RESEARCH COMMUNICATION

Vitamin B_{12} and Folate Status in Head and Neck Cancer

Gira N Raval[1], Rachana N Sainger[1], Rakesh M Rawal[1], Jayendra B Patel[1], Beena P Patel[1], Franky P Jha[1], Devendra D Patel[2], Prabhudas S Patel[1]

Abstract

Deficiency of vitamin B_{12} and folate is associated with causation of certain precancerous conditions and cancer. The present study was carried out on 56 controls, 167 patients with oral precancerous conditions (OPC) and 214 head and neck cancer patients, to evaluate the plasma vitamin B_{12} and folate levels to determine their association with tobacco habits and vegetarianism and several sociodemographic factors. The subjects were interviewed using a health habit and diet questionnaire at the time of blood collection. Simultaneous estimations of plasma vitamin B_{12} and folate were done by Dual Count Radioassay. It was found that the habit of tobacco consumption, lower education and low income were among the risk factors. A decrease in the plasma vitamin B_{12} and folate levels with respect to tobacco habits, disease progression, and vegetarian diet was also observed. The individuals in the ower quartile for vitamin B_{12} and folate were at a higher risk of developing OPC, as compared to those in higher quartiles. Similarly, the patients with OPC in lower quartiles were found to be at a higher risk of developing cancer than their counterparts. There was a significant positive correlation between vitamin B_{12} and folate levels in the subjects consuming tobacco, and more so in patients with OPC (r=0.4330, p=0.000). Folate levels were significantly lower in patients with advanced as compared with early disease (ANOVA p=0.006 and Spearman's Rho = -0.211 and p=0.01). The results suggest, potential significance of plasma vitamin B_{12} and folate levels in head and neck malignancies which needs to be confirmed by further studies on a large population.

Key Words: Cancer - folate - vitamin B_{12} - head and neck - leukoplakia - submucus fibrosis - tobacco

Asian Pacific J Cancer Prev, **3**, 155-162

Introduction

Head and neck cancer is one of the 10 most frequent malignancies worldwide with an annual incidence of around 6,15,800 cases (Parkin et al., 2001). The highest incidence of head and neck cancer has been generally observed in South Asian countries, with almost no improvement in mortality from this cancer during last three decades. In India, head and neck cancer still remains a major public health hazard, where habit of tobacco consumption and other life style factors are considered major etiologic factors. At The Gujarat Cancer & Research Institute, head and neck cancer is the leading malignancy (Annual report, 1997) .

Diet is considered to be a major contributory factor in the etiology of cancer (Levi et al., 2001; Munoz et al., 2001; Winn et al., 1984). It is believed that dietary components influence individuals' susceptibility to cancer. Among these, micronutrients and antioxidant vitamins have drawn considerable interest among the researchers. Several studies have reported that vitamin B_{12} and folate have a significant role in chemoprevention (Eto and Krumdieck, 1986; Butterworth et al., 1992; Kamei et al., 1993; Heimburger et al., 1988; Choi and Mason, 2000). The habit of tobacco consumption is an established risk factor in the causation of head and neck cancer (Rassekh 2001; Johnson 2001; Banoczy et al., 2001). Earlier studies have documented alterations in circulating levels of vitamin B_{12} and folate in humans due to the habit of tobacco smoking or chewing (Piyathilake et al., 1994; Khaled et al., 1986; Hecht, 1999).

Tobacco contains nearly 50 known carcinogens apart

[1]Biochemistry Research Division, Department of Cancer Biology, [2]Chief, Department of Surgical Oncology (Speciality Clinic) & Director, The Gujarat Cancer & Research Institute

Reprint Request and Correspondence to: Dr. Prabhudas S. Patel, In-charge, Biochemistry Research Division, The Gujarat Cancer & Research Institute, Asarwa, Ahmedabad-380 016, INDIA. Phone: ++91 79 2681451 (Ext. 303) Fax: ++91 79 2685490 Email: gcriad1@sanchar.net.in, prabhudas_p@hotmail.com

from other harmful compounds. These carcinogens can induce cancer via generation of free radicals and reactive oxygen species, formation of DNA adducts and causing activation of oncogenes and/or deactivation of certain tumor suppressor genes (Hecht, 1999). Low systemic levels of vitamin B_{12} and folate are found to be associated with an increased risk of cancer and precancers in the epithelial tissues (Eto and Krumdieck, 1986; Sankaranarayanan et al.,1989). The deficiency of vitamin B_{12} and folate leads to impaired synthesis, repair and methylation of DNA, which further causes hypomethylation thereby activating certain oncogenes (Choi and Mason, 2000; Blount et al., 1997). Decreased plasma vitamin B_{12} and folate levels are reported due to certain exogenous compounds like tobacco smoke (Khaled et al., 1986). Tobacco components like cyanates and isocyanates have been hypothesized to act as antagonist of vitamin B_{12} and folate and inhibit their biological activity (Piyathilake et al., 1994; Giovannucci et al.,1993). Apart from these, other antagonists and inhibitors are also reported to reduce plasma levels of vitamin B_{12} and folate. It has been repeatedly shown by several investigators (Dastur et al.,1972) that types of diet consumed also affect circulating vitamin B_{12} and folate levels, in addition to habit of tobacco consumption, however; clear conclusions have not been drawn.

Head and neck cancer is often preceded by precancerous conditions like oral submucus fibrosis and oral leukoplakia. It has been reported that oral leukoplakia show malignant transformation rate, ranging from 0.13% to 6% and rising to 14% or higher when dysplasia is present (Tradati et al., 1997). Earlier reports have suggested role of vitamin B_{12} and folate supplementation in the treatment of precancerous states like cervical dysplasia and bronchial metaplasia (Butterworth et al.,1992; Kamei et al.,1993). Ramaswamy et al., (1996) have reported low levels of vitamin B_{12} and folate in oral leukoplakia. Several investigators have suggested that deficiency of folate enhances development of preneoplastic conditions and cancer, which are suppressed by folate supplementation (Eto and Krumdieck, 1986). Heimburger et al., (1988), have also reported that simultaneous administration of vitamin B_{12} and folate, significantly decreased atypical cells in the sputum of cancer patients.

These facts including, rising incidence and mortality rates of head and neck cancer, increasing use of tobacco as well as paucity of reports on plasma vitamins and micronutrient levels, need a serious and urgent attention. Therefore, the present study evaluated plasma vitamin B_{12} and folate status and its association with the habits of tobacco consumption and dietary pattern (vegetarianism vs. non-vegetarianism) in patients with oral precancerous conditions (OPC) and head and neck cancer patients.

Subjects and Methods

Subjects:

The patients were classified into two groups, cancer patients and patients with OPC.

Suspected cases of head and neck cancer were enrolled from the out patients' department of The Gujarat Cancer & Research Institute, Ahmedabad. Final diagnosis was made depending upon their routine investigations as well as clinical, radiological and histopathological findings. In all, 214 untreated head and neck cancer patients were included in the study. Clinical details of the cancer patients are provided in Table-1. The sites included were oral cavity (n=143), pharynx (n=58), larynx (n=11) and maxilla (n=2). The tumors were classified according to the UICC norms (UICC, 1987). Histopathologically, 205 patients represented squamous cell carcinoma. Complete clinical details of a few patients were not available. There were more number of patients with advanced disease (stage III & IV) as compared to early disease (stage I & II).

One hundred sixty seven patients with OPC were enrolled from the Government Dental College and Hospital, Ahmedabad. Out of 167 patients with OPC, 106 patients had oral submucus fibrosis, 46 patients were having oral leukoplakia and 15 subjects represented other precancerous diseases. Fifty-six healthy individuals were also included in the study as the controls.

All the subjects were classified into two groups as: (i) with habit of tobacco (WHT) and (ii) no habit of tobacco (NHT).

Table 1. Clinical Details of Head and Neck Cancer Patients

Clinico-Pathologic Parameters		No. of Patients N=214
TNM Stage	I	8 (3.7 %)
	II	28(13.1%)
	III	31(14.5%)
	IV	124(58.0%)
	NA	23(10.7%)
Tumor Size	T1	18(8.4%)
	T2	52(24.3%)
	T3	48(22.4%)
	T4	70(32.7%)
	NA	26(12.2%)
Tumor Differentiation	Well	67(31.3%)
	Moderate	104(48.6%)
	Poor	25(11.7%)
	Undifferentiated	18(8.4%)
Histopathology	Squamous cell ca.	205(95.8%)
	Others	9(4.2%)
Nodal Status	Node negative	75(35.1%)
	Node positive	112(52.3%)
	NA	27(12.6%)

NA-Not Available

Health, Habit and Diet Questionnaire (HHDQ):

All the subjects were interviewed face to face using a HHDQ designed specially for this study. The information gathered through the HHDQ at the time of sample collection included socio-demographic details, habit of tobacco consumption, occupational details, family history of cancer, medical information and detailed dietary patterns. The information skillfully collected by trained researchers also included type, duration and frequency of tobacco consumption as well as food type (Vegetarian, non-vegetarian, boiled, moderate, oily), food taste, nutrient supplementation and food frequency details.

Blood Collection:

The blood samples were collected between 9 and 11 a.m. by venipuncture into foiled vials. Plasma were separated and stored at -80^{o}C.

Assays:

Estimations of vitamin B_{12} and folate from plasma samples were carried out using radioactive Dual Count Solid Phase No Boil Assay kit (obtained from Diagnostics Product Corporation (Louisiana, U.S.A). The assay was carried out according to manufacturer's instructions. The tracers used were I^{125} folate and Co^{57} B_{12}. Radioactivity was detected using a Gamma Counter. The plasma levels of vitamin B_{12} and folate were calculated using the standards provided with the kit.

Statistical Analysis:

Statistical analysis was performed using window based SPSS software (Version 10). Student "t" test was carried out to compare the mean values of plasma levels of vitamin B_{12} and folate. $p<0.05$ was considered statistically significant. One way Analysis of Variance (ANOVA) was used to compare vitamin B_{12} and folate levels in early and advanced malignant disease. Pearson's correlation coefficients of vitamin B_{12} and folate were also evaluated to find out the correlation between plasma vitamin B_{12} and folate levels in the subjects. Relative risk was estimated by computing Odds ratio and 95% confidence interval. Mann Whitney U test was used for non parametric comparison of transformed plasma vitamin levels into quartiles.

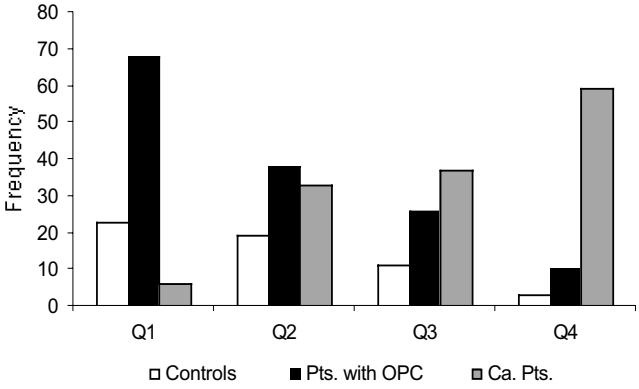


Figure 1. Age Distribution of the Subjects

Age Range
Q1 = 15 –32 Years
Q2 = 33 – 48 Years
Q3 = 49–64 Years
Q4 = 65 –80 Years

Pts. with OPC : Patients with Oral Precancerous Conditions
Ca Pts. : Cancer Patients

Results

HHDQ Analysis

Age Distribution of the Subjects:

Age distribution of the subjects included in the study is shown in figure–1. The subjects were divided into quartiles of age. Age distribution chart revealed that patients with OPC were predominant in the early age group of lower quartiles (Q1 and Q2), whereas, more number of cancer patients were observed in the late age group of higher quartiles (Q3 and Q4).

Odds Ratio Analysis :

The details obtained from HHDQ analysis were further analyzed and odds ratio were calculated to find out association of various sociodemographic factors with risk of cancer (tables 2A–2D). As evident from table–2A, the odds ratio were increased with the higher quartiles of age indicating increased risk of cancer with the increase in age. Odds ratio for education are provided in table–2B. The subjects were divided into three groups including: (i) uneducated (Q1), (ii) education upto 12th standard (Q2), (iii) more than 12th standard (Q3). As indicated in the table, higher education revealed protective effect on risk of cancer with decrease in odds ratio. Table–2C provides odds ratio for area of residence of the subjects. The subjects living in rural area showed higher risk of developing cancer. Evaluation of income as a risk factor is depicted in table–2D. The subjects were divided into four groups including: (i) <1000 Rs. (Q1), (ii) 1000 – 2000 Rs. (Q2), (iii) >2000 – 5000 Rs. (Q3) and (iv) >5000 Rs. (Q4). We found that the subjects in higher quartiles of income had lower risk of cancer.

Tobacco Habits of Subjects:

95.2% of the patients with OPC and 87.6% of the head and neck cancer patients were found to have the habit of tobacco consumption which is an established risk factor in the etiology of head and neck cancer. It was observed that chewing tobacco was the common mode of tobacco consumption in the patients with OPC (54%) while, smoking was common in cancer patients (43.6%). Among the controls, tobacco chewing habits were more common than smoking. Habit of both tobacco smoking and chewing were seen in about 28.3% of the patients with OPC, 20.74% of the cancer patients, and 14.28% of the controls (table–3A).

Table–3B shows the details of duration of tobacco habits

Table 2A-D. Odds Ratios for Various Sociodemographic Factors

	Odds Ratio		95% C.I.		Significance	
	Group A	Group B	Group A	Group B	Group A	Group B
2A: Odds Ratios for Age						
Q1	1	1				
Q2	8.53	10.48	3.67 - 19.81	5.14 - 21.38	0.000	0.000
Q3	39.08	32.08	13.18 - 115.35	14.77 - 69.70	0.000	0.000
Q4	49.63	52.18	5.92 - 416.16	13.34 - 204.05	0.000	0.000
2B: Odds Ratios for Education						
Q1	1	1				
Q2	0.206	0.353	0.07 - 0.604	0.214 - 0.583	0.001	0.000
Q3	0.016	0.210	0.004 - 0.064	0.07 - 0.634	0.000	0.005
2C: Odds Ratios for Area						
Urban	1	1				
Rural	2.70	10.29	1.48 - 4.92	6.28 - 16.87	0.001	0.000
2D: Odds Ratios for Income						
Q1	1	1				
Q2	0.246	0.095	0.092 - 0.661	0.048 - 0.187	0.008	0.000
Q3	0.169	0.052	0.057 - 0.499	0.024 - 0.111	0.003	0.000
Q4	0.146	0.136	0.07 - 0.305	0.076 - 0.242	0.000	0.000

Group A = Comparison between Controls and Cancer patients
Group B = Comparison between Pts. with OPC and Cancer patients

in all the subjects. It is clear from the table that average duration of tobacco consumption was more than 10 years in all the three groups of the subjects. The habit of snuff use (oral or nasal) was more common in the female subjects than that observed in the males in whom chewing and/or smoking were more commonly seen. Duration of oral and nasal snuff inhalation habits varied from 5-15 years among these subjects. It was also observed that the frequency of tobacco consumption in subjects with chewing habits was more than 5 packets per day and for smoking it was 25 bidies/ 10 cigarettes or more per day. Habit of tobacco chewing alone or with areca-nut and lime (e.g. gutkha, pan masala) and bidi smoking as well as cigarette smoking was the predominant mode of tobacco consumption in the subjects.

Plasma Vitamin B_{12} and Folate Levels in the Subjects:

The mean plasma levels of vitamin B_{12} and folate are previously reported to be lower in cancer patients as compared to the healthy individuals (Heimburger et al., 1988; Ramaswamy et al., 1996). Table–4A shows mean plasma levels in WHT and NHT subjects of all the three groups. Median values of plasma vitamin B_{12} and folate were also calculated for each group. It was found that the mean levels of vitamin B_{12} and folate were lower in patients with OPC and cancer patients as compared to the controls. However, the differences were not statistically significant. Plasma vitamin B_{12} and folate values were transformed into quartiles. Non parametric analysis by Mann Whitney U test was carried out (table–4B). It revealed that the individuals in lower quartile (Q1) are at a higher risk of developing OPC, as compared to those in higher quartiles. Similarly, the patients with OPC in lower quartiles were found to be at a higher risk of developing cancer than their counterparts.

Pearson's correlation coefficients were calculated to

Table 3A. Details of Tobacco Habits

		Controls	Patients with OPC	Cancer Patients
Habit	No	28 (50.0%)	8(04.8 %)	26(12.2%)
	Yes	28(50.0%)	159(95.2 %)	188(87.6%)
Type of Tobacco Habit	Smoking	8(28.57%)	26(16.35%)	82(43.61%)
	Chewing	15(53.57%)	86(54.05%)	47(25.0%)
	Both	4(14.28%)	45(28.30%)	39(20.74%)
	Snuff	1(0.46%)	2(0.93%)	20(10.63%)

Table 3B. Duration of Tobacco Consumption by Subjects

	Duration	Controls	Patients with OPC	Cancer Patients
Smoking	<5 Yrs	2(16.66%)	11(15.5%)	11(9.1%)
	5-9 Yrs	2(16.66%)	10(14.1%)	2(1.7%)
	10-19 Yrs	1(8.33%)	12(16.9%)	16(13.2%)
	>20 Yrs	7(58.33%)	38(53.5%)	92(76.0%)
Chewing	<5 Yrs	2(10.52%)	47(35.9%)	15(17.4%)
	5-9 Yrs	6(31.57%)	37(28.2%)	11(12.8%)
	10-19 Yrs	8(42.10%)	30(22.9%)	28(32.6%)
	>20 Yrs	3(15.78%)	17(13.0%)	32(37.2%)
Snuff	<5 Yrs	-	1(50.0%)	4(20.0%)
	5-9 Yrs	-	1(50.0%)	2(10.0%)
	10-19 Yrs	-	-	5(25.0%)
	>20 Yrs	1(100%)	-	9(45.0%)

OPC: Oral precancerous conditions

determine the association between vitamin B_{12} and folate levels in the subjects. Plasma vitamin B_{12} and folate levels showed positive correlation in tobacco habitués (table–5).

Relation between Stage of Disease and Plasma Vitamin B12 and Folate Levels:

The association between alterations in the plasma vitamin B_{12} and folate levels with the extent of malignant disease was also studied (figure–2). The histograms, represent vitamin B_{12} and folate levels observed in the patients with early malignant disease (stage I & II) and advanced malignant disease (stage III & IV). Folate levels were significantly lower (p=0.005) in the advanced malignant disease as compared to the early disease. The significance was further confirmed by ANOVA (p=0.006) and non parametric correlations (Spearman's rho= -0.211, p=0.01).

Effect of Vegetarianism on Plasma Vitamin B_{12} and Folate Status:

The dietary intake of vitamin B_{12} and folate is known to affect their plasma pools (Dastur et al., 1972). Therefore, to evaluate the role of diet type, the patients were categorized

Table 4A. Plasma Vitamin B_{12} and Folate Levels in Controls, Patients with OPC and Head and Neck Cancer Patients

		Mean	±S.E.	Median
Vitamin B_{12} (pg/ml)	Controls	309.87	23.30	296.37
	NHT	278.17	22.18	244.95
	WHT	368.00	45.41	299.27
	Pts. with OPC	232.73	17.40	165.29
	NHT	414.11	113.17	352.66
	WHT	223.60	17.13	162.85
	Cancer pts.	264.37	13.3	222.71
	NHT	266.24	35.20	218.29
	WHT	263.53	14.42	223.22
Folate (ng/ml)	Controls	11.124	0.62	9.93
	NHT	10.83	0.86	9.33
	WHT	11.45	0.93	10.31
	Pts. with OPC	11.09	1.04	6.75
	NHT	20.15	3.45	20.11
	WHT	10.63	1.07	6.54
	Cancer pts.	11.083	0.45	10.2
	NHT	12.89	1.46	12.09
	WHT	10.83	0.47	9.49

Table 4B. Non Parametric Test (Mann Whitney U Test)

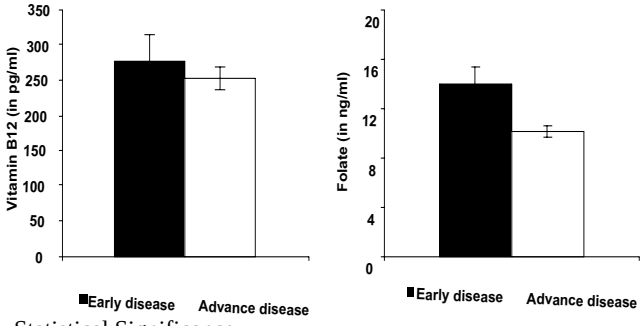
Group	U	Z	Signi-ficance
Vitamin B_{12}			
Control vs. Pts with OPC	733.5	-3.39	0.001
Control vs. Cancer Pts.	1250.5	-2.15	0.030
Pts. with OPC vs. Cancer Pts.	14831.5	-2.73	0.006
Folate			
Controls vs. Pts. with OPC	3108.0	-3.38	0.001
Control vs. Cancer Pts.	4526.5	-0.21	0.830
OPC vs. Cancer Pts.	10664.5	-4.39	0.000

OPC: Oral precancerous conditions, Pts.: Patients
WHT: with habit of tobacco, NHT: no habit of tobacco

into vegetarians and non-vegetarians. Table–6 shows mean plasma levels of vitamin B_{12} and folate in vegetarian and non-vegetarian individuals in the controls, patients with OPC and cancer patients. Plasma vitamin B_{12} levels were higher in the non-vegetarians than the vegetarian subjects of the respective group in controls, patients with OPC and cancer patients. However, folate levels did not reveal any clear pattern with respect to the diet type.

Discussion

The human body requires nearly 40 micronutrients for the normal growth and maintenance of the physiological conditions (Ames,1998). Among these micronutrients, β-carotene, vitamin C and vitamin E act as antioxidants whereas vitamin B_{12} and folate are reported to have role in chemoprevention and management of various cancers. Vitamin B_{12} and folate deficiencies, also a major cause of



Statistical Significance

	Anova		Non-Parametric Correlation	
	"F" Value	"p" Value	Coefficient	"p" Value
Vitamin B_{12}	1.33	0.250	-0.05	0.5
Folate	7.76	0.006*	-0.211	0.01*

*Statistically significant values

Figure 2. Comparison of Plasma Vitamin B_{12} and Folate Levels Between Early and Advanced Malignant Disease

Table 5. Pearson's Correlation Coefficients Between Plasma Vitamin B12 and Folate for Tobacco Consumers

All Tobacco consumers (N=375) r=0.3634 p=0.000¶	Controls (N=28)	r=0.8430 p=0.073
	OPC (N=159)	r=0.4330 p=0.000¶
	Cancer patients (N=188)	r=0.1907 p=0.018¶

¶Indicates statistically significant values
OPC: Patients with Oral precancerous conditions

DNA damage, are shown to be involved in malignant transformation. It is also documented that supplementation of these micronutrients can prevent occurrence of neoplastic changes. Studies have reported role of vitamin B_{12} and folate in oral cavity cancer (Sankaranarayanan et al., 1989), colorectal cancer (Butterworth, 1992), cervical carcinoma (Lashner et al.,1989) and in certain precancerous diseases (Butterworth, et al., 1992; Heimburger et al., 1988; Giovannucci et al., 1993, Ramaswamy et al., 1996).

Vitamin B_{12}, as a cofactor, and folate, a methyl carrier (5-methyl THF) is required for the synthesis of thymidine. The mechanism of action of vitamin B_{12} and folate is not clearly known, but reports have found its correlation with DNA synthesis and DNA methylation. Both vitamin B_{12} and folate are essential for the synthesis of deoxythymidine (a nucleotide component of DNA). The deficiency of these nutrients leads to the alterations in the nucleotide pool of cells by lowering deoxythymidine levels, this might lead to an abnormal incorporation of uracil into the DNA (Blount et al., 1997; Duthie, 1999). These cells have an increased tendency or susceptibility to undergo neoplastic transformation. Occurrence of folate sensitive fragile sites in human chromosomes is also reported by various researchers (Glover 1998; Chen et al.,1989).

In the present study, plasma vitamin B_{12} and folate status of the patients with OPC and head and neck cancer patients has been evaluated and role of tobacco habits, diet type, stage of disease is also studied in a large sample size. This is one of the few studies in the area of head and neck cancer which is yet to be explored extensively. Certain studies have reported low plasma levels of vitamin B_{12} and folate in cancers, but some studies have not found any correlation of these nutrients with cancer. In the present investigation, we observed that the mean plasma values of vitamin B_{12} and folate in patients with OPC and cancer patients were lower as compared to the controls.

Tobacco is considered to be a major risk factor for causation of head and neck cancer. The current investigation found that more than 87% of the patients were tobacco consumers. In the western countries, cigarette smoking is the most common form of tobacco consumption unlike Asian countries where tobacco chewing habits are more frequent. In our region (Gujarat, the western part of India), tobacco is consumed in variety of forms including, tobacco chewing (with or without pan, gutkha and other ready pouch eating),

Table 6. Comparison of Plasma Vitamin B_{12} and Folate Levels between Vegetarians and Non-Vegetarians

	Group	Vitamin B_{12} (pg/ml)	±S.E.	Folate (ng/ml)	±S.E.
Controls	Vegetarians	298.560	29.45	10.542	0.72
	Non- vegetarians	360.158	37.57	12.140	1.20
Patients with OPC	Vegetarians	225.931	21.12	10.442	1.27
	Non–vegetarians	237.667	27.50	11.777	1.65
Cancer Patients	Vegetarians	239.377 *	15.25	11.564	0.54
	Non–vegetarians	314.886 *	25.07	10.068	0.80

*p=0.007 (vegetarians vs. non-vegetarians)

smoking (bidi, cigarette, hukko, chilam etc.), naswar (nasal snuff), reverse smoking etc. There is a tremendous increase in the consumption of gutkha in the recent years. We have also found (unpublished data) that consumption of Gutkha, the attractively packed pouches containing tobacco alongwith other ingredients is tremendously increased among the youngsters in recent years which may be causing oral submucus fibrosis and oral leukoplakia, the highly prevalent disease in this age group (<30 years). Also the morbidity and mortality due to head and neck cancer have reached an alarming situation. One of the harmful effect of tobacco consumption, consistently been observed, is the alterations in the plasma micronutrient levels. A decrease in the plasma/serum levels of micronutrients due to tobacco, is the trend generally observed by various investigators (Piyathilake et al., 1994; Khaled et al., 1986; Ramaswamy et al., 1996; Piyathilake et al.,1992). We have also observed a decrease in the plasma vitamin B_{12} and folate levels in the patients consuming tobacco as compared to the NHT. However, the levels in controls were comparable between the NHT and WHT subjects.

In our study, the patients with OPC in lower quartiles of plasma vitamin B_{12} and folate were found to be at a higher risk of developing cancer than their counterparts. Plasma vitamin B_{12} and folate levels were positively correlated in tobacco habitués. This association was significant in the patients with OPC and cancer patients consuming tobacco. This positive association might be due to the fact that both vitamin B_{12} and folate are essentially required in the same pathway at an early step in the process of carcinogenesis (Varley, Gowenlock and Bell, 1976). In addition, some studies have shown the synergetic effect of vitamin B_{12} and folate supplementation in the treatment of certain precancers (Eto and Krumdieck ,1986 ; Butterworth et al 1992).

As the malignant disease advances, the requirement for these vitamins also increases and if this is not fulfilled properly the circulating levels are declined. Chitkara et al., (1996) have reported decrease in the vitamin levels with the advancement of disease, however, Potischman et al., (1991) found similar mean folate levels with the stage of disease. Our results showed, lower plasma levels of both vitamin B_{12} (p=0.489) and folate (p=0.005) in the advanced disease (stage III and stage IV) as compared to the early disease (stage I & stage II). Similar results were obtained by ANOVA (p=0.006) and non parametric correlation (Spearman's rho= -0.211 and p=0.01).

The dietary intake of vitamin B_{12} and folate are reflected in the plasma levels. Considering the rich sources of vitamin $B_{12,}$ it might be expected that vegetarians have lower levels of vitamin B_{12} as compared to non-vegetarians. Dastur et al., (1972) have reported lower plasma vitamin B_{12} levels in the smoker vegetarians and high levels in non-smoker non-vegetarians. The results of our study showed higher plasma vitamin B_{12} levels in the non-vegetarians whereas no clear pattern was observed for plasma folate status. Solberg et al., (1994) could not observe a significant difference in mean plasma vitamin B_{12} between lactovegetarians and controls, however, higher folate levels were seen in the vegetarians.

In conclusion, plasma vitamin B_{12} and folate levels might have an important role in the process of head and neck carcinogenesis. There are no previous reports from Gujarat (the western part of India) on these potent chemopreventive agents in spite of the fact that the lack of these nutrients might have synergetic effect, alongwith habit of tobacco consumption on the process of carcinogenesis. The involvement of vitamin B_{12} and folate in the initiation phase of malignant transformation has been reported suggesting their role in the early neoplastic changes. In Gujarat, the major part of population is rural, comprising of poor and strict vegetarians who consume the diet which is particularly deficient in vitamin B_{12} and folate as well as other important nutrients. The subjects were also found to be unaware about the association of dietary factors and habit of tobacco consumption with cancer which may be due to their poor educational status as well. Thus, it can be suggested from the present study that the diet deficient in vitamin B_{12} and folate along with habit of tobacco consumption play an important role in etiology of head and neck cancer in Gujarat. However, the results need to be confirmed on a larger population and the work is in progress in our laboratory.

Acknowledgements

The authors are sincerely thankful to the Cancer

Treatment Research Foundation, Washington, D.C., USA, for providing financial support (Grant No. G–96–134) for the study.

References

Ames BN, (1998). Micronutrients prevent cancer and delay ageing. *Toxicol Lett*, **102-103**, 5-18.

Annual report of Population Based Cancer Registry and Hospital Based Cancer Registry of The Gujarat Cancer & Research Institute (1997). Ahmedabad, India.

Banoczy J, Gintner Z, Dombi C (2001). Tobacco use and oral leukoplakia. *J Dent Edu*, **65**, 322-7.

Blount BC, Mack MM, Wehr CM , et al (1997). Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications of cancer and neuronal damage. *Proc Natl Acad Sci USA*, **94**, 3290-5.

Butterworth CE Jr., Hatch KD, Soong SJ, et al (1992). Oral folic acid supplementation for cervical dysplasia: A clinical intervention trial. *Am J Obstet Gynec* , **166**, 803-9.

Butterworth, CE Jr. (1992). Effect of folate on cervical cancer. Synergism among risk factors. *Ann NY Acad Sci* , **669**, 293-9.

Chen AT, Reidy JA, Annest JL, et al (1989). Increased chromosome fragility as a consequence of blood folate levels, smoking status and coffee consumption. *Environ Mol Mutagen*, **13**, 319-24.

Chitkara N, Dadoo RC, Bansal S, et al (1996). Plasma vitamin E levels in carcinoma breast. *Indian J Clin Biochem*, **11**, 162-4.

Choi SW, Mason JB,(2000). Folate and carcinogenesis: an integrated scheme. *J Nutr*, **130**, 129-32.

Dastur DK, Quadros EV, Wadia NH, et al (1972). Effect of vegetarianism and smoking on vitamin B12, thiocyanate, and folate levels in the blood of normal subjects. *Br Med J*, **3**, 260-3.

Duthie SJ (1999). Folic acid deficiency and cancer: mechanisms of DNA instability. *Br Med Bull*, **55**, 578-92.

Eto I, Krumdieck CL (1986). Role of vitamin B_{12} and folate deficiencies in carcinogenesis. *Adv Exp Med Biol*, **206**, 313-30.

Giovannucci E, Stampfer MJ, Colditz GA, et al (1993). Folate, methionine and alcohol intake and risk of colorectal adenoma. *J Natl Cancer Inst*, **85**, 875-84.

Glover TW (1988). Instability at chromosomal fragile sites. *Rec Res Cancer Res*, **154**, 185-99.

Hecht SS (1999). Tobacco smoke carcinogens and lung. *J Natl Cancer Inst*, **92**, 1194-210.

Heimburger DC, Alexander CB, Birch R, 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. *J Am Med Assoc*, **259**, 1525-30.

Johnson N (2001). Tobacco use and oral cancer: A global perspective. *J Dent Educ*, **65**, 328-9.

Kamei T, Kohno T, Ohwada H, et al (1993). Experimental study of the therapeutic effects of folate, vitamin A, and vitamin B_{12} on squamous metaplasia of the bronchial epithelium. *Cancer*, **71**, 2477-83..

Khaled MA, Walkins CL, Krumdieck CL (1986). Inactivation of B12 and folate coenzymes by butyl nitrite as observed by NMR: implications of one carbon transfer mechanism. *Biochem Biophy Res Comm*, **135**, 201-7.

Lashner BA, Heidenreich PA, Su GL, et al (1989). Effect of folate supplementation on the incidence of dysplasia and cancer in chronic ulcerative colitis: A case-control study. *Gastroenterology*, **97**, 255-9.

Levi F, Pasche C, Luccihini F, Vecchia CL (2001). Dietary intake of selected micronutrients and breast cancer risk. *Int J Cancer*, **91**, 260-3.

Munoz N, Plummer M, Vivas J, et al (2001). A case control study of gastric cancer in Venezuela. *Int J Cancer*, **93**, 417-23.

Parkin DM, Bray F, Ferlay J, Pisani P (2001). Estimating the world cancer burden: GLOBOCAN 2000. *Int J Cancer*, **80**, 153-6.

Piyathilake CJ, Hine RJ, Dasanayake AP, et al (1992). Effect of smoking on folate levels in buccal mucosal cells. *Int J Cancer*, **52**, 566-9.

Piyathilake CJ, Macaluso M, Hine RJ, et al (1994). Local and systemic effects of cigarette smoking on folate and vitamin B12. *Am J Clin Nutr*, **60**, 559-66..

Potischman N, Brinton LA, Laiming VA, et al (1991). A case-control study of serum folate levels and invasive cervical cancer. *Cancer Res*, **51**, 4785-9.

Practical Clinical Biochemistry (1976). In Varley, H, Gowenlock, AH and Bell, M (eds) Vitamins, The Whitefriers Press, London, **Vol 2**, p no. 215-59.

Ramaswamy G, Rao VR, Kumaraswamy SV, Anantha N (1996). Serum vitamins' status in oral leukoplakias–a preliminary study. *Oral Oncol, Eur J Cancer*, **32B**, 120-2.

Rassekh, CH (2001). Tobacco, cancer of the oral cavity and pharynx. *W V Med J* , **97**, 8-12.

Sankaranarayanan R, Duffy SW, Day NE, et al (1989). A case control investigation of the oral cavity, tongue and floor of mouth in Southern India. *Int J Cancer*, **44**, 617-21.

Solberg EE, Magnus E, Sander J, Loeb M (1994). Vegetarians and vitamin B_{12}. A controlled trial of vitamin B_{12} status in 63 lactovegetarians. *Tidsskr Nor laegeforen*, **114**, 2601-2.

Tradati N, Grigotat R, Calabrese I, et al (1997). Oral leukoplakias: to treat or not? *Oral Oncol*, **33**, 317-21.

UICC (Union Internationale Centre Le Cancer) (1987). In Hermanik P, Sobin LH (eds) TNM classification of malignant tumours, UICC Publication Geneva.

Winn DM, Ziegler RG, Pickle LW, et al (1984). Diet in the etiology of oral and pharyngeal cancer among women from the Southern United States. *Cancer Res*, **44**, 1216-22.