Journal of Applied Biomedicine 16 (2018) 387–393

Contents lists available at ScienceDirect

Journal of Applied Biomedicine

journal homepage: www.elsevier.com/locate/jab

Original research article

Genetic association of Tumour necrosis factor alpha, Interleukin-18 and Interleukin 1 beta with the risk of coronary artery disease: A case-control study outcome from Kashmir

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ARTICLE INFO

Article history:
Received 4 June 2017
Received in revised form 27 January 2018
Accepted 20 February 2018
Available online 27 February 2018

Keywords:
Inflammation
Coronary artery disease
Kashmiri
Polymorphism
Biomarkers

ABSTRACT

Coronary artery disease (CAD) is a clinical manifestation of atherosclerosis in the arteries supplying myocardium. Inflammation is the cornerstone in the development and progression of atherosclerosis. Amongst the various biomolecules tumour necrosis factor-α (TNF-α), interleukin-18 (IL-18) and interleukin-1β (IL-1β) build an inflammatory bionetwork in developing the disease. In this study we investigated the association of TNF-α SNPs [−308G/A (rs1800629), −1031T/C (rs1799964), −863C/A (rs1800630)]; IL-18 [−137G/C (rs187238)] and IL-1β SNPs [+3954C/T (rs1143634), −31C/T (rs1143627), and −511C/T (rs16944)] with coronary artery disease risk in Kashmiri population. A total of 200 cases and 260 controls were recruited in the study. Logistic regression analysis was done to investigate the association between SNPs and CAD risk. In case of TNF-α, the −308G/A-A/A and −863A/A showed an association with disease while −1031T/C was found to have an inverse relation. The IL-18–137G/C showed no statistically significant difference between controls and cases. For IL-1β the +3954C/T and −31C/T SNP variants showed no disease association while −511T/T showed significant association. Haplotypic analysis revealed the haplotype ATCGCC and GTACCTC to be associated with CAD risk and GTCGTTT, in particular, showing a profound association. Overall, our study suggests that TNF-α and IL-1β promoter polymorphisms may act as genetic risk factors in developing the coronary artery disease.

Introduction

Coronary heart disease is the leading cause of death worldwide, accounts for over seven million deaths annually. In Indian Asians, a fifth of all the global deaths are due to coronary heart disease (CHD) (Lozano et al., 2012). A cross sectional study conducted in the state of Jammu and Kashmir shows the prevalence of CAD to be 7.54% (rural – 6.7%, urban – 8.37%, males – 7.88%, females – 6.63%) (Kamili et al., 2007). Kashmir lies in the north of India and is more predisposed to developing CAD because of the ongoing lifestyle modifications and the increased exposure to traumatic and distressful conditions (Dharmender et al., 2012). The eight common risk factors that explain more than 90% of incident acute myocardial infarctions in South Asian and Indian patients are dyslipidemia, smoking, hypertension, Type-2 diabetes, central obesity, physical inactivity, low fruits and vegetable intake, and psychosocial stress (Joshi et al., 2007). Amongst them, diabetes is an independent and a major risk factor for CAD (Antoniades et al., 2004). The increased amount of reactive oxygen species and hyperglycemia in diabetes causes LDL modification, endothelial damage and dysfunction.

Coronary artery disease is a polygenic and multifactorial disease of the arteries supplying blood to the muscles of heart. It is characterized by loss of elasticity and partial or complete

Abbreviations: CAD, coronary artery disease; TNF-α, tumour necrosis factor alpha; IL-18, interleukin-18; IL-1β, interleukin 1 beta; bp, base pair.

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https://doi.org/10.1016/j.jab.2018.02.004
1214-021X/

decrease in the lumen of coronary arteries. There is impairment in the myocardial blood flow resulting in ischemia and myocardial infarction. Coronary artery disease is now considered to be an outcome of ongoing inflammatory processes (Libby, 2002a,b; Libby et al., 2002). TNF-α, and IL-1β have been of much attention because of their role in orchestrating the inflammatory responses. TNF-α is a pleiotropic cytokine with pivotal role in acute phase reaction and inflammatory cascade (Bruunsgaard et al., 2000). TNF-α causes endothelial dysfunction and vascular remodelling (Galis et al., 1994; Hotamisligil et al., 1996; Nawroth and Stern, 1986). The proinflammatory effects of TNF are mediated through NF-kB–regulated proteins, such as cytokines, chemokines, cyclo-oxygenase-2 (COX-2), and 5- lipoxygenase (5-LOX), adhesion molecules etc. The IL-1β and IL-18 belong to the same structural family (IL-1 family, or IL-F) (Mauviel, 1993). They have a similar 3D structure, and their respective precursors are activated on cleavage by the intracellular cysteine protease (Dinarello, 2011; Nakanishi et al., 2001). IL-1β stimulates proliferation of endothelial cells and vascular smooth muscle cells (Kim et al., 2010; Yang et al., 2012). It increases the expression of cell adhesion molecule on the endothelial cell surface (Yang et al., 2012), modifies the endothelium to promote coagulation and thrombosis (Dinarello, 1991). Interleukin-18 is a pleiotropic proinflammatory cytokine known to be involved in atherosclerotic plaque progression and rupture (Mallat et al., 2001). IL-18 in combination with IL-2 stimulates a Th2 response and a Th1 response when acts synergistically with IL-12 to produce IFN-g (Nakanishi et al., 2001) which has a proatherogenic effect.

The minor allele frequencies of the TNF-α, IL-18 and IL-1β SNPs as reported from earlier studies are 0.1% (Banday et al., 2016), 0.11%, 0.14% (Gupta et al., 2015) for TNF-α −308G/A, −1031T/C and −863C/A respectively; 0.19% (Birbian et al., 2013) for IL-18–137G/C; 0.25% (Daing et al., 2015), 0.54% and 0.57% (Bhat et al., 2014) for IL1β +3954C/T, −511C/T, and −31C/T respectively. Single SNP evaluation gives only a feeble idea of the risk associated with the genes while haplotypes gives a broader coverage of the gene and its impact on disease risk. Therefore in this study, we investigated the polymorphisms and carried haplotyping of TNF-α at positions −308G/A (rs1800629), −1031T/C (rs1799964), −863C/A (rs1800630); IL-18–137G/C (rs187238); lL-1β at positions +3954C/T (rs1143634), −31C/T (rs1143627) and −511C/T (rs16944) in coronary artery disease patients of Kashmir.

Materials and methods

Collection of blood samples

We carried out a case-control study to investigate the association between TNF-α, IL-1β and IL-18 polymorphisms and CAD in Kashmiri population. We recruited 200 unrelated cases with documented coronary artery disease. Age and sex-matched healthy controls (260) without any apparent diseases were selected randomly to compare with the patient data. All studies were carried out according to the declaration of Helsinki guidelines. The study protocol was approved by the institutional ethics committee, SKIMS, Soura. The characteristics of the study population are given in Table 1. Peripheral blood samples were collected in EDTA vial under sterile conditions.

Extraction of DNA

Genomic DNA was extracted from the blood samples by using the modified phenol-chloroform method of DNA extraction (Sambrook and Russell, 2001). Quality of extracted DNA was checked on 0.8% Agarose gel.

Table 1
Characteristics of the study population.

Parameters	Cases (200)	Controls (260)
Age		
>50 (%)	66%	69%
≤50 (%)	34%	31%
Gender		
Male (%)	78%	68%
Female (%)	22%	32%
Smoking status		
Smoker (%)	59%	–
Non-smoker (%)	41%	
Family history		
Yes (%)	83%	–
No (%)	17%	
ECG report		
NSTEMI[1] (%)	30%	–
STEMI[2] (%)	70%	
Diabetes		
Diabetic (%)	20%	–
Non diabetic (%)	80%	
Troponin T		
Positive (%)	32%	–
Negative (%)	68%	

Note: NSTEMI[1]: Non ST-elevated myocardial infarction; STEMI[2]: ST-elevated myocardial infarction.

Polymerase chain reaction

The genomic DNA isolated served as template and the region encompassing variants were amplified by specific reverse and forward primers given in Table 2. The genotyping of TNF-α and IL-1β was done by RFLP-PCR while that of IL-18 by Allele-specific primer PCR (AS-PCR). The Primers were designed by using Primer3, version 0.4.0 software. The reaction was carried out in a final volume of 25 μl reaction containing about 50 ng of genomic DNA, 0.5 ul of 10 mM deoxynucleotide triphosphate (dNTP) (Thermo Fischer Scientific Inc. EU, Lithuania), 0.1–1.0 mM of forward and reverse primers (Integrated DNA Technologies, Coralville, Lowa), 1.5 ul of 25 mM $Mgcl_2$, 2.5 ul of 10 X Taq Buffer and 1 U of Taq Polymerase (Thermo Fischer Scientific Inc. EU, Lithuania). The PCR conditions were as follows: initial denaturing at 97 °C for 4 min, then 35 cycles of denaturation at 94 °C for 30 s, primer-dependent annealing (Table 2), and extension for 1 min at 72 °C followed by a final extension step at 72 °C for 7 min. The reaction was performed in Applied biosystem 2320 thermal cycler. The amplicons were run on 2% ethidium-bromide stained agarose gel and checked under UV light. The size of amplicons corresponding to each of the SNP is given in Table 2.

Allele-specific PCR (AS-PCR)

In case of IL-18–137G/C, two complementary reactions were established for each allele along with the common reverse primer and two allele-specific primers, according to the original protocol used by Giedraitis et al. (2001). A control forward primer was used to amplify a 446-bp fragment covering the polymorphic site to serve as an internal positive amplification control.

Restriction fragment length polymorphism (RFLP)

The amplicons of TNF-α and IL-1β SNPs were subjected to restriction digestion by using SNP specific restriction enzymes. The list of restriction enzymes specific to SNPs is given in Table 2. All

Table 2
Primers, PCR conditions and genotyping.

SNP	Primer sequence/Amplicon size (bp)	Annealing (°C)	Restriction enzyme	Alleles corresponding to digestion products (bp)
TNF-α −308G/A				
F	5′- GAG GCA ATA GGT TTT GAG GGC CAT −3′	60 °C	*Nco*I	A/A; 97 and 20 bp
R	5′- GGG ACA CAC AAG CAT CAA G−3′			G/G; 117 bp
Amplicon	117bp			G/A; 117, 97, 20bp
TNF-α −1031T/C				
F	5′ – TAT GTG ATG GAC TCA CCA GGT −3′	63 °C	*Bbs*I	T/T; 251 bp
R	5′ CCT CTA CAT GGC CCT GTC TT −3′			C/C; 180 and 71 bp
Amplicon	251bp			T/C; 251, 180, 71bp
TNF-α −863C/A				
F	5′- GGC TCT GAG GAA TGG GTT AC-3′	59 °C	*Tai*	A/A; 104, 21 bp
R	5′- CTA CAT GGC CCT GTC TTC GTT ACG −3′			C/C; 125 bp
Amplicon	125bp			C/A; 125, 104 and 21bp
IL-18 −137G/C				
	5′- CCA ATA GGA CTG ATT ATT CCG CA 3′			
CF	5′- AGG AGG GCA AAA TGC ACT GG −3′	56 °C	–	–
CR	5′- CCC CAA CTT TTA CGG AAG AAA AAC −3′			
AC	5′ −CCC CAA CTT TTA CGG AAG AAA AAG −3′			
AG	261 bp specific to locus			
Amplicon	446 bp internal amplification control			
IL-1β +3954C/T				
F	5′-GTTGTCATCAGACTTTGACC-3′	59 °C	*Taq*I	CC; 136, 114 bp
R	5′-TTCAGTTCATATGGACCAGA-3′			TT; 250 bp
Amplicon	250bp			TC; 250, 136 114bp
IL-1β −31C/T				
F	5′-AGAAGCTTCCACCAATACTC-3′	59 °C	*Alu*I	TT; 137 and 102bp
R	5′-ACTAACTTTAGGGTGTCAG-5′			C/T; 239, 137, 102 bp
Amplicon	239bp			C/C; 239 bp
IL-1β −511C/T				
F	5′-TGGCATTGATCTGGTTCATC-3′	50 °C	*Dde*I	C/C; 160, 114, 32 bp
R	5′-GTTTAGGAATCTTCCCACTT-3′			C/T; 160, 146, 114, 32 bp
Amplicon	302bp			T/T; 60 and 146bp

the restriction enzymes were supplied by Thermo Fischer Scientific Inc. EU, Lithuania. A 10 ul of amplified products were digested with corresponding restriction enzymes in a total of 20 ul reaction volume containing 1 X buffer as supplied by the manufacturer. The reactions mixtures were incubated overnight at 37 °C and the restriction fragments were resolved on 3% ethidium-bromide stained agarose gel and visualized under UV. The restriction fragments corresponding to different alleles are given in Table 2.

Statistical analysis

The chi-square (χ^2) was used for assessment of categorical data and student's *t*-test for continuous variable analysis. Logistic regression analysis was performed to adjust age, gender, and other characteristics which significantly differed between the cases and controls in univariate analysis. Statistical analysis was carried out using the SPSS 20 software. Haplotype analysis and Linkage disequilibrium were performed by using the Iconcologia software (Catalan Institute of Oncology). The coefficient of linkage disequilibrium (D') >0.8 and a *P*-value of <0.05 was considered to be statistically significant.

Table 3
Allele frequency.

SNP	All subjects (%)	Cases (%)	Controls (%)
TNF-α −308G/A			
G	0.77	0.69	0.84
A	0.22	0.31	0.16
TNF-α −1031T/C			
T	0.73	0.82	0.69
C	0.27	0.18	0.31
TNF-α −863C/A			
C	0.82	0.74	0.93
A	0.18	0.26	0.07
IL-18 −137G/C			
G	0.55	0.55	0.56
C	0.45	0.45	0.44
lL-1β +3954C/T			
C	0.73	0.77	0.71
T	0.27	0.23	0.29
lL-1β −31C/T			
C	0.67	0.68	0.67
T	0.33	0.32	0.33
lL-1β −511C/T			
C	0.7	0.62	0.72
T	0.3	0.38	0.28

Results

The allele frequencies and genotypic distribution in the control and patient groups are shown in Tables 3 and 4. The Odds Ratios (ORs) for each genotype was tested as a three-class variable such as codominant, dominant and recessive models. In case of the TNF-α −308G/A (rs1800629) polymorphism we found G/A-A/A genotype to be associated with an increased risk of CAD in dominant model (OR = 0.43; 95% CI = 0.23–0.82; *P* = 0.0099). Its role in disease association is also clear from its allele frequency (0.31% in cases and 0.16% in controls). For −1031T/C (rs1799964) the frequency of heterozygous T/C was 18.3% in cases and 44.4% in controls. It was found to have a protective or an inverse association with the

Table 4
Association of SNPs with response status (adjusted for age and gender).

SNP	Model	Genotype	Cases	Controls	OR (95% CI)	*P*-value*
TNF-α −308G/A	Dominant	G/G G/A-A/A	54.3% 45.7%	72.1% 27.9%	1.00 0.43 (0.23–0.82)	0.0099
TNF-α −1031T/C	Over dominant	T/T-C/C T/C	81.7% 18.3%	55.6% 44.4%	1.00 4.08 (1.99–8.37)	<0.0001
TNF-α −863C/A	Recessive	C/C-A/C A/A	79.7% 20.3%	95.8% 4.2%	1.00 0.10 (0.03–0.38)	1e-04
IL-18 −137G/C	Over dominant	G/G-C/C G/C	27.5% 72.5%	17.4% 82.6%	1.00 1.78 (0.74–4.29)	0.2
IL-1β +3954C/T	Over dominant	C/C-T/T C/T	73.3% 26.7%	56.4% 43.6%	1.00 1.68 (0.83–3.41)	0.15
IL-1β −31C/T	Over dominant	C/C-T/T C/T	56.2% 43.8%	61.1% 38.9%	1.00 0.83 (0.43–1.59)	0.58
IL-1β −511C/T	Recessive	C/C-C/T T/T	75.3% 24.7%	90.1% 9.9%	1.00 0.32 (0.13–0.81)	0.018

Note: Chi square test (χ^2) test used for genotypic analysis and *P* value of <0.05 at 95% confidence interval considered to be statistically significant.

disease in an overdominant model (OR = 4.08; 95% CI = 1.99–8.37; $P < 0.0001$). The allele frequency of variant C was seen to be higher in controls (0.31%), than in cases (0.18%). For TNF-α −863C/A (rs1800630) the frequency of variant genotype A/A was 20.3% in cases and 4.2% in controls. It was found to have a significant association with the development of disease in recessive model (OR = 0.10; 95% CI = 0.03–0.38; $P = 0.0001$). The allele frequency of A was 0.26 in cases and 0.07 in controls. In case of IL-18–137G/C (rs187238) the frequency of variant genotype in cases and controls was 72.5% and 82.6% respectively. There was no significant association in an overdominant model (OR = 1.78, 95% CI = 0.74–4.29 and $P = 0.2$. For IL-1β +3954C/T (rs1143634) the frequency of variant genotype C/T was found to be 26.7% and 43.6% in cases and controls respectively. The allele frequencies were 0.23% and 0.29% in cases and controls respectively. No significant difference was seen in an overdominant model (OR = 1.68; 95% CI = 0.83–3.41; $P = 0.15$). For IL-1β −31C/T (rs1143627,) the frequency of variant genotype C/T in cases and controls was 43.8% and 38.9% respectively. There was no significant association in an overdominant model (OR = 0.83; 95% CI = 0.43–1.59; $P = 0.58$). Also, no significant difference between allele frequencies in cases (0.32%) and controls (0.33%) was found. In case of IL-1β −511C/T (rs16944), the frequency of homozygous variant T/T was found to be 24.7% and 9.9% in cases and controls respectively. A significant association was seen in recessive model (OR = 0.32; 95% CI = 0.13 −0.81; $P = 0.018$). A significant difference was seen in allele frequencies within cases (0.38%) and controls (0.28%).

Haplotype analysis revealed the haplotype GTCGTTT of TNF-α at positions −308 (rs1800629), −1031 (ra1799964), 863 (rs1800630), IL-18–137 (rs187238) and of IL-1β at positions +3954 (rs1143634), −31 (1143627) and −511 (16944) to be highly associated with CAD risk with a *P* value of 6e-04 followed by ATCGCC and GTACCTC with *P* values of 0.019 and 0.028 respectively as shown in Table 5. None of the polymorphic sites of the genes was in linkage disequilibrium with each other as shown by D' statistics in Table 6.

Coronary artery disease clinicopathological factors and TNF-α, IL-18 and IL-1β genotypes by logistic regression analysis

The TNF-α −308G/A was seen to be associated with diabetic status. The G/A – A/A genotype of TNF-α −308G/A polymorphic site was seen to be higher in non-diabetics with an OR of 3.52; 95% CI 1.54–8.07 and *P* value 0.0019. From the recessive model the C/C genotype of TNF-α −1031T/C was observed to be highly associated with the NSTEMI (non ST-elevated Myocardial infarction) with an OR 0.11; 95% CI 0.03–0.41 and *P* value 4e–04. Also, from the recessive model the C/C genotype of TNF-α −1031T/C was seen to be higher in the individuals having no family history of coronary artery disease, OR 4.46; 95% CI 1.35–14.81 and *P* value 0.018. From the recessive model, the C/C genotype of IL-18–137G/C was found to be higher in the individuals having no history of coronary artery disease with a *P* value of 0.005. From the overdominant model the C/T genotype of IL-1β + 3954 was seen to be higher in individuals with family history of CAD, OR 0.32; 95% CI 0.10–0.99 and *P* value 0.029. From the overdominant model the T/T genotype of IL-1β −511 was seen to be higher in individuals with family history of CAD, OR 0.31; 95% CI 0.10–0.95 and *P* value 0.024.

Discussion

Traditional risk factors have played an attributable role in the development of coronary artery disease however these conventional factors can only contribute 50% to the total risk for the development of CAD (Edmondson et al., 2013; Gibson et al., 1997;

Table 5
Haplotype association with response status (adjusted by Age + Gender).

TNF α −308G/A	TNFα −1031T/C	TNFα −863C/A	IL-18 −137G/C	IL-1β+3954C/T	IL-1β −31C/T	IL-1β −511C/T	Freq.	OR (95% CI)	*P*-value*
G	T	C	C	C	C	C	0.132	1.00	–
A	T	C	G	C	C	C	0.0371	0.03 (0.00–0.55)	0.019
G	T	C	G	T	T	T	0.0233	0.00 (0.00–0.03)	6e-04
G	T	A	C	C	T	C	0.0197	0.01 (0.00–0.56)	0.028

Note: OR – odds ratio; CI – Confidence interval. *$P < 0.05$, *P-value was computed by adjusting age and gender as covariates.

Table 6
Linkage disequilibrium by coefficient of Linkage disequilibrium (D').

	TNF-α −308G/A	TNF-α −1031T/C	TNF-α −863C/A	IL-18 −137G/C	IL-1β +3954C/T	IL-1β −31C/T	IL-1β −511C/T
TNF-α −308G/A	–	0.0259	0.024	0.1467	0.0329	0.1151	0.077
TNF-α −1031T/C	–	–	0.0218	0.0926	0.0098	0.1909	0.2276
TNF-α −863C/A	–	–	–	0.0828	0.0051	0.0234	0.4424
IL-18 −137G/C	–	–	–	–	0.0994	0.3009	0.0404
IL-1β + 3954C/T	–	–	–	–	–	0.4444	0.2437
IL-1β −31C/T	–	–	–	–	–	–	0.4469
IL-1β −511C/T	–	–	–	–	–	–	–

Note: D' value > 0.8, statistically significant.

Muhlestein, 2002; Nilsson et al., 2006; Ohira and Iso, 2013; Simon and Vijayakumar, 2013; Yusuf et al., 2004).

Genetic factors might contribute to the other half of the total risk factors, and many polymorphisms are considered to be associated with the onset and development of CAD (Lieb and Vasan, 2013; Lin et al., 2014; Simon and Vijayakumar, 2013; Wang et al., 2013; Wu et al., 2014; Xu et al., 2014). The new insights in understanding the role of inflammation in the pathogenesis of disease not only improve our understanding of the disease but also have clinical significance in risk stratification and designing of therapy for this menace of growing importance. The role of TNF-α −308G/A in the development of CAD is both contradictory and inconclusive. A meta-analysis and case-control conducted by Chu et al. (2012) in Asian and Caucasian showed no relation between TNF-α polymorphism and CHD/MI risk.

Our study showed a significant association of TNF-α −308G/A with the susceptibility to CAD. Our study is in accordance with that of Elahi et al. (2008) who showed 308A genotype to be related to more severe form of coronary atherosclerosis in British CAD patients. Also, in the Italian population a case-control study conducted by Szalai et al. (2002) identified TNF-α −308G/A associated with CHD/MI risk.

The TNF-α −308A allele has been found to be associated with higher inducible and constitutive TNF-α expression than TNF-α −308G allele (Abraham and Kroeger, 1999; Wilson et al., 1997). The reporter gene assay conducted by Wilson et al. (1997) showed the presence of TNF-α −308A allele within the extended MHC haplotype HLA-A1-B8-DR3-DQ2 which is responsible for high TNF-α production.

It has further been shown that this allele alters a transcription factor binding site by the formation of an altered composite transcriptional element. Thus, the binding of transcriptional factors to the −323 to −285 composite element is affected which is associated with the higher transcriptional activity of TNF-α gene (Abraham and Kroeger, 1999).

Moreover, TNF-α −308G/A showed a significant association with the development of CAD with much preponderance in non-diabetics.

For TNF-α −1031T/C our research findings showed an inverse role of T/C under an overdominant model. The TNF-α −1031C frequency was higher in controls (0.31%) than in cases (0.18%). Our results are in accordance to that of Oda et al. (2007) who reported TNF-α −1031C allele acts as a protective factor in atherosclerotic severity. The TNF-α −1031T/C showed an inverse or a protective role in the CAD development and was common in patients with NSTEMI (non ST-elevated myocardial infarction) and with those having family history of CAD.

We observed a significant difference in the TNF-α gene −863C/A polymorphism between the patients and control group ($P < 0.0001$). The −863A allele was more common in patients (0.26%) as compared to (0.07%) healthy controls. Higher frequency of the variant allele TNF-α −863A indicates a significant association of this polymorphism with CHD in the population studied. Our study is in agreement with that of Xiang et al. (2004) who also revealed a significant difference in the frequency of −863A allele between patients and controls. Some studies however reported a nonsignificant difference in the −863C/A genotype frequency between patients with CHD, myocardial infarction, and cardiomyopathy (Bennet et al., 2006; Koch et al., 2003; Liang et al., 2010). Also, TNF-α −863C/A was found to be strongly influencing the development of disease especially in individuals with no family history.

In case of IL-18–137G/C, no significant association was found between polymorphism and the overall risk of disease. The IL-18–137C allele frequency between cases (0.45%) and (0.44%) was non-significant. It does not contribute to the risk of CAD development as inferred from the co-dominant, dominant, overdominant and recessive models. Our study is in agreement with that of Thompson et al. (2007) and Hernesniemi et al. (2010a,b). The SNP was more common in individuals with no family history of the disease.

IL-1β +3954C/T polymorphism did not show any significant association with the disease. There was no significant difference in allele frequency between cases and controls 0.23% and 0.29% respectively. The meta-analysis conducted by Zhou et al. (2012) have also shown no association between IL-1β +3954C/T polymorphic site and Coronary Heart Disease Risk. In IL-1β −31C/T none of the genetic models confirmed its role in disease development. Also, the allele frequency of T in cases (0.32%) and controls (0.33%) was nonsignificant. Although various studies have highlighted the role of IL-1β −31C/T in the inflammatory responses and disease risk (Lind et al., 2005; Chen et al., 2006; Haukim et al., 2002) our study could not confirm the association. Both of the SNPs showed association with the development of disease in individuals with the family history of the CAD.

IL-1β −511T/C polymorphism showed a significant association with the coronary artery disease. The frequency of T allele was found to be 0.38% in cases and 0.28% in controls. Our study is in agreement with that of Dziedzic et al. (2005) conducted in Poland population. Moreover, the association of the IL-1β −511C/T and risk of MI and ischemic stroke at a young age has been found in Italian population (Iacoviello et al., 2005). The association of this SNP with coronary artery disease risk has been reported in African-Brazilians as well (Rios et al., 2010). The SNP was also found associated with the development of disease in individuals with family history.

Conclusions

Conclusively, the present findings emphasize the role of TNF-α −308A and −863A allele in the pathogenesis of coronary artery disease. The TNF-α −1031C allele showed an inverse relation with the disease and it seems to have a protective role against the development of disease in the Kashmiri population. IL-18–137C allele didn't show any association with the disease. IL-1β +3954 T and IL-1β −31T allele showed no contribution to the disease while IL-1β −511T allele showed significant association with the disease and are especially threatening in the individuals who are having

the family history of coronary artery disease. The haplotype GTCGTTT was found more common in the CAD individuals. These polymorphisms in synchrony with specific risk factors could serve as potential biomarkers for risk stratification and disease evaluation. The gene–environment interaction in specific populations could help in identifying the vulnerable populations. The observations need to be evaluated in a larger cohort for the better understanding of their role in the development of coronary artery disease at ethnicity level and for possible therapeutic strategies and prevention of its secondary evolution.

Conflict of interests

The authors have no conflicts of interests to declare.

Acknowledgements

The study was supported by a grant from the University grants commission (UGC) India.

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