STUDIES ON HOMOCYSTEINE DEMONSTRATING ITS SIGNIFICANCE AS A POSSIBLE TOOL FOR DIFFERENTIAL DIAGNOSIS IN OCCLUSIVE VASCULAR DISEASE

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ABSTRACT

Homocysteine (Hcy) is a non-protein forming amino- acid, whose metabolism is at the intersection of two metabolic pathways: remethylation and transsulfuration which are dependent on the vitamins folic acid, B₁₂ and B₆, and the enzymes methylenetetrahydrofolate reductase and cystathionine-β-synthetase. A deficiency of any of these vitamins or enzymes results in hyperhomocysteinemia. This causes oxidative and other damage to blood vessels, thus affecting various organ systems of the body. As part of our ongoing research on cardiovascular risk factors, we have studied the Hcy levels in the plasma of normal controls and those suffering from vascular diseases. It was observed that Hcy is significantly higher in patients of vascular diseases ($21.59 \pm 1.28 \mu\text{mol/L}$, mean $\pm$ SEM), as compared to normal controls ($11.33 \pm 0.18 \mu\text{mol/L}$). This significance was more pronounced in cases of venous thrombosis ($26.77 \pm 2.43 \mu\text{mol/L}$) as opposed to cases of arterial block ($17.27 \pm 0.84 \mu\text{mol/L}$). This signifies that Hcy estimation would be beneficial in obtaining a differential diagnosis in addition to being a modifiable vascular risk factor.

KEY WORDS

Hyperhomocysteinemia, Vascular Disease.

INTRODUCTION

Homocysteine (Hcy) is a branch-point metabolite derived from exogenous as well as endogenous methionine. Once formed, it either re-enters the activated methylation cycle or gets transsulfurated towards protein synthesis.

The rate of these two processes (remethylation and transsulfuration) adjusts the level of plasma Hcy within the reference range. But when any of the enzymes in the various steps of these two processes is defective or deficient, or the patient is suffering from a deficiency of the vitamins folic acid, B₁₂ or B₆, plasma levels of Hcy rise. Interestingly, it has been observed that hyperhomocysteinemia is treatable by the simple administration of the Vitamins folic acid, B₁₂ and B₆, even if the cause lies in the enzymes (1,2).

Homocysteine (Hcy) has been implicated as the causative factor for many disorders, but its association with vascular disease has been the longest; in fact, since its discovery as a pathogenic factor in premature atherosclerosis in cases of homocysteinuria with mental retardation in children(3).

The normal value for homocysteine in plasma/serum has been reported to be in the range of 5-15 $\mu\text{mol/L}$ in

healthy adults(4-6). These published figures have been arrived at in various global populations. However, the data on normal Indian population are few(7,8).

Most of the experimental findings implicate damage to the endothelium by Hcy. The mechanism of action of Hcy in vascular pathology has been found to differ for arteries and veins. In arteries it acts through platelet function abnormalities and in veins it involves abnormalities of coagulation and/or fibrinolysis(9). Hence, an evaluation of Hcy levels in arterial and venous occlusive disease would be required to understand the process fully.

MATERIALS AND METHODS

Hcy levels were measured in 205 normal individuals above 18 years of age who had no history of cerebrovascular disease, coronary artery disease (CAD) or any other chronic illness (eg. diabetes, chronic renal failure, hypertension, etc.), nor any present ailments.

Further, the Hcy levels were estimated in the serum of 101 patients of vascular disease. On the basis of clinical findings, angiography and doppler studies of the affected vessels by Duplex Sonography, the patients were divided into two subgroups – those who had arterial occlusion and those who had venous thrombosis.

Hcy was estimated quantitatively by ELISA (Axis Hcy Enzyme Immunoassay, which is a solid-phase enzyme immunoassay(10).

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Statistical analysis of the results was done and the p value calculated. A p value of <0.05 was considered as significant.

RESULTS AND DISCUSSION

The levels in normal subjects were found to be $11.33 \pm 0.18 \mu\text{mol/L}$ (mean $\pm$ SEM), the range being 3.8-15 $\mu\text{mol/L}$. Subjects with vascular disease were found to have a Hcy levels of 21.59 ± 1.28 , which was significantly higher ($p < 0.001$) than in the normal subjects (Table 1).

Table 1 : Homocysteine in normal subjects and patients of vascular disease

	Normal	Patients
Number of Cases	205	101
Homocysteine $\mu\text{mol/L}$ (Mean $\pm$ SEM)	11.33 ± 0.18	$21.59 \pm 1.28^*$

* $P < 0.001$

Table 2: Homocysteine levels in the two subgroups of patients of vascular disease

	Normal controls	Venous Thrombosis	Arterial Occlusion
Number of Cases	205	46	55
Homocysteine $\mu\text{mol/L}$ (mean $\pm$ SEM)	11.33 ± 0.18	$26.77 \pm 2.43^*$	$17.27 \pm 0.84^*$

* $p < 0.001$ in comparison with normals as well as with each other

reports. Some corroborate the previous observations of a positive correlation between increased levels of Hcy and occurrence of CAD, and also state it as an independent risk factor(13,14). On the other hand, one report from North India and one report from South India have not found any correlation between raised Hcy and CAD(7,8). In our study, we found raised Hcy levels were associated with an increased occurrence of vascular disease. Also, we observed that Hcy levels were higher in cases of venous thrombosis than in cases of arterial occlusion.

Although various other factors also affect Hcy levels (eg. age, sex, smoking, heterozygous enzyme deficiencies of Cystathionine-b-synthetase or thermolabile MethyleneTetraHydroFolate Reductase), these would influence both categories of patients equally and the same would also be reflected in normal controls(15,16).

The published investigations, so far, regarding the effects of raised Hcy levels in plasma on vessels and their atherothrombotic predisposition has encompassed both types of vessels – arteries and

The levels of Hcy were found to vary in these two subgroups of arterial occlusion and venous thrombosis. In patients with arterial occlusion the Hcy was found to be $17.27 \pm 0.84 \mu\text{mol/L}$, which was significantly higher than the levels in normal subjects ($p < 0.001$). Moreover, the levels in patients with venous thrombosis were found to be $26.77 \pm 2.43 \mu\text{mol/L}$ which was significantly higher than the normals ($p < 0.001$) and also than the patients with arterial occlusion ($p < 0.001$) (Table 2).

Our results show that normal Hcy values (3.8-15 $\mu\text{mol/L}$) in the North Indian urban population were in the similar range as has been reported in the previous studies pertaining to western populations(4,5,6).

In most studies conducted so far, a positive correlation has been established between elevated levels of Hcy and the incidence of CAD, and it has been stated as an independent risk factor for this condition(1,3,11,12).

In the Indian population, there have been variable

veins. But in our study we have classified and observed the difference in the plasma levels of Hcy in patients with vascular disease, depending upon whether the arteries or the veins are affected. As mentioned earlier, the mean value of Hcy in the serum of patients with venous involvement was significantly higher than that of the patients with arterial involvement, suggesting that arteries were affected at a lower level of Hcy than veins.

A possible mechanism of hyperhomocysteinemia affecting arteries more than veins could be its stimulation of smooth muscle cell proliferation [whether by its direct mitogenic effect(17) or by the activation of transcription factor NF-kB by ROS generated by autooxidation of Hcy(18,19)] which can mostly affect arteries (which have a thick layer of concentric smooth muscle cells in their tunica media), but not veins (which have only a few scattered smooth muscle cells in their media).

Smooth muscle cell proliferation will cause increased thickness of the arterial wall and therefore a reduction in the lumen of the artery. This will lead to a sluggish

flow of blood through the lumen, thus contributing to the atherogenicity of increased levels of plasma Hcy.

It may also be possible that the symptoms of arterial occlusion (CAD, stroke, ischaemia, gangrene) are much more alarming than those of venous occlusion (swelling and pain), so that the patients with arterial occlusion come to the clinician at a much earlier stage than do patients of venous occlusion.

Whether the anatomical difference between arteries and veins or the clinical difference between arterial occlusion and venous occlusion are responsible for our observations in this limited study; and whether these mechanisms can totally account for the difference in the levels of Hcy in patients of arterial and venous disease would require further investigation.

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