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Original Article

Vitamin C status in cancer patients and healthy subjects from the local population of Pokhara

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Abstract
Objectives: To assess vitamin C status by determining plasma ascorbic acid level in 55 cancer patients and 55 matched normal subjects serving as control.
Methods: The proven cancer patients were selected from those attending Manipal Teaching Hospital, Pokhara. Matched controls were from the staff of Manipal Teaching Hospital or attendants of the patients. Plasma ascorbic acid was determined by the method of Natelson[1]. Unpaired student't' test was used for the statistical evaluation. Statistical analysis was done by SPSS version 9 software.
Results: The mean level of vitamin C in normal subjects and patients was 1.03±0.26mg/dl and 0.90±0.30mg/dl respectively. None of the subjects in either group had deficient status (<0.2 mg/dl). Although its status was normal in both the groups but patients had lower level than normal subjects. Smokers and alcohol consumers had significantly lower level than non-smoker and non-alcoholics.
Conclusion: In the local population, vitamin C deficiency is not an etiologic factor in malignancy. Smoking and alcohol adversely affects the status of this vitamin.

Key words: Antioxidants, ascorbic acid, cancer, alcohol, smoking

Ascorbic acid is highly versatile nutrient metabolite. It is involved in the absorption of iron from intestine by converting ferric into ferrous state, hydroxylation reaction of procollagen, collagen synthesis independent of hydroxylation, regulation of cholesterol synthesis and its elimination, detoxification processes, inhibition of mutagenesis and carcinogenesis, post translational hydroxylation, synthesis of carnitine, alleviating immune strength, prevention of CVD and age related diseases, wound healing and for maintaining normal body function including brain, heart, lung and kidney activities. Lately, its activity as a strong antioxidant to prevent adverse effect of ROS has been centre of attraction. While its recommended daily intake is about 40mg/day for adults, there is ample evidence to suggest that higher intake is better for health by providing protection against free radical damage and for homeostasis and that there are numerous studies suggesting that this vitamin lowers risks of many chronic diseases such as cardiovascular disease, cancer, cataracts, Alzheimer's disease and many others. An elaborate recent study shows that a daily intake of ≤ 2000 mg ascorbic acid is safe for adults[2]. Since ascorbic acid is water-soluble and is excreted efficiently, its serum level does not exceed 2mg/dl even when taken in excess. As such, serum or plasma level also reflects its status in tissues[3, 4]. Obviously for clinical evaluation, the determination of serum ascorbic acid level is considered to be a convenient and reliable adjunct to decipher its status and clinical outcome. The present paper addresses this aspect in cancer patients and matched controls.

Material and methods

Fifty-five patients suffering from different types of cancer and 55 normal subjects, without any known disease, serving as controls were included in this study. Prior verbal consent was taken from all the participants. The mean age of patients and control was 60.4±12.8 years and 62.0±11.7 years respectively. In both patient group and control group 28 were males (patients-60.8±13.1 years, controls-64.4±12.3 years) and 27 were females (patients-59.8+12.7 years, controls-59.5+10.6 years).

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Three ml of blood was collected from each subject by venipuncture with standard collection technique and was transferred to vials containing EDTA (10mg). Sample was gently mixed and centrifuged at 3000 rpm for 10 minutes. The plasma was transferred to another vial and was analysed either immediately or within three days of collection by the procedure described by Natelson[1]. In case of latter, the samples were preserved in deep freeze at – 8°C because on checking we found that preservation for three days in deep freeze did not alter/change the plasma ascorbic acid values. Statistical significance was determined by students 't' test for unpaired data. The values of significance were evaluated with 'p' values. The differences were considered significant at $p<0.05$.

Results

Table1: Vitamin C levels in cases and control of various age groups

Age (Yrs)		N	Age ± SD	Vit. C ± SD	
				mg/dl	µ mol/ L
Case (55)	≤ 50	10	38.9 ± 8.1	0.85 ± 0.25	48.28 ± 14.20
	51-65	27	60.1 ± 3.8	0.81± 0.30	50.5 ± 17.04
	> 65	18	72.6 ± 5.6	0.94 ± 0.32	53.3 ± 18.10
Control (55)	≤ 50	10	42.9 ± 6.7	1.1 ± 0.38	62.48 ± 21.50
	51-65	19	58.6 ± 3.6	1.07± 0.21	60.7 ± 11.90
	> 65	26	71.6 ± 3.5	0.98 ± 0.23	55.6 ± 13.00
Total patients Vs total control P= < 0.01					

Table 2: Vitamin C levels compared in male & female for cases and controls

Sex		N	Age ± SD	Vit. C ± SD		P- value
				Mg/dl	µ mol/ L	
Male	Case	28	60.8 ± 13.10	0.86 ± 0.30	48.8 ± 17.00	0.02
	Control	28	64.3 ± 12.30	1.04 ± 0.26	59 ± 14.70	
Female	cases	27	59.89± 12.70	0.94± 0.30	53.3 ± 16.40	0.20
	Control	27	59.9 ± 10.60	1.03 ± 0.27	58.5 ± 15.30	

Table 3: Percentage of cases and controls having different levels of vitamin C

		Vitamin C± SD (µmol/ L)		
		<28.4	28.4 - 40	>40
Case	Male	1 (3.5%)	10 (35.7%)	17 (60.7%)
	Female	1 (3.7%)	7 (25.9%)	19 (70.3%)
Control	Male	0 (00%)	4 (14.2%)	24 (85.7%)
	Female	1 (3.7%)	3 (11.1%)	23 (85.1%)

Table 4: Vitamin C levels in cases and controls in various socioeconomic groups

Socioeconomic status		N	Age ± SD	Vit. C ± SD	
				mg/dl	µ mol/ L
Case	Poor	19	58.8 ± 15.00	0.9 ± 0.30	51.5 ± 17.00
	Middle	33	60.7 ± 11.60	0.9± 0.31	51.1 ± 17.00
	Upper middle	3	66.6 ± 11.30	0.9 ± 0.01	51.1± 0.50
Control	Poor	12	63 ± 10.80	1.05 ± 0.20	59.6 ± 11.30
	Middle	34	61.8 ± 11.60	1.06± 0.24	60.2 ± 13.60
	Upper middle	9	60.6 ± 13.40	0.91 ± 0.40	51.1 ± 22.70
Total patients Vs total control P= < 0.01					

Table 5: Comparison of vitamin C levels in smokers and non-smokers

		N	Age ± SD	Vit. C ± SD		P- value
				mg/dl	µ mol/ L	
case	smokers	41	63.9 ±9.60	0.87 ± 0.27	49.4 ± 15.30	<0.05
	Non-smokers	14	49.8 ± 15.30	0.99 ± 0.36	56.2 ± 20.40	
	Smokers+alcoholics	16	65.3± 9.7	0.71± 0.20	40.80± 11.83	<0.002
	Non-smokers+Non-alcoholics	26	59.9 ±16.6	0.99± 0.29	56.66± 16.88	
Control	smokers	33	64.8± 11.6	0.96± 0.20	54.5± 11.30	<0.05
	Non-smokers	22	57.4 ±10.10	1.10± 0.31	62.4 ±17.60	
	Smokers+ alcoholics	21	65.57±10.41	0.95±0.20	54.15 ±11.57	<0.01
	Non-smokers+ Non-alcoholic	15	58.60± 12.5	1.24± 0.0.14	70.64± 8.44	

Table 6: Vitamin C level compared in alcoholic and non-alcoholic cases and controls

Drinking habit		N	Vit. C ± SD		P- value
			mg/dl	µ mol/ L	
Case	alcoholic	26	0.81 ± 0.0.29	46 ± 16.40	0.04
	Non-alcoholic	29	0.97 ± 0.29	55 ± 16.40	
Control	Alcoholic	26	0.96± 0.28	54.5 ± 15.90	0.03
	Non-alcoholic	29	1.10 ± 0.23	62.4 ± 13.00	

Discussion

Ever since its Nobel Prize winning discovery by Szent Georgy in 1928, ascorbic acid has received increasing attention for its diversified participation in metabolic activities. By virtue of its interconvertible property into oxidized and reduced state, ascorbic acid activity supervises several biological oxidation-reduction reactions. One of them is to act as an antioxidant in water-soluble medium by accepting unpaired electron from free radicals especially from tocopheryl radical in membranes to generate tocopherols so that antioxidants are regenerated in membranes for protection against free radical damage[13]. There is a body of evidence that ascorbic acid is singularly most important nutrient antioxidant in cytosolic medium. In view of its enormous physiological and biochemical importance in the body, and reports that it confers protection against cancer[14], that it reduce the side effects of cancer

therapies and that no report is available on vitamin C status in normal population and cancer patients in the local population, we undertook this study to examine its plasma level in 55 patients of cancer and the same number of controls.

The mean ascorbic acid level in both patient and normal groups was within the normal range and age did not show significant relationship (Table 1) and this difference was visible in both male and female groups (Table-2). However, statistical evaluation showed that patients had significantly lower level than normal subjects ($p < 0.01$). Since majority of patients were taking B-complex supplementation, this lower level in them seems to be due to greater metabolic consumption of this vitamin. Tumour cells are documented to consume more ascorbic acid than normal cells[5].

Serum or plasma level of vitamin C is said to be a good marker of its tissue status. The accepted normal range is from 0.4-2.0mg/dl[3,6] but is believed to exert its optimal influence when present in concentration >40μmol/l[4] whereas deficient status is taken as <0.2mg/dl (11.5μmol/l). Accordingly, we divided normal subjects and patients into three categories viz- deficient normal but not having the optimal level and optimal level (Table-3). None of the subjects in either group was deficient. Notably 60.7% and 70.3% male and female patients had optimal level. The percentage of this category was higher in control group (males 85.7% and females 85.1%).

Since vitamin C is a nutrient metabolite, its intake is expected to be influenced by diet, which in turn largely depends on socioeconomic status. Lower serum levels have been reported in poor individuals[7]. In this series socioeconomic wise no difference was observed among patients. Surprisingly the upper strata of normal group had lower level of this vitamin compared to poor or middle group people. Whether this finding is fortuitous or of real significance requires a larger series to be investigated.

In the antioxidant and prooxidant equation in humans, the two important influencing factors are smoking and alcohol intake and both are known to influence vitamin C status adversely[8, 9, 10, 11]. This influence is very much evident in this study. The smokers in both patients and normal group had significantly lower level than non-smoker counterparts. A significant observation is that alcohol further decreased vitamin C status among smoker patients, but not in the normal subjects, thereby suggesting that cancer patients develop alternative routes or exacerbated consumption of this vitamin. Lastly, the effect of alcohol was examined. It can be seen from the table 6 that alcohol consumed in both groups had significantly lower level of the vitamin. These observations are conformity to those repeated by other workers [8, 12]. Recent estimates in Nepal have shown that heavy smoking and alcohol consumption are very high in this country and oral questionnaire revealed that alcohol consumers were consuming more then 80 ml ethanol per day which placed them in the categories of alcoholics. While most of the patients had stopped smoking or had drastically reduced it after detection of disease but otherwise almost all of them were heavy smokers before hand. In this connection, it would be worthwhile to mention that heavy smoking and heavy drinking, at the verge of binging, is highly common in Nepal. A recent statistics revealed by the World Society Reforms and Overall Development Services Centre Nepalgunj states that around 4 million families in this country spend a staggering sum of Rs. 24 billion per year on cigarettes and smoking. Distressingly, each family in the country spends about Rs. 20 thousand on cigarettes and Rs. 40 thousand on liquor annually. To make the things worse, the local brew is easily available at cheaper cost and so are local made bidis and cigarettes. A very recent article by Go et al (2005) abundantly highlights the carcinogenic effects of alcohol through multiplex processes including free radicals. Valdes et al (2005)[15] have concluded from their an elaborate study that smoking through heightened oxidative stress acts as an important risk factor in ageing and age related diseases. They observed a dose dependent relationship of smoking on telomerase length which is a marker of chromosome protection from degradation and end - to- end fusion and noted that each pack-year smoked was equivalent to a 5 base pair (bp) of telomere length lost compared with the non-smoker.

Conclusion

The strength of the present paper lies in three observations: first, a large number of normal subjects and patients did not have adequate level of vitamin to exert optimal antioxidant property, the percentage being higher in patient group, second, smoking and alcohol emfeebled its status and third, both smoking and alcohol appear to be cogent risk factors in carcinogenesis in local population.

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References

1. Natelson S- Techniques of clinical chemistry, 3rd Ed. publisher Charles, C. Thomas, USA, 1971 :288, 751 and 162.
2. Hatchcock JN, Azzi A, Blumberg J, Bray T, Dickinson A. et. al.- Vitamins E and C are safe across a broad range of intakes. 2005. Am J Clin Nutr; 81: 736.
3. McCormick DB, and Greene HL- Vitamins. In: Burtis KA. and Ashwood RA. Editors- Tietz Text Book of Clinical Chemistry. 3rd ed., W.B Saunders Company, 1998:1023- 1024.
4. Daga MK, and Mohan A- Antioxidants and disease-current status. JAPI.1996; 44(10): 703-710.
5. Kuragupuran R, Subapriya R, Kabalimoorthy J, Nagini S- Antioxidant profile in the circulation of patients with fibro adenoma and Aden carcinoma of the breast. Clin Biochem .2002; 35: 275-279.
6. Varley H, Gowenlock AH and Bell M- Vitamins. In: Practical Clinical Biochemistry. Vol. 2. 5th ed.1980. William Heinemann Medical Books Limited, 19: 249-255.
7. Singh PP, Purabia P, Purabia SL.-Nutritional studies on the population of southern Rajasthan: III tribal adolescents, adults and elderly.In-Free radicals and antioxidants, sort out facts from fiction.1999: 249-259.
8. Das SK and Vasudevan DM. -Biochemical diagnosis of Alcoholism. Indian J Clin Biochem. 2005; 20 (1): 35-42.
9. Kim HS and Lee BM- Protective effects of antioxidant supplementation on plasma lipid peroxidation in smokers. J Tox and Env Health part A. 2001; 63(8):583-98.
10. Lykkesfeldt J, Christen S, Wallock LM, Chung HH, Jacob RA et al editors- Ascorbic acid is significantly decreases by smoking and repleted by moderate supplementation. Am J Clin Nutr. 2000; 71(2): 530-36.
11. Banerjee KK, Marimuthu P, Sarkar A, and Chaudhari RN- Influence of cigarette smoking on vitamin C, Glutathione and Lipid peroxidation status. Indian J Public health.1998; XXXXII (1):20-23.
12. Go VL, Gutzovekaya A.and Pandol SI- Alcohol and pancreatic cancer. Alcohol. 2005; 35:205.
13. Packer JE, Slater TF, Darrow RM- Nature (London). 1979: 278: 737-738.
14. Block G- Vitamin C and Cancer prevention: The epidemiologic evidence. Am J Clin Nutr. 1991: 53: 270S-282S.
15. Valdes AM, Andrew T, Gardner JP, Kimura M, Oelsner E et al- Obesity, cigarette smoking and telomerase length in womwn. Lancet. 2005: 366: 662.