Evaluation of CD36 polymorphisms as predisposition factors for Myocardial infarction among population from Turabah province, Saudi Arabia

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Abstract: Acute myocardial infarction (MI) is typically precipitated by thrombosis superimposed upon a ruptured coronary plaque. CD36 is a multifunctional integral-membrane glycoprotein that acts as a receptor for thrombospondin, collagen, long-chain fatty acids, and oxidized LDL and it was be shown that CD36 can account for up to 40% of the binding and internalization of oxidized LDL by macrophages. In a case-control study we evaluate the role of cd36 polymorphisms in predisposition to Myocardial Infarction among population of Tarabah province. Using PCR-RFLP three reported CD36 mutations (C478T, Del539AC and ins1159A) were screening in 120 MI Cases and 120 Healthy controls. None of the three mutations were detected in this study population (MI cases or controls). We concluded that these polymorphisms were not detected in study population and have no role in genetic susceptibility to MI in Saudi population from Turabah in western region of Saudi Arabia. We recommend further large studies are needed to confirm the absence of these polymorphism in Saudi population in Turabah and other regions.

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1. Introduction

Atherosclerotic Coronary Diseases (CAD) and myocardial infarction (MI) remain the most significant causes of death in developed countries and by 2020 it is expected to be the first cause of death worldwide. Several epidemiologic studies have documented hypertension, hypercholesterolemia, diabetes, obesity, hypertriglyceridemia, sedentary lifestyle and smoking as the most important risk factors for cardiovascular disease [1]. Myocardial infarction (MI) is usually caused by occlusion of a coronary artery, which is induced by thrombosis and/or rupture of plaque based on atherosclerosis of the coronary arteries [2]. In spite of screening and the treatment for these major risk factors, still small portion of patients with significant cardiovascular disease who do not have any of these risk factors suffer events, such as stroke and MI. Numerous epidemiologic studies have documented that stroke has a significant genetic component. Family studies reported that the incidence of MI in individuals whose first-degree relatives died of MI is 2 to 4 times higher than that in the general population [3]. Twin studies in three populations demonstrated that the concordance of MI was higher among monozygotic twins than among dizygotic twins, especially in females [4]. Based on these observations, it has been suggested that genetic factors may participate in the development of CAD and/or MI, in addition to environmental factors. Several studies have examined the association between the polymorphisms in the candidate genes for CAD or MI, and it has been reported that several gene polymorphisms are associated with CAD/MI in certain populations..

Acute myocardial infarction (MI) is typically precipitated by thrombosis superimposed upon a ruptured coronary plaque and can be effectively treated by timely fibrinolytic therapy. These observations highlight the central role of the coagulation and fibrinolytic systems in the pathogenesis of acute MI and suggest that a patient's vulnerability to an MI may be modulated by individual variations in the balance between coagulation and fibrinolysis. Large numbers of proteins interact within the complex, regulated systems of coagulation and fibrinolysis. Polymorphisms in genes that encode these circulating or cell-bound proteins may affect their structure, concentration, or function, and thereby alter the balance between coagulation and fibrinolysis. Polymorphisms in factor V, factor VII, prothrombin, plasminogen activator inhibitor I, and platelet glycoproteins, have been investigated in several studies for their association with coronary disease [5, 6].

CD36 or glycoprotein IIIb/platelet glycoprotein IV is a mediator of platelet adhesion to collagen and a receptor for thrombospondin 1 (TSP-1) [7]. On platelets, CD36 recognition of oxidized LDL contributes to their activation and provides a mechanistic link between hyperlipidemia, oxidant stress, and the prothrombotic state. Furthermore, Cell-derived micro-particles are major ligands for CD36 and could contribute to thrombus formation in a CD36-dependent manner even in the absence of hyperlipidemia. Studies in mice models have reported that CD36 deficiency in mice is associated with inhibition of thrombus formation and with a reduction in micro-particles accumulation in thrombi [8]. In humans, CD36 deficiency was first identified in patients with refractoriness to multiple platelet transfusions and is relatively common (2–7%) in persons of Asian and African descent [9]. CD36 deficiency is divided into two subgroups: type I deficiency, in which neither platelets nor monocytes express CD36, and type II deficiency, in which monocytes express CD36 despite the lack of platelet CD36. Two mutations that are reported to be responsible for CD36 deficiency, a substitution of T for C at nt 478 of *CD36* cDNA in codon 90 (proline90→serine) and a dinucleotide deletion at nt 539 in codon 110 [10, 11]. Studies in C478T demonstrated that subjects with type I deficiency are homozygous for the Pro90-to-Ser mutation, whereas subjects with type II deficiency are heterozygous for this mutation. The type I CD36 deficiency is most commonly detected in subjects who are homozygous or compound heterozygous for the following three mutations: *C268T*, *949insA*, and *329-330delAC*. A survey of the CD36 mutations in type I CD36 deficiency has revealed that *C268T* is the most common mutation, and is responsible for more than 50% of the mutated allele frequency in Asians [12]. Thus we hypothesize that cd36 deficiency may contribute in initiating and progressing of thrombosis at atherosclerotic lesions and participating Myocardial infarction in cd36 deficiency subjects.

2. Material and Methods

In a case-control study was conducted to evaluate the role of cd36 (C478T, Del539AC and ins1159A) polymorphisms in predisposition to Myocardial Infarction among population of Tarabah province. Blood sample were collected and DNA was extracted from 120 patients who recruited to Turabah General Hospital and were diagnosed to have myocardial infarction; all the selected patients had gave their informed consent to participate in the study. The control group (120) were selected from general representing a general, unrelated subjects from population of Tarabah who never have a history of MI or angina, clinical evidence of Coronary artery disease (CAD), stroke or any atherosclerotic disease in the past and are matching in age or older, sex, and tribe were selected for the study after accepting to participate in the study.

Screening of cd36 (C478T, Del539AC and ins1159A) polymorphisms PCR-Restriction Enzyme Length Polymorphisms (PCR-RFLP):

Genomic DNA was extracted from peripheral blood using standard procedures[13]. In this study, we evaluated that if 478C→T substitution (Pro90, wild homozygote) of the gene CD36 as a risk factor for predisposition to MI in study population.

Screening of codon 90 polymorphism in CD36 gene was done by RFLP method using ***Sau96 I*** restriction enzyme since the polymorphism abolished the normal existent recognition site for the enzyme. PCR amplification of a 153 bp DNA segments of CD36 gene was carried out in a volume of 10 ml containing 100 ng of DNA using the following primer pair; forward primer 5`-GGCACAGAAGTTTACAGACAG-3` and reverse primer was 5`-ATGGTCAAGGTAAGAGTGTC-3`. PCR amplification program as follows; denaturation at 98 ˚C for 20 s, followed by annealing at 55 ˚C for 20 s and polymerization at 72 ˚C for 30 s for 40 cycles. The amplified products was digested with 0.4 U of restriction enzyme ***Sau96 I*** at 37 8C for 10 h. Digestion of the 153-bp PCR product with ***Sau96 I*** yielded the following three distinct patterns: 94- and 59-bp products will define the Pro90 homozygote, 153-, 94-and 59-bp products will define the Pro90rSer90 heterozygote and a 153-bp product will define the Ser90 homozygote **(Fig. 1)**.

Screening of the **Ins1159A** polymorphism was done by RFLP method using ***Xmn I*** restriction enzyme since the polymorphism abolished the normal existent recognition site for the enzyme. While Screening of **CD36 Δ539AC** was done by RFLP method using ***Ssp I*** restriction enzyme since the mutation has created a new recognition site for the enzyme at the polymorphic site. PCR amplification in a total volume of 30 µl in GeneAmp PCR System 9600 (Perkin-Elmer) using the following primer pairs were used to amplify polymorphic regions.;

For **Ins1159A**: XMNI(+): 5`- CGTTAAT-CTGAAAGGATTCC -3` and XMNI(-): 5` TGTACAATTTTTTGAGAGAA-3`.

For **Δ539AC**: ID(+): 5`- AGATCTA-ATGTTCACATATG-3` and SSPI(-): 5`-CCATTGGGCTGCAGGAAAGAGAATAT-3`

PCR products were digested with the restriction endonucleases, ***Xmn* I** (New England BioLabs, Beverly, MA) and ***Ssp* I** (TaKaRa), respectively. For genotyping, all the three Digested DNA were mixed with 5µl loading buffer before being loaded on a 10 %

Non-Denaturating Polyacrylamide gel and electrophoresed at 140 V for one hour. Then the gel was stained in 0.1µg/ml ethidium bromide solution for 10-15 minutes and visualized under UV light in Gel Documentation System (GDS) **(Fig. 2).**

Statistical analysis

The Statistical Package for the Social Science (SPSS for Windows 14) was used for statistical analysis. *T*- test was used for comparing the distribution of a variable between cases and controls groups. The Chi-square test had been applied in comparing of categorical data. Genotypes distributions among the MI cases and controls were determined using a continuity-adjusted Chi-square or Fisher's exact test for each genotype compared with the homozygous wild-type for that locus. Odds ratios were calculated with 95%confidence intervals. *P* values of less than 0.05 were considered as significant.

3. Results

Study subjects

A total of 120 patients, who were diagnosed as having MI, were selected for the study. With The mean age of the study subjects was (66.87 ± 14.46) years old; the minimum age is 43 years old and the maximum one is 85 years old. 72 (60%) were males and 48 (40%) were females. *Table 1* is showing the demographic and laboratory finding of study subjects.

Table 1. Comparison of characteristics findings in MI patients and control groups

Characteristic	Patients (mean ± SD)	Control (mean ± SD)	*P value*
Age (Yr)	66.97±13.70	62.00±20.00	*0.095*
Body weight (Kg)	81.00±15.65	80.32±16.01	*0.469*
Body Mass Index (kg/m^2)	28.90±2.39	27.90±5.39	*0.222*
Systolic BP	138.67±25.43	137±25.43	*0.886*
Diastolic BP	78.50±12.39	77±8.2	*0.431*
Hb (gm/dl)	13.46±2.1	13.98±2.59	*0.697*
RBS (mmol/l)	11.71±5.44	9.82±3.60	*0.006*
Total Cholesterol Level (mmol/l)	4.21±1.29	4.08±0.88	*0.899*
Serum Triglycerides (mmol/l)	2.315±0.692	1.468±0.645	*0.001*
HDL (mmol/l)	1.170±0.380	1.265±0.380	*0.599*
LDL mmol/l)	3.662±1.3	2.432±1.3	*0.001*

The codon 90 polymorphism of the human CD36 gene was not detected among study population:

A total of 240 subjects (MI cases N=120 and healthy control N=120) were screened for the mutation (Fig. 2). The only genotype observed was the normal variant (Pro90 homozygote) and no mutant variant Ser90 homozygote nor heterozygote genotype were detected in all subject **(Fig. 1)** The allele frequency in this population was 100% for the normal allele pro90 and 00% for the mutant allele (Table 2). This indicates that the codon 90 pro/Ser polymorphism may not exist in this population and has no role in genetic predisposition for MI in this population.

CD36 Ins1159A (+/-) and polymorphism was not detected among study population:

Screening of the polymorphism was done by PCR-RFLP method in a total of 240 subjects (MI cases N=120 and healthy control N=120) **(Fig. 2)**. No insertion variant **Ins1159A** homozygote **(+/+)** nor heterozygote genotypes **(+/-)** were detected in all subjects. All study subject (cases and control) were **Ins1159A (-/-)** homozygote. The allele frequency in this population was 100% for the normal allele **Ins1159A (-)** and 00% for the mutant allele **Ins1159A (+)** (Table 2). This indicates that the CD36 Ins1159A (+/-) polymorphism not exist in this population and has no role in genetic predisposition for MI in this population.

CD36 Δ539AC polymorphism was not detected among study population:

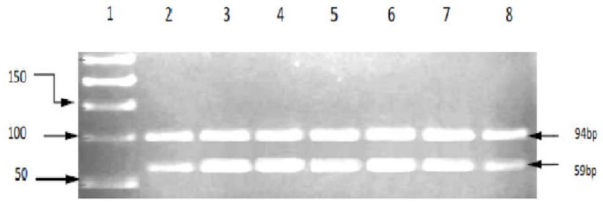

Figure 1. PCR-RFLP screening of CD36 478 C > T among study subjects
Legand: Lane 1: 100bp DNA marker, lanes 2, 3 , 4, 5, 6, 7 and 8 are normal homozygote genotypes (CC , Pro / Pro)

Figure 2. Screening of CD36 Del539AC and ins1159A mutations by PCR-RFLP method

Legand: a: Showing PCR-RFLP for Ins1159A: Lane 1 a 50bp DNA marker, lane 2 undigested product for internal un-digestion control while lane 3, 4, 5, 6, 7, 8, 9 and 10 complete digestion of PCR product (Ins1159A(-/-). **b:** Showing PCR-RFLP for Del539AC: Lane 1 a 50bp DNA marker, lane 2 undigested product while lane 3, 4, 5, 6, 7, 8, 9 and 10 no digestion of PCR product (homozygous wild-type variant)

Screening of **CD36 Δ539AC** was done by RFLP method using ***Ssp I*** restriction enzyme since the mutation has created a new recognition site for the enzyme at the polymorphic site. A total of 240 subjects (MI cases N=120 and healthy control N=120) were screened for the mutation (Fig. 3). The only genotype observed was the wild-type variant. The mutant variant **Δ539AC** homozygote nor heterozygote genotype were not observed in all subject. The allele frequency in this population was 100% for the wild-

type allele and 00% for the mutant allele (Table 2). This indicates that this polymorphism not exist in this population and has no role in genetic predisposition for MI in this population.

4. Discussions

CD36 is a multifunctional integral-membrane glycoprotein that acts as a receptor for thrombospondin, collagen, long-chain fatty acids, and oxidized LDL and it was be shown that CD36 can account for up to 40% of the binding and internalization of oxidized LDL by macrophages [14, 15]. Two known mutations cause CD36 deficiency, ie, a 478C → T substitution in codon 90 (proline90 → serine) and a dinucleotide deletion at nucleotide 539 in codon 110 [11, 16]. a new mutation was identified in Japanese which is a single nucleotide insertion at nucleotide 1159 in codon 317. This mutation leads to a frameshift and the appearance of a premature stop codon [17]. In this study we studied the association of these mutations and genetic susceptibility to MI among Saudi population from Turabah province in Western region of Saudi Arabia. None of the three mutations were detected in this study population. This indicated that all these mutation, the 478C→T substitution in codon 90, the AC dinucleotide deletion at nucleotide 539 in codon 110 and the A single nucleotide insertion at nucleotide 1159 in codon 317. This may be due to the fact that these mutation are not exist in Saudi population in Turabah province or exist but at very low frequencies and not detected due to smaller study sample (N=120). The same result was reported in study from north Indian population. where they screened 300 subjects but failed to detect any of 478C → T substitution in codon 90, the AC dinucleotide deletion at nucleotide 539 in codon 110 or the A single nucleotide insertion at nucleotide 1159 in codon 317[18].

CD36 polymorphism vary between different racial and ethnic populations although, in this study, We conclude that the study CD36 polymorphisms were not detected in Saudi Arabia population in from Turabah. Although, this does not exclude the presence of other mutations at CD36 gene locus in this population and further studies needed to screen for the polymorphism of CD36 and to evaluate their relation to Myocardial infarction and other diseases.

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References

1. Aboderin, I.e.a., *Life Course Perspectives on Coronary Heart Disease, Stroke and Diabetes: Key Issues and Implications for Policy And Research.* WHO, 2002.
2. P., L., *Coronary artery injury and the biology of atherosclerosis: inflammation, thrombosis, and stabilization.* Am J Cardiol 2000(86): p. 3J-9J.
3. Shea S, O.R., Gabrieli C, et al., *Family history as an independent risk factor for coronary artery disease.* J Am Coll Cardiol, 1984(4): p. 793-801.
4. Marenberg ME, R.N., Berkman LF, et al., *Genetic susceptibility to death from coronary heart disease in a study of twins.* N Eng J Med 1994(330): p. 1041-6.
5. Ye Z, L.E., Higgins JPT, et al., *Seven haemostatic gene polymorphisms in coronary disease: meta-analysis of 66,155 cases and 91,307 controls.* Lancet, 2006(367): p. 651-8.
6. Boekholdt SM, B.N., Moons AHM, et al., *Genetic variation in coagulation and fibrinolytic proteins and their relation with acute myocardial infarction. A systematic review.* Circulation, 2001(104): p. 3063-8.
7. Collot-Teixeira, S., et al., *CD36 and macrophages in atherosclerosis.* Cardiovasc Res., 2007. 3(75): p. 468-77.
8. Silverstein, R.L., *Inflammation, atherosclerosis, and arterial thrombosis: role of the scavenger receptor CD36.* Cleve Clin J Med, 2009. suppl 2(76): p. S27-30.
9. Hirano, K., et al., *Pathophysiology of human genetic CD36 deficiency.* Trends Cardiovasc Med, 2003. 4(13): p. 136-41.
10. Kajihara, S., et al., *Association of the Pro90Ser CD36 mutation with elevated free fatty acid concentrations but not with insulin resistance syndrome in Japanese.* Clin Chim Acta, 2001. 1-2(314): p. 125-30.
11. Kashiwagi H, H.S., Tomiyama Y, Mizutani H, Take H, Honda Y, Kosugi S, Kanayama Y, Kurata Y, Matsuzawa Y., *A novel polymorphism in glycoprotein IV (replacement of proline-90 by serine) predominates in subjects with platelet GPIV deficiency.* Thromb Haemost., 1993(69): p. 481-484.
12. Watanabe, K., et al., *Different patterns of 123I-BMIPP myocardial accumulation in patients*

with type I and II CD36 deficiency. Kaku Igaku, 1997. 12(34): p. 1125-30.
13. J. Sambrook, D.W. Russell, and J. Sambrook, *Molecular cloning: a laboratory manual, Cold Spring Harbor Press,*. Cold Spring Harbor, NY, 2001.
14. Nozaki S, K.H., Yamashita S, Nakagawa T, Kostner B, Tomiyama Y, Nakata A, Ishigami M, Miyagawa J, Kameda-Takemura K, Kurata Y, Matsuzawa Y., *Reduced uptake of oxidized low density lipoproteins in monocyte-derived macrophages from CD36 deficient subjects.* J Clin Invest., 1995(96): p. 1859-1865.
15. Love-Gregory, L., et al., *Common CD36 SNPs reduce protein expression and may contribute to a protective atherogenic profile.* Hum Mol Genet. 20(1): p. 193-201.
16. Kashiwagi H, T.Y., Honda S, Kosugi S, Shiraga M, Nagao N, Sekiguchi S, Kanayama Y, Kurata Y, Matsuzawa Y., *Molecular basis of CD36 deficiency: evidence that a 478C T substitution (proline90 serine) in CD36 cDNA accounts for CD36 deficiency*. J Clin Invest., 1995(95): p. 1040-1046.
17. Hirokazu Kashiwagi, Y.T., Shuichi Nozaki, Shigenori Honda, Satoru Kosugi, Masamichi Shiraga, Tsutomu Nakagawa, Nobuo Nagao, Yuzuru Kanakura, Yoshiyuki Kurata, Yuji Matsuzawa, *A Single Nucleotide Insertion in Codon 317 of the CD36 Gene Leads to CD36 Deficiency*. Arteriosclerosis, Thrombosis, and Vascular Biology, 1996(16): p. 1026-1032.
18. Gautam, S., et al., *Preliminary studies on CD36 gene in type 2 diabetic patients from north India.* Indian J Med Res, 2011. 134: p. 107-12. arid ecosystem. Global Ecology and Biogeography 2003;10(2):77-100.

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