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Estimation of Interleukin-1β Promoter (−31 C/T and −511 T/C) Polymorphisms and Its Level in Coronary Artery Disease Patients

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ABSTRACT

Interleukin-1β (IL-1β) is an inflammation-causing cytokine that exerts several unique biological effects and could lead to future adverse events of CAD. The piece of work presented herein is aimed at investigating possible association of IL-1β levels to its polymorphic site viz. −511 and −31 at promoter region in Saudi CAD patients. The study included 155 confirmed CAD patients and 80 healthy control individuals both men and women. Concentration of IL-1β in the patients' serum was measured by ELISA method. For single nucleotide polymorphism (SNP) analysis, sanger method of DNA sequencing was followed. We observed variable numbers of SNPs at −31 C/T and −511 T/C promoter regions in Saudi patients suffering from CAD in comparison to the control set of individuals. However, the changes in the number of SNP-hotspots were determined to be non-significant with reference to the control set. The haplotype analysis at −31 and −511 also did not show any significant changes between control and CAD patients. Moreover, serum IL-1β levels were observed to be expressively higher in patients suffering from CAD ($P < 0.001$) and its associated complications viz. STEMI ($P < 0.001$), NSTEMI ($P < 0.001$), and UA ($P < 0.001$). Our study provides the status of SNPs at IL-1β promoter in Saudi population. As per our information, ours is the first article that shows the genetic diversity in IL-1β promoters and its level in the Saudi CAD patients. J. Cell. Biochem. 9999: 1–6, 2017.

KEY WORDS: CORONARY ARTERY DISEASE; ELISA; INFLAMMATION; INTERLEUKIN-1β; SINGLE NUCLEOTIDE POLYMORPHISM

Coronary artery disease (CAD) is marked by progressive appearance of atherosclerotic plaques in the coronary arteries [Jabir and Tabrez, 2016]. The pathophysiological condition in atherosclerosis involves several metabolic process viz. lipid-accumulation and also accumulation of cells involved in the process of inflammation [Jabir et al., 2016b; Neelofar et al., 2016]. Despite the different demographic locations and lifestyle profiles of individuals all across the world, continuous rise in CAD cases have been reported in the scientific literature which suggests an important role of genetic factor(s) [Biswas et al., 2013]. Growing evidence also indicates the critical involvement of several pro-inflammatory cytokines in the CAD pathogenesis [Kofler et al., 2005; Briasoulis et al., 2016; Yin et al., 2017].

Interleukin-1β is a potential inflammation-causing cytokine. It is released predominantly by immune-derived cells associated with a variety of activities such as proliferation of endothelial cells, complement activation, and adhesion molecules expression on the arterial wall [Dinarello, 2011; Alfaidi et al., 2015]. Some studies also indicate the synthesis and expression of IL-1β in atherosclerotic coronary arteries within the endothelium [Galea et al., 1996; Chibana et al., 2017]. Moreover, in considerable amount of literature authors have discussed the role played by IL-1β with reference to atherosclerotic process and suggested it as a crucial cytokine that induced a network of signaling pathways [Tedgui and Mallat, 2006; Calkin and Tontonoz, 2010; Kim et al., 2010; Ait-Oufella et al., 2011]. Also, IL-1β has a significant say in several aspects of

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vascular-inflammation, such as recruitment of monocytes to the aortic intima, formation of neointima, and foam cells [Chamberlain et al., 2006]. The association of atherosclerosis and augmented risk of thrombo-embolic complications has also been attributed to several factors related to IL-1β [Singh et al., 2002].

The genetic disparity could affect pro-inflammatory gene regulation. This in turn could obviously influence the inflammatory responses and development of atherosclerotic lesions [Chen et al., 2006]. IL-1β is considered as candidate gene for polymorphism study because of their association with inflammatory responses and CAD. Earlier studies pointed possible association of IL-1β promoter polymorphism at −511 and −31 sites with CAD and associated conditions [Zhang et al., 2006; Oda et al., 2007; Rios et al., 2010]. However, the location of SNP-hotspots in IL-1β promoter is not consistent among the population of different racial and ethnic origins [Zeybek et al., 2011].

In this piece of work, we have attempted to explore the possible association of IL-1β levels and its polymorphic site viz. −511 and −31 at promoter region in the Saudi CAD patients. As stated in the abstract section, ours is the first article that shows the genetic diversity in IL-1β promoters and its level in the Saudi CAD patients.

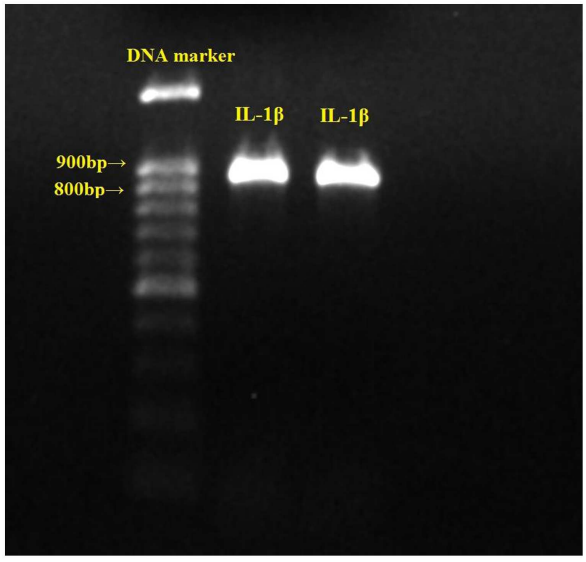

Fig. 1. A representative of 1% agarose gel photograph of IL-1β promoter. Lane A: DNA marker; Lanes B and C: PCR product of IL-1β promoter.

MATERIALS AND METHODS

The department of cardiology within King Abdulaziz University Hospital, Jeddah, Kingdom of Saudi Arabia was the site of this research work. A total of 155 confirmed CAD patients and 80 healthy control individuals were selected for this research-study after due endorsement from ethical committee of the concerned hospital. Informed consent was acquired from each of the individuals involved. The patients' selection criteria was followed as described earlier by us [Firoz et al., 2015].

SAMPLE COLLECTION

The blood samples were collected in EDTA coated and/or free tubes from selected cardiovascular patients and healthy control individuals. Serum separation was done by centrifugation-process at an acceleration of 2,000*g* performed for 5 min and this was stored at −80°C for further analysis.

DNA ISOLATION

DNA isolation was carried out from blood samples as described earlier by Jabir et al. [2016a].

POLYMERASE CHAIN REACTION (PCR)

Extracted DNA samples were amplified by conventional PCR method by the use of manually designed forward (5′ TGGCATTGATCTGGTTCATC 3′) and reverse (5′ AATAAGCCATCATTTCACTGGCGAG 3′) primers specific for IL-1β promoter. We performed PCR-amplification experiments as reported previously by our group [Jabir et al., 2016a]. Briefly, we used an annealing temperature of 58°C for PCR. The PCR products were visualized by running agarose gel (Fig. 1) and were stored at 4°C until next procedure.

DNA SEQUENCING AND ANALYSIS

The purification of PCR products was carried out by using PCR purification kit (Thermo Scientific). The subsequent positive DNA clones were sequenced and analyzed as described in Jabir et al. [2016a, 2017].

STATISTICAL ANALYSIS

Statistical analysis was performed by GraphPad Instat 3.05 (GraphPad Software Inc., San Diego, CA). The serum concentration of IL-1β in our studied population is reported as mean ± SD of duplicate value. Correlation of these variables among patients and control was determined by chi-square and two tailed *t*-test. The genotype of IL-1β promoter at −31 and −511 and allelic frequency were measured by the chi-square test. The comparison of the association of genotypes at −31 and −511 in IL-1β assembly were measured using Fisher's exact test (Two tailed). A probability of $P < 0.05$ was considered as a criterion of significance.

RESULTS

The basic and clinical characteristics of the individuals involved in the current study are summarized in Table I. Risk factors of CAD showed statistically significant association with age ($P < 0.05$) and gender ($P < 0.01$). Other biochemical risk markers viz. total cholesterol, low-density lipoprotein (LDL) cholesterol, and fasting blood glucose also showed an increase in the level from 3.84 to 4.16 ($P < 0.05$), 2.40 to 2.65 ($P < 0.05$), and 5.4 to 8.35 ($P < 0.001$), respectively. On the other hand, the triglycerides and high-density lipoprotein (HDL) cholesterol levels did not show any significant change compared to healthy control individuals.

TABLE I. Basic and Clinical Characteristics of Study Population

Parameter	Control individuals (n = 80)	CAD patients (n = 155)	*P*-value
Age (years)	47.0 ± 5	59.0 ± 10	<0.05
Male (n)	50	124	<0.01
Fasting glucose (mmol/L)	5.4 ± 0.47	8.35 ± 3.67	<0.001
Hb1Ac	4.55 ± 0.49	7.90 ± 1.79	<0.001
Total cholesterol (mmol/L)	3.84 ± 0.54	4.16 ± 1.11	<0.05
Triglycerides (mmol/L)	1.60 ± 0.29	1.75 ± 1.02	NS
LDL cholesterol (mmol/L)	2.40 ± 0.6	2.65 ± 0.79	<0.05
HDL cholesterol (mmol/L)	1.06 ± 0.19	1.06 ± 0.24	NS

Hb1Ac, glycosylated hemoglobin; HDL, high density lipoprotein; LDL, low density lipoprotein.

The analysis of IL-1β by ELISA method showed a significant rise in its level by 66% in CAD group compared with control individuals (4.31 vs. 2.59). Among the different forms of myocardial infarction (MI), ST-elevation myocardial infarction (STEMI), non ST-elevation myocardial infarction (NSTEMI), and unstable angina (UA) groups also showed a highly significant increase by 54%, 64%, and 100% in IL-1β level, respectively. Moreover, the highest pronounced increase in IL-1β level was recorded in UA group (Table II).

A total 650 bp upstream of IL-1β gene was targeted for variant analysis in Saudi population. We subjected 155 samples of CAD and 80 samples of healthy control individuals for PCR amplification and its sequencing. After pre-processing of trace files, we obtained 227 high quality sequences, and 8 trace files were completely rejected. Cumulatively, we obtained 177,468 bp sequence data with an average sequence length of 742 bp. The shortest sequence was of 104 bp and the longest high quality amplicon sequence was of 906 bp. The sequence obtained was analyzed for the existence of SNPs at two loci, that is, −511 T/C (rs16944) and −31 C/T (rs114627). Both loci were aimed to be covered in all the samples, however due to trimming of low quality sequences, −511 locus was covered in 152 CAD and 75 control samples whereas −31 locus was covered in 134 CAD and 57 control samples.

IL-1β −511 T/C (rs16944) locus was found to be covered in 20%, 44%, and 36% in TT, TC, and CC genotype, respectively, in control individuals and showed a similar ratio of genotypic distribution in CAD samples (Table III). This clearly suggests that there is no clear genotypic or allelic association with CAD for this locus at least in our studied population. Furthermore, high level of heterozygosity was recorded at this locus and TT genotype was in least percentage. Similarly, −31 C/T (rs114627) locus of IL-1β also showed polymorphisms in our samples but genotypic diversity observed in both control and CAD group was found to be similar (Table IV). At this site, CC was found to be the minor genotype in both group. The lesser percentage of CC genotype in control individuals compared with CAD group may be attributed to comparatively lesser number of control samples. Other two alternate genotypes, that is, TT and TC were recorded almost equally in this population (Table IV). As

TABLE II. Comparison of Serum IL-1β Level (pg/mL) Between Control and Different CAD Conditions

Group	n	IL-1β level (pg/mL)	*P*-value
Control	80	2.59 ± 0.62	
CAD patients	155	4.31 ± 1.40	<0.001
STEMI patients	64	3.98 ± 1.20	<0.001
NSTEMI patients	62	4.25 ± 1.42	<0.001
UA patients	29	5.19 ± 1.38	<0.001

STEMI, ST segment elevation myocardial infarction; NSTEMI, non ST segment elevation myocardial infarction; UA, unstable angina.

TABLE III. Distribution of Genotypic, Allelic Frequencies, and Carriage Rates of IL-1β at −511 T/C (rs16944) Among Control and CAD Patients

	Control individuals (%) (n = 75)	CAD cases (%) (n = 152)
Genotypic frequency		
TT	15 (20)	31 (20.39)
TC	33(44)	63 (41.44)
CC	27 (36)	58 (38.15)
P-value	0.93	
Allelic frequency		
T	63 (42)	125 (41.11)
C	87 (58)	179 (58.88)
P-value	0.85	
Odd's ratio (95%CI)	0.964 (0.648–1.434)	
Carriage rates		
T(+)	48 (64)	94 (61.84)
T(−)	27 (36)	58 (38.15)
P-value	0.75	
Odd's ratio (95%CI)	0.911 (0.514–1.618)	
C(+)	60 (80)	121 (79.60)
C(−)	15 (20)	31 (20.39)
P-value	0.94	
Odd's ratio (95%CI)	0.976 (0.490–1.945)	

TABLE IV. Distribution of Genotypic, Allelic Frequencies, and Carriage Rates of IL-1β at −31 C/T (rs114627) Among Control and CAD Patients

	Control individuals (%) (n = 57)	CAD cases (%) (n = 134)
Genotypic frequency		
CC	7 (12.28)	28 (20.89)
CT	26 (45.61)	53 (39.55)
TT	24 (42.10)	53 (39.55)
P-value	0.36	
Allelic frequency		
C	40 (35.08)	109 (40.67)
T	74 (64.91)	159 (59.32)
P-value	0.3	
Odd's ratio (95%CI)	1.268 (0.804–2.0)	
Carriage rates		
C(+)	33 (57.89)	81 (60.44)
C(−)	24 (42.10)	53 (39.55)
P-value	0.74	
Odd's ratio (95%CI)	1.112 (0.592–2.086)	
T(+)	50 (87.71)	106 (79.10)
T(−)	7 (12.28)	28 (20.89)
P-value	0.15	
Odd's ratio (95%CI)	0.530 (0.217–1.296)	

TABLE V. IL-1β Promoter Compound Genotype and Haplotype Frequency Among Control and CAD Patient

	Promoter site						
	−511	−31	Control individuals (%)	CAD cases (%)	OR[a]	CI	*P**-value
Genotypic frequency							
	TT	CC	7 (12.7)	28 (21.4)	1		
	TT	CT	0	1 (0.8)	0.7	(0.02–21.4)	0.22**
	TT	TT	1 (1.8)	1 (0.8)	0.2	(0.01–4.5)	0.39**
	TC	CT	24 (43.6)	47 (35.9)	0.5	(0.2–1.3)	0.14
	TC	TT	3 (5.5)	6 (4.6)	0.5	(0.1–2.5)	0.4**
	CC	CT	1 (1.8)	2 (1.5)	0.5	(0.04–6.3)	0.518**
	CC	TT	19 (34.5)	46 (35.1)	0.6	(0.2–1.6)	0.31
Total			55	131			
Haplotype							0.35***
	T	C	38 (34.55)	104 (39.69)	1		
	T	T	5 (4.55)	9 (3.44)	0.6	(0.2–2.1)	0.533**
	C	T	66 (60)	147 (56.1)	0.8	(0.5–1.3)	0.39
	C	C	1 (0.91)	2 (0.76)	0.7	(0.06–8.2)	1**
Total			110	262			

OR, odd ratio; CI, confidence interval.
[a]OR using the −511 T/T with −31 C/C as the reference group (OR = 1) in the analysis of genotype and the −511 T with −31 C as the reference group in the analysis of haplotype.
*P-value for chi-square test, unless otherwise labeled.
**P-value for Fisher's exact test.
***P-value for chi-square test for trend.

diversity of genotype in both group was found to be similar, no significant association ($P = 0.36$) was recorded at this locus. We also analyzed the compound genotype and haplotype distribution of IL-1β promoter at −31 and −511 in both CAD patients and control sets (Table V). The compound genotype and haplotype distribution also showed non-significant changes.

DISCUSSION

CAD pathogenesis has been implicated with multiple genetic and inflammatory pathways that modulate various inflammation-causing cytokines especially at different stages of atherosclerosis [Zakynthinos and Pappa, 2009]. Genetic association of IL-1β with different pathological conditions of CAD have also been suggested in the literature [Zhang et al., 2006; Oda et al., 2007; Rios et al., 2010; Rai et al., 2016]. In the present study, we observed some differences in the genotypic and allelic distribution in IL-1β promoters between CAD and control individuals however the diversity did not show any statistically significant correlation.

The present study also showed a significant rise in the IL-1β serum level in CAD patients and other MI conditions (Table II). Our findings are in agreement with previous reports that suggest promotion of cytokine-mediated inflammatory reactions in CAD [Hasdai et al., 1996; Tsimikas et al., 2014]. Increased level of IL-1β in CAD and other MI events noted by us also suggests its potential role in post-apoptotic necrosis and accelerated atherosclerosis.

The −511 T/C (rs16944) polymorphism in IL-1β promoter has been associated with several disease phenotype associated with inflammatory component [Hall et al., 2004]. Their association had been reported with various pathological conditions such as *Helicobacter pylori*-induced chronic hypochlorhydric response, gastric cancer, Alzheimer's, and meningococcal disease [Read et al., 2000; Hwang et al., 2002; Payão et al., 2012]. Moreover, IL-1β variants and its association have also been reported with type 2 diabetes (an important co-expressing CAD pathology) in different ethnic populations [Banerjee and Saxena, 2014]. On the other hand, there are also conflicting reports related with the expression pattern of IL-1β gene variants in CAD patients [Oda et al., 2007; Rios et al., 2010; Bashour et al., 2013]. Zhang et al. [2006] reported the association of IL-1β promoter polymorphism at −511 C/T site with the severity of CAD, and also suggested their possible role in the secretion of IL-1β and aggravation of inflammation and dyslipidemia [Zhang et al., 2006]. In one study, Oda et al. [2007] also reported −511 T allele as a risk factor for atherogenesis in the Japanese population [Oda et al., 2007]. Furthermore, Rios et al. [2010] reported the presence of more prevalent CC genotype at −511 position and suggested a twofold increase in the risk of CAD among African-Brazilians population [Rios et al., 2010]. Higher percentage of CC genotype and their association with CAD has also been reported in Japanese population [Momiyama et al., 2001]. However, we did not record any significant association at polymorphic sites −31C/T (rs114627) and −511 T/C (rs16944) with CAD in Saudi population (Tables III and IV). Our study results are in agreement with the previous findings in different ethnic populations [Miranda-Malpica et al., 2008; Rios et al., 2010; Gorący et al., 2011; Bashour et al., 2013].

Discrepancies between studies might be because of different patterns of linkage disequilibrium between this polymorphism and other functional mutations in the IL-1β gene that can differ among different ethnic groups. Environmental factors could also affect the gene expression pattern which strongly depends on the ethnicity and regional location [Tabassum et al., 2013]. Therefore, we recommend further investigations to envisage the presence of functional mutations in IL-1β gene.

CONCLUSION

The piece of work presented herein reports the significant rise in IL-1β serum level with CAD and other MI complications. Although, we observed variable number of SNPs at both the sites but the changes were found to be non-significant. Future studies on larger sample size are suggested to establish the exact role of IL-1β in pathogenesis of CAD at genetic level.

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