

Homocysteine as a Biomarker for Thrombosis: What is the Learning Curve?

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Homocysteine (Hcy) is a sulfur-containing amino acid and not present in our diet. It is synthesized as an intermediate product during transsulfuration of cysteine from methionine requiring cystathionine β synthase (CBS) and a cofactor-vitamin B₆. It can be remethylated to methionine by the enzyme methylene tetrahydrofolate reductase (MTHFR) with vitamin B₁₂ and folic acid as cofactors. Hcy is normally present in plasma in a concentration of 5-15 $\mu\text{mol/L}$ with 75% being protein bound. Mildly high levels may be seen in 5-7% of general population.¹ Hyperhomocysteinemia (HHcy) may be moderate, intermediate and severe referring to plasma Hcy levels of 16-30, 31-100 or >100 $\mu\text{mol/L}$, respectively; and may be inherited or acquired. Genetic defects with mutations of MHTFR enzymes result in intermediate (50-100 $\mu\text{mol/L}$), while CBS deficiency will lead to severe hyperhomocysteinemia (>100 $\mu\text{mol/L}$).² Besides being inherited, HHcy may be the consequence of smoking, nutritional deficiencies (of vitamin B₆, B₁₂ and folic acid), intake of anti-cholesterol agents, renal failure and thyroid disorders.

Hcy has been identified as an independent risk factor for venous thrombosis; besides, its association with vascular events like myocardial infarction and stroke. Its role in osteoporosis, macular degeneration, adverse pregnancy outcome and dementia is supported in some but not all studies. A number of reports described thrombosis in unusual sites such as retinal vein as a consequence of high Hcy levels. Heijer *et al.* in 1996 studied 269 patients with first episode of deep venous thrombosis with an equal number of matched controls; and observed high homocysteine levels in 13% cases as compared to 10% controls with an odd ratio of 2.5 (95% confidence interval; 1.2 - 5.2).³ The study demonstrated that the risk of thrombosis was significant at Hcy level above 22 $\mu\text{mol/L}$ at a mean age of 44 years (range 16-70). This study is important as it was the first to show the risk of thrombosis with moderate homocysteinemia.

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The relationship between Hcy and thrombosis is complex and multifactorial. Blood vessels (vascular injury and endothelial dysfunction), platelets (increased platelet activity), and coagulation factors (increased tissue factor expression, increased factor V activity, amplified thrombin generation, reduced anticoagulants and fibrinolysis)³ are all affected by high levels of homocysteine. Molecular mechanisms are poorly understood and involve oxidative stress, DNA hypomethylation, and pro-inflammatory effects.³ It is also suggested that homocysteine may not cause thrombosis by itself but does so in the presence of other factors such as methionine.

Should Hcy be measured? Current understanding is that Hcy should not be measured in non-selected general population. However, there is a substantial evidence that homocysteine levels may be checked in young patients who present with unexplained thromboembolic disease. Lussana *et al.* studied 19,678 patients with thrombosis who had thrombophilia screening over 12 years in six Italian thrombosis centers. Median level of homocysteine was 130 $\mu\text{mol/L}$ (range 101-262) in patients with a median age of 47 years (range 19-83). Venous thromboembolism (71%) was seen more frequently than arterial thromboembolism (26%), while recurrent thrombosis was seen in 42% patients.⁴ Similarly, a meta-analysis of 23 cohort and 33 case-control studies demonstrated significant association between cerebral venous thrombosis and inherited thrombophilia and hyperhomocysteinemia.⁵ In Denmark, a cohort of patients (n=184) with hyperhomocysteinemia was studied from 1996 to 2011. A MTHFR c.677TT genotype was identified in 49%, while CBS c.833T>C (p.I278T) in 4% patients. It was remarkable that patients having a CBS mutation had thrombosis at a young age of 25 years, approximately. The authors concluded that Hcy should be part of the thrombophilia screening for unexplainable arterial or venous thrombosis in individuals below 40 years.²

Since high Hcy levels may result from vitamin B or nutritional deficiencies, it is reasonable to argue if vitamin B supplementation will lower the risk of thromboembolism. Several randomized homocysteine-lowering therapy trials failed to show that vitamin B supplementation substantially modifies the risk of thrombosis or prevents its recurrence despite lowering Hcy levels. Interestingly, B-vitamins and betaine therapy

may be beneficial in reducing the thrombosis risk in patients with homocystinuria, a metabolic syndrome that is characterized by severe hyper-homocysteinemia, and was first described in 1962. Since thrombosis may be the first clinical manifestation of homocystinuria in patients that may be silent otherwise, evidence suggests that Hcy measurement may be helpful for identifying subjects at a greater risk of disease. Such patients may benefit from a more aggressive management of other modifiable risk factors, as recently demonstrated by result of the 5-year Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) trial.⁶

The current literature suggests that homocysteine is a biomarker for thrombosis but is not involved in the etiology of thrombosis by itself. There are other cryptic key players that act in synergy with homocysteine to potentiate thrombosis. In a local study in 2006, mild hyperhomocysteinemia (15-25 $\mu\text{mol/L}$) was frequent in patients with acute myocardial infarction. Though, this study failed to establish any association of hyper-homocysteinemia and MHTFR 677C>T mutation with coronary artery disease, the synergistic effect of other risk factors with hyperhomocysteinemia could not be ruled out.⁷

Mean plasma homocysteine levels in Pakistani normal adults were reported high at $16.4 \pm 4.9 \mu\text{mol/L}$,⁷ while still higher levels were reported in young patients with coronary artery disease (mean levels $18.1 \pm 5.3 \text{ mmol/L}$).⁸ Secondly, poor dietary habits with absent meat intake can further aggravate hyperhomocysteinemia, making them vulnerable to cardiac disease.⁹ In addition to nutritional deficiencies, Mohsin *et al.* reported that individuals having MTHFR 677CT or TT genotypes were at greater risk of developing hyperhomocysteinemia.¹⁰

All these findings indicate that Pakistanis are more prone to homocysteinemia. It is amazing that simple folic acid fortification (140 $\mu\text{g}/100\text{g}$) of the US grain supply by the Food and Drug Administration (FDA) in 1996 is expected to have decreased the US populations' plasma homocysteine levels by 53%. With a human develop-

ment index of 0.538, Pakistan ranks 147th of 188 countries. Poverty, lack of education, and dearth of health provisions are the major barriers to proper nutrition. High homocysteine levels in our population mandate the need for mass micronutrient supplementation for preventing coronary artery disease and other thrombotic events.

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