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Original Article

Genetics Section

Chemokine Receptor Gene (CCR5) Polymorphism in Acute Coronary Syndrome: A Cross-sectional Study

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ABSTRACT

Introduction: Acute coronary syndrome is a multifactorial disease with a complex pathogenesis, mainly resulting from the interplay of genetic and environmental risk factors. Chemokines and their receptors play crucial roles in the initiation and progression of atherosclerosis. Chemokine Receptor 5 (CCR5) is an important mediator of leukocyte recruitment and leukopedesis. Most studies conducted on the relationship between CCR5 gene polymorphism and coronary artery disease in different regions and populations worldwide show conflicting results.

Aim: To investigate the genetic polymorphism of CCR5 genes associated with patients with acute coronary syndrome in the Vijayapura population.

Materials and Methods: A cross-sectional study was conducted at BLDE (Deemed to be University) Shri B.M. Patil Medical College Hospital and Research Centre, Vijayapura, Karnataka, India, involving patients admitted for acute coronary syndrome. A total of 100 patients were admitted with acute coronary syndrome. Nineteen patients with diabetes mellitus were excluded from the study based on the exclusion criteria. Clinical history, examination, electrocardiographic assessments, laboratory profiles, and blood samples were taken for the analysis of CCR5 gene polymorphism as part of the work-up. Patients were classified into two groups: one with the presence of CCR5 polymorphism as Group A (n=6), and the other without polymorphism as Group B (n=75). Parameters such as age, sex, occupation, lipid profile, renal function tests, and CCR5 polymorphism were studied between the groups. The data were statistically analysed. Categorical variables between the two groups were compared using the Chi-square test. Normally distributed continuous variables were compared using independent t-test, and non normally distributed variables were compared using the Mann-Whitney U test.

Results: In the present study, the most common age group was 50-70 years with a male predominance of 60.7%. Most of the patients in the study group were farmers (34.7%), followed by housewives (32%) and businessmen (14.7%). The most common risk factors observed in both study groups were smoking and tobacco chewing. Gene sequencing revealed CCR5 gene polymorphism in six out of 81 patients who were labelled as Group A, indicating an incidence of 7.5% ($p<0.001$). Out of the six positive patients in Group A, three were males and three were females. One patient was 45 years old, while the remaining five were above 60 years old.

Conclusion: The present study demonstrates a positive association between CCR5 polymorphism and acute coronary syndrome, indicating that the study population is genetically susceptible to the disease. By screening for high-risk individuals, better and more effective early interventions can be planned, thereby reducing the social burden, morbidity, and mortality associated with the disease.

Keywords: Atherosclerosis, Coronary artery disease, Diabetes mellitus, Inflammatory mediators

INTRODUCTION

As per the Global Burden of Disease study, it is estimated that 24.8% of all deaths in India are attributable to Cardiovascular Disease (CVD). According to the present study, the age-standardised CVD death rate in India is 272 per 100,000 people, which is higher than the global death rate of 235 per 100,000 people [1]. Many predisposing risk factors have been identified for Acute Coronary Syndrome (ACS), including non modifiable factors such as age, sex, ethnicity, family history, and genetic factors, as well as modifiable factors such as hypertension, diabetes mellitus, smoking/tobacco use, obesity, and diet [2].

Atherosclerosis is a chronic inflammatory condition that worsens over time. It is characterised by the accumulation of lipids in the intima of blood vessel walls, endothelial dysfunction, and vascular inflammation [3]. When the endothelium is damaged, inflammatory cells, particularly monocytes, migrate into the subendothelium where they differentiate into macrophages. Macrophages release chemoattractants, cytokines (such as chemokines and interleukins), and matrix metalloproteinases, enzymes that break down the extracellular matrix and contribute to plaque disruption [4]. Chemokines play a crucial role in the pathogenesis of atherosclerosis, which is a risk factor for coronary heart disease. Detecting CCR5 polymorphism can help establish its role in acute coronary syndrome [5,6].

Leukocytes produce soluble proteins called chemokines, which bind to G-protein-coupled receptors located in the lipid layer of the cell surface. These receptors consist of seven transmembrane domains (7TM) [7]. The CCR5 gene is predominantly found in endothelial and immune cells and is located on chromosome 3P21.3 [8]. Chemokines are produced by immune cells such as macrophages, neutrophils, mast cells, eosinophils, dendritic cells, and epithelial cells. After recognising their receptors, chemokines bind to the N-terminus part of the chemokine and activate the receptor [9].

The CCR5delta32 allele has been associated with reduced susceptibility to coronary artery disease, delayed onset of coronary heart disease in women, and protection against myocardial infarction [10]. According to a study conducted by Hyde CL et al., in 2010, the CCR5delta32 polymorphism is linked to higher plasma levels of high-density lipoprotein cholesterol and lower levels of triglycerides, both of which contribute to a decreased risk of cardiovascular disease [11].

Fractalkine, acting at CX3CR1, appears to support chronic monocyte adherence and survival within the plaque, while CCL5, acting at CCR5, is thought to be essential for monocyte recruitment during the development of atherosclerosis [12]. In an in-vivo study, it was observed that suppression of CCL2, CX3CR1, and CCR5 had

additive effects in reducing atherosclerosis. Targeting all three systems was necessary for nearly complete eradication of the disease in an atherosclerotic mouse model. CCR5 signalling controls the recruitment of monocytes to the plaques [13].

Monocytes play a significant role in atherosclerosis, and there is a CCR2-CX3CR1++Ly-6Clo monocytosis that is independent of CCR2 and CX3CR1 but relies on CCR5 signaling for monocyte entry into lesions and recruitment of T cells into established plaques [14,15]. Patients with coronary artery disease have peripheral blood mononuclear cells with elevated expression of CCL3 and CCL4, which act on CCR5 to worsen atherosclerosis. This effect can be reduced by statin medication [16]. However, the available studies on CCR5 polymorphism and coronary artery disease have shown conflicting results [17-20].

Two similar studies conducted on the Indian population were carried out in the North Indian population [21]. These studies influenced us to investigate the genetic polymorphism of CCR5 genes associated with patients with acute coronary syndrome in the Vijayapura population.

MATERIALS AND METHODS

The present cross-sectional study was conducted in the Department of General Medicine at BLDE (Deemed to be University) Shri B.M. Patil Medical College, Hospital, and Research Centre in Vijayapura, Karnataka, India. The study spanned from January 2020 to June 2022 and involved 100 patients admitted to the hospital with acute coronary syndrome. Prior to conducting the study, it received approval from the Institutional Ethical Committee (IEC/NO-09/2021 dated-22/01/2021), and it is registered in the Clinical Trial Registry-India (CTRI/2021/04/032889 dated-16/04/2021). The patients were given detailed explanations about the procedure, and consent was obtained.

Inclusion and Exclusion criteria: The inclusion criteria for the study were patients admitted with ST segment elevation myocardial infarction, non ST segment elevation myocardial infarction, and unstable angina. Patients with diabetes mellitus were excluded from the study.

Sample size calculation: The sample size was calculated based on an anticipated proportion of 5.2% of CCR5 among acute Myocardial Infarction (MI) patients, resulting in a required sample size of 81 patients, with a 95% level of confidence and 10% absolute precision [5]. The formula used for sample size calculation was:

$$n=\frac{z^2p^*q}{d^2}$$

where Z is the Z statistic at the α level of significance,

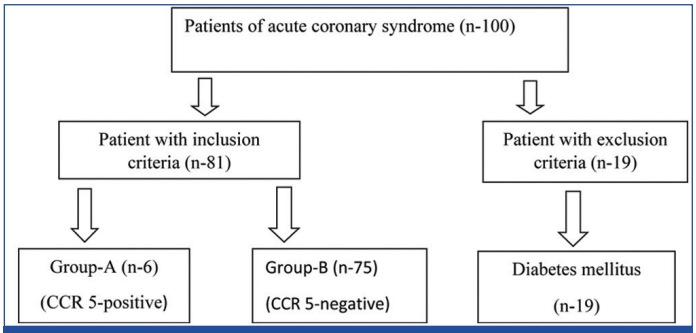
d^2=Absolute error

P=Proportion rate

q=100-p

Study Procedure

Baseline investigations, including complete blood count, random blood glucose, renal function tests, lipid profile, serum electrolytes, and urine examination, were conducted. Additionally, cardiac-specific investigations such as Troponin I, Creatinine Phosphokinase-MB, Electrocardiogram, Chest X-ray, and 2-Dimensional Echocardiography were performed. A peripheral blood sample of 1 mL was collected from each patient for analysing CCR5 polymorphism. Out of the 100 patients admitted with acute coronary syndrome, 19 patients with diabetes mellitus were excluded based on the exclusion criteria. The patients were then divided into two groups: Group A (n=6) with the presence of CCR5 polymorphism, and Group B (n=75) without the polymorphism, as shown in [Table/Fig-1].



[Table/Fig-1]: Algorithm showing the study protocol.

Genotyping: From 300 µL of peripheral blood, genomic Deoxyribonucleic Acid (DNA) was isolated using a commercial DNA isolation kit (Bangalore Genei, India). The primer sequences used for amplification are shown below:

Forward: 5'-CTCCCAGGAATCATCTTTACC-3'

Reverse: 5'-TCATTTCGACACCGAAGCAG-3' [22].

The Polymerase Chain Reaction (PCR) reaction was conducted in a 20 µL reaction volume, which included 2 µL of genomic DNA (ranging from 50 ng/µL to 100 ng/µL), 0.4 µL of each primer (5 pmol), 0.4 µL of dNTP (10 pmol), 0.1 µL of Taq DNA polymerase (5 units/µL), 2 µL of Taq Buffer (5X) (Takara, Japan). The total volume was adjusted to 20 µL using molecular biology grade water. After PCR amplification, the amplicons were subjected to 1% agarose gel electrophoresis, and the DNA bands were observed using gel documentation.

Sequencing run: The prepared samples were analysed on an ABI 3730 genetic analyser (Applied Biosystems, USA) to generate DNA sequences. After the sequencing reaction was completed, the quality of the generated sequences was checked using Sequencing Analysis v5.4 software (Applied Biosystems, USA).

Sequence alignment: The generated sequences were aligned with their respective reference sequences using Variant Reporter software (ABI v1.1). This software performs sequence comparisons to identify novel mutations, known variants, insertions, and deletions. The results from the variant reporter were tabulated in PDF format, which is the default output format of the software program.

STATISTICAL ANALYSIS

The obtained data was entered into a Microsoft excel sheet, and statistical analysis was conducted using the Statistical Package for the Social Sciences (SPSS, version 20.0). The results were presented as Mean (Median)±Standard Deviation (SD), counts and percentages, and diagrams. For normally distributed continuous variables, a comparison between two groups was performed using an independent t-test. For non normally distributed variables, the Mann-Whitney U test was utilised. Categorical variables between the two groups were compared using the Chi-square test. A p-value of less than 0.05 was considered statistically significant. All statistical tests were performed as two-tailed tests.

RESULTS

Out of the 81 patients studied, the most common age group in Group-A was 60-69 years, while in Group-B it was 50-59 years, with a significant p-value of 0.001, as shown in [Table/Fig-2]. Among the study participants, the majority were farmers (34.7%), followed by housewives (32%) and businessmen (14.7%). Other parameters such as haemoglobin, lipid profile, serum creatinine, serum electrolytes, and blood sugar were compared between the study groups and are tabulated in [Table/Fig-3]. The present study observed a high incidence

of smoking and tobacco chewing as risk factors for acute coronary syndrome in both study groups. The overall sex distribution showed a male predominance, with 53 male patients (60.7%) and 28 female patients (39.3%), as shown in [Table/Fig-4].

Age (Years)	Group-A (n=6)		Group-B (n=75)		p-value
	n	%	n	%	
30-39	0	0	4	5.3	0.001*
40-49	1	16.7	12	16.0	
50-59	0	0	30	40.0	
60-69	3	50.0	23	30.7	
70-80	0	0	6	8.0	
80-90	2	33.3	0	0	
Total	6	100.0	75	100.0	

[Table/Fig-2]: Distribution of patients according to age.

mutations. The distribution of polymorphism among males and females was equal among the six positive cases in Group-A. The base position in genomic DNA for all six positive mutations ranged from 8250 to 340, within a narrow range, and no novel polymorphism was observed.

Regarding Electrocardiogram (ECG) findings, out of the 81 patients, Group-A had three patients with ST elevation in the inferior leads (II, III, aVF), and one patient each with Left Bundle Branch Block (LBBB), Non-ST-elevation Myocardial Infarction (NSTEMI), and lateral wall STEMI. In Group-B, the most common ECG finding was NSTEMI (22 patients, 29.3%), followed by ST elevation in the inferior leads (II, III, aVF) (20 patients, 26.7%), ST segment elevation in anterolateral leads (V3-V6, I, aVL) (12 patients, 16%), and unstable angina (2 patients, 2.7%).

In Group-A, consisting of six cases, Left Ventricular Ejection Fraction (LVEF) was observed to be <40% in 3 patients (50%) and >40% in

Parameters	Group-A (n=6)		Group-B (n=75)		p-value
	Mean±SD	Median	Mean±SD	Median	
Age (years)	65.83±13.92	64.00	55.75±9.146	57.00	0.001*
Pulse rate (beats per minute)	85.00±11.64	85.00	86.67±15.61	86.00	0.942
Respiratory rate (cycles per minute)	18.83±1.60	18.00	18.60±2.42	18.0	0.817
Temperature (degree Celsius)	37.17±0.75	37.00	37.32±0.498	37.00	0.620
Haemoglobin (gm%)	12.00±1.60	12.00	13.12±2.205	13.00	0.177
Total count (cells/cu.mm)	11186.6±1184.39	10575.00	11121.81±3502.18	10400.0	0.465
ESR (mm/hr)	23.33±17.17	19.50	16.08±9.548	15.00	0.425
RBS (mg/dL)	115.33±10.57	114.00	121.60±30.20	112.00	0.864
Blood urea (mg/dL)	29.00±9.69	28.00	28.48±9.39	27.00	0.470
Sr. Creatinine (mg/dL)	1.67±0.21	1.00	0.97±0.23	1.00	0.002*
Sr. Sodium (mmol/L)	137.50±3.50	137.50	136.32±3.82	136.00	0.435
Sr. Potassium (mmol/L)	4.33±0.81	4.50	4.17±0.47	4.00	0.366
Total cholesterol (mg/dL)	144.17±37.26	146.50	161.00±0.312	160.00	0.312
Triglycerides (mg/dL)	131.67±44.59	129.50	148.36±64.35	140.00	0.658
High-density lipoprotein (mg/dL)	34.00±12.94	34.50	35.56±7.96	34.00	0.928
Low-density lipoprotein (mg/dL)	76.67±27.59	61.50	84.80±26.64	82.00	0.170

[Table/Fig-3]: Background parameters between study groups.
*significant at 5% level of significance (p<0.05). ESR: Erythrocyte sedimentation rate; RBS: Random blood sugar

Risk factors		Group-A (n=6)		Group-B (n=75)		p-value
		No.	%	No.	%	
Non modifiable	Sex					
	Male	3	50.0%	50	63.3%	0.4088
	Female	3	50.0%	25	33.3%	
Modifiable	Smoking	2	33.3%	28	37.3%	0.8452
	Alcohol	0	0	8	10.6%	0.259
	Tobacco chewing	2	33.3%	29	36.0%	0.7959
	Hypertension	0	0	20	26.6%	0.1786

[Table/Fig-4]: Distribution of risk factors between study groups.

Out of the 81 patients, blood samples were analysed for CCR5 polymorphism. Six patients (7.4%) tested positive, while 75 patients (92%) tested negative, as shown in [Table/Fig-5]. Among the six positive patients in Group-A, five of them had a frameshift mutation, as shown in [Table/Fig-6,7], which displays one of the frameshift

CCR5 polymorphism	Group-A		Group-B		p-value
	No.	%	No.	%	
Absent	0	0	75	100.0	0.0001*
Present	6	100.0	0	0	
Total	6	100.0	75	100.0	

[Table/Fig-5]: Distribution of CCR5 polymorphism in study patients.
*significant at 5% level of significance (p<0.05)

S. No.	Base position in genomic DNA	Mutation type	Nucleotide change	cDNA Ref. ENST00000445772	Variants
1	g.8320T>G	Transversion	T-G	C.559T>G	Frameshift
2	g.8293T>A	Transversion	T-A	C.532T>A	Missense
	g.8429G>A	Transition	G-A	C.668G>A	Frameshift
3	g.8334G>T	Transversion	G-T	C.573G>T	Frameshift
4	g.8332A>G	Transition	A-G	C.571A>G	Frameshift
5	g.8334G>T	Transversion	G-T	C.573G>T	Frameshift
6	g.8334G>C	Transversion	G-C	C.573G>C	Frameshift

[Table/Fig-6]: Details of ccr5 polymorphism analysis.

[Table/Fig-7]: Showing nucleotide base guanine replaced by thymine leading to frame shift mutation.

3 patients (50%). In Group-B, consisting of 75 cases, LVEF was <40% in 42 patients (56%) and >40% in 33 patients (44%).

DISCUSSION

In the present study, the most common age group was 60-69 years, which was similar to a study conducted by Kobayashi A et al., on 190 patients hospitalised with acute coronary syndrome between January 2007 and December 2013. They observed that the common age group was 60-70 years [23]. Age is an important non modifiable risk factor and indicates that atherosclerosis is a disease that primarily affects the elderly.

In the present study, the sex distribution showed a male predominance of 53 patients (60.7%) and female patients of 28 (39.3%), which is similar to a study conducted by Sharma R et al., in 2014 on 1562 South Indian patients. They found that the majority were male, with 1242 (79.5%) and the remaining being females, with 320 (20.5%) [24]. This can be attributed to the fact that modifiable risk factors such as smoking and alcohol consumption are more prevalent in males.

Regarding modifiable risk factors, the present study found that smoking was present in 30 patients (37%) and tobacco chewing in 31 patients (38.2%), similar to a study conducted by Rao V et al., in 2017 on 100 patients with acute coronary syndrome. They found that smoking was present in 61% of patients and alcohol consumption in 29% of patients [25]. Therefore, there is a need for policies to control tobacco use, promote a healthy diet, and educate patients regarding the adverse effects of tobacco use, which can help improve the life expectancy of patients with acute coronary syndrome.

Out of the six positive cases in Group-A, there was an equal distribution of the polymorphism among males and females. In a 2006 study by Sharda S et al., on CCR5 deletion polymorphism in North Indian patients with coronary artery disease, they found a three times higher frequency of the polymorphism in CAD patients compared to normal individuals [21]. Similarly, a 2011 study by Singh N et al., showed similar results with a four times higher frequency of the polymorphism in acute myocardial infarction patients [5]. In a 2008 study by Afzal AR et al., conducted in the Bruneck population, the polymorphism was associated with significantly lower carotid intima-media thickness in the common carotid artery and a reduced incidence of cardiovascular disease [17]. In 2001, González P et al., discussed genetic variation at the CCR5/CCR2 in myocardial infarction and found that patients carrying the Δccr5-allele would be protected against an early episode of MI [18].

Similarly, other studies conducted outside of India in Spain, Czech Republic, Germany, and Hungary have shown a lower frequency of the polymorphism in CAD patients, concluding a protective role in their respective ethnicities and populations [6]. The two Indian studies conducted in the North Indian population, as explained above, showed a significant positive association between CCR5 polymorphism and coronary artery disease, with no protective role [21]. The present study is the first to be conducted in the South Indian population, showing evidence of CCR5 polymorphism in acute coronary syndrome patients.

Limitation(s)

The present study was conducted with a smaller study group. Future studies with a larger group can provide a better understanding of CCR5 polymorphism in acute coronary syndrome.

CONCLUSION(S)

Genetic studies of diseases are gaining more popularity and importance in order to study the diseases in detail. The present study shows the expression of CCR5 polymorphism in patients with acute coronary syndrome. Screening for CCR5 polymorphism in high-risk individuals helps in risk modification and effective early intervention for these individuals, thereby minimising the social burden, morbidity, and mortality associated with the disease.

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