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Diet, Antioxidants and Risk of Cancer: A Case-control Study

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A total of 3810 patients were seen in hospital over a 2-year period. There were 101 patients with cancer of various organs of the body. These 101 cancer patients were compared with 100 age- and sex-matched control subjects selected from the same population. The aim was to find the relation between dietary and plasma levels of antioxidants and minerals and the risk of cancer. Dietary intakes were assessed by a 7-day food intake assisted questionnaire to find out previous intakes. The study showed that the plasma levels of antioxidant vitamins A, E and C and β-carotene and the zinc:copper ratio and zinc levels were significantly and inversely associated with the cancer group compared to the control subjects. The serum calcium level was also lower in the cancer group but the magnesium level was higher. Underlying these findings, the consumption of antioxidant vitamins A, E and C and β-carotene, calcium and magnesium was significantly lower in the cancer group compared to the control subjects. The differences in intake indicated a higher risk of cancer with a lower consumption of antioxidant vitamins and fruit and vegetables and with a higher consumption of flesh foods and total visible fat. Lipid peroxides, malonyldialdehyde and diene conjugates, which are indicators of free radical stress and cell damage, were significantly higher in the cancer group compared to the control subjects. The findings suggest that the increased consumption of antioxidant and mineral-rich fruit and vegetables, in conjunction with a low fat diet, may provide protection against cancer. However, more studies are necessary to confirm our suggestions.

Keywords: antioxidant vitamins, dietary fat, zinc, copper, magnesium, fruit and vegetables.

INTRODUCTION

It has been estimated that 35% of the cancer mortality in the US population is attributable to diet [1]. Population-based cancer records in India, which cover 3.5% of the population, indicate a crude cancer incidence rate of 6.6 per 10 000, while the crude mortality rate from cancer is estimated to be 3.8 per 10 000 [2]. The age-adjusted incidence rates of cancer in India for the period 1980–1987 indicate that cancers of the oral cavity, pharynx, oesophagus and of the trachea and bronchus are common in males, while cancers of the genital tract, in particular the cervix and the breast, are important in females [2]. It has been estimated by the Indian Council of Medical Research that, in India, the number of cancer patients by the year 2000 will be over 0.8 million per annum [2]. The total number of cancer patients in India appears to be 2 million. The cancer incidence rates vary from one area to another.

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There is evidence that smoking, tobacco and betel chewing and dietary factors are important risk factors of cancer in India [2–7]. Epidemiological studies suggest that a higher dietary fat and animal foods intake increase the risk, whereas fruit and vegetables may have protective effects [8–10]. The protective effects may be due to the consumption of vitamins A, C and E and β-carotene, zinc and selenium [10, 11]. The risk of cancer may increase due to dietary excess and a deficiency of certain nutrients [10]. The survey conducted by the National Nutritional Monitoring Bureau in ten Indian states documents a poor consumption of green leafy vegetables and a poor intake of vitamins C and A, folate and riboflavin in different population groups [12]. In a number of epidemiological studies [10, 11], inverse associations have been observed between antioxidants and the risk of various cancers, most notably cancer of the upper aerodigestive tract, lung, breast, intestinal tract and cervix. There is a lack of evidence on the relation between dietary factors and cancer in India. In the present case-control study, we examine whether dietary factors and plasma levels of antioxidants are associated with cancer.

SUBJECTS AND METHODS

Over a period of 2 years, 3810 subjects attended the Centre of Nutrition which provides medical services to 0.432 million city dwellers, for the treatment of various health problems. Of the 3810 subjects studied on entry to this study, 101 had cancer of various parts of the body. Clinical, radiological, laboratory and ultrasonographic data were obtained in all the subjects at the centre. Computerized axial tomography and histopathological tests were also performed wherever indicated to confirm the diagnosis of cancer. The controls ($n = 100$) were matched for age (within 5 years of the mean) and sex and were drawn from non-family attendants sharing a similar socio-economic status to the cancer patients. Of the 172 subjects considered, those with coronary artery disease ($n = 8$), diabetes ($n = 6$) and those who did not cooperate in blood tests ($n = 48$) were excluded. All patients were classified based on TNM (tumour, nodes, metastasis) classification into five stages (Table 1).

Dietary intakes were obtained from all the patients and the control subjects while in the hospital using 7-day diet diaries, assisted by a questionnaire completed by the dietitian using food models and food measures. All patients and control subjects were asked to follow their usual diets and recollect their food consumption in the last 6 months before recording the dietary intakes by means of the questionnaire. The questionnaire was designed and validated [3] to obtain information regarding dietary habits before the diagnosis of cancer. Probing questions were asked to ascertain changes in dietary intake after the diagnosis of cancer and the quantity of different food groups consumed by the subjects. The dietary intake of spouses was also considered while completing the questionnaire for the dietary assessment of the patients. Nutrient intakes were calculated through the questionnaires by computation of Indian food composition tables [13].

Laboratory data were obtained on entry to the study for all the patients and the control subjects. A venous blood sample was obtained after an overnight fast and analyzed for blood count, haemoglobin, urea glucose, vitamins C, E and A and β-carotene [14, 15] and lipid peroxides (thiobarbituric acid reactive substances), malonyldialdehyde (MDA) and diene conjugates in all the patients and controls within 3 h of blood collection [16–18]. A vitamins A, E and C and β-carotene assay was performed in the plasma obtained from heparinized blood within 1 h of collection by ultra violet visible spectrophotometry. The plasma was stored in a refrigerator after centrifugation. Zinc, copper, magnesium, calcium and iron were also assayed by colorimetric methods [19–27].

A recently modified colorimetric method for a quantitative assay of thiobarbituric acid reactive substances (TBARS) in the plasma, free of interference from sialic acids, was used. The thiobarbituric acid was dissolved in sodium sulphate and both the liberation of TBARS and a colour reaction were performed simultaneously. Lipid peroxidation begins with the

TABLE 1. Clinical data and stage of the disease: mean (standard deviation)

	Cancer		Controls	
Clinical data	Male	Female	Male	Female
Subjects	53.0	48.0	52.0	48.0
Mean age (years)	51.5 (7.5)	43.0 (5.8)	50.2 (7.0)	42.4 (5.4)
Weight	55.2 (4.6)*	46.4 (3.5)	59.5 (4.8)	50.2 (3.7)
BMI (kg m^{-2})	20.5 (1.4)	19.3 (1.2)*	21.4 (1.5)	20.6 (1.1)
Tobacco users (%)	38.0 (71.7)*	10.0 (20.7)	26.0 (50.0)	12.0 (25.0)
Site of cancer (%)				
Oral cavity and pharynx	20.0 (37.7)	8.0 (16.7)		
Trachea bronchus and lungs	8.0 (15.0)	2.0 (4.2)		
Larynx	4.0 (7.5)	1.0 (2.1)		
Oesophagus	4.0 (7.5)	3.0 (6.2)		
Stomach	6.0 (11.3)	3.0 (6.2)		
Cervix	–	15.0 (31.2)		
Ovary	–	4.0 (82)		
Breast	–	10.0 (20.8)		
Prostate	5.0 (9.4)	–		
Liver	6.0 (11.7)	2.0 (4.1)		
Number of cases by TNM classification				
$T_1N_0M_0$	5.0 (9.4)	4.0 (8.2)		
$T_1N_1M_0$ $T_2N_0M_0$	10.0 (18.8)	8.0 (16.4)		
$T_2N_1M_0$ $T_2N_2M_0$	15.0 (28.2)	12.0 (24.6)		
$T_2N_1M_1$ $T_2N_2M_1$	13.0 (24.5)	14.0 (29.1)		
$T_3N_1M_0$ $T_3N_3M_0$	10.0 (18.8)	10.0 (20.8)		

The p-value was obtained by a comparison of the cancer group with the control group in each sex: $*p < 0.05$.

formation of a lipid-free radical which rearranges to form a diene. Partial oxidation results in the formation of a lipid peroxy radical which takes up a hydrogen atom resulting in the formation of TBARS. Malonyldialdehyde (MDA) is a breakdown product of unsaturated fatty acids. Increases in the plasma levels of TBARS and MDA and diene conjugates are the indicators of enhanced oxidative stress and cell damage. However, there is considerable disagreement regarding the reliability of various tests to measure oxidant and antioxidant effects.

Ascorbic acid was assayed at 520 nm with 2,4-dinitrophenyl hydrazine forming red bis-hydrazone having a coefficient variation of 5.2%. Vitamin A and β-carotene were separated in diethylether and the β-carotene level was obtained at 46 nm using a standard curve. The colorimetric estimation of vitamin A was made from the concentrate of diethylether using trifluoroacetic acid as the reagent with correction for the colour contribution by β-carotene. Vitamin E was extracted into n-hexane and analyzed with ferric chloride/D α-α dipyridyl reagent. The coefficient of variations for vitamins A and E and β-carotene were 4.7, 4.3 and 2.3%, respectively.

One-way analysis of variance and t-test were used for the analysis of data. Only a p-value of <0.05 and two-tailed t-tests were considered to have statistical significance. Differences between the cancer patients and the control subjects were obtained for different data and 95% confidence interval (CIs) calculated. The intake of nutrients per 1000 kcal/day was

TABLE 2. Food consumption pattern by average intake of foods: mean (standard deviation)

Foods (g/day)	Men			Women		
	Cancer ($n = 53$)	Controls ($n = 52$)	Difference (95% CI)	Cancer ($n = 48$)	Controls ($n = 48$)	Difference (95% CI)
Wheat, rice and millet	272.0 (23)	243.0 (27)	21.0 (9–32)	249.0 (21)	269.0 (24)	20.0 (8–34
Roots and tubers	68.0 (6)	70.0 (7)	2.0 (9.5–6.5)	61.0 (5)	66.0 (6)	5.0 (1.6–11)
Pulses	32.0 (4.3)	39.0 (6.4)	7.0 (1.2–22)	29.0 (4)	34.0 (6)	5.0 (1.4–14)
Vegetables	63.0 (4.7)*	90.0 (7)	27.0 (12–41)	58.0 (4.5)	81.0 (6.2)	23.0 (9–42)
Fruits	55.0 (7.3)	74.0 (9)	19.0 (9–28)	47.0 (7)*	67.0 (9)	20.0 (11–30)
Milk and milk products	231.0 (16)	216.0 (12)	15.0 (9–26)	218.0 (15)	196.0 (11)	12.0 (5–42)
Sugar and jaggery	23.0 (3.4)	26.3 (3.5)	3.3 (0.5–7.5)	21.0 (3.1)	24.0 (3.2)	3.0 (0.2–6)
Total visible fat	38.0 (5.7)*	23.6 (3.6)	14.4 (8–22)	35.0 (5.5)*	21.2 (3.2)	13.8 (0–14)
Flesh foods and eggs	29.8 (4.3)*	17.0 (2.9)	12.8 (5–19)	27.5 (4)*	15.1 (3.6)	12.4 (7–19)
Total fruit and vegetables	117.0 (9)*	163.0 (13)	46.0 (22–68)	107.0 (8)*	147.0 (72)	40.0 (19–65)
Fruit and vegetable : visible fat ratio	3.1 (0–7)	6.9 (1.2)	3.8 (1.6–6)	3.1 (0.6)*	6.9 (1.1)	3.8 (1.2–6.1)

The p-value was obtained by a comparison of the cancer group with the controls by the t-test using analysis of variance: $*p < 0.05$.

TABLE 3. Nutrient intake in cancer group and controls: mean (standard deviation)

Nutrient (mg 1000 $kcal^{-1}$/day)	Men			Women		
	Cancer ($n = 53$)	Controls ($n = 52$)	Difference (95% CI)	Cancer ($n = 48$)	Controls ($n = 48$)	Difference (95% CI)
Vitamin A	176.0 (23)*	265.0 (35)	89.0 (50–130)	162.0 (19)*	250.8	88.0 (42–120)
Vitamin E	4.7 (1.3)*	7.1 (2.7)	2.4 (0.5–5.4)	4.3 (1)*	6.5 (2.5)	2.2 (0.5–4)
Vitamin C	26.0 (4.6)*	36.6 (7.8)	10.6 (4–16)	22.1 (4.3)*	33.6 (7.2)	11.5 (6.5–18)
β-Carotene	638.0 (110)*	918.0 (138)	280.0 (150–375)	585.0 (92)	832.0 (125)	247.0 (150–356)
Calcium	655.0 (93)*	857.0 (112)	202.0 (110–312)	595.0 (81)*	781.0 (98)	186.0 (102–280)
Magnesium	163.0 (21)*	236.0 (32)	73.0 (34–110)	146.0 (15)*	216.0 (28)	70.0 (41–105)
Iron	17.3 (3.7)	19.6 (3.6)	2.3 (0.4–5.8)	15.0 (3.5)	18.0 (3.4)	3.0 (0.6–7)
Zinc	5.9 (1.3)	6.8 (1.4)	0.9 (1.0–3.6)	5.2 (1)	6.1 (1.2)	0.9 (0.2–1.9)
Copper	0.9 (0.2)	1.0 (0.8)	0.1 (0.02–0.4)	0.8 (0.2)	1.0 (0.8)	0.2 (0.05–0.5)
Fibre (g day)	11.0 (2.8)*	17.0 (3.7)	6.0 (1.1–12)	9.0 (2.6)*	14.2 (3.2)	5.2 (1.5–12)

The p-value was obtained by a comparison of the cases and controls by the t-test using analysis of variance: $*p < 0.05$.

TABLE 4. Laboratory data on the cancer group and control group in men and women: mean (standard deviation)

	Men		Women	
Data	Cancer	Control	Cancer	Control
Subjects	53.00	52.00	48.00	48.00
Vitamin A (mg dl^{-1})	28.00 (4.5)*	42.40 (7.2)	26.50 (3.6)*	39.30 (6.3)
Vitamin E (mg dl^{-1})	0.38 (0.09)**	0.68 (0.15)	0.35 (0.08)**	0.66 (0.16)
Vitamin C (mg dl^{-1})	0.18 (0.06)**	0.33 (0.08)	0.16 (0.04)**	0.38 (0.09)
β-Carotene (mg dl^{-1})	16.50 (2.8)*	22.80 (4.1)	14.00 (2.6)*	21.00 (3.8)
Iron (mg dl^{-1})	120.00 (12)	125.00 (14)	115.00 (11)	118.00 (13)
Calcium (mg dl^{-1})	7.10 (1.6)*	9.40 (2.5)	6.60 (1.5)*	9.20 (2.7)
Magnesium (mg dl^{-1})	2.20 (0.4)*	1.70 (0.3)	2.10 (0.4)*	1.60 (0.3)
Zinc (mg dl^{-1})	72.00 (6.6)**	110.00 (8.7)	77.00 (6.7)*	105.00 (8.8)
Copper (mg dl^{-1})	102.00 (8)	108.00 (7)	98.00 (6)	102.00 (7)
Zinc:Copper ratio	0.70 (0.16)*	1.01 (0.18)	0.78 (0.16)*	1.02 (0.18)
Lipid peroxides (nmol ml^{-1})	2.60 (0.4)*	1.10 (0.12)	2.40 (0.4)*	1.20 (0.15)
Malonyldialdehyde (nmol ml^{-1})	1.50 (0.33)*	0.87 (0.13)	1.40 (0.32)*	0.86 (0.12)
Diene conjugates	33.60 (4.2)*	24.60 (3.5)	32.60 (4.5)*	23.50 (3.4)

The p-value was obtained by a comparison of the cancer group from the control group by the t-test using analysis of variance: $*p < 0.05$, $**p < 0.01$.

calculated for both sexes. The cut-off points were based on the values in the control subjects. The percentages in the groups were compared by χ^2 test.

RESULTS

The mean age and mean sex were comparable between the cancer and the control groups (Table 1). Tobacco consumption was very high among the cancer patients, especially males. The mean body weights and body mass indexes (BMIs) were lower in both men and women cancer patients, compared to the controls. The relative frequency of cancers of the aerodigestive tract was the major cause of cancer in males and of the genitals in females. The consumption of vegetables and fruit was significantly lower and the consumption of visible fat and flesh foods was higher in the cancer group compared to the controls. The ratio of total fruit and vegetables with the total visible fat intake was inversely associated with the risk of cancer (Table 2). When calculated separately, the food intakes were slightly higher in men than in women. The visible fat intakes in the higher social classes were mainly Indian ghee and vegetable oils and in poor people were *trans* fatty acids.

Table 3 shows that the antioxidant vitamins A, E and C and β-carotene intake were significantly lower in the cancer group compared to the control subjects. The intakes of calcium, magnesium and fibre were also lower in the cancer group than in the control subjects. The differences in intake indicate a higher risk with the lower than required intake of these nutrients (Table 3). Lipid peroxides, diene conjugates and the MDA levels were significantly higher in the cancer group compared to the control group (Table 4).

The plasma concentrations of the antioxidant vitamins A, E and C and β-carotene were inversely associated with cancer. Calcium, zinc and the zinc:copper ratio were also significantly associated with cancer compared to the control subjects. However, the serum magnesium level was significantly higher in the cancer patients compared to the controls (Table 4). After an adjustment for smoking, the association of the plasma levels of antioxidant vitamins and minerals remained significant.

DISCUSSION

The present study shows that lower plasma concentrations of the antioxidant vitamins A, E and C and β-carotene were significantly associated with the risk of cancer in men and women. Low levels of zinc and the zinc:copper ratio were also associated with a higher risk of cancer (Table 4). We also found that a lower consumption of antioxidant vitamins A, E and C and β-carotene as well as fruit and vegetables was significantly associated with the cancer group compared to the control subjects (Tables 3 and 4). The difference indicates a higher risk with a lower consumption of antioxidant vitamins and fruit and vegetables and higher intakes of flesh foods and total visible fat (Table 2). Smoking was more common among the cancer group than the controls in men but not in women. Evidence from all over the world indicates that tobacco consumption is an important risk factor in cancer [24–26]. Tobacco smoke is a rich source of carcinogens [25] and smoking may also cause a deficiency of vitamin C and β-carotene, which may enhance the risk of cancer [3, 25]. This study also showed higher levels of lipid peroxides, MDA and diene conjugates in the cancer group which are indicators of oxidative stress.

Other studies from India have also demonstrated a significant association between a lower intake of antioxidant vitamin-rich foods and the risk of cancer [3–6, 24]. In a recent case-control study in 45 patients with oral cancer, 90% were smokers and lower plasma levels of vitamins A and E and zinc were associated with cancer [24]. In an excellent review [11] on antioxidants (carotenoids and vitamins C and E) and cancers of the lung, upper aerodigestive tract, uterine cervix, colon, breast and prostate, the data concerning carotenoids and lung cancer risk were most consistent. Of eight diet studies, four showed protection and of six serum studies, five showed protection with carotenoids against lung cancer with strong associations that tended to follow a dose–response pattern [11]. There was weaker evidence of protection by vitamins C and E for lung cancer. However, for upper aerodigestive tract cancers, there was evidence of the protective effect of carotenoids in three of the four diet studies and of vitamin C in four of the five diet studies. For cancer of the uterine cervix, there was only suggestive evidence of protection by vitamin C in four of the five diet studies and possibly from carotenoids in two of the five diet studies. In the present study, the majority of males had either oral cancer or aerodigestive tract cancer, whereas among the females more than 50% had cancer of the female genitals including the breast. A subgroup analysis showed that patients with breast, liver and prostate cancer were associated with lower plasma levels of the antioxidant vitamins A, E and C and β-carotene as well as lower zinc levels. Therefore, the analysis of the relation of cancer and antioxidants in all types of cancer in our study appears to be justified.

A lower consumption of fruit and vegetables was associated with cancer in the present study; fruit and vegetables are rich sources of the antioxidant vitamin C, β-carotene, fibre, flavinoids and minerals which protect against cancer [8, 10]. In more than 200 case–control or cohort studies [8, 10], those consuming higher amounts of vegetables and fruit were less prone to developing various cancers such as cancers of the stomach, colon, oral cavity, larynx, pancreas, bladder, cervix and lung. In a large prospective study, the breast cancer incidence was 25% higher among women with a lower intake of vegetables [8]. There is evidence that the nutrients and non-nutrients found in fruit and vegetables can block the formation of carcinogens, induce detoxifying enzymes and antagonize the effects of endogenous oestrogens [27–30]. We also observed a higher risk of cancer with a higher intake of flesh foods and total visible fat, although within desirable limits, which is consistent with other studies [9, 10].

Low levels of zinc in cancer of the oesophagus and oral cavity have been described by several investigators and zinc deficiency has been implicated in the mechanism of carcinogenesis [24, 27, 28]. Our study also showed that the zinc levels were low in all types of cancer of the aerodigestive tract, uterine cervix and breast. Zinc intake is usually lower in Indians and the higher content of phylates in the Indian diet can inhibit the absorption

of zinc resulting in zinc deficiency [13]. In the control subjects, the serum zinc levels were on the lower side of the normal range (Table 4). It has been demonstrated that zinc has a significant beneficial effect on glutathione-5 transferase and also on DNA repair mechanisms [29]. Experimental studies indicate that zinc deficiency enhances carcinogenesis, in particular nitrosamine-induced cancers [29]. We observed higher serum magnesium levels in the cancer group which may be due to cell damage and transport from intracellular stores. Copper and zinc are also important constituents for superoxide dismutase enzyme and iron is the constituent of catalase enzyme. Both enzymes are antioxidants and the antioxidant activity of antioxidants may be enhanced by magnesium [31].

It is possible that the disease may cause changes in the plasma levels of antioxidant vitamins and minerals rather than these factors causing the cancer. Although we made an attempt to measure the pre-diagnosis food intake, this too may presumably have been affected by changes before the cancer was clinically manifest. However, at least the differences in the cases and controls in relation to vitamins and minerals may in part be the risk factors of cancer.

In conclusion, low plasma levels of vitamins A, E and C and β-carotene and zinc were inversely associated with the risk of cancer. A lower consumption of antioxidant vitamins, fruit and vegetables and a higher intake of flesh foods and visible fat, although within desirable limits, were also significantly associated with cancer. A higher intake of fruit and vegetables in conjunction with a low fat diet may protect Indians against cancer and heart disease [30, 31].

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