

## Association study between rs2275913 genetic polymorphism and serum levels of IL-17A with risk of coronary artery disease

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### ABSTRACT

Coronary artery disease (CAD) is now considered as a main cause of disability and mortality in Iranian population. Inflammatory processes are the initial events in the development of CAD. Interleukin-17A (IL-17A) is a pro-inflammatory cytokine and its genetic variation may contribute to the development of CAD. This study investigated serum levels and the G-197A polymorphism of IL17A in a group of patients with CAD and healthy controls. The study population included 220 angiographically verified CAD patients and 220 healthy controls. Genotyping of G-197A polymorphism of IL17A was done by PCR-RFLP method and serum level of IL-17 was measured by enzyme immunoassay. Results indicated that serum concentration of IL-17A was significantly higher in CAD group than control group ( $P < 0.001$ ). Also, serum levels of IL-17A was significantly higher in carriers of GA and AA genotype relative to carriers of GG genotype in both study population ( $P < 0.05$ ). The G-197A polymorphism of IL17A increased the risk of CAD in mutant homozygous ( $P = 0.007$ ) but not heterozygous ( $P = 0.104$ ) genotype. Moreover, this polymorphism was associated with higher risk of CAD development in allelic ( $P = 0.041$ ) model. However, no significant association was observed between genotypic distribution of G-197A polymorphism and the number of stenotic vessels ( $P > 0.05$ ). In conclusion, the present study indicated G-197A polymorphism of IL17A as a significant contributor to the development but not to the severity of CAD. Moreover, elevated serum levels of IL-17A were identified as a susceptibility marker of CAD.

**Keywords:** Coronary artery disease; Interleukin 17A; rs2275913 Polymorphism

### INTRODUCTION

Coronary artery disease (CAD) is a disease with a growing incidence and a high rate of debility and mortality. The initial event in the CAD pathogenesis involves the formation of an atherosclerotic plaque which gradually narrows and occludes the lumen of artery [1]. The initiation and progression of atherosclerotic plaque is affected by acquired, genetic and epigenetic factors [2, 3]. Oxidative stress and inflammatory process are among the most important contributor of atherosclerotic plaque formation and destabilization [4]. Inflammatory cytokines

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plays pivotal role in actually all stages of CAD development. Inflammatory cytokines causes endothelial damage associated with overexpression of adhesive molecules and release of chemo attractive substances which collectively leads to progression of inflammation [5]. Also, variations in genes involved in inflammatory cytokine signaling pathways may contribute to the development of CAD [6]. Interleukin-17 (IL-17) is produced by Th17 cells and acts as a pro-inflammatory cytokine. IL-17 is consisted of six members (from IL-17A to IL-17F), which are involved in pathogenesis of numerous inflammatory disease [7-9]. The G-197A (rs2275913) promoter polymorphism of IL17A has been implicated in pathogenesis of various inflammatory diseases [10, 11]. However, inconsistent results have been published regarding the role of rs2275913 polymorphism in the CAD pathogenesis [12-14]. So, the current case control study was aimed to assess the serum concentration of IL-17A as well as the frequency of G-197A genetic polymorphism of IL17A in the study population.

## MATERIALS AND METHODS

**Study population:** The present study investigated 220 CAD patients admitted to angiography center of Moussavi teaching hospital of Zanjan and 220 healthy control subjects. The presence of CAD was verified by coronary angiography conducted by a cardiovascular specialist. The CAD patients with vessel stenosis of more than 50% were included in the study and were categorized as single-, double-, and triple-vessel stenosis based on the number of stenotic vessels. CAD patients under treatment with lipid controlling drugs or anti-inflammatory drugs and with coronary lumen stenosis of less than 50% were not included in the study. Also, CAD patients with a previous history of metabolic, inflammatory, autoimmune, malignancy and congenital heart defects were debarred from the study. The control subjects were included in the study following precise examination by a cardiovascular specialist. Control subjects were debarred from the study if they had cancer, metabolic, autoimmune, renal, hepatic, cardiac and inflammatory disease. Information regarding the hypertension (BP>140/90 mmHg), diabetes (blood glucose level $\geq$ 126mg/dl), smoking, hyperlipidemia and family history of any disease was obtained for all of the study population. The study was approved by ethical committee of Zahedan University of Medical Sciences (IR. ZAUMS. REC.1396.43), Zahedan, Iran.

**Biochemical assays:** Following 12 hours fasting, blood samples were drawn into EDTA vacutainer tubes. The plasma fraction was separated and stored at -20°C until analysis for biochemical parameters. Lipid levels were measurement using a Mindray BS-200 auto-analyzer. IL-17A was assayed by an ELISA kit (Bioassays Technology, China), according to the instruction and recommended protocol of assay kit. The sensitivity of assay protocol was 2.38 pg/ml and the coefficient of variation (CV) for intra-assays and inter-assays was <8% and <10%, respectively.

**Genetic analysis:** The EDTA anti-coagulated blood samples was used for DNA extraction by a commercially available kit (Yekta Tajhiz Azma, Iran) using the guideline of kit. The IL17A G-197A (rs2275913) gene polymorphism was genotyped by PCR-RFLP technique. The following primer pairs F:5'-GCC AAG GAA TCT GTG AGG AA-3' and R:5'-TGC CTG CTA TGA GAT GGA CA-3' was used for amplification of target sequence using a standard PCR protocol. The digestion of amplified product (424 bp) with 5 unit of *XagI* restriction enzyme created two bands (260bp and 164bp) in G allele carrier and a single undigested band (424bp) in the carrier of A allele.

**Statistical analysis:** The numerical data and categorical data were examined by independent *t*-test and Chi-square test or Fisher's exact test, respectively and odd ratio and 95% confidence intervals (CI) were determined using SPSS-18 (SPSS Inc., Chicago, Ill., USA) with a P value significant levels of less than 5%.

## RESULTS

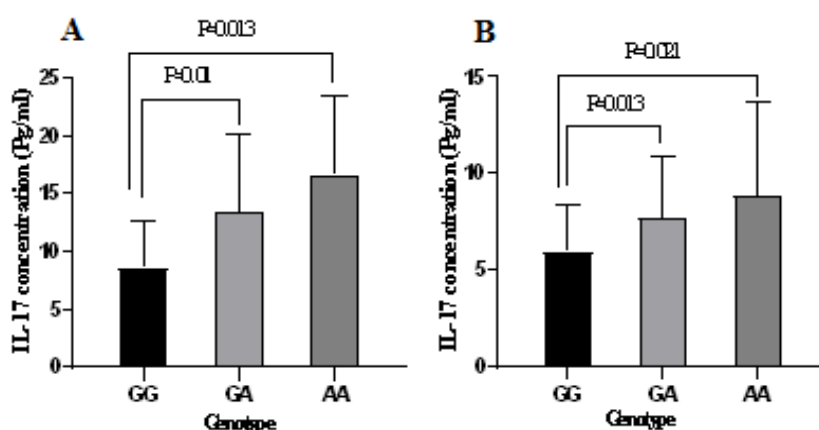
The demographic, biochemical and clinical features of study population indicated, hyperlipidemia, diabetes, hypertension, smoking and elevated levels of IL-17A was more common in the CAD group in relation to control group (supplementary Table 1).

The genotype distribution of the rs2275913 polymorphism was in accordance with the Hardy-Weinberg equilibrium in both CAD group ( $\chi^2=0.27$ ,  $df=1$ ,  $P=0.602$ ) and control group ( $\chi^2=0.003$ ,  $df=1$ ,  $P=0.954$ ). The genotype frequency in CAD group (AA, 15.46%; GA, 44.54%; GG, 40.0%) was significantly different than that of in control group (AA, 08.19%; GA, 40.45%; GG, 51.36%) ( $\chi^2=8.466$ ,  $df=2$   $P=0.014$ ). As indicated in Table 1, the homozygous AA genotype was more common among CAD group than control group (15.46% vs. 8.19%) which significantly increased the risk of CAD by 2.42 (95% CI: 1.26-4.54,  $P=0.007$ ). However, no such association was found for heterozygous GA genotype ( $P=0.104$ ). Moreover, the mutant A allele significantly increased the risk of CAD by 1.53 (OR=1.53, 95% CI:1.16-2.03;  $P=0.041$ ).

**Table 1:** Genotypic and allelic frequency of IL17A G-197A polymorphism in CAD and control groups

IL17A	CAD group	Control group	OR (95% CI)	P
Genotypes/alleles	n (%)	n (%)		
GG	88 (40.00)	113(51.36)	Ref.	-
GA	98 (44.54)	89 (40.45)	1.41 (0.95-2.10)	0.104
AA	34 (15.46)	18 (8.19)	2.42 (1.26-4.54)	0.007
G	274 (62.27)	315 (71.60)	Ref.	-
A	166 (37.73)	125 (28.40)	1.53 (1.16-2.03)	0.041

Figure 1 indicated the association between different genotypes and serum levels of IL-17A in study population. As shown, carriers of GA and AA genotypes had significantly higher levels of IL-17A relative to carriers of GG genotype in both studied group ( $P<0.05$ ). However, as presented in Figure 2, no significant association was seen between genotype distribution of this common polymorphism and the number of diseased vessels ( $P>0.05$ ).

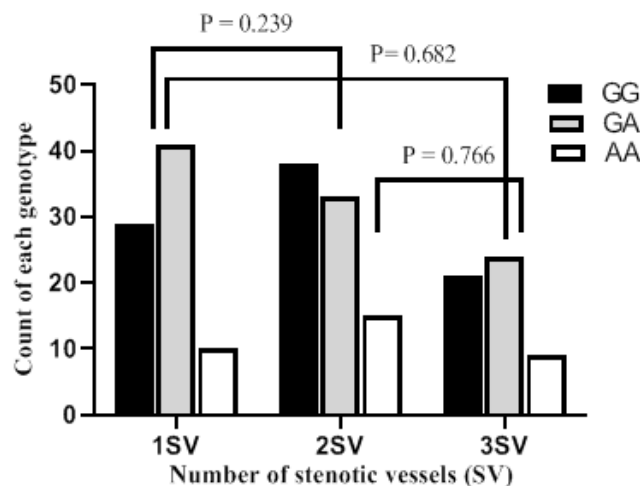


**Figure 1:** Association of the IL17A G-197A genotypes with serum levels of IL-17A in CAD group (A) and control group (B).

## DISCUSSION

CAD is now considered as an inflammatory process initiated as soon as early childhood. The present study identified elevated levels of IL17A pro-inflammatory cytokine in CAD group as compared with control group, which was in agreement with some previous studies [15, 16]. Moreover, the higher frequency of mutant homozygous AA genotype and mutant A allele in

CAD group relative to control group, may suggest a possible role of this common polymorphism in the development of CAD [12, 13]. Recently, a study done by Geng et al., reported that AA genotype and A allele of rs2275913 genetic variation significantly increased the risk of CAD development [12]. However, some other studies did not confirm the role of this genetic polymorphism in the CAD development [14, 17]. Numerous reasons may be involved in the inconsistency of association studies such as interactions between genes, the influence of environmental factors, sample size variability and ethnic differences [18]. The frequency of the minor A allele in our population was 28.40% which was in accordance with a reported frequency in a China (29.64%) [19] and Brazilian (28.30%) [20] population. However, it was higher than the reported frequency of A allele in Mexican (19.04%) [21] population and lower than that of in a Middle Eastern Chinese (46.94%) population [13].



**Figure 2:** the frequency of IL17A G-197A genotypes between patients with one, two and three stenotic vessel (SV).

Results of the present study found no significant association between the rs2275913 polymorphism and the number of stenotic vessels, indicating the ineffectiveness of this genetic polymorphism in determining the severity of CAD. The present study revealed higher levels of IL-17A in carriers of AA and GA genotypes relative to GG genotype in both studied group, which was in accordance with Espinoza et al study [22]. The rs2275913 polymorphism located in the promoter region of IL17A gene is adjacent to a binding motif for nuclear factor activated T cells (NFAT) transcription factor which significantly enhances the NFAT binding activity in the presence of A allele of IL17A rs2275913 polymorphism [22]. Recently, a functional in vitro study by Espinoza indicated higher levels of IL17A in the carriers of GA and AA genotypes of rs2275913 polymorphism relative to carriers of GG genotype [22]. In contrast, a recently published study, reported elevated levels of IL17A in carriers of GG and GA genotypes in patients with ischemic stroke [23]. So, it seems that the rs2275913 polymorphism exerts a context dependent effect on the serum levels of IL17A. In conclusion, this study identified AA genotype and A allele of the rs2275913 polymorphism as well as elevated levels of IL-17A cytokine as susceptibility markers for development of CAD.

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**Conflict of Interest:** There is no conflict of interests to be declared regarding the publication of this paper.

**REFERENCES**

1. Korosoglou G, Lehrke S, Mueller D, Hosch W, Kauczor HU, Humpert PM, Giannitsis E, Katus HA. Determinants of troponin release in patients with stable coronary artery disease: insights from CT angiography characteristics of atherosclerotic plaque. *Heart* 2011;97:823-831.
2. Abi Khalil C. The emerging role of epigenetics in cardiovascular disease. *Ther Adv Chronic Dis* 2014;5:178-187.
3. Dai X, Wiernek S, Evans JP, Runge MS. Genetics of coronary artery disease and myocardial infarction. *World J Cardiol* 2016;8:1-23.
4. Galkina E, Ley K. Immune and inflammatory mechanisms of atherosclerosis. *Annu Rev Immunol* 2009;27:165-197.
5. Ramji DP, Davies TS. Cytokines in atherosclerosis: Key players in all stages of disease and promising therapeutic targets. *Cytokine Growth Factor Rev* 2015;26:673-685.
6. Seidi A, Mirzaahmadi S, Mahmoodi K, Soleiman-Soltanpour M. The association between NFKB1 -94ATTG ins/del and NFKB1A 826C/T genetic variations and coronary artery disease risk. *Mol Biol Res Commun* 2018;7:17-24.
7. Gaffen SL. Structure and signalling in the IL-17 receptor family. *Nat Rev Immunol* 2009;9:556-567.
8. Cătană CS, Neagoie IB, Cozma V, Magdaş C, Tăbăran F, Dumitraşcu DL. Contribution of the IL-17/IL-23 axis to the pathogenesis of inflammatory bowel disease. *World J Gastroenterol* 2015;21:5823-5830.
9. Bulat-Kardum LJ, Etokebe GE, Lederer P, Balen S, Dembic Z. Genetic polymorphisms in the toll-like receptor 10, interleukin (IL) 17A and IL17F genes differently affect the risk for tuberculosis in Croatian population. *Scand J Immunol* 2015;82:63-69.
10. Hammad A, Mosaad YM, Hammad EM, Elhanbly S, El-Bassiony SR, Al-Harrass MF, Eid R, Sharaf Eldein OA, Alsawah GA, Yahia S, Fawzy IM. Interleukin-17A rs2275913, interleukin-17F rs763780 and rs2397084 gene polymorphisms as possible risk factors in Juvenile lupus and lupus related nephritis. *Autoimmunity* 2016;49:31-40.
11. Han L, Lee HS, Yoon JH, Choi WS, Park YG, Nam SW, Lee JY, Park WS. Association of IL-17A and IL-17F single nucleotide polymorphisms with susceptibility to osteoarthritis in a Korean population. *Gene* 2014;533:119-122.
12. Geng GY, Liu HL, Zhao YJ, Wu L, Mao L, Ba N. Correlation between polymorphisms in the IL-17A and IL-17F genes and development of coronary artery disease. *Genet Mol Res* 2015;14:11488-11494.
13. Zhao Q, Jiang H, Ma T, Qiu H, Guo M, Zhang X. The association between IL-17A and IL-23R polymorphisms and coronary artery disease risk in a Middle Eastern Chinese population. *J Clin Lab Anal* 2019;33:e22893.
14. Bao MH, Luo HQ, Xiang J, Tang L, Dong LP, Li GY, Zeng J, Li JM. Meta-analysis for the association between polymorphisms in interleukin-17A and risk of coronary artery disease. *Int J Environ Res Public Health* 2016;13.
15. Min X, Lu M, Tu S, Wang X, Zhou C, Wang S, Pang S, Qian J, Ge Y, Guo Y, Xu D. Serum cytokine profile in relation to the severity of coronary artery disease. *Biomed Res Int* 2017;2017:4013685.
16. Taleb S, Tedgui A, Mallat Z. IL-17 and Th17 cells in atherosclerosis: subtle and contextual roles. *Arterioscler Thromb Vasc Biol* 2015;35:258-264.
17. Zheng XS, Wang S, Ni M. Association between interleukin 17A gene polymorphisms and risk of coronary artery disease. *Genet Mol Res* 2016;15.
18. Miller J, Schwarz W. Aggregate and individual replication probability within an explicit model of the research process. *Psychol Methods* 2011;16:337-360.
19. Shuang L, Li Z, Chen F, Cui X, Ning Y, Su Y, Dong M. Association between interleukin-17 gene polymorphisms and risk of coronary artery disease. *Int J Clin Exp Pathol* 2015;8:11653-11658.

20. Zacarias JM, Sippert EA, Tsuneto PY, Visentainer JE, Sell AM. The influence of interleukin 17A and IL17F polymorphisms on chronic periodontitis disease in Brazilian patients. *Mediators Inflamm* 2015;2015:147056.
21. Vargas-Alarcón G, Angeles-Martínez J, Villarreal-Molina T, Alvarez-León E, Posadas-Sánchez R, Cardoso-Saldaña G, Ramírez-Bello J, Pérez-Hernández N, Juárez-Rojas JG, Rodríguez-Pérez JM, Fragoso JM. Interleukin-17A gene haplotypes are associated with risk of premature coronary artery disease in Mexican patients from the genetics of atherosclerotic disease (GEA) study. *PLoS One* 2015;10:e0114943.
22. Espinoza JL, Takami A, Nakata K, Onizuka M, Kawase T, Akiyama H, Miyamura K, Morishima Y, Fukuda T, Kodera Y, Nakao S. A genetic variant in the IL-17 promoter is functionally associated with acute graft-versus-host disease after unrelated bone marrow transplantation. *PLoS One* 2011;6:e26229.
23. Huang HT, Lu YL, Wang R, Qin HM, Wang CF, Wang JL, Xiang Y, Guo J, Lan Y, Wei YS. The association of IL-17A polymorphisms with IL-17A serum levels and risk of ischemic stroke. *Oncotarget* 2017;8:103499-103508.