diet questionnaire analysis)

Several striking observations regarding dietary habits of the subjects obtained from analysis of Health, habit and diet questionnaire were as follows: More than 80 % of the subjects consumed moderate to spicy food. Green leafy vegetables/cruciferous, the rich source of β-carotene and vitamin-E were consumed only 1-2 times per week in inadequate quantities. Consumption of carrots and tomatoes, the rich source of β-carotene and vitamin-A was also irregular and inadequate. Potatoes, brinjal, onions, cabbage, lady's finger etc. were frequently consumed, due to low cost and easy availability of these vegetables. It was also observed that around 80% of the subjects were unaware of the association between dietary habits and cancer. Data of dietary habits of current population clearly indicated that diet was not balanced, and lacking in several essential micro-nutrient sources due to the consumption of cereals, pulses, green leafy vegetables, cruciferous vegetables, fruits, milk and milk products etc. in inadequate amounts and not on regular basis.

Discussion

The study of various etiological factors in oral and pharyngeal cancer is of prime importance in reducing cancer incidence. Tobacco consumption in any form is believed to be a potential etiological factor for development of oral and pharyngeal cancer. Etiology of tobacco related cancers, such as oral and pharyngeal cancer, in Indian population is different from that in Western population. Tobacco chewing, bidi/cigarette smoking, reverse smoking, snuffing, etc. are common in Indian population whereas, cigarette smoking is more prevalent in Western countries. In current study, we have gathered details regarding duration and type of tobacco habit among healthy individuals, patients with OPC and patients with oral and pharyngeal cancer. Majority of the patients with malignancy had history of tobacco consumption on an average for more than 10 years. The individuals with OPC such as oral leukoplakia and submucous fibrosis are more likely to develop cancer later on (Paterson et al., 1996). Cox and Walker have reported that oral submucous fibrosis affects approximately 2.5 million people per year, in Indian subcontinent (Cox, 1996). Further, Gupta et al. have reported that oral precancerous lesions are more prevalent in the younger (below 35 years) age group (Gupta et al., 1998). In the current study, we found that more than 80% of patients having OPC were from age range between 16-30 years. Further, we also found that tobacco chewing was frequently associated with oral precancerous diseases whereas, smoking habits, in addition to chewing, were more common in the oral and pharyngeal cancer patients.

Various micronutrients are reported to play a preventive role against cancer. Hence, the diet plays an important role in the etiology of cancer. Importance of dietary intake of vitamins and their role as anti-carcinogenic agents has been reported by several investigators (Peto et al., 1981; Zeigler 1989; Ibrahim et al., 1977). Dietary sources rich in carotene (mainly β-carotene), vitamin-A and tocopherols are believed to provide a protective effect against cancer. It is essential to study the role of diet as well as other factors like socio-demographic details, tobacco habits and their effects on the development of cancer in Indian population. Poor nutritional status together with habit of tobacco consumption poses a major risk factor for developing cancer. It has been reported that diet of the rural population of India is not sufficient and lacks most of the essential nutrients (Vijayraghavan and Rao, 1998). There are several reports in which role of diet among OPC (oral leukoplakia, oral submucous fibrosis) as well as oral and pharyngeal cancer has been individually studied among Indian population (Gupta et al., 1998; Gupta et al., 1999; Rao and Desai, 1998). However, to the best of our knowledge there is no report from Gujarat where majority of the population is vegetarian and habit of tobacco consumption is more prevalent. Further, in this region 34 % cancer cases are of oral cavity and pharynx (GCRI Registry, 1995) Majorities of the subjects in current study were from economically poor group and did not consume green leafy vegetables and fruits on regular basis. In their diet, the vegetables frequently used were potatoes, brinjal, onions etc., which are cheap in price and easily available. Further, majority of the subjects were unaware of the importance of proper dietary habits and its association with cancer as they were only primarily educated. As reported by Rao et al, illiterates had 50-60% excess risk for pharyngeal cancer (Rao et al., 1999). The lack of balanced diet and poor economic status is suggestive of poor nutritional status among population of Gujarat, India.

Various epidemiological studies from other parts of India and world have reported that dietary intake of β-carotene, vitamin-A, vitamin-E and blood levels of these micronutrients are inversely associated with the risk of cancer (Palan et al., 1996; Zheng et al., 1993; Stahelin et al., 1991; Chitkara et al., 1996). Assessment of serum/plasma micronutrient status is more reliable and biologically meaningful than dietary estimates of nutrients, as plasma levels reflect the dietary intake, absorption, utilization and

other metabolic aspects including depletion of serum/plasma and tissue nutrients due to oxidative stress (Handelmann et al., 1996). Our data revealed a decline in the mean plasma levels of β-carotene and vitamin-E in the patients with OPC and untreated oral and pharyngeal cancer patients as compared to the controls, which are in agreement with previous studies (Chitkara et al., 1996; Peng et al., 1998). The current findings that the patients exhibited lower vitamin levels than the controls have been statistically strengthened by ROC curve analysis. The ROC curve revealed that the vitamin levels have efficacy to discriminate between controls and untreated cancer patients. The decrease in plasma levels of β-carotene and vitamin-E in cancer patients could be due to the possibility that they react very rapidly with molecular oxygen and free radicals, the role of which has been implicated in carcinogenesis (Chitkara et al., 1996). It is also suggested that vitamin-E acts as a scavenger and protects polyunsaturated fatty acids from peroxidation reactions in head and neck cancer (Lal et al., 1996). Findings from the two major trials; CARET (Omenn et al., 1996) and ATBC (The Alpha-Tocopherol, Beta Carotene cancer prevention study group, 1994), have reported that supplemental β-carotene alone or in combination with retinol appears to increase the risk of lung cancer particularly in current smokers. Further, Copper et al. (1999) have reported no difference in plasma vitamin-A levels in untreated oral and pharyngeal cancer patients as compared to the controls. In the current study, we have observed elevated levels of plasma vitamin-A in OPC and cancer patients as compared to the controls. However, plasma Vitamin-A levels were comparable between patients with OPC and untreated cancer patients. Nuclear retinoid receptors (RAR & RXR) are mediators of retinoid actions and it is reported that retinoic acid receptor-β (RAR-β) is down regulated in premalignant and malignant head and neck cancer tissues (Xu et al., 1994). This might be the reason for higher plasma vitamin-A levels in the patients of our study group.

Tobacco along with poor nutritional status may cause a synergetic effect and pose higher risk of malignancy. The decrease in the mean plasma levels of β-carotene and vitamin-E in the subjects having tobacco habit when compared to the subjects with no habit of tobacco consumption has been reported by various investigators (Lee et al., 1998; Ramaswamy et al., 1996; Stryker et al., 1988). In the current study, we also found lower levels of β-carotene and vitamin-E among tobacco consumers then those who were not consuming tobacco. Odds ratio analysis revealed that habit of tobacco consumption and lower plasma β-carotene were significantly associated with higher risk for cancer in healthy individuals and patients with OPC. The decline in the plasma vitamin levels in the individuals having habit of tobacco consumption may be due to their aberrant dietary habits and increased demand of these nutrients due to tobacco habit. The increased free radical load due to tobacco habit is reported, which in association with lower vitamin intake may shift the normal free radical-vitamin balance in the body, initiating the process of deterioration, which thereby may increase the risk of cancer (Faruque et al., 1995).

Chitkara et al. (1996) reported that the decrease in plasma vitamin-E concentrations is directly related with the increase in stage of breast cancer. In the present study, regression analysis revealed that there was negative correlation between the mean plasma levels of β-carotene (p=0.000) with extent of malignant disease. The results were strengthened by ANOVA analysis. There was a significant decrease in mean levels of β-carotene with the increase in the stage of the malignant disease (F=0.000).

In conclusion, our study clearly indicates that habit of tobacco consumption, poor nutritional status and lack of awareness regarding association of diet with malignancy are major risk factors in the etiology of oral and pharyngeal cancer in Gujarat. Individuals with habit of tobacco consumption had lower levels of β-carotene and vitamin-E, which might increase the risk for developing cancer. Both malignancy and tobacco consumption may have caused the lower level of β-carotene and vitamin-E. The results are in agreement with various studies from various other countries. Increased intake of β-carotene and vitamin-E may prevent the formation of precancerous lesions, induce their remission or inhibit the progression of precancerous lesions to malignant disease. The trends provide interesting clues to the causes and prevention of oral and pharyngeal cancer whose etiology is not fully understood. The current investigation clearly warrants more studies in this region and attention of the researchers and policy makers.

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