

Hyperhomocysteinemia and Coronary Artery Disease in Pakistan

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Abstract

The relative risk of developing coronary artery disease (CAD) in Pakistani men is highest in early ages. Majority of those suffering from CAD belong to the lower middle socioeconomic stratum of the society. Mild hyperhomocysteinemia (concentration of plasma homocysteine between 15-25 $\mu\text{mol/l}$) is very commonly seen in Pakistani patients with acute myocardial infarction (AMI) as well as in normal healthy subjects. There appears to be a lack of association between hyperhomocysteinemia and CAD in Pakistani population. There is also no evidence of association of methylenetetrahydrofolate reductase 677C>T mutation with CAD in this population. High prevalence of deficiency of folate and vitamin B6 appears to be the major causes of hyperhomocysteinemia in Pakistani population.

Deficiencies of micronutrients (folate, vitamin B12 and possibly vitamin B6) along with mild hyperhomocysteinemia, perhaps, act synergistically with other classical risk factors in Pakistani population to further increase the risk of CAD.

Introduction

Coronary artery disease in Pakistan

Pakistani people belong to the South Asian population which has the highest known rate of coronary artery disease (CAD).¹ According to the careful estimates based on scientific studies nearly 100,000 individuals suffered from acute myocardial infarction (AMI) in calendar year 2002.² The relative risk of developing CAD in Pakistani men is highest in early ages.³ In a recently published study, 16% of AMI patients at the Aga Khan University Hospital (AKUH) between 2000-2002 were found to be younger than 45 years of age and 93% of them were men.⁴ Earlier reports on AMI patients from other cities of Pakistan presented a similar picture.^{5,6} In a relatively larger study, the incidence of AMI was found to be 192.8/100,000 males during 1994.⁷ This figure places Pakistan at par with those countries which have the highest known rates of CAD. This shows that both intrinsic and extrinsic factors contribute to the development of CAD in Pakistani population. Since majority of those suffering from CAD belong to the lower middle socioeconomic stratum of the society, it has a devastating impact on the family and the

society.² CAD, therefore, has become a major health problem of this country.

Homocysteine and atherosclerosis

Homocysteine is a sulfur containing amino acid which is formed during metabolism of methionine. It is metabolized by two pathways, remethylation and transsulfuration.⁸ In remethylation, homocysteine acquires a methyl group either from N⁵, methyltetrahydrofolate (in all tissues) or from betaine (in liver and kidneys) to form methionine. The former methylation step requires folate and vitamin B12 as cofactors. In transsulfuration pathway, homocysteine condenses with serine to form cystathionine with the help of a B6-dependent enzyme. Cystathionine is then enzymatically catabolized to sulfate and excreted in the urine. Transsulfuration pathway is effective when there is excess of methionine or cysteine synthesis is required. A defect in any one of the enzymes involved in these two pathways or deficiency of folate, vitamin B12, or vitamin B6 would lead to hyperhomocysteinemia which is now recognized as an independent risk factor for atherosclerosis.⁸

Hyperhomocysteinemia and normal levels of homocysteine

Hyperhomocysteinemia is usually defined by using arbitrary cut-off points, for example, above the 95th percentile or more than two standard deviations above the mean values obtained from fasting normal healthy subjects.⁹ Most studies so far have indicated normal plasma homocysteine level to be in the range from 5 to 15 $\mu\text{mol/l}$.^{10,11} However, this does not imply that there is no risk associated with homocysteine levels between 10 to 15 $\mu\text{mol/l}$. Robinson and colleagues have shown that risk for CAD is represented by a continuum of total homocysteine concentration, with substantial risk occurring between 10 to 15 $\mu\text{mol/l}$.¹² Perhaps the "desirable level" should be 10 $\mu\text{mol/l}$ or below.

The definition of elevated levels of homocysteine is not standardized. According to Jacobsen, subjects with CAD, cerebrovascular disease and peripheral vascular disease usually present with "mild hyperhomocysteinemia" (15-25 $\mu\text{mol/l}$),¹³ while individuals with homocysteinuria because of rare inborn errors of homocysteine metabolism have "severe hyperhomocysteinemia" with homocysteine concentrations in the range of 50-500 $\mu\text{mol/l}$.¹⁴ On the other

hand, subjects with impaired renal function usually present with "intermediate hyperhomocysteinemia" with homocysteine concentrations between 25-50 $\mu\text{mol/l}$.¹⁵

Hyperhomocysteinemia and coronary artery disease

CAD leading to AMI or angina arises because of a combination of atherosclerosis and thrombosis. Homocysteine has been found to induce endothelial cell damage, oxidize low-density lipoprotein and stimulate smooth muscle cell proliferation.¹⁶ Through these effects, it has been shown to be associated with development of atherosclerosis. In addition, high concentration of homocysteine has been found to enhance thrombogenesis by increasing the activities of factor XII and factor V depressing the activation of protein C, inhibiting the expression of thrombomodulin and suppressing the expression of heparan sulfate by endothelium.^{16,17} Association of homocysteine with both of these pathological processes (atherosclerosis and thrombosis) makes it a potential risk factor for CAD. While, several case-control studies have provided consistent evidence of the role of homocysteine as a risk factor for CAD, quite a few have also shown a complete lack of association between the two.^{16,17} The Hordaland Homocysteine Study, involving more than 12,000 subjects showed that elevated plasma homocysteine level was associated with major components of cardiovascular risk profile, such as, male sex, old age, smoking, high blood pressure, elevated cholesterol level and lack of exercise.¹⁸ More recently, Wald et al have provided evidence that the association between homocysteine and cardiovascular disease is causal and lowering homocysteine concentration by 3 $\mu\text{mol/l}$ from current levels through folic acid supplementation, would reduce the risk of ischaemic heart disease by 16%.¹⁹

Hyperhomocysteinemia and CAD in Pakistan

So far 3 studies have been published regarding hyperhomocysteinemia and CAD in Pakistan.²⁰⁻²² While studies by Salahuddin et al²⁰ and Aamir et al²¹ have reported positive association between elevated plasma homocysteine and CAD, a recent study from the AKUH laboratory showed lack of association between hyperhomocysteinemia and CAD in a Pakistani population of patients with AMI.²² The difference appears to be due to the design of the studies, size of the population, and socioeconomic background of the subjects. Mean plasma homocysteine levels in AMI patients and controls in the studies by Salahuddin et al, Aamir et al and Iqbal et al were $14.97 \pm 1.13 \mu\text{mol/l}$, $10.57 \pm 0.31 \mu\text{mol/l}$, $18 \pm 5.9 \mu\text{mol/l}$, $10.8 \pm 4.1 \mu\text{mol/l}$, $18 \pm 8.36 \mu\text{mol/l}$ and $16.4 \pm 4.9 \mu\text{mol/l}$, respectively. While mean concentration values of plasma homocysteine in AMI patients in 3 studies were not statistically different ($p=0.09$), mean concentration of homocysteine in controls (16.4 ± 4.9

$\mu\text{mol/l}$) in the AKUH study was significantly different from the values reported in the other two studies ($p=0.0001$). This could be due to the fact that AKUH study matched the controls with cases not only on the basis of age and gender but also on socio-economic background. Moreover, those taking B-complex vitamin (folate, B12 and B6) supplements during the last six months were excluded from the study. One third (32.5%) and almost half (49%) of the controls were folate-deficient and B6-deficient respectively. Compared to folate-normal individuals (AMI patients as well as controls), folate-deficient and B6-deficient subjects were having significantly ($P<0.002$) elevated levels of plasma homocysteine indicating a positive association between deficiency of folate and B6 and hyperhomocysteinemia in Pakistani population.²² This is in line with the observation of Selhub and colleagues that inadequate plasma concentrations of one or more B vitamins contribute to approximately two thirds of all cases of hyperhomocysteinemia.²³

Nutritional deficiency of B-vitamins, therefore, appears to be the major cause of hyperhomocysteinemia in Pakistani population. Such high plasma levels of homocysteine in normal healthy subjects are not unique to our population. Similar high levels of plasma homocysteine in normal controls have also been reported in certain populations in South India ($18 \pm 10.7 \mu\text{mol/l}$)²⁴, Thailand ($19.9 \pm 8.1 \mu\text{mol/l}$)²⁵, Turkey ($15.6 \pm 10 \mu\text{mol/l}$)²⁶, and Syria ($14 \pm 6.2 \mu\text{mol/l}$ in males and $11.2 \pm 3.6 \mu\text{mol/l}$ in females).²⁷ In another study conducted on population from Karachi and Rawalpindi involving 397 AMI patients and 225 normal healthy controls, mild hyperhomocysteinemia (mean concentration of homocysteine about $17 \mu\text{mol/l}$) was found in both patient and control groups with hardly any difference between the two mean homocysteine values.²⁸ The results of this study further substantiate AKUH initial observation regarding lack of association between hyperhomocysteinemia and CAD in Pakistani population.

Factors causing hyperhomocysteinemia in Pakistani population

Elucidation of the mechanism of hyperhomocysteinemia in Pakistani population is one of the major challenges for the local scientific community. Both intrinsic and extrinsic factors will have to be taken into account. An enzyme, 5, 10-methylenetetrahydrofolate reductase (MTHFR), catalyzes the formation of 5-methyltetrahydrofolate which is involved in the methylation of homocysteine to methionine.⁹ Another local study have recently shown lack of association of methylenetetrahydrofolate reductase 677C > T mutation with CAD in Pakistani population.²⁸ The frequency of homozygous mutant gene which is associated with

hyperhomocysteinemia in most populations appears to be quite low (2% in AMI patients and 3% in normal healthy subjects) in Pakistani population.²⁸ Therefore, mild hyperhomocysteinemia observed in Pakistani AMI patients and normal controls, appears unlikely to be due to 677C > T mutation in MTHFR gene. Role of other mutations in MTHFR, however, cannot be discounted in Pakistani population.²⁹ Mutations in vitamin B12-dependent methionine synthase have been identified, however, the prevalence of these mutations in general population and their contribution to hyperhomocysteinemia in heterozygous individuals is still not known.¹³ Similarly, in the gene for vitamin B6-dependent cystathionine B-synthase nearly 40 mutations have been identified, but the role of cystathionine B-synthase polymorphism in atherosclerosis remains to be determined.¹³

High prevalence of deficiency of folate and vitamin B6 appears to be the major cause of hyperhomocysteinemia in Pakistani population. The deficiency of these vitamins could be due to lower intake of fresh fruits and vegetables, overcooking of our food and a high prevalence of parasitic enteric infections (especially amoebiasis and giardiasis) in our population.³⁰ In addition to these, there could be other factors, yet unknown, affecting various enzymes of remethylation and transsulfuration pathways of homocysteine metabolism leading to hyperhomocysteinemia in Pakistani population.

So far, no large scale "community-based" studies have been conducted to assess the prevalence of folate, vitamin B6 and B12 deficiencies in Pakistan. In the absence of these studies, it would be difficult to ascertain the enormity of the problem due to these micronutrients deficiency in our general population. A recent local study showed a causal relationship between nutritional deficiency of folate, B12 and B6 and AMI in our population.¹⁻¹ Whether this association is through "mild hyperhomocysteinemia" (observed in our population) or not, remains to be ascertained.

Conclusion

It is possible that deficiencies of micronutrients along with mild hyperhomocysteinemia could be acting synergistically with other highly prevalent classical risk factors, such as, low HDL-cholesterol, hypercholesterolemia, hypertriglyceridemia, hypertension, diabetes mellitus; waist-hip ratio, obesity, tobacco use, parental history of ischaemic heart disease etc., thereby, further increasing the risk of CAD in Pakistani population.¹⁻³¹⁻³⁵ There is a need for supplementation trials at the community level with folate, vitamin B6 and possibly vitamin B12 to lower the high levels of homocysteine followed by assessment of the impact of this intervention on

the development of CAD in our population.

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Case Series

Angiokeratoma of tongue: a series of 14 cases

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Abstract

Angiokeratomas (AC) are vascular lesions which are defined histologically as one or more dilated blood vessels lying directly subepidermal and showing an epidermal proliferative reaction with ectatic capillaries in the papillary dermis. Only three other cases of isolated mucosal angiokeratoma have been reported in the indexed literature. We reviewed all cases of angiokeratoma located on the tongue, diagnosed in our department during a study period of 10 years (1995-2005).

Histologically all 14 cases showed dilated and congested blood vessels in the upper papillary dermis. They lack deep dermal involvement. Hyperkeratosis and acanthosis were also seen in most of the cases. No clinical data was available to assess systemic disease. A higher incidence of AC in tongue is seen in our study.

Introduction

Angiokeratomas (AC) represent ectasia of superficial blood vessels associated with secondary epidermal changes, especially acanthosis and hyperkeratosis. Five variants have been described. They are: 1) the generalized systemic type-angiokeratoma colporis diffusum of Fabry; 2) multiple papular, and plaque like - angiokeratoma circumscriptum (naeviforme); 3) the bilateral form occurring on the dorsal areas of the fingers and toes- angiokeratoma of mibelli; 4) angiokeratoma of Fordyce- the localized scrotal form; and 5) solitary papular angiokeratoma, although usually single, multiple lesions may occur between the age of 10 and 40 years and the legs

are the site of predilection. Multiple lesions have been reported in zosteriform distribution. Association of angiokeratoma circumscriptum with angiokeratoma of the scrotum, angiodyplasia (Klippel-Trenaunay-Weber syndrome) Cobb syndrome, and other mixed vascular malformations have been reported.^{1,5}

Methods and Results

We reviewed all cases of AC located on the tongue (n=14) diagnosed in our department during a study period of 10 years (1995-2005). Location of the lesions was tip of tongue in 2 cases, anterior two third in 2 cases, posterior one third in 2 cases, dorsum of tongue in 1 case and location was not mentioned in 7 cases. In 2 cases, the clinical clue

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