ABSTRACT

Homocysteine, a thiol amino acid, is present at a branch point in the methionine metabolism. The intracellular homocysteine metabolism is well regulated and its concentration in circulation is less than 12 µmol/L. However, improper metabolism of homocysteine due to genetic and/or environmental factors (including diet) may result in high plasma homocysteine levels. An elevated level of homocysteine (hyperhomocysteinemia) has been associated with various diseases and/or clinical conditions. It has also been implicated as an independent risk factor for cardiovascular disorders. Further, hyperhomocysteinemia has been found to have graded effect on the risk of coronary artery disease (CAD) and the extent and severity of the disease. In CAD patients, homocysteine levels are a significant predictor of mortality, independent of traditional risk factors.

CAD is one of the cardiovascular diseases that is probably the largest cause of mortality and morbidity worldwide and is reaching epidemic proportions in developing countries. It has been predicted that deaths due to CAD is likely to be more than any other disease in India. Furthermore, in Indians, CAD tends to occur much earlier than any other ethnic groups. This is surprising considering the fact that a majority of Indian population adheres to a vegetarian diet which is supposed to be a healthier alternative especially in the context of cardiovascular disorders. However, vegetarian diet lacks important micronutrient like vitamin B12 and thus it can be presumed that vegetarians have low levels of vitamin B12, an important micronutrient, and as a consequence elevated homocysteine levels. Apart from the nutritional factors, many single nucleotide polymorphisms (SNP) especially those present in the genes involved in the homocysteine metabolism have been shown to be associated with the altered levels of homocysteine. Very few studies have been done to elucidate the role of various polymorphisms on the levels of various biochemical parameters like homocysteine, cysteine, folate and vitamin B12 in Indian population.

We have studied the impact of biochemical parameters on CAD in Indian population and checked the effect of several non-synonymous SNPs (nsSNPs) on these biochemical parameters. The study was done in two different study groups. First was a case-control group i.e. AIIMS study group
where 876 individuals (mainly Indo-European population from northern part of India) were recruited at a tertiary care centre from New Delhi. Out of these 876 individuals, 448 were CAD patients as confirmed by coronary angiography and 428 individuals with negative treadmill test were considered as controls. In the second study group i.e. Indian Genome Variation Consortium (IGVC), more than 2000 individuals from 55 sub-populations were recruited from various parts of the country based on their geographical locations and linguistic lineages. Samples collected in this study group were divided into four groups based on their linguistic lineage i.e. Austro-Asiatic, Dravidian, Indo-European and Tibeto-Burman.

In the AIIMS study group, percentage of vegetarians in CAD patients was found to be significantly higher as compared to controls. Studies done in western populations have shown that vegetarian diet reduces the incidence of CAD up to 24%. The contradictory results obtained in our study might be due to the fact that a vegetarian diet lacks vitamin B12, an important micronutrient that can be obtained only from animal source. Among the vegetarians, the median B12 concentration was found to be 140 pmol/L which is below normal range (150 pmol/L) while among the non-vegetarians, the median concentration was 157 pmol/L. Since the effect of vitamin B12 on CAD might be mediated through the modulation of thiol concentrations, we determined the concentrations of homocysteine and cysteine (thiols associated with CAD) in the studied individuals. The total thiol concentration was found to be significantly higher in CAD patients than controls. We also found that among the thiols, cysteine alone was significantly associated with CAD but not homocysteine. This may be due to rapid conversion of homocysteine to cysteine in individuals deficient in vitamin B12 where remethylation of homocysteine to methionine is expected to be lower.

In the background of vitamin deficiency, we studied 45 nsSNPs from 12 genes involved in homocysteine metabolism. Out of these 45 nsSNPs, only 15 were found to be polymorphic (Minor Allele Frequency ≥ 1%) in our study population. These 15 nsSNPs were genotyped in 546 individuals (243 patients and 303 controls). We ascertained the effect of these 15 nsSNPs on homocysteine, cysteine, vitamin B12 and folate in these individuals. Only two SNPs from methylenetetrahydrofolate reductase (MTHFR C677T) and choline
Abstract
dehydrogenase (CHDH A119C) genes were found to significantly modulate the levels of homocysteine both at genotypic as well as allelic levels. MTHFR C677T was associated with elevated homocysteine levels under the assumption of a recessive model while CHDH A119C was significantly associated with decrease in homocysteine levels under the assumption of both dominant and recessive models. These two SNPs were then further genotyped in an additional 330 individuals. The two polymorphisms remained significant even after increasing the sample size. Three way diet genotype interactions was observed where vegetarian diet with MTHFR C677T and CHDH A119C significantly increases the levels of homocysteine.

We checked the basal frequencies of these polymorphisms in Indian population as a whole by genotyping individuals from IGVC study group. We observed in AIIMS study as well as IGVC study that MAF for MTHFR C677T is far less than what is reported in three HapMap populations (Caucasian, Japanese and Chinese) and almost similar to African-Americans. Interestingly, the MTHFR 677TT genotype was present mostly in all the populations of North India (consisting of both Indo European and Tibeto-Burman linguistic lineage) and almost absent in other population studied. When other nsSNPs that were studied in AIIMS study group were genotyped in IGVC study individuals, differences were observed in the MAF between IGVC and dbSNP. 9 SNPs were found to be of lower MAF as compared to dbSNP while other two were found to be higher. Another polymorphism MTHFR A1298C is also known to be associated with decreased enzyme activity. Interestingly, MAF for this polymorphism was found to be highest in both of our study groups as compared to the four HapMap populations as well as MAF reported in dbSNP.

All the exons and exon-intron boundaries from three genes i.e. CBS, MTHFR (that play a major role in homocysteine metabolism) and TCN2 (required for vitamin B12 transport) were screened using dHPLC in 95 individuals from AIIMS study to find out novel mutations/polymorphisms. 33 novel mutations were observed in CBS, 12 in MTHFR and 6 in TCN2 gene. Frequencies of 13 polymorphisms were also determined in CBS and MTHFR genes (selected on the basis of their high frequency, presence in HapMap data and equal spacing) by genotyping more than 1800 individuals from IGVC study including two novel polymorphisms that were present at higher frequencies.
A total of 62 genes that were linked to structural and functional abnormalities of the cardiovascular system caused by clinically relevant genetic and environmental stimuli were also selected. 350 SNPs from these genes were genotyped in about 550 individuals recruited in IGVC study from 24 sub-populations to generate the basal frequency in Indian population. Out of 350 SNPs, 334 were successfully genotyped and studied for their differences in MAF in four linguistic groups. 20 SNPs were found to be non-polymorphic. MAF for the 314 SNPs that were found to be polymorphic were compared among four linguistic groups. Significant differences were observed revealing the genetic heterogeneity among the four linguistic groups. 70 SNPs showed significant differences in MAF between any two linguistic groups while 21 SNPs showed differences among all the four groups. 70 SNPs did not show any difference in MAF among the groups. Furthermore, 3 SNPs were non-polymorphic in 3 linguistic groups, 5 SNPs were non-polymorphic in 2 linguistic groups and 7 SNPs were non-polymorphic in only one linguistic group.

Recently, one SNP present upstream of INSIG2 gene was found to be associated with obesity in a genome wide scan. We also genotyped this polymorphism in AIIMS and IGVC study individuals to check whether the SNP is significant in Indian scenario keeping in mind the difference in genetic and environmental modifiers among various populations. This SNP was not found significant in our population. Even after segregating the individuals on the basis of sex, no significant difference in BMI were observed as a function of age.

In conclusion, in our study cohort we found CAD patients having vitamin B12 deficiency with high levels of thiol. Our study suggests that two polymorphisms MTHFR C677T and CHDH A119C are associated with elevated levels of homocysteine. Furthermore, diet-gene interaction between the MTHFR C677T, CHDH A119C and vegetarian diet was also observed resulting in hyperhomocysteinemia. These three factors may play an important role in CAD for Indian population. Various novel mutations were observed in the three genes (CBS, MTHFR and TCN2). Further, SNPs present in genes playing an important role in cardiovascular structure and function showed varying MAF among four linguistic groups thereby revealing the genetic heterogeneity in these groups. SNP present upstream of INSIG2 gene was not found to be significantly associated with BMI in our study groups.