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"SERUM ANTIOXIDANT VITAMIN C CONCENTRATION AND LIPID PROFILE IN CORONARY HEART DISEASE (CHD) PATIENTS".

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ABSTRACT:

BACK GROUND AND OBJECTIVES: *Free radicals, reactive oxygen species play an important role in the pathogenesis of atherosclerosis causes Coronary Heart Disease (CHD). Antioxidant present in our diet such as vitamin C, vitamin E and b-Carotene may act against the free radicals. Thus they can prevent the process of atherosclerosis and CHD. Object of this study was to determine the serum antioxidant vitamin C and lipid profile in CHD patients.*
DESIGN AND SETTING FOR STUDY: *At Shaikh Zayed Hospital and Punjab Institute Of Cardiology, Lahore.*
SUBJECT AND METHODS: *We have measured the Antioxidant vitamin C concentration and lipid profile in the case controlled study of 80 patients of CHD; and 21 Healthy Control Subjects by matching their age, sex, weight and height with respect to those of CHD patients.*
RESULTS: *No significant (P> 0.05) difference was observed in the mean serum vitamin C level in the CHD patients and control subjects. Regarding lipid profiles; significant raised triglycerides, total cholesterol and LDL-cholesterol and significant decreased HDL-cholesterol were observed when compared CHD patients with normal subjects.*
CONCLUSION: *No doubt nutrient oxidants are better for preventing CHD, but this study showed no correlation between antioxidant vitamin C level and Coronary Heart Disease (CHD). So there is need to carry out a wide study of antioxidant (Vitamin C) level in Coronary Heart Disease.*

KEY WORDS: Coronary Heart Disease (CHD), Atherosclerosis, lipid metabolism, free Radicals, Reactive oxygen species (ROS), Oxidative stress, Antioxidants and Vitamin C.

INTRODUCTION:

Many studies have shown that the free radicals, reactive oxygen species (ROS), are involved in the pathogenesis of atherosclerosis and Coronary Heart Disease (CHD) (Hayn *et.al*., (1996)[1]; Nordberg and arner (2001)[2]). ROS are responsible for oxidative modification of low-density lipoprotein (LDL) particles (Bellomo *et al*., (1995)[3]; Halle Well (1995)[4] in the arterial subedothelium that results in structural changes of LDL, which are postulated to make them more etherogenic than native LDL (Lusis, 2000)[5]. The Anti-oxidants, both dietary as well as endogenous are an important protective factors (Lissi *et al*., 1995)[6].The most readily available naturally occurring antioxidant in food are Vitamin E and Vitamin C. Vitamin C (ascorbic acid) is generally considered to be a key aqueous- phase antioxidant and ascorbic acid deficiency may contribute to oxidative stress and lipid per-oxidation in the CHD patients (Vivekananthan *et.al*., (2003)[7]; Bleys et.al (2006)[8]. Therefore study was planned to access the serum concentration of Vitamin C and lipid profile in CHD Patients.

SUBJECTS AND METHODS:

This study included eighty (80) patients of Coronary Heart Disease with first and second episode of Myocardial Infarction, and twenty one (21) normal healthy persons as control subjects, matched for age, sex, weight and height.

Sample: About 10ml venus blood samples, were taken after over night fast from the patients and control subjects. Blood serum samples were separated after centrifugation at 200g. Serum samples were stored at 4°C for immediate analysis and at –20°C for later assay. For vitamin C analysis serum was mixed with equal volume of 0.75M solution of Meta-phosphoric acid and centrifuged supernatant was frozen at -20°C for later assay.

METHODS:

Vitamin C was measured by the method of Brewster and truly [9] (Cited by pesce and Kaplan 1987). Cholesterol was determined after enzymatic hydrolysis and oxidation. Liberated H_2O_2 reacts with 4-aminoantipyrine in the presence of phenol and peroxidase, and form a red coloured indicator quenoneimine, which was measured at 546 nm (Lie *et al.*, 1976)[10]. Low density Lipoprotein (LDL), very low density lipoprotein (VLDL) and chylomicron fractions were precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium chloride. After centrifugation HDL-cholesterol concentration in supernatant was determined by total cholesterol method as described above. Moreover triglyceride was determined after enzymatic hydrolysis of trigliceride with lipase, phosphorylation of glycerol with Gylcerol phosphokinase, and oxidation of glycerol-3-phosphate with glycerophosphate oxidase. Thus liberated H_2O_2 reacts with 4-aminoantipyrine, in the presence of phenol and peroxidase; and form red coloured quinoneimine, measured at 546 nm (Fossati and prencipe 1982)[11]. However LDL Cholesterol was calculated by the formula of Friede-wald *et al*., (1972)[12] as;

LDL-cholesterol = total cholesterol-(Triglyceride + HDL-cholesterol)

RESULTS:

Present study included 80 Coronary Heart Disease (CHD) patients from Shaikh Zayed Hospital and Punjab Institute of Cardiology Lahore, and 21 healthy control subjects, matched for age and Sex. Male to Female ratio in the patients was 1:1 and in controls was 1.1:1 (Table: 1).

There was no significant difference in the mean age, height and weight of the patients and the control subjects (Table: 2). According to occupation out of 21 control subjects were 8 house wife, 2 service person, 1 unemployed, 1 student, 6 business person, 1 zamindar and 2 doctors; where as out of 80 CHD patients 31 were house wives, 9 service persons, 9 unemployed, 1 student, 25 business persons, 2 zamindars and 3 doctors (Table 3).

Table 4 shows the lipid and vitamin C comparison among CHD and control subjects. Finding shows significantly higher ($p<0.05$) level of total cholesterol, LDL-cholesterol and triglycerides; where as no significant difference was observed in CHD when compared with control subjects.

Out of Twenty Nine CHD patients with raised triglyceride level, thirteen patients had vitamin C level, of =6mg/L and sixteen patients with vitamin C level of <6mg/L, while out of fifty one with normal triglyceride level, thirty patients showed vitamin C level of =6mg/L and twenty one patients showed vitamin C level of <6mg/L (Table: 5).

Out of fifty five CHD patients with raised total cholesterol level, 30 patients had vitamin C level of =6mg/L and 25 patients had vitamin C level of <6mg/L, and showed no significant (P>0.05) difference, when compared with twenty five patients with normal total cholesterol level, out of which 13 patients had vitamin C level of =6mg/L and 12 patient had vitamin C level of <6mg/L (table: 5).

Out of fifty eight CHD patients with decreased HDL-cholesterol level, 30 patients had vitamin C level =6mg/L and 28 patients had vitamin C level of <6mg/L. The incidence was not significantly (P>0.05) different when compared with twenty two patients with normal HDL-cholesterol level, out of which 13 patients had vitamin C level of =6mg/L and 9 patients had vitamin C level of <6mg/L (Table: 5).

Out of thirty five CHD patients with raised LDL-cholesterol level, 19 patients had vitamin C level of =6mg/L and 16 patient had vitamin C level of <6mg/L The incidence was not significantly (P>0.05) different when compared with forty five patients with normal LDL-cholesterol level, out of which 24 patients had vitamin C level of =6mg/L and 21 patients had vitamin C level of <6mg/L

TABLE 1

The age and sex distribution of control group and CHD patients is given.

Age (years)	Control			Patients		
	Male	**Female**	**Total**	**Male**	**Female**	**Total**
20-40	2	2	4	7	8	15
40-60	5	3	8	16	14	30
> 60	4	5	9	17	18	35
Total	**11**	**10**	**21**	**40**	**40**	**80**
Male : Female ratio	1.1 : 1			1 : 1		

TABLE 2

Age, height and weight of control subjects and CHD patients (Mean ± SE is given. Figure in the parenthesis show number of cases)

	Control (21) Mean ±SE	**Patients (80) Mean ± SE**
Age (years)	57.4 ± 1.9	56.4 ± 1.98
Height (feet)	5.53 ± 0.052	5.56 ± 0.04
Weight (Kg)	58.48 ± 1.75	59.3 ± 0.69

TABLE: 3

The distribution of controls and CHD patients according to occupational group is given (Figure in parenthesis indicate total number of control and patients).

Occupation	**Control (n = 21)**	**Patients (n = 80)**
House Wife	08	31
Service	02	09
Unemployed	01	09
Student	01	01
Business	06	25
Zamindar	01	02
Doctor	02	03
Total	**21**	**80**

(Table: 5).

DISCUSSION:

Present study included eighty Patients of coronary Heart Disease (CHD) and twenty one healthy control subjects, matched for age and Sex. Male and female ratio of patients was 1:1 and that of control subjects was 1.1:1 (Table 01). Sixty five patients of CHD and seventeen persons from the control group were in the age group of greater than forty years. The mean age, height and weight of the patients showed no significant (P> 0.05) difference when compared with the age, height and weight of the control subjects (Table 02). Most of the studies have shown that the majority of the CHD patients were obese males, over forty years of age (Massie 2000)[13]. Record of the profession showed that 31 Patients were house wife, 9 were service persons, 9 were unemployed, 1 was student, 25 were business Persons, 2 were Zamindar and 3 were doctors (Table 03).

The present study shown that the mean cholesterol level of the Patients (226.3 ± 6.3 mg/dL) was significantly higher ($p < 0.05$), when compared with the control subjects level (198.05±5.37mg/dL) (Table 04). The level of total cholesterol in healthy subjects has been reported to be 150 to 200mg/dL (Gey *et al*., 1991)[14]. In the previous studies Ma *et al*., (1992)[15] had also reported that the higher cholesterol levels are directly related with the CHD.

CHD Patients had shown significantly ($p<0.05$) higher level of triglyceride (159.5+5.03 mg/dL) (Table 04). Triglyceride level in healthy persons has been reported to be less than 165 mg/dL (Hultquist *et al*., 1997)[16] and Ma *et al*., (1992)[15] had also reported that high triglyceride level is associated with the development of CHD.

Mean level of HDL cholesterol of the CHD Patients (36.8 + 0.99 mg/dL) was significantly lower when compared with the control level (39.90+0.48 mg/dL) (Table 04). Hultquist *et al*., (1997)[16] had reported that the HDL Cholesterol is inversely correlated with the development of CHD and healthy subjects HDL Cholesterol level < 40mg/dL.

In the present study, the LDL Cholesterol level of the CHD Patients (157.6 +5.97 mg/dL) was seen to be significantly higher as compared to the control subjects (128.81+5.97 mg/dL) (Table 04). Previous studies had also shown that the high level of LDL Cholesterol is directly related with the development of the CHD (Ma *et al*., 1992)[15]. Normal LDL Cholesterol level in the healthy persons has been reported to be less than 180 mg/dL.

Mean vitamin C level of the CHD patients was 6.21±0.27mg/L as compared with that of control subjects, 6.5 ±0.42mg/L (Table 04). No significant difference was seen among the patients control subject (Table 04). The level of serum vitamin C in the healthy subjects has been reported to be 6-20mg/L (Jackson *et al*., 1995, Riemersma *et al*., 1991)[17,18]. Vitamin C concentrations < 4.1mg/L (23.4mM) are considered to be critically low and scurvy may develop if the concentrations of vitamin C are < 1.93mg/L (11mM) (Hultquist *et al*.,1997)[16]. No reports on vitamin C levels in CHD patients were available.

Present study has shown that the vitamin C has no significant correlation with the lipid profile (Total Cholesterol, Triglyceride, HDL-Cholesterol and LDL-Cholesterol) of the CHD patients (Table 5). No comparable observation has been previously reported.

TABLE: 4

Levels of lipid profile and vitamin E in controls and patients of Coronary heart disease (CHD) (Mean ± SE is given)

Group	Total cholesterol (mg / dl)	Triglyceride (mg / dl)	HDL-c (mg/dl)	LDL-c (mg/dl)	Vitamin C (mg/L)
Control (n = 21)	198.05±5.37	146.43±4.10	39.90±0.48	128.81±5.14	6.5 ± 0.42
Patients (n = 80)	226.3±6.3*	159.5±5.03*	36.8±0.99*	157.6±5.97*	6.21 ± 0.27

*P < 0.05

TABLE: 5

Correlation of Vitamin C levels with lipid profile in Coronary Heart Disease (CHD) patients.

Group	**No. of patients with Vitamin C ³ 6mg/L**	**No. of patients with Vitamin C < 6mg/L**	**Total**
Raised triglyceride	13	16	29
Normal triglyceride	30	21	51
Total	43	37	80
Raised total cholesterol	30	25	55
Normal total cholesterol	13	12	25
Total	43	37	80
Decreased HDL cholesterol	30	28	58
Normal HDL cholesterol	13	09	22
Total	43	37	80
Raised LDL-cholesterol	19	16	35
Normal LDL-cholesterol	24	21	45
Total	43	37	80

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