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Plasma Vitamin C, β-Carotene, Lipids and Lipoproteins in Young Adult Smokers and Non-Smokers

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Abstract: Cigarette smoke has been implicated as a significant contributing factor in the development of chronic diseases. The adverse effect of smoking may elicit oxidative stress. This study was designed to assess the role of cigarette smoke on plasma vitamin C, β-carotene, lipids and lipoproteins in smokers. Sixty subjects consisting of thirty male active smokers with mean age of 37±1.84 years and thirty male non smokers with mean age of 34.6±2.3 years were selected for this study. The anthropometric indices and biochemical parameters were determined using standard procedures. The result shows that body mass index (BMI) ($p<0.05$) and plasma vitamin C ($p<0.001$) were significantly reduced in the smokers when compared with non-smokers. The plasma Total Cholesterol (TC), High Density Lipoprotein Cholesterol (HDLC), Low Density Lipoprotein (LDLC), Triglyceride (TG), and β-carotene were however not significantly different in smokers when compared with non Significant decreases were obtained in BMI and plasma vitamin C ($p<0.001$), β-carotene ($p<0.01$) Significant correlations were obtained between vitamin C and HDLC ($r = 0.335$, $p<0.05$), TC and LDLC ($r = 0.923$, $p<0.01$), body weight and BMI ($r = 0.770$, $p<0.05$) and TG and BMI ($r = 0.570$, $p<0.02$) in the smokers. Age was significantly correlated with TG ($r = 0.601$, $p<0.05$) and BMI ($r = 0.362$, $p<0.01$). This study that showed that reduced plasma vitamin C is associated with smoking. Because vitamin C plays important role in the maintenance of cellular antioxidants, active smokers should take vitamin C supplement to ameliorate the effect of cigarette smoke on cellular antioxidants.

Key words: β-carotene, HDLC, LDLC, smokers, TC, TG, vitamin C

INTRODUCTION

Cigarette smoking was reportedly associated with increasing risk of death from chronic pulmonary and cardiovascular diseases (Frei *et al*., 1999). Suggested possible explanations for this association including altered blood coagulation, impaired integrity of arterial wall, changes in blood lipids and lipoprotein concentrations as well as low antioxidant vitamins in smokers (Murata *et al*., 1989). Available studies have showed that smoking is higher among younger adults (Morrow *et al*., 1995; Doll *et al*., 2004). Long-term smoking has been reported to potentiate endothelial dysfunction in hyperchol- esterolemic patients by enhancing the oxidation of LDLC (Cohan *et al*., 1995). Study has shown that cigarette smoke contains small amounts of free radicals along with other toxins (Pourcelot *et al*., 1999).

Vitamin C, an antioxidant prevents free radical induced tissues damage by suppressing pro-oxidant states (Health Education and Welfare, 1974). Beta-carotene is especially noted in the class of nutrients that has potential chemo-preventive agents (the carotenoids) due to its anti-oxidant properties (Epstein, 2003). Some conditions commonly associated with reduced plasma vitamin C level are poor sperm quality (Ebesunun *et al*., 2009), increase gastric cancer and damage to DNA of germ cells (Farinatia *et al*., 1998) among others.

Study has also shown that chronic vitamin C deficiency leads to severe hypercholesterolemia and accelerated atherosclerosis in smokers (Health Education and Welfare, 1974). This study was designed to assess plasma lipids, lipoproteins β-carotene and ascorbic acid in young adult smokers in Nigeria.

MATERIALS AND METHODS

A total number of sixty (60) free living young adult males were recruited for the study. This comprises of thirty (30) male active smokers of age range 35-40 years (37.7±1.8) and thirty (30) male non-smokers of age range 30-40 years (34.6±2.3) served as the controls. The participants were selected from commercial blood donors in the University College Hospital Ibadan Nigeria.

The weight and height were measured using standard methods and BMI was calculated using H^2/W (Rowland, 1989).

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Table 1: Physical and biochemical parameters in smokers and non-smokers (Mean±S.E)

Variables	SmokersN = 30	Non-smokers N = 30	t-value	p-value
Age (yrs)	37.7±1.8	34.6±2.3	1.061	NS
Weight (kg)	61.4±1.7	64.4±1.8	1.235	0.05
BMI (kg/m^2)	21.0±0.5	23±0.8	2.001	NS
TC (mg/dl)	153±9	136±7	1.587	NS
HDLC (mg/dL)	59±3	52±3	1.709	NS
LDLC (mg/dL)	89±9	74±7	1.348	NS
TG (mg/dL)	94±0.06	86±0.05	1.086	NS
Vitamin C (mg/dL)	0. 86±0.05	1.16±0.05	3.962	0.01
β-carotene (μg/dL)	2.69±0.17	3.16±0.32	1.302	NS

NS: not significant

Table 2: Physical and biochemical parameters in smokers and non-smokers aged <30 years (Mean±S.D)

Variables	Smokers N = 30	Non-smokers N = 30	t-value	p-value
Weight (kg)	57±9	62±9	1.300	NS
BMI (kg/m^2)	22±4	21±2	0.910	NS
TC (mg/dL)	147±65	126±29	0.950	NS
HDL-C (mg/dL)	64+24	54±15	1.940	NS
LDL-C (mg/dL)	70±39	62±28	0.437	NS
TG (mg/dL)	67±44	59±20	0.509	NS
Vitamin C (mg/dL)	0.66±0.6	1.12±0.3	2.311	0.03
β-carotene (μg/dL)	3.57±1.5	2.52±0.9	1.702	NS

Blood sample collection: Overnight fasting (10-14 h) venous blood samples were collected from the ante-cubital vein of all the subjects by standard venipuncture technique into ethylene diamine tetra-acetic acid (EDTA) bottles and immediately placed in a dark wrapper to prevent oxidation of vitamin C and β-carotene. The samples were centrifuged at 3000 rpm for 5 min and the plasma were aspirated into a clean vial and stored at -4ºC until analyzed.

Analytical procedures: Analysis of vitamin C (Ascorbic acid) was based on the method postulated by Kyaw (1977). This is based on the principle of the formation of a light-blue supernatant when ascorbic acid reacts with sodium tungstate in an alkaline solution; the intensity of the colour produced is proportional to the concentration of ascorbic acid in the plasma.

Analysis of β-carotene was based on Neeld and Pearson (1942) in which trifluoroacetic acid reacts with the conjugated bond system of vitamin A to form a faint blue, short-lived compound. The TC was estimated using the enzymatic method of Allain *et al*. (1974) while TG analyzed based on the modified enzymatic method of Bucolo and David (1973). The HDLC was determined after precipitating with heavy metal of MgCL-sodium Tungstate solution to remove other lipoproteins fractions. The supernant was assayed for HDLC using the same method for TC.

Accuracy and precision of biochemical tests were monitored by including commercial quality control samples within each batch of test assay.

Statistical analysis: All results were subjected to statistical analysis using SPSS version 11.0. The results were expressed as mean ±SE. Student t-test for parametric data was used to compare groups. Pearson correlation coefficient was used to establish associations between variables. Results were regarded as significant at $p<0.05$.

RESULTS

Table 1 shows the mean±standard error of all the physical and biochemical parameters in active smokers and non-smokers. The mean value of BMI (21±0.5 kg/m^2) in smokers was statistically decreased when compared with the corresponding mean value in the non-smokers (23±0.8 kg/m^2) ($p<0.05$) while the mean value of 0.86±0.06 mg/dL of plasma vitamin C was also significantly decreased in the smokers compared with the corresponding value of 1.16+0.05 mg/dL in the non-smokers ($p<0.001$). The increases in the other parameters were not significant.

Table 2 shows the physical and biochemical parameters in smokers and non-smokers of <30 years of age. The plasma vitamin C level was significantly reduced in the smokers ($p<0.03$) when compared with the corresponding values in the non-smokers of the same age. Although there were decreases and increases in mean body weight, BMI, TC TG, LDLC, and β-carotene, these changes were not statistically significant when compared with the corresponding value in the non-smokers of similar age.

Table 3 shows the physical and biochemical parameters in smokers and non-smokers of >30 years of age. Plasma vitamin C ($p<0.001$) and β-carotene ($p<0.01$) showed significant reductions when compared with the corresponding non-smokers of the same age. No significant changes were obtained in the other parameters.

Table 4 shows the correlation coefficient of all parameters in smokers. Plasma vitamin C was positively

Table 3: Physical and biochemical parameters in smokers and non-smokers aged >30 years (Mean±S.D)

Variables	Smokers N = 30	Non-smokers N = 30	t-value	p-value
Weight (kg)	62±9	68±12	1.640	NS
BMI (kg/m^2)	21±3	25±5	3.661	0.001
TC (mg/dL)	154±49	147±35	0.450	NS
HDL-C (mg/dL)	59±17	49±15	1.730	NS
LDL-C (mg/dL)	91±49	86±42	0.273	NS
TG (mg/dL)	97±66	99±52	0.151	NS
Vitamin C (mg/dL)	0.88±0.28	1.21±0.27	3.518	0.001
β-carotene (μg/dL)	2.59±0.86	3.90±2.17	2.739	0.01

NS: not significant

Table 4: Correlation coefficient of physical and biochemical parameters in smokers

Variables	Vit C (mg/dL)	β –carotene (μg/dL)	TC (mg/dL)	HDLC (mg/dL)	TG (mg/dL)	LDLC (mg/dL)	Weight (Kg)	BMI (kg/m^2)
Age(years)					0.382*			0.601**
Vit C(mg/dL)				0.375*				
β –carotene (μg/dL)								
TC(mg/dL)						0.923**	0.412*	0.393*
HDLC (mg/dL)	0.375*							
TG(mg/dL)								0.362*
LDLC(mg/d)			0.923**					
Weight(Kg)			0.412*					0.847**
BMI(kg/M^2)			0.393*	0.085*	0.362*		0.847**	

*: Significant at 0.05 level; **: Significant at 0.01 level

significantly correlated with plasma HDLC ($r = 0.400$, $p<0.05$) while plasma TC was positively correlated with plasma LDLC ($r = 0.894$, $p<0.01$), weight and BMI ($r = 0.770$, $p<0.05$) respectively in the smokers. There was also significant correlation between plasma TG and BMI ($r = 0.570$, $p<0.01$) in all the smokers.

DISCUSSION

This study demonstrated a marked decrease in plasma vitamin C in smokers. A number of studies have indicated reduced plasma vitamin C levels in smokers (Pelletier, 1970; Health Education and Welfare, 1974).The presence of injurious substances such as nicotine, carbon monoxide, carbon particles, acetaldehyde and nitrogen dioxide in cigarette smoke have been implicated in reduced bioavailability of vitamin C (Pelletier, 1970).

The plasma β-carotene was not significantly altered in all the smokers, interestingly however, there was decreased plasma β-carotene in the age group >30 years, and whether differences in age are important contributing variable in this regard remains uncertain. There is the possibility that increasing free radicals in cigarette smoke may considerably decrease the bioavailability of vitamin C and β-carotene as antioxidant nutrients with increasing years of smoking. Whether this increased is related to duration of smoke could not be ascertained from this study. The reduced plasma vitamin C concentration in smokers in the present study suggests that these individuals may be at the risk of developing chronic obstructive pulmonary disease. A positive correlation of BMI and weight in smokers further support this possibility.

Plasma β carotene is especially noted among the class of nutrients that is regarded as potential chemo preventive agents due to its antioxidant properties (Epstein, 2003). Knekt *et al*. (1994) had earlier reported that serum concentration of vitamin C and β-carotene appears to play some important roles in the prevention of pulmonary damage initiated by oxidants. These two antioxidants as evident from epidemiological study are required in increased amount by smokers. (Aderson *et al*., 1991). The smokers in this study appear to be at risk of these oxidative damages associated with smoking, even more at risk are age group >30 years who showed marked reduction in plasma vitamin C.

There is accumulated evidence in support of a strong association between ischemic heart disease and hypercholesterolemia (Krichevsky *et al*., 1995). Although the increased in the mean plasma LDLC among smokers in this study was not statistically significant, there is the likelihood that these smokers may shift toward risk of developing atherosclerosis if the smoking habit is not controlled. Increasing evidence has it that the high risk of Coronary Heart Disease (CHD) in smokers is medicated through increased plasma LDLC (Scott, 1995). Inhaled cigarette smoke enhances lipid peroxidation and this may lead to damage of the endothelium (Frei *et al*., 1991). The positive correlation between TC and LDLC indicates that increase in TC leads to corresponding increase in LDLC thus subjecting the smokers to possibly premature CVD risk. An earlier report (Jack, 1995) showed elevated plasma HDLC in smokers contrary to our finding which showed reduced mean HDLC.

Elevated plasma triglyceride was obtained in our smokers. Elevated plasma TG has been weakly

association with CHD as an independent risk factor (Grundy and Vega 1992). While the plasma vitamin C and β-carotene varied with age in the smokers of this study is largely unknown. However, it could be speculated that this age group may not have paid attention to healthy eating vis a vis balance diet. Thus the likelihood to develop atherosclerosis vascular disease is high particularly with cigarette smoking.

CONCLUSION

The low plasma vitamin C in smokers in the present study is an indication that smokers are most liable to develop chronic diseases associated with reduced antioxidant nutrients. Dietary supplementation of vitamin C in smokers will reduce the risk of developing early atherosclerotic heart disease. Further study will be required on larger number of samples.

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AUTHOR'S CONTRIBUTION

Ebesunun M.O., was involved in the conception, design, interpretation and drafting of the manuscript, Adetunji R.A., was involved in the conception, collection of data and analysis of result as well as drafting and Umahoin K.O. was involved in the interpretation and drafting of the manuscript.