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EFFECT OF SMOKING ON HOMOCYSTEINE, VITAMIN B_{12} AND FOLIC ACID LEVLES IN THE PATIENTS OF ACUTE MYOCARDIAL INFARCTION

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ABSTRACT

Objectives: To evaluate levels of serum homocysteine, vitamin B_{12} and folic acid in the smoker and non smokers of acute myocardial infarction.

Material and Method: A case control study of 200 patients of acute myocardial infarction between the age of 20-60 years and an equal number of age and sex matched controls were carried out during January 2007 to December 2008. Serum homocysteine levels were determined on Abbott Immunoassay Analyzer (IMX) and determination of B vitamins was done on HPLC.

Results: Mean age of the smoker patients of acute myocardial infarction was 41.72 ± 2.43 years, while that of never smoker patients was 50.56 ± 3.28 years. In both cases and controls current smokers had higher homocysteine levels but patients showed intermediate hyperhomocysteinemia (31.83 ± 1.09 and 15.94 ± 2.65 μ mol/L) respectively in the patients and controls. Never smoker patients had lower homocysteine levels as compared to smokers and showed moderate hyperhomocysteinemia (19.62 ± 2.14 μ mol/L). Never smoker controls showed normal homocysteine levels. Current smokers had lower levels of vitamin B_{12} and folic acid (103.43 ± 1.39 pg / mL and 3.48 ± 2.31 ng/mL respectively) as compared to smoker controls and the patients who never smoked.

Conclusion: Smokers tend to have high homocysteine concentrations and low levels of vitamin B_{12} and folic acid, which may result in sudden cardiovascular events.

Key Words: Smoking, homocysteine, B vitamins, acute, myocardial infarction.

INTRODUCTION

Elevated homocysteine levels have been established as a risk factor for occlusive vascular disease.[1-2] The sulfhydryl containing amino acid homocysteine is produced by the demethylation of methionine. Methylation of homocysteine catalyzed by the enzyme Methionine Synthase converts homocysteine to methionine. In this way homocysteine is continuously removed from the circulation.[3-4] In humans vitamin B_{12} acts as a coenzyme while folic acid provides the methyl essential for the reaction to take place. Vitamin B_{12} and folic acid deficiency can cause reduction in Methyl tetrahydro folate reductase (MTHFR) activity leading to decrease in methionine synthesis and homocysteine accumulation.[5]

In late 60's Dr. Kilmer McCully determined through extensive research that taking adequate amounts of folic acid (vitamin B_9) along with vitamin B_{12} and B_6 can normalize homocysteine levels as it is either converted to methionine or to cysteine by metabolic pathways requiring these vitamins.[6] Vitamin deficiencies in ethnic groups can result not only from poor dietary intake but also from life style factors. These deficiencies in addition to other harmful affects are known to increase the levels of circulating homocysteine. Total homocysteine reflects homocysteine metabolism and normal serum/plasma homocysteine is taken from 5-15 μ mol/L. Moderate and Intermediate hyperhomocysteinemia refers to homocysteine concentrations in the range of 16–30 μ mol / L and 31-100 μ mol / L respectively.[7]

Smoking is a dominant risk factor affecting homocysteine metabolism by affecting the cofactors

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of its metabolic pathway.[8] In addition to this some other mechanisms involving pro- oxidant properties e.g. redox changes in glutathione can explain that smoking contributes to atherosclerotic vascular disease.[9] In the present study we determined Serum total homocysteine, Vitamin B_{12} and Folic acid levels of current smokers and never smoker patients of acute myocardial infarction and compared it with control subjects.

MATERIAL AND METHODS

The study was carried out at Pakistan Medical Research Council, Khyber Medical College, Peshawar in collaboration with Abbott Laboratories Research Division and Cardiology Departments of Khyber Teaching Hospital and Post Graduate Medical Institute Lady Reading Hospital, Peshawar. Two hundred fully diagnosed patients of acute myocardial infarction and an equal number of age and sex matched control subjects were included in the study. Age range for the subjects was from 20-60 years. Exclusion criteria from the study was subjects with a history of hypertension and coronary artery disease or with signs of cardiovascular disease, malignancy, megaloblastic anemia, renal failure and thyroid disorders. Informed consent was obtained from the subjects, General physical examination was done and their brief history was recorded in a questionnaire. Smoking habits were defined as current smokers and never smokers. Subjects categorized as smokers were those who gave a history of light smoking (5-10 cigarettes per day regularly since the last 1-2 years). Smokers were told not to change their smoking habits during the study.

Sample Collection: Blood samples from all the subjects were taken in the morning after an over night fast and collected in a plain tube. Serum was separated from it with in one hour by ultra centrifugation at 1000g for one minute at room temperature. Serum from each subject was divided in three separate epindorph bottles and stored frozen at -20°C for the estimation of homocysteine, vitamin B_{12} and folic acid. Serum total homocysteine was analyzed in batches by Fluorescence Polarization Immunoassay (IMX) (ABBOTT). B vitamins were analyzed by High Performance Liquid Chromatography (HPLC) using a standard solution of these vitamins.

Data Analysis: Data analysis was done on SPSS 10 software expressing numerical and categorical data as means. Means of serum total homocysteine, | vitamin B_{12} and folic acid of current smoker and never smoker patients and controls were compared using 'z' test and P values were calculated. The values having probability less than 0.05 were considered statistically significant.

RESULTS

There was no female patient in the present study who gave the history of smoking. Mean age of the patients and control, levels of serum total homocysteine are shown in Table 1. The difference in the homocysteine levels of smoker and non smoker controls is more alarming (15.94 ± 2.65 μ mol / L and 8.19 ± 3.07 μ mol / L respectively) as moderate elevation of homocysteine levels in apparently healthy individuals may predict future cardiovascular events. Figure 1 shows vitamin B_{12} levels of patients and never smoker patients. The serum folic acid levels of current and never smokers are shown in Fig. 2.

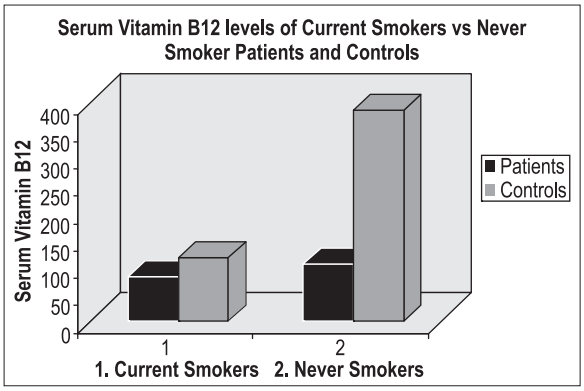

Fig. 1: Normal Serum Vitamin B_{12} : 100 – 700 pg / mL

DISCUSSION

Accumulating data from epidemiological studies suggests that individuals with elevated blood

Table 1: Serum Homocysteine levels of Smoker / Non smoker patients of Acute Myocardial Infarction (AMI) and Control subjects

Variable	Group/Subjects	Mean Age ± SD	Serum Homocysteine (mean ± SD) (μ mol/L)
Current Smokers	A-Patients (n=100)	41.72 + 2.43***	31.83 + 1.09***
	B-Controls (n=100)	47.10 + 4.63	15.94 + 2.65
Never Smokers	C-Patients (n=100)	50.56 + 3.28**	19.62 + 2.14***
	D-Controls (n=100)	49.05 + 1.61	8.19 + 3.07

*** P < 0.001: Highly significant

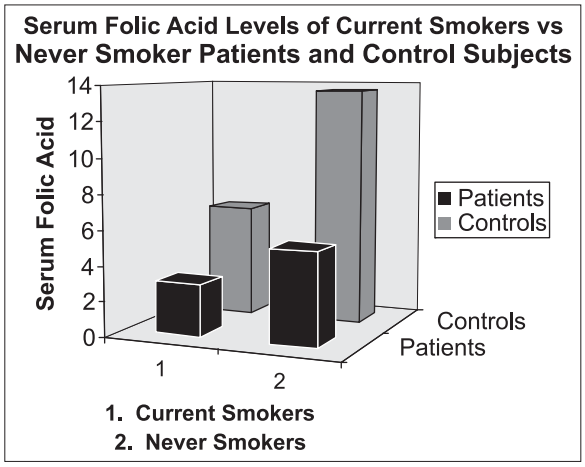

Fig. 2: Normal Serum Folic Acid : 3 – 16 ng / mL

levels of homocysteine have increased risk of cardiovascular diseases.[10,11,12] Elevated blood homocysteine levels can result from genetic errors e.g. Methyl tetrahydrofoalte reductase polymorphism or nutritional deficiencies of vitamin B_{12} and folic acid.[13] Some cardiovascular risk factors especially smoking interact in a complex manner with these water soluble vitamins resulting in a deficiency of these vitamins in the body.

We reviewed the currently available evidence of this association between homocysteine and acute myocardial infarction and also determined whether it is affected by smoking and circulating blood levels of vitamin B_{12} and folic acid. Elevated homocysteine levels in the present study associated with low B vitamin status in the patients of acute myocardial infarction can be explained on theoretical grounds by the effect of vitamin B_{12} and folic acid as cofactors on homocysteine metabolism.[14] Chandalia et al[15] are of the view that nutritional deficiencies in the people of South East Asian region occur because they are infrequent meat and dairy product consumers (may be due to increasing poverty) and some are habitual vegetarians, these effects are comparable to our results. Previous studies on hyperhomocysteinemia from other parts of our country report that Pakistani people belong to an ethnic group which has the highest incidence of coronary artery disease, as are in our results also substantial nutritional deficiencies of B vitamins result in hyperhomocysteinemia perhaps through an interplay with classical cardiovascular risk factors (highly prevalent in this population) which could be a further aggravating factor in the causation of cardiovascular accidents in Pakistani population.[16], as in our study the poor class was low in B Vitamins. The results of present study have shown intermediate hyperhomocysteinemia with significantly low levels of vitamin B_{12} and folic acid in the current smoker patients of acute myocardial infarction. Even in the control subjects, smokers have shown homocysteine levels slightly above normal, and their B vitamin levels were in the lower limits of normal. The reason why homocysteine concentrations are raised in smokers is not fully known in a study by Nygard et al.[10] The same effect of smoking was noted which may be due to the formation of reactive Oxygen species. Further more reduced intake of nutrients and vitamins and lower levels of plasma folate, red blood cell folate vitamin B_{12} and plasma pyridoxal phosphate have been demonstrated in smokers. The reason why vitamin B_{12} deficiency is more common in smokers than in general population is that principal co-enzyme form of vitamin B_{12} is 5^2- deoxyadenosyl cobalamine.The notable feature of the molecule is the cobalt-carbon bond between 5^2 carbon of the 5^2- deoxyadenosyl moiety (the sugar part) and the cobalt of cobalamine. In smoking cyanide absorbed from the smoke into the blood causes the replacement of 5^2- deoxyadenosyl group by a cyanide. Vitamin B_{12} there fore cannot act as a co-enzyme for the metabolic removal of homocysteine from the body. Smoking also enhances platelet aggregability, increases blood viscosity and shifts the antithrombotic balance towards increased coagulability. This increases insulin resistance and increases homocysteine levels of the blood, this effect was also noted by Iqbal MP et al.[16] Because elevated homocysteine may be prothrombotic, it is conceivable that the homeostatic balance that should reduce the propencity to form large thrombi and stabilize existing atherosclerotic plaques can be tipped over to the opposite by hyperhomocysteinemia[17].

CONCLUSION

Smokers should be offered intesive advise to cease smoking to prevent cardiovascular diseases as it is directly related to Acute Myocardial infarction, with high homocysteine and decreased Vitamin B_{12} and Folic acid.

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