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Association between ATTG insertion/deletion of NFκB1 (rs28362491) Gene Promoter and Coronary Artery Diseases

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***Abstract*_Coronary artery disease (CAD) is the most common cause of death in cardiovascular diseases. Many of the genes associated with the pathogenesis of CAD are regulated by Nuclear Factor κappa light chain enhancer of activated B cells (NFκB). Proteins in NFκB family have a vital regulator for many cellular processes. NFκB transcription factors are one of the most important causes of inflammatory responses. Deletion of ATTG -94 NFκB1 gene promoters, decreases the levels of the protein subunits (P50 and p105) and as a result it affects the inflammatory responses. Those who are carrying this deletion may be at greater risk of CAD disease. In this research paper 250 people, 120 CAD patient and 130 people without any CAD symptoms, have been analyzed. Genomic DNAs from both groups have been extracted using salting out method. Then, the primer sequences were designed for NFκB1-94ATTG ins/del polymorphism using Polymerase Chain Reaction (PCR). Rs28362491 position was assessed by Restriction Fragment Length Polymorphism-Polymerase Chain Reaction (RFLP-PCR) methods in patients and controls. At the final stage of the project, the obtained data were analyzed using statistical Med Calc software (Version 12.1.4.0). The results showed that there was no significant difference in .The deletion and insertion ATTG alleles distribution among controls and patients. (*P* = 0.18)**

***Key words*_ CAD, NFκB1-94ATTG ins/del olymorphism, RFLP-P**

I. INTRODUCTION

Coronary artery disease (CAD) or atherosclerosis is the most common cause of death worldwide, with interactions between both metabolic risk factors and immunological and genetic determinants in the development and progression of the disease [Cliton 2004, Kop and Gottdiener 2005]. Nowadays, our understanding about the correlation between inflammatory cytokines and chemokines and an increased risk for CAD has been improved by the evidence that circulating leukocytes adhere to the endothelium in response to chemotactic cytokines secreted by cells of the blood vessels at the earliest stages in the development of atherosclerosis [Baudhuin 2009, Hansson 2005]The NFκB transcription factors have a key role in many cellular processes such as inflammatory responces and pathogenesis of CAD[De Winther et al. 2005]. Functionality of NFκB proteins is dependent on subunit's dimerization: p50/p105, p65/RelA, c-Rel, Rel-B and p52/ p100 [Lin et al. 2005]. P50 and p105 subunits of the complex are encoded by alternative splicing process from the NFκB1 gene and processed by 26S proteasome [Karban et al. 2004]. P50 subunit is involved in CAD through both pro- and anti-inflammatory responses.

In the absence of stimuli, NFκB is sequestered in the cytoplasm bound to a member of the IkB family of inhibitor proteins [Park et al. 2007]. However, in the presence of activating stimuli, IkBa is rapidly phosphorylated, leading to ubiquitination and degradation by the proteasome, thus NFκB translocates into the nucleus regulating proinflammatory gene expression [Karin and Delhase 2000].

P50 homo dimer and P50/P65 hetero-dimer of relative frequency determine the power of the inflammation [Kawamura et al. 2005, Pereira and Oakley 2008].

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P50 homo dimers suppress transcription of pro-inflammatory cytokines such as TNF-alpha and IL-12 but they stimulate transcription of anti-inflammatory cytokine such as IL-10 [Cao et al. 2006, Kawano et al. 2006]. In addition, -94 ATTG insertion (Ins) or deletion (Del) in NFκB1 gene leads to differential expression of P50 and P105 subunits [Cao et al. 2006, Karban et al. 2004]. Interestingly, the NFκB protein function is lower in carriers of the Del allele [Karban et al. 2004, Kawano et al. 2006]. Therefore, they are in more tendancy to have severe inflammatory response and at greater risk of CAD [Vogel et al. 2009, Vogel et al 2011, Vogel et al. 2011]. Taken together, considering that there are genetic variations in different ethnic groups, and just one study has been done on the association of ATTG Ins/Del of NFκB1 gene promoter with coronary artery diseases in the world up to now, in this case-control report, this association was assessed with the risk of CAD in Iranian population.

II. Subjects and Methods

A. Study population

The subjects enrolled in this study comprised 120 patients with CAD who visite coronary angiography section in Amir-almomenin hospital (Heart Center) in kordkoy city of Golestan province and 130 healthy controls from unrelated families living in the Golestan province (North of Iran). All subjects gave written informed consent to participate. In general, blood samples were taken from both groups in this study.

B. Genotyping for rs28362491

Genomic DNA samples of all individuals were extracted from whole blood collected with anticoagulant (EDTA, 15% w/v), using a 'salting out' method (16). The forward primer 5'TGGGCACAAGTCGTTTATGA3 ' and the reverse primer 5'CTGGAGCCGGTAGGGAAG3' were used to amplify a fragment containing the ATTG insertion / deletion (281 bp (Deletion) or 285 bp (Insertion)) in – 94 promoter region of NFκB1 gene (rs28362491). Amplification was performed using Taq DNA polymerase enzyme (GenetBio). PCR products were analyzed through electrophoresis on 1.5% Agarose gel in reference to a 50 bp molecular weight marker (Fermentas). Restriction Fragment Length Polymorphism (RFLP) technique was used by Van91I (PflMI) restriction enzyme (Fermentas), to identify the genotype of rs28362491. Then, the horizontal electrophoresis (3% Agarose gel) and vertical electrophoresis (12% Polyacrylamide gel) were carried out to evaluate the RFLP products. Finally, Ins/Ins, Ins/Del and Del/Del genotypes were scored blindly and analysis of half of the samples was repeated twice for checking the accuracy of the results.

C. Statistical analysis

To interpret the results and significance levels of genotypic and allelic frequencies, Chi-Square (χ^2) test, *P*-value, parameters, Odd Ratio (OR)s and Confidence Interval (CI)s were calculated using a statistical software Med Calc (Version 12.1.4.0).

III. Results

The CAD study group (n=120) and unrelated healthy controls (n =130) were of similar sex and age with 25-75 years. Allele and genotype frequencies of the analyzed samples of the ATTG insertion / deletion of NFκB1 (rs28362491) polymorphism are depicted in the tables below (I, II).

Gene	NFκB1	NFκB1
Alleles	Ins	Del
Cases n (%)	140(58.33)	100(41.67)
Controls n (%)	168(64/62)	92(35.38)
OR	1.0	1.30
95% CI	Reference	0.91-1.87
P	0.18	0.15
χ^2	1.82	

Table I: Results of allele frequencies of NFκB1 polymorphism

Gene	NFκB1	NFκB1	NFκB1
Genotype	Ins/Ins	Ins/Del	Del/Del
Cases n (%)	38(31.67)	64 (53.33)	18 (15)
Controls n (%)	49 (37.69)	70(53/85)	11 (8.46)
OR	1.0	1.18	2.11
95% CI	Reference	0.68-2.03	0.89-4.99
P	0.23	0.55	0.08
χ^2	2.95		

Table II: Results of genotype frequencies of NFκB1 polymorphism

Deviation from Hardy–Weinberg equilibrium was not found with regard to genotypes distribution in the control group (p >0.05). We observed that the Del/Del genotype was not common in CAD patients (15%) as compared with control individuals (8.46%) and the difference was not statistically significant (p> 0.05). The observed difference was calculated with a $2 * 2$ χ^2 test.

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So, it cannot be considered that there is a relation between mutant genotype (Del/Del) and Iranian patients with CAD (OR = 2.11, 95% CI = 0.89-4.99). Patients with CAD also did not commonly show the Del allele (41.67%) compared to controls (35.38%) (p> 0.05, OR =1.30, 95% CI = 0.91-1.87). Therefore, it can be regarded that there is no association between the mutant allele and CAD in patients of Iranian origin. Gel pictures depicted below show Ins/Ins, Ins/Del and Del/Del genotypes in our study.

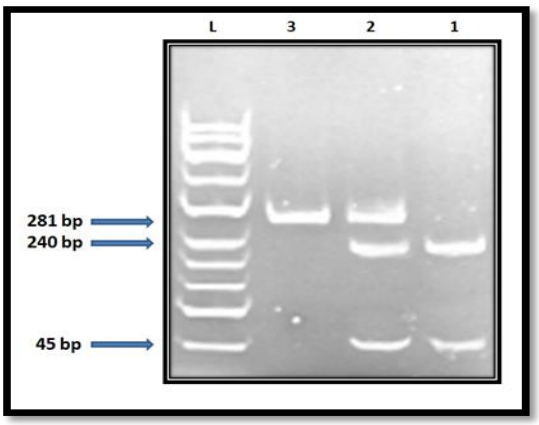

Figure 3-1: Picture of polyacrylamide gel 12%, for the study NFκB1 gene promoter polymorphism. Lane L: 50-bp Ladder, Lane 1 with two fragments in relation to ATTG insertion/insertion, Lane 2 with three fragments in relation to ATTG insertion/deletion, Lane 3 with one fragment in relation to ATTG deletion/deletion.

IV. Discussion

Several studies in more recent years revealed the critical contribution of inflammatory response in the incidence of CAD and many of the genes associated with the pathogenesis of the disease are tuned-up by NFκB1 [Lusis and Fogelman 2004, Pai et al. 2004]. However, the NFκB1 gene encodes two proteins, a 105 kDa non DNA-binding cytoplasmic molecule (p105) and a 50 kDa DNA-binding protein (p50) that corresponds to the N-terminus of p105 [Frantz et al. 2006]. Homo dimer proteins of P50 subunit NFκB1 are involved in anti-inflammatory reactions [Baudhuin 2009] .Additionally, relationships between the -94 insertion / deletion ATTG of NFκB1 gene and the lower promoter activity and p50/p105 NFκB1 depletion were investigated in functional efforts, suggesting the higher risk of CAD [Borm et al. 2005, Bourcier 1997]. Furthermore, in vitro evidence attempted by Karban et al. 2004 demonstrated that the deleted ATTG sequence of NFκB1 gene promoter disrupted binding of an unknown nuclear factor, leading to a reduction in transcriptional function of the promoter, and P50 protein biosynthesis. Accordingly, a lower frequency of the Deletion allele was found in patients affected by CAD compared to control subjects. These indicate a potential protective role of the D allele on CAD susceptibility [Tak and Firestein 2001]. Given that, the association between the ATTG insertion/deletion of NFκB1 gene promoter and different disorders, such as oral, gastric and prostate cancers [Lin et al. 2005, Lo et al. 2009, Cao et al. 2006] have been reported. Interestingly, "Boccardi et al.," in a case/control study on Caucasians affected by myocardial infarction, living in southern Italy, showed that -94 Deletion ATTG of NFκB1 gene is associated with reduced risk of the disease [Boccardi et al. 2011]. Vogel et al. 2009 findings in a cohort study on patients with CAD, of the American and European white races indicated that individuals carrying the deletion ATTG of NFκB1 gene promoter are at greater risk of the disease. Our study showed that there were no significant differences in genotype and allele frequencies of the ATTG insertion / deletion of NFκB1 gene promoter between patients and controls in the study population (P > 0.05). The results obtained in this work suggest that the mutation (ATTG deletion of NFκB1 gene promoter) in our study population was not associated with coronary artery disease and it does not increase the risk of CAD in our population. This means that there was no significant difference in the deletion and insertion ATTG alleles' distribution among controls and patients in this population.

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