Is There Any Association between CD_{14}Polymorphism and Coronary Artery Disease?

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Abstract

Background:
Several pro-inflammatory single nucleotide polymorphisms have been associated with the development of atherosclerosis and coronary artery disease (CAD). CD_{14}+ monocytes are one of these molecules due to their ability to produce variable cytokines. Association betweenCD_{14}+ monocytes and carotid intimal-medial thickness has been previously identified. In this study, we tried to evaluate the relation between CD_{14} polymorphism and CAD.

Methods:
We evaluated 200 patients divided into two matched groups based on the result of their coronary angiography: 100 patients who had significant lesion(s) in their coronary arteries were allocated to the case group, and the other 100 without CAD were categorized as the control group. The polymorphism of CD_{14} was determined via allele-specific polymerase chain reaction (PCR).

Results:
The frequency of the genotypes (CC, TT, and CT) was 45%, 7%, and 48% in the case group and 24%, 38%, and 38% in the control group:there was a significant difference in the allele genotype frequency between the two groups (p value <0/001).

Conclusion:
CD_{14} polymorphism was associated with CAD. ***(Iranian Heart Journal 2012; 13(3):6-10).***

Keywords: CD_{14}■Polymorphism■PCR■Coronary artery disease■Atherosclerosis

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Introduction

Coronary artery disease (CAD) is one of the common health problems burdening health care services around the world.CAD is a complex phenomenon characterized by the presence of atherosclerosis in epicardial coronary arteries.Variable genetic and environmental factors can influence the development and prognosis of atherosclerosis(1).

Despite the importance of traditional factors such as diabetes mellitus and dyslipidemia, 20% of all cardiovascular events occur without any of the major classic vascular risk factors(2).A great deal of research over the past 10 or 15 years has focused on the identification and evaluation of novel atherosclerotic risk factors. Some of these novel factors that can predict future cardiovascular events are:LP-PL(A_2)(3),LP(a)(4),ICAM-1(5),and myeloperoxidase(6). Nevertheless, the association between CAD and the other factors such as CD_{28} has yet to be fully established(7).The role of the inflammatory process in the formation and development of atherosclerotic plaque has been documented. The uptake of oxidized LDL by subintimal macrophages characterizes the formation of the atherosclerotic plaque, initiating a local inflammatory reaction, increasing the expression of leukocytes adhesion molecules, entering blood monocytes in the artery wall in response to chemo attractant cytokines, and leading to foam cell formation. The latter cells are a source of meditators that lead to the migration and proliferation of smooth muscle cells and ultimately the development of a fatty streak(2).

CD_{14} is a monocyte surface receptor that acts as co-receptor (along with the toll-like receptor) for the detection of bacterial lipopolysaccharides (LPS) in the innate immune system(8).CD_{14}+ monocyte are pro-inflammatory molecules due to their ability to produce variable cytokines such as TNFα. Previous studies have already established the association between C polymorphism of CD_{14} and carotid intimal-medial thickness, but both polymorphisms have not been linked with the risk of atherosclerotic cerebral ischemia(9).

Moreover, the role of CD_{14} polymorphism in other diseases such as chronic obstructive pulmonary disease (COPD) (10), colorectal cancer(11), rheumatoid arthritis(12),and inflammatory bowel disease (IBD)(11) has been investigated before.

The aim of this study was to evaluate the polymorphism of CD_{14} as a probable risk factor for CAD.

Methods

This observational case-control analytical study,approved by the institutional Review Board,was performed between June 2009 and March 2011 in Ekbatan Hospital, Hamedan, Iran. From symptomatic (chest pain and exertional dyspnea) patients who had positive non-invasive tests and accepted to participate in this study by signing an informed written consent, 200 patients were selected. The study population was divided into two matched groups in terms of the major risk factors for CAD and based on the result of their coronary artery angiography: those with significant stenoses (stenosis more than 70% of the coronary artery diameter) in one, two, or three main coronary arteries were allocated to the case group, and those with only CAD symptoms but with normal coronary angiography were allocated to the control group.

Patients with COPD, rheumatoid arthritis, renal failure, or other systemic diseases were excluded from the study. For the selected patients, peripheral blood samples were drawn during angiography. DNA extraction was conducted via the salting out method. Polymorphism was identified via allele-specific PCR, using an allele-specific primer for C and T in CD_{14} genes. The primers used to detect C and T alleles were as follows:

5' CTC CAG AAT CCT TCC TGA TAC GAC-3' (C prime)
5' TTC TTT CCT ACA CAG CGG CAC CC-3' (reverse C prime)
5' TGT AGG ATG TTT CAG GGA GGG GTA-3' (T prime)
5'TTG GTG CCA ACA GAT GAG GTT CAC-3' (reverse T prime)

The PCR process was performed in the following manner:
After DNA extraction from the patient's peripheral blood sample and target hybridization (DNA probe formation), denaturation of DNA (melting and separating double strands of DNA) was performed at 95℃ for one minute. Then a specific probe was added to the denatured DNA, and annealing was done at 60℃ for one minute. At the end, the extension process was performed at 72℃ for one minute. This cycle was repeated several times. The PCR product was separated on 2% agarose gel and visualized by ethidiumbromide staining (13).
All the datawere analyzed with SPSS 13 software.

Results

The study population was comprised of 20 (20%) females and 80 (80%) males in the case group and28 (28%) females and 72 (72%) males in the control group.
As regards vessel involvement in the case group, there was 35% single-vessel disease,33% two-vessel disease, and 32% three-vessel disease. The frequencies of C allele in the case and control groups were 64.8% and 35.3%, respectively, and the frequencies of T allele in the case and control groups were 35.2% and 64.7%, respectively. Consequently, comparison of these data revealed a significant difference with respect to the frequency of C and T alleles between the two groups (p value <0/001),Table 1.
The frequencies of allele genotypes (CC,CT, and TT) were 45%,48%, and 7% in the case and 24%, 38%, and 38% in the control group, respectively. Analysis of these data showed a significant difference in regard to the allele genotype frequency between the two groups (p value <0/001)(Table 2).

Table 1- The frequency of C and T allele in the case and control groups

Allele / Group	C	T
Case	64/8%	35/2%
Control	35/3%	64/2%

Table 2- The frequency of allele genotypes (CC,CT, and TT)

Genotypes / Group	CC	CT	TT
Case	45%	48%	7%
Control	24%	38%	38%

Discussion

CAD is a major cause of death and disability in developed countries and it is responsible for about one-third of all deaths in individuals over the age of 35(14). The role of the inflammatory process in atherosclerosis has been previously documented, and so has the relationship between various inflammatory factors and CAD.CD_{14} is a pro-inflammatory molecules expressed strongly on the surface of monocytes (one of the first molecules to infiltrate vulnerable atherosclerotic plaques) (15) . One study (9)reported the association between C polymorphism of CD_{14} and carotid intimal-medial thickness but suggested that both polymorphisms were not related to atherosclerotic cerebral ischemic events.
In recent years, a few studies have probed into the relationship between $CD_{14}$and CAD, directly; some of them, however, have failed to establish such correlation (16,17). A meta-analysis (18) revealed that T allele and TT genotype were associated with ischemic heart disease in East Asian populations but not in Europeans and

Indians(18). Another study(19) performed in Switzerland documented a relationship between CRP and CD_{14}TT genotype and coronary plaque volume (assessed by IVUS), independently of concomitant cardiovascular risk factors (19).
Similar studies have not been undertaken in our country thus far. In this study, we tried to match both case and control groups with respect to the major risk factors for CAD so as to eliminate the potential effect of confounding factors. We showed an association between CD_{14} polymorphism and CAD. Our results could, therefore, be helpful to identify and predict future cardiovascular events particularly in individuals without any major or traditional risk factors for CAD.

Conclusion

Among the non-traditional risk factors of atherosclerosis, CD_{14} polymorphism was associated with CAD in our study population.

Conflict of Interest Statement

The authors have no conflict of interest to declare.

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