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**BIOCHEMICAL PARAMETERS OF SMOKERS AND NON-SMOKERS-A CASE
CONTROL STUDY**

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ABSTRACT

Coronary artery disease is a leading cause of death all over the world and plasma total homocysteine level is a strong, graded, and independent risk factor for coronary heart disease. Smoking is one of the powerful risk factors for coronary artery disease and is having an association with the raised levels of plasma homocysteine, low levels of folate and vitamin B12. Keeping in view the association of smoking, folate and Vitamin B12 deficiency and hyperhomocysteinemia, a prospective case control study was conducted with the objective, to evaluate the levels of serum folate, serum B12 and plasma total homocysteine in smoker and non-smoker patients suffering from documented coronary artery disease and normal healthy subjects of local population. Study was carried out in IM&DC (Islamabad), SSH (Rawalpindi) and CUPIMS (Islamabad) from March-2007 to June-2008. One hundred and fifty age and sex matched persons were included in this control study. Serum was analyzed for folic acid, Vitamin B12 using radioimmunoassay, lipid parameters by enzymatic colorimetric method, while total homocysteine in plasma using Fluorescence polarization immunoassay (FPIA) and plasma glucose by enzymatic colorimetric method. Mean plasma total homocysteine in smokers (Patients and controls) was found significantly higher ($23.90 \pm 79.20 \mu\text{mol/L}$, 17.07 ± 2.28

$\mu\text{mol/L}$), where as the levels of serum folate and Vitamin B12 were found significantly low (3.5 ± 1.07 ng/mL, 4.28 ± 1.18 ng/mL) and (240.36 ± 79.20 pg/mL, 331.71 ± 109.80 pg/mL) respectively in smoker patients and controls. It is concluded that smokers have lower concentrations of serum folate, Vitamin B12 and high levels of total homocysteine than non-smokers.

Keywords: Smoking, Hyperhomocysteinemia, Coronary Artery Disease, Cyanocobalamine, Folic acid

INTRODUCTION

Hyperhomocysteinemia is one of the major and independent risk factor for the development of atherosclerotic coronary artery disease [1]. The disease seems to follow an accelerated course in the developing countries as compared to the developed world [2, 3]. In our country 47% of all cardiac deaths are due to coronary heart disease [4]. Smoking is one of the powerful risk factor for coronary artery disease which in turn is associated with the deranged levels of lipids and higher levels of the plasma homocysteine [5, 6]. In the Framingham Heart study, cardiovascular mortality increased 18% in men and 31% in women for each ten cigarettes smoked per day [7]. The use of tobacco products decreases high-density lipoprotein cholesterol (HDL-C). In an observational epidemiological study, HDL-C was 12% lower in each group of female smokers and 7% lower in each group of male smokers than in non-smokers [8]. A prospective observational study showed that

homocysteine level were $1.1 \mu\text{mol/L}$, higher in men, who smoked, than those who did not smoke [8]. A strong correlation has been seen among fasting total homocysteine and smoking [9]. In Hordaland homocysteine study a strong and graded association between the daily number of cigarettes smoked and homocysteine higher and lower levels was observed. The association was stronger in women than in men and stronger among elderly subjects [10, 11]. Multiple detailed analyses have shown that smoking is associated with a 1.5 to 2.0- $\mu\text{mol/L}$ elevation of homocysteine levels [12]. This is compatible with the reports of low folate and cobalamin status in smokers with the non-smokers [13]. A decrease in the levels of serum and erythrocytes folate was also observed in smokers compared to non-smokers [14]. Vitamin deficiency is by far the leading cause of hyperhomocysteinemia. It may be due to inadequate intake, reduced absorption from gastrointestinal tract,

increased consumption, and drug interaction [15]. Folate deficiency is the most common Vitamin deficiency in Europe, partly because of lack of fresh fruits and vegetables [16]. Risk factor for atherosclerosis, thrombosis and other vascular diseases is hyperhomocysteinemia. In the pathogenesis of these diseases, auto-oxidation of homocysteine is crucially involved [17]. Vitamin B12 deficiency in elderly is frequently due to inadequate absorption resulting from an age related decrease in gastric acid secretion or a slight increase in gastric pH, or to intrinsic factor deficiency, and may affect as many as 30-40 % of the elderly population [18]. Keeping in view the association of smoking, folate and Vitamin B12 deficiency and hyperhomocysteinemia, a prospective case control study was conducted with the objective, to evaluate the levels of serum folate, serum B12 and plasma total homocysteine in smoker and non-smoker patients suffering from documented coronary artery disease and normal healthy subjects of local population.

MATERIALS AND METHODS

The present study was carried out at Department of Biochemistry (Islamabad Medical and Dental College Islamabad), Social Security Hospital Rawalpindi in collaboration with Cardiac Units of Pakistan

Institute of Medical Sciences Islamabad, from March-2007 to June-2008. Informed consent was obtained before enrolment from patients. Subjects were divided into two groups, Group X and Group Y.

Group X Include 75 subjects with no history of disease and served as control. They were age and sex matched to group.

Group Y Include 75 Patients having established coronary artery disease diagnosed by coronary angiography, in the Cardiac Unit of Pakistan Institute of Medical Sciences, Islamabad. The controls were free of overt disease. There was no evidence of diabetes mellitus, hypertension, obesity, and hypercholesterolemia in any of these individuals. The subjects in the groups who were taking complex multivitamins, antiepileptics, methotrxate, or suffering from hypothyroidism, renal failure and rheumatoid arthritis were excluded from the study. The questionnaire provided information about type of work, smoking habit, medical history of cardiovascular disease, hypertension and family history of coronary heart disease. Smoking habits of subjects were grouped into current smokers and non-smokers; weight was measured on a balance while participants were without shoes and heavy outer garments, and height was measured in the standing position following weight measurement.

Blood pressure of subject was measured twice in the right arm with a 5-minute interval of rest, using a standard mercury sphygmomanometer. The values were recorded in the Performa. Angiographically diagnosed cases of coronary artery disease were selected from Social Security Hospital Rawalpindi, Cardiac Units of Pakistan Institute of Medical Sciences, Islamabad. The subjects were asked to fast for 4-8 hours and 10 ml of blood was collected from the antecubital vein (after all aseptic measures) while the subjects were sitting up right. Sampling was done between 08.00 and 09.00 hours. Samples were analyzed in one run at the end of the study (to omit analytical variations between run) except plasma glucose that was analyzed within four hours of sample collection by enzymatic colorimetric method. Serum was analyzed for Folic acid, Vitamin B using radioimmunoassay. Lipid parameters were analyzed by enzymatic colorimetric method, while total homocysteine in plasma by using Fluorescence polarization immunoassay (FPIA). Low-density lipoprotein Cholesterol was calculated by the Friedewald formula [19].

Statistical Analysis

Comparison of difference of means between Control and Subject groups was made by

using Student's t -test. P values less than 0.05 i.e., 0.001 were considered significant.

RESULTS

Table 1 shows the comparison of means values of demographic and clinical characteristics of control (Group-X) and patients (Group-Y). The control group comprised of 61 males and 14 females. The mean age and body mass index was [52.52±5.62 years and 23.64±4.02 Kg/m² respectively] in the group X. 22.7% of them had a positive family history of ischemic heart disease and 18.7% were the smokers. The patients group (group-Y) also comprised of 14 females and 61 males with a mean age of 50.92± 5.73. The mean body mass index was 24.03 Kg/m² which is very close to the mean body mass index of control group. 28% of them were having positive family history of ischemic heart disease, 45.3% had positive history of hypertension, 45.3% diabetes and 65.3% were smoker. The percentage of smokers was significantly higher (p<0.0001) in patients group as compared to the control group.

Along with the number of observations and units, percentages are given in parenthesis.

Table 2 shows comparison of Plasma glucose, total homocysteine, Serum triglyceride, total Cholesterol, high-density lipoprotein cholesterol (HDL-C) and low-

density lipoprotein cholesterol (LDL-C), folic acid and Vitamin B₁₂] levels in control subjects and group Y patients. Plasma total homocysteine level in patients (21.32 ± 7.63 $\mu\text{mol/Lit}$) when compared with mean plasma total homocysteine level in controls (13.60 ± 3.36 $\mu\text{mol/Lit}$) was found to be significantly high ($p < 0.0001$). Similarly the mean serum values of total cholesterol (208.40 ± 50.98 mg/dL), triglyceride (152.20 ± 14.14 mg/dL) and LDL-C (126.14 ± 39.90 mg/dL), of patients were found significantly high ($p < 0.0001$, $p < 0.006$ and $p < 0.002$ respectively) as compared to control subjects levels (184.78 ± 17.42 mg/dL, 140.17 ± 23.36 mg/dL and 111.74 ± 19.00 mg/dL respectively). The levels of HDL-C, serum folic acid and serum Vitamin B₁₂ [33.40 ± 5.75 ng/dL, 3.37 ± 1.91 ng/dL and 248.69 ± 98.19 ng/dL] were found to be significantly lower as compared to levels of control subjects [42.60 ± 6.42 mg/dL, 5.44 ± 2.08 ng/mL and 427.30 ± 139.89 pg/mL]. When the mean plasma values of glucose (96.08 ± 14.94 mg/dL) of patients was compared with that of control group (94.25 ± 20.24 mg/dL). The difference was found to be non-significant statistically.

Table 3 shows the comparison of plasma glucose, total homocysteine, serum cholesterol triglyceride, HDL-C and LDL-C,

folic acid and Vitamin B₁₂ levels between smokers and non-smokers of control and patients. Plasma total homocysteine, serum triglycerides, and LDL-C were found significantly higher in smokers of control and patients. Plasma total homocysteine, serum triglycerides, and LDL-C were found significantly higher in smokers as compared to the non-smokers of both control and patients group. The levels of serum folic acid, Vitamin B₁₂ and HDL-C was found significantly lower in smokers as compared to non-smokers of both groups where as the plasma glucose was found not significantly different among smokers and non-smokers of both the groups while serum total cholesterol was significantly higher in patients group as compared to the non-smoker patients. The difference in the levels of serum total cholesterol among smokers and non-smokers was found not significant in control group.

Table 4 shows the comparison of plasma total homocysteine, serum folic acid and Vitamin B₁₂ levels between control and patients by smoking status. Plasma total homocysteine levels of smoker patients was found significantly higher than the levels of smokers control [23.90 ± 7.46 $\mu\text{mol/L}$, $p < 0.0001$]. The levels of folate and vitamin B₁₂ of smokers patients were found be lower than the levels of smokers control 4.28 ± 1.18 ng/mL,

$p < 0.0001$ and 331.71 ± 109.80 pg/mL 240.36 ± 79.20 pg/mL $p < 0.003$ respectively.

DISCUSSION

Hyperhomocysteinemia is being recognized as serious and independent risk factor for the development of atherosclerosis and thromboembolism [16]. Atherosclerosis is considered the primary cause of death in industrialized countries as it represents the underlying pathomechanism responsible for the majority of cases of myocardial cerebral infarction [20]. Homocysteine seems to promote atherothrombogenesis [21]. Nutritional deficiencies of cobalamin (Vitamin B₁₂), folate or pyridoxine (Vitamin B₆) can result in blockade of homocysteine metabolic pathways, as these vitamins are essential cofactors for the enzymes in these pathways [6]. An epidemiological studies has shown that elevated plasma total homocysteine is an independent risk factor for atherosclerotic disease in the coronary, cerebrovascular, and peripheral vessels [22]. In the present study, it has been also found significantly { $p < 0.001$ } high values of plasma total homocysteine in patients with coronary artery disease as compared to control subjects. This is in agreement with the above mentioned observations indicating that hyperhomocysteinemia may be considered as a major risk factor for coronary artery disease.

The percentage of smokers in our study was significantly high ($p < 0.001$) in patients with coronary artery disease as compared to control subjects. In this study it has been found a significantly high levels of plasma total homocysteine in smokers controls (17.07 ± 1.28 $\mu\text{mol/L}$) and smokers patients (23.90 ± 7.46 $\mu\text{mol/L}$) as compared to non-smokers control (12.81 ± 3.06 $\mu\text{mol/L}$) and patients (16.45 ± 5.26 $\mu\text{mol/L}$). It correlates to the findings who have reported that history of smoking is independently is associated with premature coronary heart disease [4] and who have reported an increase level of total homocysteine in smokers as compared to non-smokers [10, 23]. It is found a significantly high ($p < 0.001$) percentage of history of hypertension in patients with coronary artery disease as compared to control subjects [24]. It also conform finding in which it was reported that elevated blood pressure was a significant, strong and independent risk factor for coronary artery disease, both in men and women [2]. In our study, we could not find any significant difference of plasma glucose between controls and patients of coronary artery disease. The dyslipidemia, most clearly associated with increased risk for coronary artery disease is hypercholesterolemia, particularly elevated plasma levels of cholesterol carried as LDL [25]. In the present

study, it is reported a significant difference between the total cholesterol and triglyceride level of cases (patients with coronary artery disease) and control subjects. Similarly, lower means level of high-density lipoprotein cholesterol, serum folate and serum vitamin B₁₂ with a significant difference in group-Y subjects as compared to control. We have also

observed that, the patients with coronary artery disease have significantly high level of low density-lipoprotein cholesterol ($p < 0.002$) as compared to control. It is observed that smoking is related to higher levels of the plasma homocysteine and with low level of folate and Vitamin B₁₂ [26-29].

Table 1: Comparison of Demographic and Clinical Characteristics of Group-X and Group-Y Subjects: (The Values are Expressed as Mean±SD)

Characteristics	Control (Group-X) (n=75)	Patients (Group-Y) n=75	P-Value
Male	61 (81.3%)	61 (81.3%)	1.00
Female	14 (18.7%)	14 (18.7%)	1.00
Age (years)	52.25± 5.62	50.92±5.73	0.399
Body Mass Index Kg/m ²	23.64± 4.05	24.03±3.15	0.517
Smokers	14 (18.7%)	49 (65.3%)	<0.0001
Family history of IHD	17 (22.7%)	21 (28.00%)	0.574
History of diabetes mellitus	Nil	34 (45.3%)	Nil
History of Hypertension	Nil	45 (45.3%)	Nil

Table 2: Comparison of Biochemical Parameters of Group X (Controls) and Group Y (Patients with Coronary Artery Disease) Subjects (The Values are Expressed as Mean±SD)

Parameters	Control (Group X) (n=75)	Patients (Group Y) (n=75)	P-Value
Glucose (mg/dL)	94.25± 20.24	96.08± 14.49	0.533
Total Cholesterol (mg/dL)	184.78± 17.24	208.41± 50.98	<0.0001
Triglyceride (mg/dL)	140.17± 23.36	152.20± 14.14	<0.006
HDL-C (mg/dL)	42.60± 6.42	33.40± 5.75	<0.0001
LDL-C (mg/dL)	111.74± 19.00	126.14± 39.90	<0.002
Total Homocysteine (µmol/L)	13.60± 3.36	21.32± 7.63	<0.0001
Folate (ng/mL)	5.44± 2.08	3.37± 1.91	<0.0001
Vitamin B ₁₂ (pg/mL)	427.30± 139.89	248.69± 98.19	<0.0001

Table 3: Comparison of Plasma Glucose, Total Homocysteine, Serum Cholesterol, Triglyceride, HDL-C, LDL-C, Folic Acid and Vitamin B₁₂ Levels of Smokers and Non-Smokers Among Control and Patients (The Values are Expressed as Mean±SD)

Variable	Control (n=75)		P-value	Patients (n=75)		P-value
	Smokers (n=14)	Non-Smokers (n=61)		Smokers (n=49)	Non-Smokers (n=26)	
Glucose (mg/dL)	95.50±1 2.34	93.96±21.96	0.802	95.51± 13.61	96.41± 217.45	0.899
Total Cholesterol (mg/dL)	178.71± 17.34	186.18± 17.28	0.150	220.51± 58.00	185.61± 20.18	<0.0001
Triglyceride (mg/dL)	150.69± 14.23	137.76± 25.08	<0.005	161.67± 14.28	134.35± 14.09	<0.0001
HDL-C (mg/dL)	37.57± 6.02	43.76± 6.55	<0.0001	30.41± 5.58	39.05± 6.14	<0.0001

LDL-C (mg/dL)	12.58± 18.74	105.81± 19.64	<0.0001	139.48± 18.41	119.83± 22.52	<0.0001
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Table 4: Comparison of Serum/Plasma Folate, Vitamin B₁₂ and Total Homocysteine Levels Among Control and Patients by Smoking Status (The Values are Expressed as Mean±SD)

Variable	Smokers		P-value	Non-Smokers	
	Control (n=14)	Patients (n=49)		Control (n=61)	Patients (n=26)
Folate (ng/mL)	4.28± 1.18	3.50± 1.07	<0.003	5.70± 2.16	4.30± 1.54
Vitamin B ₁₂ (pg/mL)	331.71± 109.80	240.36± 79.20	<0.003	449.24± 137.49	264.38± 126.91
Total Homocysteine (µmol/L)	17.07± 2.28	23.90± 7.46	<0.0001	12.81± 3.06	16.45± 5.26

CONCLUSION

It is concluded that the smokers were more prone to the development of coronary artery disease and it has an association with high levels of total homocysteine, low-density lipoprotein cholesterol, total cholesterol and triglyceride. Similarly, smoking is also associated with the low levels of folate, Vitamin B₁₂ and high-density lipoprotein cholesterol which ultimately results in deranged pathology of cardiovascular system.

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