International Journal of Life Sciences, Biotechnology and Pharma Research Vol. 13, No. 11, November 2024 Online ISSN: 2250-3137
Print ISSN: 2977-0122

DOI: 10.69605/ijlbpr_13.11.2024.69

ORIGINAL RESEARCH

Association of CD14 gene polymorphism with coronary artery disease

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Received date: 16 November, 2024 Acceptance date: 18 November, 2024

ABSTRACT

Background: A major global cause of morbidity and death, coronary artery disease (CAD) is impacted by both environmental and genetic factors. One important immune response regulator that has been linked to CAD susceptibility is the CD14 gene. In a case-control population, this study examines the relationship between CAD and a polymorphism in the CD14 gene. **Methods**: The CD14 gene polymorphism was genotyped in 90 participants, including both healthy controls and CAD patients. To evaluate the risk association, genotype and allele frequencies were examined, and odds ratios (OR) were computed. **Results**: In CAD cases, the frequencies of CD14 genotypes CC, CT, and TT were 24.44%, 51.11%, and 24.44%, respectively, while in controls, they were 15.55%, 40%, and 45.44%. The ORs for the genotypes CC, CT, and TT were, respectively, 0.57 (P = 0.292), 0.64 (P = 0.29), and 2.47 (P = 0.046). CAD cases had 100% and 75.44% frequencies of the C and T alleles, while controls had 71.1% and 84.44% frequencies, respectively. With an OR of 0.55 (P = 0.05), the C allele provided protection, whereas the T allele was linked to a higher risk of CAD (OR of 1.81).**Conclusion**: There is a strong correlation between the CD14 gene polymorphism and CAD. While the C allele seems to be protective, the TT genotype and T allele are linked to an increased risk of CAD. These results suggest that CD14 polymorphism may play a part in the pathophysiology of CAD and call for more research to determine its potential therapeutic implications.

Keywords: CD14 gene, Coronary Artery Disease, Polymorphism

INTRODUCTION

Coronary artery disease (CAD) is one of the leading causes of mortality worldwide, contributing significantly to the global burden of cardiovascular diseases. It is a multifactorial condition characterized by the narrowing or blockage of coronary arteries due to the build-up of atherosclerotic plaques, which leads to ischemic heart disease [1]. The development of atherosclerosis is driven by a complex interplay of genetic, environmental, and lifestyle factors, including dyslipidemia, hypertension, smoking and diabetes mellitus. Recent advances in genomics have highlighted the role of genetic susceptibility in the pathogenesis of CAD, leading to an increased focus on identifying genetic polymorphisms that may influence disease risk [2].

Among the numerous genetic factors implicated in CAD, polymorphisms in genes associated with the immune response have garnered particular interest. Inflammation plays a critical role in all stages of atherosclerosis, from the initiation of plaque formation to the rupture of unstable plaques, which can result in acute coronary events such as myocardial infarction [3]. Cluster of differentiation 14 (CD14) is a key molecule involved in the innate immune response and plays an essential role in recognizing bacterial endotoxins, such as lipopolysaccharides (LPS) , which activate inflammatory signaling pathways [4].

The CD14 gene, located on chromosome 5q31.1, encodes for the CD14 receptor, which exists in both membrane-bound (mCD14) and soluble (sCD14) forms. These forms are pivotal in the Toll-like receptor 4 (TLR4) signaling pathway, which mediates the body's response to microbial infection and triggers a cascade of pro-inflammatory cytokines [5]. Studies

DOI: 10.69605/ijlbpr_13.11.2024.69

have shown that CD14 is involved in chronic inflammatory processes, including those seen in atherosclerosis. Elevated levels of sCD14 have been associated with increased inflammatory activity and higher cardiovascular risk, linking CD14 to the pathogenesis of CAD [6-8] .

One of the most studied polymorphisms in the CD14 gene is the C-260T polymorphism (rs2569190), located in the promoter region. This polymorphism influences the transcriptional activity of the CD14 gene, potentially leading to altered expression of CD14 protein. The T allele has been reported to be associated with higher levels of circulating sCD14, which may enhance the inflammatory response and increase susceptibility to CAD [9].

Several studies have investigated for the association between the C-260T polymorphism and CAD risk, but the findings have been inconsistent across different populations. Some studies have reported a significant association between the T allele and increased risk of CAD, while others have found no such link. These discrepancies may be attributed to differences in ethnic background, environmental factors, and sample sizes across studies.

Given the pivotal role of inflammation in the development of atherosclerosis and the involvement of CD14 in immune regulation, the CD14 C-260T polymorphism represents a promising candidate for genetic association studies in CAD. The present study aims to evaluate the association between the CD14 gene polymorphism and the risk of CAD in north Indian populations, with the goal of better understanding the genetic determinants of CAD susceptibility.

MATERIALS AND METHODS

Study Design and Population: This was a case-control study designed to investigate the association between CD14 gene polymorphism and coronary artery disease (CAD). The study included a total of 45 patients diagnosed with CAD and 45 age and gender-matched healthy controls with no history of CAD. All participants were recruited from the Department of Cardiology Era's Lucknow Medical College and Hospital, Lucknow, Uttar Pradesh.

The inclusion criteria for CAD patients were the confirmed diagnosis of CAD through coronary angiography (≥50% stenosis in one or more major coronary arteries), Age between 40 -65 years old and had no history of any other significant chronic diseases. The healthy controls were selected from individuals undergoing routine medical check-ups and matched for age, sex, and ethnicity with the patient group. Informed written consent was obtained from all participants, and the study was approved by the Institutional Ethics Committee, Era's Lucknow Medical College and Hospital, Lucknow, Uttar Pradesh.

DNA Extraction and Genotyping: Peripheral blood samples (5mL) were collected from each participant in EDTA tubes. Genomic DNA was extracted from the blood samples using standard phenol-chloroform DNA extraction method for genomic DNA isolation. The CD14 gene polymorphism (rs2569190, C-260T) was genotyped using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The primers used for amplification of the target region were:

- Forward primer: *5'*ATCATCCTTTTCCCACACC 3'
- Reverse primer: 5'AACTCTTCGGCTGCCTCT 3'

The PCR conditions were as follows:

- Initial denaturation at 94°C for 5 minutes.
- 35 cycles of denaturation at 94°C for 30 seconds , annealing at 55°C for 30 seconds , and extension at 72°C for 1 minute.
- Final extension at 72°C for 10 minutes.

The standard phenol-chloroform DNA extraction method was used for genomic DNA isolation. Detailed procedure used for DNA isolation is as follows,

Procedure

- At room temperature, the frozen whole blood samples that had been kept in EDTA vials at -20°C were thawed.
- After mixing 1 ml of blood with 1 ml of Lysis buffer, the mixture was centrifuged for 5 minutes at 11,000 rpm.
- Supernatant was thrown away.
- 400 µl of Lysis buffer was added once more, and the ingredients were combined before being centrifuged for two minutes at 11,500 rpm.
- Supernatant was thrown away.
- 400 µl of Double Distilled (DD) water was added, and the pellet was mixed until it became miscible. It was then centrifuged for two minutes at 11,500 rpm.
- Supernatant was thrown away.
- Mixed after adding 100 µl of proteinase buffer. carefully mixed in 10 µl of 10% sodium dodecyl sulphate (SDS).
- added 120 µl of 5M NaCl and thoroughly mixed. added 300 µl of DDWater and thoroughly mixed.
- 400 µl of phenol:chloroform (4:1) was then added, mixed, and centrifuged for 10 minutes at 12,000 rpm.
- Moved the aqueous layer to a fresh tube, mixed, and added a milliliter of chilled absolute alcohol. Centrifuged for 4 minutes at 11,000 rpm.
- Throw away the supernatant.
- Added 100 µl of 70% alcohol, separated the pellet, and centrifuged for two minutes at 11,500 rpm.
- Supernatant was thrown away.

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- Then dried overnight at 37°C or for two to three hours at 56°C with the cap open. 100 µl of DD water was added.
- Then, incubated in an Eppendorf with a closed cap at 37°C for the entire night or for two to three hours at 56°C.

After being separated, the DNA samples were combined with bromophenol blue, a DNA tracking dye, and put onto a 1.0% agarose gel that had been stained with 0.5µg/ml EtBr. After that, the samples were electrophoresed between 60 and 90 volts. In a gel documentation system (UVP Bioimaging system, Cambridge, U.K.), DNA was seen and recorded. A nanodrop spectrophotometer (Thermo 2000C model, Thermo Fisher, USA) was used to estimate the amount of DNA, and the A260/A280 ratio was used to assess the DNA's quality. Ultimately, the extracted DNA was kept at -20 degrees Celsius.

Statistical Analysis

All the data was presented as mean ± SD. The genotype data was compare between cases and controls using the Chi-Square test. Other Variables will be compared using student's t-test for normally-distributed using student's t-test for normally distributed variables. Statistical significance was set at $p < 0.05$. All statistical analyses were performed using SPSS 26.0

RESULTS

This case-control study included 90 subjects, *45* CAD cases and *45* ethnicity-matched healthy controls. The mean ages were *53.89* ± 10.47 and 44.30 ± *9.53* years in CAD cases and control, respectively. Clinical and biochemical parameters of CAD cases and controls are shown in Table 1

Table 1: Clinical and Biochemical Parameters of CAD cases and controls

Parameters	Control (45)	Cases (45)
Age	44.30±9.53	53.89±10.47
BMI	23.73±2.58	24.65±3.00
BP SYS	118.70±8.22	146.33±18.50
BP DYS	73.85±13.01	82.09±4.63
Blood sugar	115.68±18.11	203.59±58.97
S.CHOL	169.72±26.76	164.60±31.68
TG	141.9±41.68	129.46±35.26
HDL	55.76±10.49	32.02±10.84
VLDL	35.70±10.73	29.25±11.35
LDL	91.69±21.55	89.12±16.85

CD14 (rs2569190) **polymorphism analysis:** The CD14 gene CC, CT, and TT genotype frequencies were 24.44, 51.11, and 24.44% in CAD cases and 15.55, 40, and 45.44% in healthy controls, respectively. OR for CC, CT, and TT genotypes were 0.57, 0.64, and 2.47 (P = 0.292, 0.29, 0.046), respectively. The frequencies of C and T alleles were 100 and 75.44% in CAD eases as compared to 71.1 land 84.44% in the controls. OR for C was 0.55 (P = 0.05), and for T allele OR = 1.81 (P = 0.05) (details shown in Table 2).

Table 2: The genotype, allele frequencies of CD14 (rs2569 190) genes and their statistical analysis among CAD cases and controls.

CD14 (rs2569190) Genotypes	Cases (N=45)	Freq. (%) Cases	Controls (N=45)	Freq. (%) controls	OR	P-Value
CC	11	24.44	7	15.55	0.57	0.292
CT	23	51.11	18	40	0.64	0.29
TT	11	24.44	20	45.44	2.47	0.046
Alleles						
C	45	100	32	71.11	0.55	0.05
T	34	75.44	38	84.44	1.81	0.05

DISCUSSION

The current study examined genotypic frequencies and clinical characteristics in a sample of CAD patients and controls to examine the relationship between the CD14 gene polymorphism (rs2569190) and coronary artery disease (CAD). The distribution of the CD14 genotypes and a number of clinical parameters showed significant differences between the two groups, according to the study.

Individuals with CAD had higher average systolic (146.33 mmHg) and diastolic (82.09 mmHg) blood pressure than controls (118.70 mmHg and 73.85 mmHg, respectively), according to the clinical characteristics of the cases and controls, which also show significant differences in blood pressure and blood sugar levels. Furthermore, compared to controls (115.68 mg/dL), the blood sugar levels in cases were significantly higher (203.59 mg/dL), which is in line

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with the established link between diabetes and CAD. It's interesting to note that HDL levels were considerably lower in the CAD group (32.02 mg/dL) than in the control group (55.76 mg/dL), indicating that dyslipidemia may be a risk factor for CAD. Other lipid parameters, such as LDL, VLDL, triglycerides, and total cholesterol, did not, however, differ substantially between the groups.

The TT genotype was more common in cases (24.44%) than in controls (15.55%) in terms of CD14 gene polymorphisms; this difference was statistically significant, with an odds ratio (OR) of 2.47 (p=0.046). This implies that the TT genotype might increase inflammatory responses, which could make it a risk factor for CAD. However, there was no discernible link between the CC and CT genotypes and CAD. With an OR of 1.81 (p=0.05), the T allele was more common in cases (75.44%) than in controls (71.11%), which further supports the link between the T allele and an increased risk of CAD. The C allele, on the other hand, was more common in controls (100%) than in cases (71.11%), indicating a protective tendency.

The findings are consistent with earlier research that links inflammatory processes linked to CAD to polymorphisms in the CD14 gene, specifically the T allele [10,11]. Primarily found on monocytes and macrophages, the CD14 receptor is important for identifying bacterial lipopolysaccharides (LPS) and starting an inflammatory response, which can accelerate the development of atherosclerosis and CAD [12]. Higher CD14 expression may result from the TT genotype's increased frequency in CAD patients, which would promote chronic inflammation and aid in the pathophysiology of CAD [13].

CONCLUSION

According to this study, there may be a link between CAD and the CD14 gene polymorphism (rs2569190), with a higher risk associated with the TT genotype and T allele. The results emphasize how CAD susceptibility is influenced by genetic factors, especially those related to inflammation. Furthermore, conventional risk factors like high blood pressure, hyperglycemia, and low HDL levels continue to be important predictors of CAD risk. These findings lend credence to the theory that CAD risk is increased by a combination of metabolic abnormalities and genetic predisposition. To confirm these results and comprehend the molecular processes by which CD14 polymorphisms affect the risk of CAD, more research with bigger sample sizes and a wider range of demographics is advised.

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