Association of MTHFR, IL-6 and ICAM-1 gene Polymorphisms with Coronary Artery Disease in South-Indian Ethnic Subset: A Case-Control Study

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INTRODUCTION
Cardiovascular disease (CVD) is the leading cause of mortality and morbidity all over the world. Among these cardiovascular deaths, half result from coronary artery disease (CAD). The prevalence of CAD in India is rising with time in both rural and urban areas.1 CVDs are showing an escalation among the Indian population with a trend of affecting the younger age groups.

The most striking feature of premature CAD in Indians is low prevalence of traditional risk factors. Increased prevalence of CAD is associated with increased levels of inflammatory markers which damage the endothelium and increase the development of atheromatous plaques.2,3 Phenotypic variations of these markers may depend on physiological stress or genetic variations.

Genetic susceptibility to CAD may depend on genes related to inflammatory process. Hence in the present study we studied Methylenetetrahydrofolate reductase (MTHFR), Interleukin-6 (IL-6) and Intercellular adhesion molecule-1 (ICAM-1) genes in which genetic variations lead to hyperhomocysteinemia, release of inflammatory markers such as hsCRP, fibrinogen, LP[a] and elevated expression of soluble ICAM-1 levels respectively.4,5 Methylenetetrahydrofolate reductase (MTHFR) gene catalyzes the reaction that reduces 5, 10-methylene tetrahydrofolate to 5-methyltetrahydrofolate which acts as a carbon (C1) donor in the remethylation of Homocysteine into Methionine. A variant of this enzyme called ‘thermolabile MTHFR’ that has reduced activity at higher temperatures is produced by a common single nucleotide mutation in the exon-4 of MTHFR gene and is incapable of reduction of 5, 10-methylene tetrahydrofolate to 5-methyltetrahydrofolate resulting in the elevated levels of homocysteine (hyperhomocysteinemia).6 Homocysteine acts as a corrosive agent and causes oxidative damage to endothelium and results in atherosclerosis.7

IL-6 has been shown to contribute to both, atherosclerotic plaque development and plaque destabilization via a variety of mechanisms. IL-6 stimulates the expression of tissue factors, matrix degrading enzymes, LDL receptors in macrophages, as well as the aggregation of the platelets and the production of CRP, fibrinogen, LP[a] and serum amyloid A (SAA) by hepatocytes. These agents cause damage to the endothelium, increased occurrence of thrombosis, decreased the HDL-cholesterol and increased macrophage uptake of lipid which are events in CAD. This stimulation of IL-6 on acute phase proteins shows a link between IL-6 and CVD.10,11 ICAM-1 is expressed on vascular endothelium and plays a key role in trans-endothelial migration of leukocytes. ICAM-1 functions as a ligand for β2 integrin molecules (LFA-1 and Mac-1) present on leukocytes12 and encodes a cell surface glycoprotein which is typically expressed on endothelial cells and cells of immune system and is involved in cell-cell adhesive interactions of the immune system. ICAM-1 expression on endothelium is regulated by cytokine stimulation.13 ICAM-1 functions as a natural receptor for lymphocytes and is involved in the binding of leukocytes to the arterial endothelium which leads to the formation of strong plaques by the segregation of fat molecules and leukocytes on arterial walls.14 In view of lack of polymorphism studies on these genes in our ethnic population the present study was aimed to examine the polymorphisms...
of MTHFR, IL-6 and ICAM-1 genes in young CAD patients of South-Indian ethnicity.

MATERIALS AND METHODS

This was a prospective Case-Control study conducted in the department of Cardiology, SVIMS, Tirupati. This study was approved by the Institutional Ethics Committee of our institute with IEC approval no. 220. 50 CAD patients aged between 18 and 50 years, diagnosed with acute coronary syndromes [Unstable angina (USA), Myocardial infarction (ST-elevation myocardial infarction (STEMI) and Non-STEMI (NSTEMI)] were recruited in the CAD group. In addition, 50 healthy subjects aged between 18 and 50 years, without any known risk factors (diabetes mellitus, hypertension, smoking, obesity) and without family history of CAD (confirmed by history and physical examination) were recruited in control group. Patients with chronic stable angina, having other co-morbid conditions such as renal, lung, liver and other systemic illness, pregnant women were excluded from the study. A written informed consent was obtained from all the study participants following ethical guidelines of the 1975 declaration of Helsinki.

Biochemical Analysis

Fasting lipid profile including total cholesterol, triglycerides, HDL cholesterol were estimated using commercial kits on DNX600 Beckman auto analyzer. LDL cholesterol and VLDL cholesterol were calculated using Friedwald formula.13 Homocysteine levels were estimated using Dialab kit using enzymatic recycling method on DNX600 Beckman auto analyzer. High sensitivity C-reactive protein (hsCRP) levels were estimated on Beckman system pack by immunoturbidimetry assay method. Protein C,14 Protein S15 and anti-cardiopulmonary antibodies were estimated by ELISA method.

Genetic Analysis

Genomic DNA was isolated from blood samples using QIAamp DNA Mini spin-column [Qiagen] DNA extraction kit.16,17 Extracted DNA samples were analyzed on 1% agarose gel electrophoresis.18 The following Oligonucleotide primers were designed using Oligo-6, NCBI blast and Primer-3 softwares and synthesized at Eurofins genomics India Pvt. Ltd. Bengaluru, India.20 Polymerase chain reaction (PCR) amplification was done in the Eppendorf Mastercycler nexus gradient-flexlid model, Hamburg, Germany, in a 50 µL reaction volume comprising of 1x assay buffer, 1.5 mmol MgCl₂, 500 ng template DNA, 50 pico mole forward primer, 50 pico mole reverse primer, 100 µmol dNTPs mix and 1U Taq DNA polymerase (Thermo Scientific, USA).

PCR amplification was performed with following conditions: Denaturation at 94°C; Annealing at 60°C, 55°C and 56°C for MTHFR (exon-4), IL-6 (exon-4) and ICAM-1 (exon-6) respectively; and Extension at 72°C. The amplified PCR products were analyzed on 2% agarose gel in 1X TAE buffer comprising of ethidium bromide for 1 hour.

MTHFR: Methylenetetrahydrofolate reductase; IL-6: interleukin-6; ICAM-1: intercellular adhesion molecule 1.

<table>
<thead>
<tr>
<th>Forward Primer [5’ to 3’]</th>
<th>Reverse Primer [5’ to 3’]</th>
<th>Amplicon Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTHFR, Exon-4</td>
<td>GGCACGGACAGCTGGGAGTT</td>
<td>546 bp</td>
</tr>
<tr>
<td>IL-6, Exon-4</td>
<td>TACATGGGGCCCTCTGATTGTC</td>
<td>428 bp</td>
</tr>
<tr>
<td>ICAM-1, Exon-6</td>
<td>CCTCTGCGCTCGGTGGCTAGT</td>
<td>449 bp</td>
</tr>
</tbody>
</table>

by Sanger’s dideoxynucleotid sequence termination method at Eurofins Genomics India Pvt Ltd., Bengaluru, India.

The sequences were compared by performing multiple sequence alignment using ClustalX tool (Version 1.83, National Center for Biotechnology Information, Bethesda, MD) to identify the mutations in the sequences. Expert Protein Analysis System (ExPASy) analysis was used to translate nucleotide sequences into amino acid sequence and changes in amino acid sequences were noted for each sequence. All the mutated sequences were deposited at NCBI-GenBank database.21

Statistical Analysis

Data was collected in a pre-designed proforma and entered in Microsoft Excel spread sheets. Normality of distribution was checked with Kolmogorov-Smirnov test. Descriptive statistics including mean and standard deviation (SD) for continuous variables and proportions for categorical data were calculated. Continuous data was tested for statistical significance with Independent Student’s t-test. All the statistical analysis was performed with the help of Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA.).

RESULTS

Mean age of the CAD group was 36.6 (±0.7) years and control group was 34.9 (±0.7) years (p=0.11), thus the two groups are comparable. By chance majority of the cases were males (86%) in both the groups. Smoking (60%) was the most prevalent risk factor in CAD group followed by diabetes mellitus (34%), hypertension (30%) and obesity (24%). Baseline and demographic details are summarized in Table 1.

Statistically significant higher levels of total cholesterol, triglycerides, LDL-cholesterol, VLDL-cholesterol and Lp[a] were observed in CAD group compared with control group (p<0.05). There was no statistically significant difference in HDL-cholesterol (p=0.06).

Risk factors related to inflammation (homocysteine and hsCRP) and coagulation (fibrinogen, protein-C and protein-S) were estimated and values are summarized in Table 2. A statistically significant elevation of inflammatory markers homocysteine, hsCRP and fibrinogen were found in CAD group (p<0.05). Protein-C deficiency was observed in 32% of the patients whereas protein-S deficiency was not observed in any case.

Methylenetetrahydrofolate reductase (Exon-4) Mutation analysis

Multiple sequence alignment showed a single nucleotide mutation i.e., c.677 C>T in exon-4 of MTHFR in 10% of CAD group (Figure 1). This single nucleotide change (transition) causes a replacement of amino acid Alanine to Valine at position 222 (p.A222V) in the protein sequence. The identified mutation (35.8±4.7 µmol/L vs 17.5±7.7 µmol/L, p<0.0001).
Latheef, et al.: MTHFR, IL-6 and ICAM-1 polymorphism in CAD

Table 1: Baseline and Demographic details of the Study group.

<table>
<thead>
<tr>
<th>Variable</th>
<th>CAD group (n=50)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, in years</td>
<td>36.6±0.7</td>
<td></td>
</tr>
<tr>
<td>Male: Female, n</td>
<td>43:07</td>
<td></td>
</tr>
<tr>
<td>Obesity, n (%)</td>
<td>12 (24%)</td>
<td></td>
</tr>
<tr>
<td>Smokers, n (%)</td>
<td>30 (60%)</td>
<td></td>
</tr>
<tr>
<td>Alcoholics, n (%)</td>
<td>19 (38%)</td>
<td></td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>15 (30%)</td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>17 (34%)</td>
<td></td>
</tr>
<tr>
<td>Family h/o CAD, n (%)</td>
<td>12 (24%)</td>
<td></td>
</tr>
<tr>
<td>Presentation of Patients:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>STEMI</td>
<td>38 (76%)</td>
<td></td>
</tr>
<tr>
<td>NSTEMI</td>
<td>07 (14%)</td>
<td></td>
</tr>
<tr>
<td>USA</td>
<td>05 (10%)</td>
<td></td>
</tr>
<tr>
<td>Type of disease:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SVD</td>
<td>35 (70%)</td>
<td></td>
</tr>
<tr>
<td>DVD</td>
<td>09 (18%)</td>
<td></td>
</tr>
<tr>
<td>TVD</td>
<td>02 (4%)</td>
<td></td>
</tr>
<tr>
<td>Normal Coronaries</td>
<td>04 (8%)</td>
<td></td>
</tr>
<tr>
<td>Diseased Vessels:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAD</td>
<td>35 (59.3%)</td>
<td></td>
</tr>
<tr>
<td>LCX</td>
<td>07 (11.9%)</td>
<td></td>
</tr>
<tr>
<td>RCA</td>
<td>17 (28.8%)</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Comparison of Inflammatory and Coagulation Profile.

<table>
<thead>
<tr>
<th>Variables</th>
<th>CAD group</th>
<th>Control group</th>
<th>p-val</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homocysteine (µmol/L)</td>
<td>19.3±9.2</td>
<td>16.8±1.2</td>
<td>p = 0.05*</td>
</tr>
<tr>
<td>hsCRP (mg/dl)</td>
<td>2.3±2.2</td>
<td>0.4±0.1</td>
<td>p&lt;0.0001*</td>
</tr>
<tr>
<td>Fibrinogen (mg/dl)</td>
<td>382.5±175.4</td>
<td>112.4±38.0</td>
<td>p&lt;0.0001*</td>
</tr>
<tr>
<td>Anti-Cardiolipin antibodies (MPL U/ml)</td>
<td>7.96±1.09</td>
<td>6.80±4.51</td>
<td>p = NS</td>
</tr>
<tr>
<td>Protein C deficiency</td>
<td>16 (32%)</td>
<td>00</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Protein S deficiency</td>
<td>00</td>
<td>00</td>
<td>--</td>
</tr>
</tbody>
</table>

hsCRP: high sensitivity C-reactive protein; SD: standard deviation; NS: not significant. Independent sample t-test. *indicates statistical significance (p≤0.05).

Present study findings showed that the mutation c.677 C>T in exon-4 of MTHFR is associated with an increased risk of CAD (OR: 12.21, 95% CI: 0.66 to 226.98) in South-Indian ethnic population.

Interleukin-6 (exon-4) Mutation analysis

Sequence analysis with multiple sequence alignment tool showed mutations (Figure 1) in exon-4 of IL-6 in 13 CAD patients (C-5, C-9, C-10, C-12, C-14, C-15, C-21, C-24, C-28, C-29, C-32, C-45 and C-47). All the identified mutations were novel. There was no mutation in control group. Identified mutations were translated to amino acid sequences with ExPASy analysis and the changes in protein sequence was noted and deposited in GenBank. GenBank accession numbers are KX430119, KX430120, KX430121, KX430122, KX430123, KX430124, KX430125, KX430126, KX430127, KX430128, KX430129, KX430130 and KX430131 (Table 4).

A higher hsCRP and fibrinogen levels were found in patients with mutations in IL-6 gene which was statistically significant (p<0.0001 and p=0.017 respectively) (Table 3, Figures 2 and 3). Higher Lp[a] values were also found in patients with IL-6 mutations but not statistically significant (p=0.82).

Present study findings showed that the mutations in exon-4 of IL-6 gene is significantly associated with an increased risk of CAD (OR: 36.36, 95% CI: 2.09 to 631.21) in South-Indian ethnic subset.
### Table 4: Mutations identified in exon-4 of IL-6 gene.

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Nucleotide Sequence</th>
<th>Protein Sequence</th>
<th>hsCRP (mg/dl)</th>
<th>Fibrinogen (mg/dl)</th>
<th>GenBank Accession No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-9</td>
<td>c.581 G&gt;A</td>
<td>No change</td>
<td>2.52</td>
<td>711</td>
<td>KX430120</td>
</tr>
<tr>
<td>C-24</td>
<td>c.581 G&gt;A</td>
<td>No change</td>
<td>3.9</td>
<td>403</td>
<td>KX430126</td>
</tr>
<tr>
<td>C-28</td>
<td>c.581 G&gt;A</td>
<td>No change</td>
<td>3.93</td>
<td>527</td>
<td>KX430127</td>
</tr>
<tr>
<td>C-29</td>
<td>c.519 G&gt;A, c.552 G&gt;A</td>
<td>No change</td>
<td>6.3</td>
<td>384</td>
<td>KX430128</td>
</tr>
<tr>
<td>C-32</td>
<td>c.581 G&gt;A</td>
<td>No change</td>
<td>2.52</td>
<td>496</td>
<td>KX430129</td>
</tr>
<tr>
<td>C-45</td>
<td>c.581 G&gt;A</td>
<td>No change</td>
<td>5.85</td>
<td>491</td>
<td>KX430130</td>
</tr>
<tr>
<td>C-47</td>
<td>c.581 G&gt;A</td>
<td>No change</td>
<td>6.3</td>
<td>318</td>
<td>KX430131</td>
</tr>
<tr>
<td>C-14</td>
<td>c.506 G&gt;A, c.509 C&gt;T, c.538 G&gt;T, c.548 G&gt;A, c.581 G&gt;A</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>


**DISCUSSION**

Strong scientific evidence indicates that inflammatory pathway activation is important in the initiation, maintenance and progression of atherosclerosis. The inflammatory cascade has been implicated during the entire plaque formation, from the early stages of endothelial dysfunction to the development of acute coronary syndromes (ACS). This is in accordance with the presence of elevated circulating biomarkers of inflammation, which independently predicts the likelihood of adverse cardiovascular events.22-24

In the present study, a statistically significant elevation of inflammatory markers homocysteine, hsCRP and fibrinogen were found in CAD group which confirms the key role of inflammatory markers in CAD. Protein-C deficiency was observed in 32% of the patients. Protein-C and S are synthesized by the hepatocytes and endothelial cells. Protein-C upon activation by protein-S inhibits coagulation pathway by cleaving FVa and FVIIa. Therefore, Protein-C deficiency is suggestive of hypercoagulable state in CAD group.

In recent years, genomic susceptibility to diseases has attracted a growing attention to research the genetic polymorphisms involved in pathogenesis of diseases. Genetic polymorphisms can change the structure and quantity of the gene product, ultimately affecting the function of the product. The inflammatory status is an important step to start and promote the pathogenesis of atherosclerosis.

A single nucleotide mutation i.e., c.677 C>T in exon-4 of *MTHFR* gene was observed in 10% of CAD cases while it was absent in controls. This single nucleotide change (transition) causes production of thermolabile methylenetetrahydrofolate reductase enzyme which is incapable in conversion of homocysteine to methionine. The present study findings suggest that the T allele is associated with an increased risk of CAD in South-Indian ethnic population. This finding is consistent with previous reports25-29 in which *MTHFR* C677T transition was found to be a risk factor for premature myocardial infarction (MI).

The current study showed that the T allele is significantly associated with CAD (OR: 12.21, 95% CI: 0.66 to 226.98). This finding is consistent with a previous report in which *MTHFR* C677T transition was found to be a risk factor for premature MI.25-27-30 On the other hand, other authors31-32 reported insignificant differences for the T allele between MI subjects and the control group. Indian studies by SK Gupta et al.33 and Ravi Kanth et al.34 also showed no association of *MTHFR* polymorphism with CAD. In our study, the average homocysteine levels were significantly higher in the patient group than in controls. This is in agreement with observations by other investigators.25,28,35

The *MTHFR* C677T mutation is identified as a major determinant of homocysteine concentrations in Europeans, but is less prevalent and does not influence homocysteine concentrations in South Asians.36 Each 5 μmol/L rise in homocysteine levels conferred ~9% increase in the risk of coronary heart disease (CHD) events, independent of other conventional CHD risk factors.37 Various studies reported elevated levels of homocysteine with increased risk of CVD.38-40 The level of plasma homocysteine depends on the combined effects of genetic and environmental factors.41 *MTHFR* gene mutation (c.677 C>T; p.A222V) observed in our ethnic group is associated with Hyperhomocysteinemia which is a risk factor for CAD.

Cytokine-mediated inflammation accompanies atherosclerosis from its initiation to the occurrence of clinical endpoints.42 In the present study, it has been found that the *IL-6* gene polymorphism is a risk factor of CAD susceptibility. Mutations in exon-4 of *IL-6* gene was observed in 13 (26%) patients. These polymorphisms may influence the expression and function of IL-6 protein and thus affect the susceptibility to cardiovascular diseases. This study showed a statistically significant higher levels of

**Figure 4:** Comparison of mean hsCRP levels in patients with *ICAM-1* mutation and without *ICAM-1* mutation.

**Figure 3:** Comparison of mean Fibrinogen levels in patients with IL-6 mutation and without IL-6 mutation.

**Intercellular adhesion molecule-1 (exon-6) gene Mutation analysis**

*ICAM-1* (exon-6) mutation i.e., c.1405 A>G was observed in 9 (18%) patients on Multiple sequence alignment (Figure 1). This mutation causes a change of amino acid Lysine to Glutamic acid (p. K 469 E) at position 469 (ExPASy analysis). Identified mutations were submitted to GenBank and the accession numbers are - KX239886, KX239887, KX239888, KX239889, KX239890, KX239891, KX239892, KX239893 and KX258233.

A statistically significant higher level of hsCRP was found in patients with *ICAM-1* polymorphism compared to patients without *ICAM-1* mutation (4.27±2.07 vs 1.86±1.98 mg/dl, p = 0.002) indicating that this mutation increases the inflammation (Figure 4). Observations of the present study showed association of *ICAM-1* gene mutation with CAD risk (OR: 23.12, 95% CI: 1.31 to 409.15).
hsCRP and fibrinogen levels in patients with IL-6 gene mutations than in patients without mutations ($p<0.0001$ and $p=0.017$ respectively).

Elevated levels of serum hsCRP serve as a strong independent predictor of risk of MI, stroke, peripheral arterial diseases and cardiovascular mortality. In 2002, Ridker et al. reported the role of abnormal CRP values in the development of atherosclerotic CVD. Further in 2008, Ridker et al. confirmed hsCRP as a strong, independent predictor of future heart disease. The Cardiovascular Health Study evaluated hsCRP levels in men and women aged 65 years or more without a history of vascular disease. The study reported strong association between elevated levels of hsCRP with increased 10-year risk of CHD beyond traditional risk factors.

Guruprasad et al. showed that elevated serum hs-CRP levels provide a useful marker for cardiovascular risk which, when combined with traditional risk factors, may help improve global risk prediction. There is evidence that hs-CRP, a leading inflammatory biomarker for clinical application, is independently associated with the risk of incidence or recurring cardiovascular events regardless of the lipid levels.

Interleukin-6 induces the acute phase reactants such as fibrinogen, serum amyloid A, CRP and haptoglobin. Fibrinogen is produced by the liver on IL-6 induction. Fibrinogen is majorly involved in the blood coagulation cascade which results in thrombosis. Plasma fibrinogen concentrations are recognized as an independent predictor of MI. It also binds to platelet glycoproteins, facilitating platelet aggregation. In terms of atherogenesis, fibrinogen may act by binding to LDL and stimulating proliferation of vascular smooth muscle cells. In addition to its role as a nonspecific marker of inflammation, fibrinogen may also have a direct role in atherogenesis and thrombogenesis by acting as a bridging molecule for many types of cell-cell adhesion events critical for atherogenesis. It has been suggested that the association between fibrinogen haplotypes and MI is partly mediated through pleiotropic effects of the serum IL-6 concentration.

Many studies assessed the relationship between IL-6 gene polymorphisms and pathogenesis of CAD and reported conflicting results. In present study, IL-6 polymorphisms in exon-4 were studied which is not studied earlier by other researchers and found novel mutations which are significantly associated with elevated inflammatory markers in CAD patients.

ICAM-1 functions as a natural receptor for lymphocytes and is involved in the binding of leukocytes to the arterial endothelium which leads to the formation of strong plaques by the segregation of fat molecules and leukocytes on arterial walls. Current study showed ICAM-1 (exon-6) mutation i.e., c.1405 A>G in 18% (n=9) of the patients. ICAM-1 1405 A>G substitution determines change of Lysine to Glutamic acid at 469 position (K469E) in the fifth Ig-like domain. This domain is involved in binding to the LFA-1 ligand of leukocytes on arterial walls. This result suggests that the A>G genotype of the ICAM-1 gene polymorphism in codon at position 469 (K>E) is a genetic risk factor that may determine an individual’s susceptibility for CAD and MI.

Genome wide association (GWA) investigation studied four novel loci and showed that ICAM-1 (K469E) polymorphism determines the circulating concentration of sICAM-1 apart from G241R. In the current study, ICAM-1 gene K469E polymorphism showed a significant association with CAD risk in our ethnic group (OR: 23.12, 95% CI: 1.31 to 409.15).

Nakashima et al. showed up-regulation of ICAM-1 at atherosclerosis-prone sites and animal studies have shown a reduction in atherosclerosis in mice deficient in ICAM-1. ICAM-1 polymorphism (rs5498) has been suggested to have functional activity and affect mRNA splicing patterns that modify cell-cell interactions and influence inflammatory response.

In addition, this variant might have possible functional value in the etiology of atherosclerosis. The present study showed that the mutation in exon-6 of ICAM-1 gene is associated with an elevated levels of hsCRP in patients with CAD.

The present polymorphisms study of MTHFR, IL-6 and ICAM-1 genes helps us to better evaluate the prognosis of CAD in our ethnic patients. Thus future studies should focus on adopting reliable, cost effective and less time consuming methods to perform genotyping on large number of similar ethnic groups and thereby making it possible to establish the real effect of genotype for the benefit of the society.

**Limitations**

1. Sample size is relatively small.
2. This is a single center study and the study population is from few districts of Andhra Pradesh state of India only. Hence multi-centric studies with diverse ethnic populations are needed to elucidate the impact of these genetic polymorphisms in the risk of CAD.

**CONCLUSION**

Coronary artery disease a multifactorial arterial disease involves multiple genetic and environmental factors. Polymorphisms observed in MTHFR, IL-6 and ICAM-1 genes in South-Indian ethnic population which are associated with elevated levels of inflammatory markers – homocysteine, hsCRP and fibrinogen appear to be predisposing factors for atherosclerosis.

**ACKNOWLEDGEMENT**

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**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

**ABBREVIATIONS**

- ACS: Acute coronary syndromes
- bp: base pairs
- CAD: Coronary Artery Disease
- CHD: Coronary heart disease
- CI: Confidence intervals
- CVD: Cardiovascular Disease
- DVD: Double vessel disease
- ELISA: Enzyme linked immunosorbent assay
- ExPaSy: Expert Protein Analysis System
- GWA: Genome wide association
- hsCRP: High-sensitivity C-reactive protein
- ICAM-1: Intercellular Adhesion Molecule-1
- IEC: Institutional ethics committee
- IL-6: Interleukin-6
- LAD: Left anterior descending artery
- LCX: Left circumflex artery
- LP[a]: Lipoprotein [a]
- MI: Myocardial infarction
- MTHFR: Methylenetetrahydrofolate reductase
- NCSI: National Center for Biotechnology Information
- NSTEMI: Non ST-elevation myocardial infarction
- OR: ODDS ratio
- PCR: Polymerase chain reaction
- RCA: Right coronary artery
- SAA: Serum amyloid A
- SD: Standard deviation
- SPSS: Statistical Package for Social Sciences
- STEMI: ST-elevation myocardial infarction
- SVD: Single vessel disease
- TAE: Tris-Acetate-EDTA
- TVD: Triple vessel disease
- USA: Unstable angina

**SUMMARY**

Genetic polymorphisms observed in MTHFR, IL-6 and ICAM-1 genes in South-Indian ethnic population which are associated with elevated levels of inflammatory markers – homocysteine, hsCRP and fibrinogen appear to be predisposing factors for atherosclerosis and further progression of CAD. Further studies with larger sample size including different ethnic populations are needed to elucidate the impact of these genetic polymorphisms in the risk of CAD.
REFERENCES


