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Impact of Homocysteine, Folate and Vitamin B12 levels in patients of Arteriosclerosis

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ABSTRACT

Cardiovascular disease [CVD] and stroke are the most common causes of death. Several studies have demonstrated that increased plasma level of total homocysteine is associated with premature onset of CVD and stroke. Arteriosclerosis, a disease of the large arteries, is one of the primary causes of heart disease and stroke. Increased homocysteine level have been associated with more advanced extracranial carotid artery arteriosclerosis, with decreased levels of vitamin B_{12} and folic acid. In present study plasma total homocysteine, serum folic acid and serum vitamin B_{12} in patients with arteriosclerosis were measured in total 150 patients and compared with the healthy control subjects [n=200]. The level of plasma total homocysteine was significantly higher [$p<0.01$] in patients with arteriosclerosis as compared to healthy controls. Whereas significant decrease in serum folic acid [$p<0.01$] and vitamin B_{12} [$p<0.01$] was observed in patients with arteriosclerosis than normal healthy controls, the decrease in levels of vitamins may be due to dietary insufficiency, which corresponds with severity of tissue damage.

Keywords: Arteriosclerosis, Homocysteine, Folic acid, vitamin B_{12}.

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INTRODUCTION

Cardiovascular disease [CVD] remains the major cause of morbidity and death in developed and developing countries [1]. Conventional risk factors for cardiovascular disease, including hypertension, cigarette smoking, obesity, hypercholesterolemia, diabetes account for approximately 50% of all cases [2]. Over the last decade, evidence has accumulated that plasma total homocysteine [tHcy] and B-vitamins concentrations are associated with an increased risk of arteriosclerotis and thrombosis [3].

Arteriosclerosis characterized by thickening, hardening and loss of elasticity of the walls of the blood vessels due to deposition of calcium. Initially lesions are formed on the arterial walls, which results in blistering and the accumulation of low density lipoprotein cholesterol. This produces higher blood pressure, which facilitates the imbedding of cholesterol and calcium in the vessel walls.

Arteriosclerosis, the peripheral cause of CVD and stroke, is a complex and chronic process enhances sites of endothelial cell injury and culminates in the formation of stratified lesions of the arterial wall [2]. Although advanced arteriosclerotic lesions cause progressive narrowing of the vessel lumen that can lead to ischemic symptoms, acute coronary syndromes usually results from lesion rupture and thrombosis.

Genetic risks are related to the ability of the body to process like uptake and metabolize low-density lipids that contain cholesterol. Gene therapy that forces the growth of new blood vessels bypassing an area has also been used. Exercise often can inure utilization of excess low density lipids. Although the relationship between blood cholesterol levels and arteriosclerosis is not fully understood, the utilization of low-density lipids appears to be a primary indicator of the risk of arteriosclerosis [4].

Homocysteine [Hcys] is a sulfhydryl containing amino acid synthesized by demethylation of an essential amino acid methionine. Methylation of homocysteine, catalyzed by methionine synthetase produces methionine. In this reaction, vitamin B_{12} acts as a coenzyme while folic acid provides the methyl essential for the reactions to take place. Therefore, folic acid and vitamin B_{12} deficiency can cause reduction in methylene tetrahydrofolate reductase [MTHFR] activity, leading to decrease in methionine synthesis and homocysteine accumulation [5].

The exact mechanism by which homocysteine promotes cardiovascular disease are not yet fully understood. However, genetic defects in the enzymes of Hcys metabolism markedly increase Hcys level. Mutations in Methylene Tetra Hydro Folate Reductase [MTHFR] and Cystathionine beta Synthase [CBS] are associated with excess level of Hcys and an excess rate of stroke and venous thrombosis [6,7].

MATERIAL AND METHODS

The case-control study was performed in groups of 150 arteriosclerosis patients and 200 healthy control subjects. Out of 150 patients included in this study: 50 subjects had premature

CVD; 50 had experienced acute myocardial infarction and 50 had been diagnosed with cerebral stroke. Blood samples were withdrawn from patients after an overnight fast in polythene tube and spun within one hour at 3000g for 20 minutes. Then samples were analyzed for total homocysteine and B-vitamins viz. Folic acid and vitamin B_{12}.The written inform consent was obtained from each patient. The study was approved by the local ethics committee.

Plasma level of homocysteine was determined by high performance liquid chromatography [HPLC] method, coupled with fluorescence detector with internal standard [8] and plasma level of folic acid and vitamin B_{12} by competitive immunoassay using direct chemiluminescence technology. [9] The results were expressed as mean ± SD and analyzed by 'z' test.

RESULTS

A total of 350 participants were enrolled from which 150 were patients of arteriosclerosis and 200 were normal healthy controls. The mean age of the study group was 54.19 ± 8.87 years in patient group and 58.79 ± 10.51 in healthy normal controls. No significant difference was noted between the patient and control group in this regard.

The mean plasma level of total homocysteine was 23.37 ± 0.82 µmol/lit in the study group and was significantly [$P < 0.01$] higher in patients of arteriosclerosis than the normal controls which is 10.97± 0.60 µmol/lit. On the other hand both plasma folate and vitamin B_{12} levels were significantly [$p<0.01$] lower in patients of arteriosclerosis [5.17 ± 0.82 ng/mL & 263.76±113.04 ng/mL respectively] than healthy normal controls [11.56±6.4 ng/mL & 453.56±167.45 ng/mL respectively]. As depicted in the table no. 1.

DISCUSSION

Cardiovascular disease is caused by disorder of the heart and blood vessels and includes coronary heart disease; cerebrovascular disease etc. people die from CVDs particularly heart attacks and stroke every year thus making stroke, the second leading cause of death. Projection to the year 2020 indicated an increase in the number of CVD cases, majority of such cases will be from developing countries like India. The relation between elevated plasma tHcy and vascular disease risk is independent of traditional risk factors. [10] Several studies have shown that moderate and high tHcy plasma levels which increasing the risk for CVD [11].

Nonetheless, in a meta-analysis of the association between tHcy and CVD, epidemiological studies support that every 5 µmol/L increase in plasma tHcy concentration causes risk of heart disease. [12, 13]

The metabolism of tHcy is a complex system involving several enzymes and cofactors. Jakubowski H [11] et al reported that only abnormal laboratory finding was, high plasma homocysteine level, which may be important causal factor leading to premature coronary atherosclerosis and MI.

Refsum H [12] et al observed significantly increased levels of homocysteine in coronary artery disease patients as compared to normal healthy controls and showed that homocysteine appears to be an independent risk factor for coronary artery disease in young patients. Selhub J [13] et al reported abnormally high total plasma homocysteine levels in the fasting sample, investigated within 1-7 hours after their MI. They concluded that high plasma homocysteine levels may be a risk factor for MI patients.

We observed highly significant [<0.01] increased level of total homocysteine in study group than the healthy normal controls. We also found significant decrease in vitamin B12 and folate in the study group and normal healthy controls, the results are concluded in table-1.

Table No. 1: Levels of Homocysteine, Folic Acid, and Vitamin B12 in Arteriosclerotic Patients And Healthy Controls

Biochemical Parameters	Subjects	MEAN ± SD	p value
tHcy[µmol/L]	Patient	23.37 ± 8.69	<0.01
	Control	10.97 ± 5.64	
Folic acid 9 [ng/mL]	Patient	5.17 ± 3.54	<0.01
	Control	11.56 ± 6.40	
Vit B12 [pg/mL]	Patient	263.76 ± 113.04	<0.01
	Control	453.56 ± 167.45	

Similar results were reported by Mujawar S. A. [14] et al, significant decreases [p< 0.001] were observed in serum folic acid and vitamin B12 whereas, tHcy levels showed significant increase [p < 0.001] in cigarette smoker as compared to control group. Sadeghian S [5] et al, the plasma folic acid concentration was significantly lower in patients of coronary artery disease whereas plasma vitamin B12 concentrations were not significantly differed between patients and control subjects. Our results differ from Vanuzzo D [15] et al; they observed the mean plasma concentration of plasma pyridoxal phosphate; serum vitamin B12 and blood folic acid were similar in patients of stroke and controls.

According to several studies additional identified factors are age, male sex, menopause, lifestyle factors smoking, coffee intake, vitamin deficiency, gelatin and renal dysfunction, malignancies, drugs such as folate antagonist, B_{12} antagonist, anticonvulsants, and some lipid modifying treatment etc. [15, 16]. These factors causes low vitamin B_{12} and low folic acid, which is associated with an increased tHcy level as seen in patients of arteriosclerosis [11].

Lower vitamin B12 concentration in the cell can be the result of lower vitamin B_{12} intake, but they can also be attributed to a disturbance in the absorption, transport or cellular uptake of this vitamin. The analysis of the risk of low folate is a baseline for CVD and it is statistically significant. The lower B_{12} vitamin status with the risk of cardiovascular events is mediated by increased homocysteine plasma concentration [15].

Some genetic factors also contribute for the causation of cardio vascular diseases, for example the mutations in the enzyme methylene tetrahydrofolate reductase [MTHFR] [17]

gene, cystathionine-β-synthase [18], and decrease the activity of that enzyme which may causes the increased level of tHcy. The current research is continued for the genetic analysis of the few common mutations in the genes that take part in homocysteine metabolism, which may be the risk factor for cardio vascular diseases.

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