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Comparison of Serum β-carotene and Retinol in Smokers and Non-smokers

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ABSTRACT

The study under report was an attempt to evaluate the effect of smoking on serum β-carotene and retinol. A total of 50 men aged 20-25 years were selected and grouped as smokers and non-smokers (25 each). Subjects included as smoker were smoking 8-10 cigarettes per day for the last 3-4 years. Serum was prepared from 5 mL venous blood from the donors. Serum β-carotene and retinol were determined using trifluoro acetic acid (TFA) method. Proteins were precipitated with 95% ethanol. Beta-carotene and retinol were extracted into light petroleum and followed by spectrophotometric analysis to study the intensity of the yellow color present due to carotenes. After evaporating the light petroleum, the residue was dissolved in anhydrous chloroform and mixed with TFA. The intensity of the blue color produced, gave the absorbance for retinol. The data obtained was statistically analysed for descriptive statistics and comparison of means using independent sample t-test, through SPSS 400. Statistical appraisal of the data did not indicate a significant difference in the serum β-carotene of smokers and non-smokers but a significant ($P<0.05$) difference for retinol between smokers and non-smokers was observed.

Key Words: β-carotene; Retinol; Smokers

INTRODUCTION

Smoking is described as the single largest preventable cause of death. Tobacco use is very common in Pakistan; 34% of men and 12.5% of women, use some form of tobacco on regular basis (National Health Survey of Pakistan, 1998). Tobacco use is a major risk factor for diseases of the heart and blood vessels, chronic bronchitis and emphysema, cancers of the lung, larynx, oral cavity, esophagus, pancreas and bladder and other problems such as respiratory infections and stomach ulcers.

Cigarette smoke causes the production of free radicals in human body. A free radical is any molecule that contains one or more unpaired electrons e. g., superoxide (O^{-}_{2}) and hydroxyl ion ($O^{\cdot}H^{-}$) (Dubick & Keen, 1991). Free radicals are extremely reactive and can cause cell injury and death. Polyunsaturated fatty acids in cell membranes, proteins such as enzymes and membrane ion transporters and DNA are the cell components, which are damaged by these reactive species. Each free radical can initiate a series of chain reactions, which continues and damages the cell constituents. These species disappear from the body following reactions with other free radicals or more importantly, due to the actions of the antioxidant system. These radicals play a significant role in the disease process, because they cause the destruction or depletion of antioxidants during their neutralization (Al-Senaidy *et al.*, 1997). The antioxidant system consists of superoxide dismutase, glutathione peroxides, metal binding proteins, vitamin E, Vitamin C, β-carotene, vitamin A, uric acid, bilirubin, albumin, DNA repair enzymes and methionine sulphoxide reductase.

β-carotene is a precursor of retinol in human body. The beneficial effects of β-carotene are mainly due to its high pro-vitamin activity and its antioxidant nature (Hilbert & Mohsenin, 1996). They can inactivate the free radicals and singlet oxygen by a process termed as quenching (Masci, 1991). They also inhibit the lipid peroxidation in membrane but only at low O_2 concentration. Retinol is a fat soluble vitamin and in the serum, circulates largely in the form of a 1:1 complex of retinol and retinol binding protein (RBP). Vitamin A plays an important role in growth, reproduction, growth of healthy skin and epithelial cells of mucosal linings, cellular differentiation, glycoprotein synthesis, membrane stabilization and the immune response. The data on any aspect of smoking with respect to serum concentrations of β-carotene and retinol is very scanty. The main objective of the study was to evaluate the effect of smoking on serum β-carotene and retinol.

MATERIALS AND METHODS

Selection of study samples. A total of 50 volunteer young men aged 20-25 years, were selected for the study, and divided into two groups i. e., Group A: Smokers (n=25) and Group B: Non-smokers (n=25). Subjects included as smokers were those consuming 8-10 cigarette/day for the last three years.

Collection of blood samples. Venous blood (5 mL) was drawn with the help of pyrogen free, disposable syringe,

between 9-11 A.M. The blood samples were free from haemolysis and were kept protected from light.

Preparation of serum. Blood was transferred to centrifuge tube and centrifuged at 2500 rpm for 7-10 min. The supernatants (serum) were separated with the help of 3 mL venoject tube, stored at 4°C and analyzed for β-carotene and retinol within 72 h.

Determination of β-carotene and retinol in serum. Serum β-carotene and retinol were determined using TFA method.

Concentration of serum β-carotene. It was calculated by the following formula:

$$\text{Serum } \beta\text{-carotene (mg/L)} = \frac{A_{450} \text{ of Unknown}}{A_{450} \text{ of Standard}} \text{ X Concentration of Standard X 3}$$

Absorbance of each working standard (carotene) at 450 nm having different concentration is given in Table I. By putting this value to the formula finally the serum carotene value was calculated as:

$$\text{Serum Carotene} = A_{450} \text{ of Unknown X } A_1$$

Concentration of serum retinol. Formula applied to calculate the serum retinol concentration was:

$$\text{Serum Retinol (mg/L)} = \frac{A'_{620} \text{ of Unknown}}{A'_{620} \text{ of Standard}} \text{ X Concentration of Standard X 3/2}$$

To calculate of each retinol working standard was used:

$$A'_{620} = A_{620} - F \,.\, A_{450},$$

Where, A_{620} = Absorbance of each retinol working standard at 620 nm, A_{450} = Absorbance of each carotene working standard at 450 nm, F = Calculated as A_{620}/A_{450}

After substituting the value of F, A'_{620} of each retinol working standard was determined and given in the Table II. Substituted the average value A_2 to the formula to calculate the serum retinol concentration.

Statistical analysis. The data thus obtained was subjected to statistical analysis for descriptive statistics and comparison of mean using independent sample t-test, using SPSS 400.

RESULTS AND DISCUSSION

Very little data is available on any aspect of smoking under local conditions therefore interpretation of the results becomes difficult.

β-carotene. Statistical appraisal of the data (Table III) did not indicate a significant difference in the serum β-carotene of smokers and non-smokers. These findings are not in agreement with Singh *et al.* (1994), Street *et al.* (1994) and Al-Senaidy *et al.* (1997). They had reported low serum and plasma carotenes in smokers and associated it with high lipid peroxidation. However, our results are in line with those of Berr *et al.* (1998). They reported higher levels of β-carotene in female smokers due to consumption of fruits and milk. Dietry history of our study samples indicated reasonable intake of fruits (Table V). This probably explains the normal level of β-carotene as observed in the present study.

Table I. Absorbance values of carotene working standards of different concentrations and calculation of factor A_1

Sr. No.	Conc. of carotene working standards (mg/L)	A_{450} of carotene working standards	Calculations Conc./Obs X 3
1	0.5	0.17	0.5/0.17 x 3 = 8.8
2	1.0	0.3	1/0.3 x 3= 10
3	2.0	0.59	2/0.59 x 3 = 10
4	3.0	0.85	3/0.85 x 3 = 10.5
5	4.0	1.1	4.0/1.1 x 3 = 10

Average = 10.04 = A_1

Table II. Absorbance values of different retinol working standards of different concentrations and calculation of factor A_2

Sr. No.	Conc. of retinol working standards (mg/L)	A_{450} of retinol working standards	Calculations Conc./Obs x 3/2
1	0.4	0.043	0.1/0.043 x 3/2 = 13
2	0.8	0.11	0.8/0.11 x 3/2 = 10.9
3	1.2	0.16	1.2/0.16 x 3/2 = 11.2
4	1.6	0.215	1.6/0.215 x 3/2 = 11.2

Average = 11.8 = A_2

Table III. Comparison of serum β-carotene in smokers and non-smokers by t-test

Statistical parameters	Smokers	Non-smokers
Mean	134.4	156.1
S. E.	15.7	12.6
Minimum	30.0	60.0
Maximum	281	281
P (t-test)	0.283	Non-significant

Table IV. Comparison of serum retinol between smokers and non-smokers by t-test

Statistical parameters	Smokers	Non-smokers
Mean	28.9	46.9
S. E.	4.0	5.0
Minimum	11.0	13.0
Maximum	82.0	90.0
P (t-test)	0.05	Significant at P < 0.05

Retinol. Retinol is not a strong antioxidant as its precursor β-carotene (Hilbert & Mohsenin, 1996), but retinol has many other vital functions. Our results (Table IV) showed low levels of retinol in smokers and normal levels in non-smokers. Statistical analysis revealed a significant ($P<0.05$) difference between smokers and non-smokers. Our findings are fairly in line with those of Singh *et al.* (1994), who reported significantly ($P<0.05$) low serum retinol levels in smokers. Faruque *et al.* (1995) and Al-Senaidy *et al.* (1997) also reported low serum retinol levels in smokers but these low were not statistically significant. Low level of serum retinol in smokers may be due to a marginal intake of

Table V. Comparison of various factors influencing the antioxidant status between smokers and non-smokers

Parameters	Smokers	Non-smokers
No. of subjects	25	25
Average intake of fruits	Frequent	High
Average intake of tea	Seldom	Seldom
Average intake of cigarettes	8-10	0
Average intake of milk	Seldom	High

vitamin A, which is generally present in the society. Smoking perhaps shifts this from margin to deficiency (National Health Survey of Pakistan, 1998). β-carotene is the precursor of retinol, and it is used in quenching of free radicals produced by cigarette smoke, thus sufficient amount is not available for conversion into retinol (Zondervan *et al.*, 1996*)*.

CONCLUSION

Based on the results of present study, it is suggested that dietary/supplementary intake of β-carotene and retinol should be increased for smokers.

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