ANTIOXIDANT STATUS IN CORONARY HEART DISEASE (CHD) PATIENTS WITH TYPE 2 DIABETES MELLITUS

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Background: The present study was carried out to see the levels of vitamin C, vitamin E and total antioxidant (AO) in Coronary Heart Disease (CHD) patients with and without Type-2 Diabetes Mellitus (T2DM). In various previous studies it has been reported that, diabetes, hypertension and smoking are risk factors for CHD and all risk factors were common in these patients. **Methods**: Serum was tested from 80 CHD patients and 21 healthy controls, matched for age, height, and weight. **Results:** No significant difference was seen between the age, height and weight of the subjects and controls. CHD patients were mostly male, smokers, over 40 year of age and belonging upper class families. The mean plasma glucose was significantly higher ($p<0.05$) in CHD patients having T2DM as compare to CHD patient with out T2DM. Risk factors for CHD, like diabetes, hypertension and smoking were common in these patients. No significant difference was seen in vitamin C level of patients of CHD. Vitamin E level was significantly ($p<0.05$) low among the CHD patients as compared to controls and a significant ($p<0.05$) decrease in mean vitamin E level was observed among smoker CHD patients as compared to non-smoker CHD patients. But no significant difference in vitamin C and vitamin E levels of CHD patients with diabetes and hypertension were observed when compared with CHD patients having no such complaints. The CHD patients' total antioxidant level was significantly ($p<0.05$) decreased as compared to controls. **Conclusion**: The total AO (Vit. C and E) were not significantly higher in CHD patients with hypertension and diabetes as compared to those patients of CHD having no hypertension and diabetes.

Keywords: Coronary Heart Disease (CHD), Ischemic Heart Disease (IHD). Type 2 Diabetes Mellitus (T2DM), Fasting Plasma Glucose (FPG), Antioxidant (AO)

INTRODUCTION

Ischemic heart disease (IHD) is the leading cause of death in most population of the world which can be decreased by changing life style.[1] Ischemic heart disease (IHD) and CHD are the generic designation for the four forms of cardiac diseases, i.e., angina pectoris, sudden cardiac death, myocardial infarction and chronic ischemic heart disease.[2] In most cases the imbalance results from insufficient blood flow secondary to the development of atherosclerosis resulting in narrowing of the coronary arteries and hence the term 'Coronary Heart Disease'.[2,3]

Identified common risk factors and their relationship with atherosclerosis and IHD[3] are:

1) Dyslipidemia/Hypercholesterolemia
2) Diabetes Mellitus
3) Obesity
4) Type A personality
5) Hypertension
6) Cigarette smoking

The peroxidation of lipoproteins, especially low density lipoproteins plays a significant role in the pathogenesis and progression of atherosclerosis.[4] The interior of advanced atherosclerotic lesions is a pro-oxidant environment, as the entrant from lesions can promote lipid oxidation, including peroxidation of LDL and generation of highly reactive hydroxyl radicals from hydrogen peroxide.[5,6,7]

Free radicals are being continuously produced by the body by different mechanisms which can oxidize multiple fatty acid side chains to lipid peroxides.[8] These peroxidation reactions are countered by antioxidants present in plasma and interstitial fluid, and α-tocopherol (vitamin E) is the most important compound that inhibit lipid peroxidation in the presence of vitamin C, and give protection against cardiovascular disease.[9]

The clustering of insulin resistance with obesity, dyslipidemia, hypertension, and an increased risk of cardiovascular disease is known as Syndrome-X or Insulin Resistance Syndrome.[10] Mean plasma glucose level in diabetic CHD patients were observed higher as compared to non-diabetic CHD patients in most of the studies carried out by number of workers.[10,11] Cardiovascular disease is one of the major cause of death in T2DM patients and First Degree Relatives (FDRs) of such patients are also at increased risk of developing diabetes and cardiovascular disease.[11]

An absolute or relative deficiency of antioxidant defence may lead to a situation of increased oxidative stress, especially in case of obesity, dyslipidemia and insulin resistance, and this may be associated with both the causes and consequences of a variety of disorders, including CHD and cancer.[12]

The free radicals are usually removed or inactivated *in vivo* by a team of antioxidants (Vit. E and Vit. C).[12]

The most outstanding contributions to the epidemiology of the effects of dietary supplementation with vitamin E have been studied extensively, which showed that a 40% reduction in coronary artery disease is seen in those individuals who supplemented their diet with vitamin E intake.[13,14]

MATERIALS AND METHODS

A total of 80 pre-diagnosed coronary heart disease patients from Punjab Institute of Cardiology and 21 normal healthy controls from general staff of Shaikh Zayed Hospital Lahore, were studied. The patients and controls were matched for age, sex, height and weight. Socioeconomic history and habit of any addiction was also recorded.

About 10 ml of fasting venous blood sample of CHD patients and controls were taken and allowed to clot to get serum. Sera were stored at 4 ºC for immediate analysis and at -20 ºC for later analysis. For vitamin C analysis serum was mixed with equal volumes of 0.75 M Meta-phosphoric acid and centrifuged to get supernatant. The supernatant obtained was frozen at -20 ºC for later analysis of vitamin C. Another 1.5 ml of blood was placed in fluoride EDTA tubes for glucose estimation. Following assays were carried out on serum specimens of the patients and controls:

1. α-Tocopherol (Vit. E) by method of Baker and Frank 1968.[15]
2. Ascorbic Acid (Vit. C) by method of Brewster and Turly.[16]
3. Fasting Plasma Glucose was measure by hexokinase method.[17]
4. Total antioxidant

RESULTS

This cross sectional comparative study included 80 CHD pre-diagnosed patients from Punjab Institute of Cardiology and 21 healthy controls from Shaikh Zayed Hospital Lahore, and were matched for age, sex, weight and height. No significant differences in mean age, height and weight of patients and controls were seen. Twenty-two patients had shown different vitamin deficiency signs and symptoms. Out of these, 12 patients had swollen gums, 5 had glossitis, 3 patients had fissured skin, one had alopecia and one had brittle nails (Table-1).

No such complaints were seen in controls when studied for associated diseases. Twenty-two CHD patients, i.e., 12 males and 10 females, were seen to be diabetic, while 26, i.e., 13 males and 13 females were hypertensive; and 30 male and 2 female patients were smokers (Table-2).

Table-1: Number of patients with CHD having signs and symptoms associated with vitamin C and E deficiency

Signs and Symptoms	Number	Percentage
Swollen Gums	12	15.5
Glossitis	5	6.25
Dry Fissured Skin	3	3.75
Alopecia	1	1.25
Brittle Nails	1	1.25
Total	**22**	**27.5**

Table-2: Distribution of CHD patients according to the presence of risk factors

Risk Factor	Number of Patients	Duration (Years)
Diabetes		
Male	12	2–14
Female	10	1–4
Total:	**22**	
Hypertension		
Male	13	2–20
Female	13	1–18
Total:	**26**	
Smoking		
Male	30	2–10
Female	2	4–6
Total:	**32**	

Mean vitamin C levels of the CHD patients and control were 6.21±0.27 mg/L and 6.5±0.42 respectively, and showed no significant differences (Table-3).

A significant (p<0.05) difference was seen between the mean vitamin E level of CHD patients (8.69±0.19mg/L) and the controls (10.02±0.70 mg/L) (Table-3).

CHD patients with diabetes and hypertension showed no significant difference (p>0.05) in the levels of vitamin C, Vitamin E and total antioxidant as compared to the patients without diabetes and hypertension respectively (Table-4).

No significant difference was observed in mean FPG level of CHD patients (87.39±1.48) and controls (83.44±1.42), though the FPG was on higher side in CHD patients relative to controls (Table-3). A significant (p<0.05) difference in mean fasting plasma glucose of diabetic CHD patients (95.82±1.90) as compared to non-diabetic CHD patients (84.00±1.29) was observed (Table-4).

Out of 52 CHD patients with diabetes, hypertension and smoking, 50 patients had vitamin E level of ≥6 mg and only two patients have vitamin E level <6 mg/L. The incidence was not significantly different when compared with 28 patients without diabetes, hypertension and smoking, out of which 26 patients had vitamin E level of ≥6 mg/L and only 2 patients had vitamin E level of <6 mg/L (Table-5).

With respect to Vitamin C, out of 52 CHD patients with diabetes, hypertension and smoking, 32 patients had vitamin C level ≥6 mg/L and 22 patients had vitamin C level of <6 mg/L. The mean level was not significantly different, when compared with 28 patients

without diabetes, hypertension and smoking from which 13 patients had vitamin C level of ≥6 mg/L and 15 patients had vitamin C level of <6 mg/L (Table-5).

Total AO out of 52 patients with diabetes, hypertension and smoking, 27 patients had total antioxidants level of ≥612 µ mol/L and 25 patients had total antioxidant level of <612 µ mol/L. The mean levels were not significantly different when compared with 28 patients without diabetes, hypertension and smoking, out of which 17 patients had total antioxidants level of ≥612 µ mol/L and 7 patients had total antioxidant level of <612 µ mol/L (Table-5).

Mean total antioxidant level of the CHD patients (708.30±27.60 µ mol/L) was significantly (P<0.05) low as compared to the total antioxidant levels in controls (785±42.10 µ mol/L) (Table-3). The CHD patients with and without diabetes and hypertension had shown no significant difference in the levels of total antioxidants (Table-4).

Table-3: Levels of total antioxidant, vitamin C, vitamin E and FPG in Patients of CHD and Controls (Mean±SEM)

Group	Total Antioxidant (µ mol/L)	Vitamin C (mg/L)	Vitamin E (mg/L)	FPG (mg/dL)
CHD-Patients (n=80)	708.3 ± 27.60*	6.21 ± 0.27	8.69 ± 0.19*	87.39 ± 1.48
Controls (n = 21)	785 ± 42.13	6.5 ± 0.42	10.02 ± 0.70	83.44 ±1.42

*p<0.05

Table-4: Total antioxidant, Vitamin C, Vitamin E and FPG levels in Diabetic/Hypertensive and Smoker CHD patients and in non-diabetic/non-Hypertensive and non-smoker CHD patients (Mean ± SEM)

Group	Total Antioxidant (µ mol/L)	Vitamin C (mg/L)	Vitamin E (mg/L)	FPG (mg/dL)
Diabetic CHD Patients (n=22)	665 ± 48.0	6.31 ± 0.36	8.98 ± 0.23	95.82 ± 1.90*
Hypertensive CHD Patients (n=26)	723.8 ± 51.0	6.65 ± 0.62	8.70 ± 0.35	86.00 ± 1.45
Smoking CHD Patients (n=32)	660 ± 38.41	6.35 ± 0.34	8.36 ± 0.39*	80.35 ± 1.10
Non-Diabetic CHD patients (n=58)	725 ± 33.0	6.17 ± 0.35	8.56 ± 0.24	84.00 ± 1.29
Non-Hypertensive CHD Patients (n=54)	701 ± 32.96	6.0 ± 0.27	8.66 ± 0.23	82.68 ± 1.55
Non-Smoker CHD Patients (n=48)	727.54 ± 37.60	6.11 ± 0.39	8.89 ± 0.18	83.66 ± 1.42

*p<0.05

Table-5: The number of CHD patients showing Vitamin C, Vitamin E and total antioxidant levels with or without diabetes, hypertension and smoking

	Vitamin E		Vitamin C		Total Antioxidant	
Group	**≥ 6 mg/L**	**<6 mg/L**	**≥6 mg/L**	**<6 mg/L**	**>612 µmol/L**	**< 612 µmol/L**
CHD patients with Diabetes/Hypertension and Smoking (n=52)	50	2	30	22	27	25
CHD Patients without Diabetes/Hypertension and smoking (n=28)	26	2	13	15	17	11
Total: (n=80)	76	4	43	37	44	36

DISCUSSION

The present study was carried out to see the levels of vitamin C, vitamin E and total antioxidant in CHD patients with and without T2DM. In various previous studies it has been reported that, diabetes, hypertension and smoking are the risk factors for CHD and all risk factors were common in these patients[14,18]. Many of the previous studies had also shown that majority of the CHD patients were obese male over 40 years of age. No significant difference in mean age, height and weight of patients and controls were seen in the present study. [14,18]

In the present study patients had shown different vitamin deficiency signs and symptoms like swollen gums, glossitis, fissured skin, alopecia and brittle nails, while no such signs were seen in controls when studied for associated disease. Similarly in CHD patients, a number of patients, both male and female were found to be diabetic, hypertensive and smokers. These findings were in consistent with the previous studies that the diabetes, hypertension and smoking are risk factors for coronary heart disease.[18]

Mean vitamin C levels of the CHD patients and control had shown no significant difference. The serum vitamin C level in healthy subjects has been reported to be 6–20 mg/L.[19,20,21] Vitamin C level less than 4.1 mg/L (23.4µmol/L) are considered to be critically low and scurvy may develop if the level drops below 1.93 mg/L (11 µ mol/L).[19] Rimm *et al* (1993) reported that a high intake of vitamin C was not associated with a low risk of CHD.[14]

A significant difference (p<0.05) was seen between the mean vitamin E level of CHD patients (8.69±0.19 mg/L) and the controls (10.02±0.70 mg/L). Riemersma *et al* (1991)[21] have reported that vitamin E remained independently and inversely related to the risk of CHD. The level of vitamin E in healthy subjects has been reported to be 6–19 mg/dL[15,19,20]

CHD patients with diabetes and hypertension showed no significant differences (p>0.05) in the levels of vitamin C, Vitamin E and total AO as compared to patients without diabetes and hypertension respectively. Asayama *et al*[22] reported that vitamin C level was decreased in the serum of children with IDDM,[22] but the

correlation of diabetes/hypertension with vitamin C was not previously reported in CHD patients. No significant difference was observed in mean FPG level of CHD patients (87.39±1.48) and controls (83.44±1.42) though the FPG was on higher side in CHD patients relative to controls. A significant difference ($p<0.05$) in mean FPG level of diabetic CHD patients (95.82±1.90) as compared to non diabetic CHD patients (84.00±1.29) was observed . Higher FPG has been reported in diabetic CHD patients as compared to non diabetic CHD patients earlier in may studies.[10,11]

Our study also showed no sisnificant difference between total AO level, Vitamin C and Vitamin E levels in CHD patients with and without diabetes, hypertension and smoking. However when taken separately Vitamin E level had shown significantly reduced levels in smoker CHD patients as compared to non-smoker CHD patients.. Rimm *et al* had also observed that increase intake of antioxidants, primarily vitamin E, is associated with a reduced risk of CHD. This finding is also in consistent with geographic correlation between serum vitamin E, coronary mortality rates and reduced serum α–tocopherol in patients with CHD.[13,14]

Mean total AO level of the CHD patients (708.30±27.60µmol/L) was significantly low ($p<0.05$) as compared to the total antioxidant levels in controls (785±42.10 µmol/L) . The total antioxidant level in healthy subjects has been reported to be 612–1634 µmol/L.[20,22] The CHD patients with and without diabetes and hypertension showed no significant difference in the levels of total antioxidants. But the previous studies had shown that the obesity, diabetes, hypertension and smoking are the risk factors for the coronary heart disease.[18]

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