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ORIGINAL ARTICLE

Possible association of interleukin-1beta (-511C/T) and interleukin-6 (-174G/C) gene polymorphisms with atherosclerosis in end stage renal disease Egyptian patients on maintenance haemodialysis

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Abstract In end stage renal disease, inflammation is considered a critical regulator of atherosclerotic plaque formation and progression, to which many dialysis and non-dialysis-related factors may contribute. Since circulating inflammatory cytokine levels vary inter-individually, one may speculate that genetic factors, such as polymorphisms in genes encoding them, may be involved in determining the individual inflammatory reaction in response to a given insult. The present work aimed to study interleukin-1B (-511C/T), and interleukin-6 (-174G/C) gene polymorphisms and their possible association with atherosclerosis in Egyptian patients with end stage renal disease on maintenance haemodialysis. The present study was conducted on 100 Egyptian subjects, the control group (n = 30) and the patient group (n = 70) with end stage renal disease on maintenance haemodialysis which were further subdivided into two subgroups with (n = 33) and without atherosclerosis (n = 37) as evidenced by CIMT, ECG ischaemic changes, cerebrovascular insufficiency (CVI), and peripheral vascular insufficiency (PVI). All studied subjects were subjected to detailed history taking, routine laboratory investigations and molecular studies including detection of IL-1B (-511C/T) and IL-6 (-174G/C) gene polymorphisms using the Polymerase chain reaction/Restriction fragment length polymorphism (PCR/RFLP) technique. The genotype distribution and allele frequency of IL-1B (-511C/T) and IL-6 (-174G/C) showed no statistical significant

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difference among the studied groups. To conclude the development of atherosclerosis among Egyptian patients on maintenance haemodialysis cannot be attributed to these two gene polymorphisms.

1. Introduction

In end stage renal disease (ESRD), endothelial dysfunction and atherosclerosis are almost universal [1]. Patients with ESRD especially those treated with haemodialysis (HD) are at increased risk for both morbidity and mortality from cardiovascular diseases [2]. In HD patients, the high prevalence of traditional cardiovascular risk factors contributes to the excessive risk of cardiovascular diseases. Nevertheless, studies focused on non-traditional risk factors, such as oxidative stress and inflammation in ESRD [3,4].

Inflammation is considered as a critical regulator of atherosclerotic plaque formation and progression leading to the fatal clinical endpoints; myocardial infarction, stroke, gangrene and sudden cardiac death [5]. Many uraemic patients show serological evidence of an activated inflammatory response, as indicated by increased circulating levels of non-specific markers of inflammation such as C-reactive protein (CRP) and pro-inflammatory cytokines such as IL-1 and IL-6 [4]. It was found that IL-1B plays an important role in the pathogenesis of atherosclerosis [6–8]. In addition interleukin-6 (IL-6) is associated with endothelial damage and initiation of atherosclerotic events [9]. Its expression and secretion are regulated by IL-1 and TNF-α, which are highly induced in the atherosclerotic plaque [10].

In ESRD, it seems conceivable that both dialysis and non-dialysis-related factors may contribute to a state of chronic inflammation [11]. Since circulating cytokine levels vary considerably inter-individually, one may speculate that genetic factors, such as polymorphisms in genes encoding them, may be involved in determining the individual inflammatory reaction in response to a given insult [12]. Many epidemiological studies have investigated the association between atherosclerosis and inflammatory cytokine gene polymorphisms [13,14]. One important single nucleotide polymorphism (SNP) is IL-1B -511(C > T) in which the cytosine residue is substituted by thymine at position -511 at the promoter region of IL-1B gene [15]. In addition, many SNPs were determined in the 5′ flanking region of the IL-6 gene. One of these SNPs is -174G/C in which the guanine residue is substituted by cytosine at position -174 [16].

2. Aim of this work

The present work was designed to study interleukin-1 beta (-511C/T), and interleukin-6 (-174G/C) gene polymorphisms and their possible association with atherosclerosis in Egyptian patients with end stage renal disease on maintenance haemodialysis.

3. Subjects and methods

Subjects: After the approval of the Ethics Committee of the Medical Research Institute, informed consents were taken from all subjects who participated in the study.

The present study included one hundred subjects, divided into two main groups; **Control group:** which included 30 apparently healthy volunteers of comparable age and socioeconomic status to the patient group; 13 males (43.3%) and 17 females (56.7%), their mean age was 43.2 ± 10.1 years and **Patient group:** which included seventy patients with end stage renal disease on maintenance haemodialysis for more than 6 months, (35 males (50%) and 35 females (50%)), their mean age was 47.8 ± 11.7 years and their duration of dialysis ranged from 12 months to 192 months with median 47.5 months.

They were selected from the Nephrology Unit of Medical Research Institute, Alexandria University. The dialysis sessions for these patients were three times weekly and the duration range of each session was four hours. A standard dialysis prescription using a cuprophane hollow fibres dialyser was adopted. The patients were further subdivided according to the presence of atherosclerosis as evidenced by carotid intima media thickness measurement (CIMT), 12 leads standard electrocardiography (ECG), cerebrovascular insufficiency (CVI), and peripheral vascular insufficiency (PVI) into two subgroups: Group A included 33 patients (18 (54.5%) males and 15 (45.5%) females) without atherosclerosis; their mean age was 43.6 ± 11.9 years and group B included 37 patients (17 (45.9%) males & 20 (54.1%) females) with atherosclerosis, their mean age was 51.5 ± 10.4 years. Both groups were exposed to comparable haemodialysis conditions.

Both groups were exposed to detailed history with special emphasis on age and duration of dialysis and thorough physical examination with special stress on the measurement of arterial blood pressure, and body mass index. Following an overnight fasting period and immediately before dialysis session, 10 mL whole venous blood were withdrawn from each

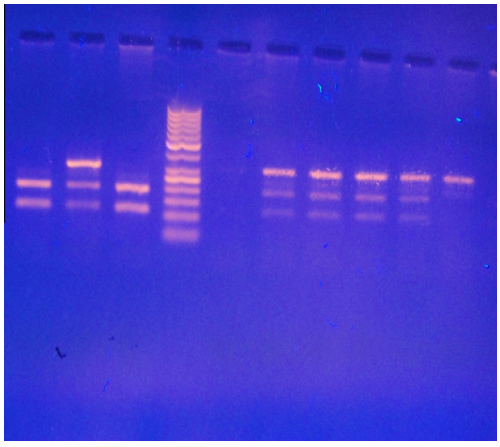

Figure 1 Agarose gel electrophoresis of PCR products digested with AvaI restriction enzyme. **Lane 4:** 50 bp DNA Ladder (50–1000 bp). **Lane 1, 3:** TT homozygote alleles (bands at 114 bp and 190 bp). **Lane 2, 6, 7, 8, 9:** CT heterozygote alleles (bands at 114 bp, 190 bp, and 304 bp). **Lane 10:** CC homozygote alleles (band at 304 bp).

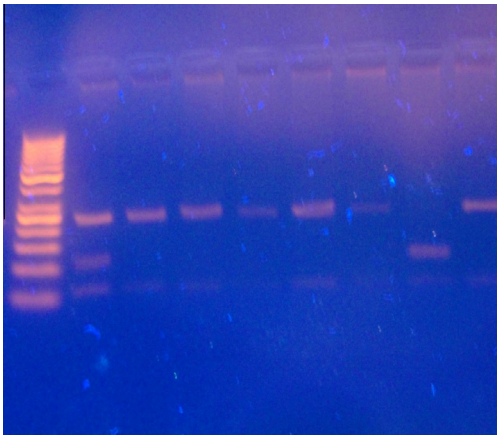

Figure 2 Agarose gel electrophoresis of PCR products digested with N1aIII restriction enzyme. **Lane 1:** 50 bp DNA Ladder (50–1000 bp). **Lane 2:** GC heterozygote alleles (bands at 54 bp, 111 bp, 122 bp and 233 bp). **Lane 3, 4, 5, 6, 7, 9:** GG homozygote alleles (bands at 54 bp and 233 bp). **Lane 8:** CC homozygote alleles (bands at 54 bp, 111 bp and 122 bp).

subject before heparinization of the line and before the patient being connected to the dialysis machine.

One mL of whole blood was mixed with 50 uL of 3.8% Ethylene Diamine Tetraacetic Acid (EDTA) for molecular studies and the rest 9 mL whole blood was left to clot and the obtained serum was used for determination of the concentrations of the traditional analytes for all subjects using an automatic auto analyser (Olympus, Germany) including fasting serum glucose, creatinine, triglycerides, cholesterol (total, high and low density lipoprotein fractions), albumin and C-reactive protein levels. Molecular studies for detection of IL-1B (-511C/T) and IL-6 (-174G/C) gene polymorphisms were done to all subjects using the Polymerase chain reaction/Restriction fragment length polymorphism (PCR/RFLP) technique and included DNA extraction from peripheral blood leucocytes using Gene JET™ Genomic DNA purification Kit (Fermentas – Thermo, USA). Purity of extracted genomic DNA was detected by agarose gel electrophoresis of the yield on 1% agarose gel. In addition, the genomic DNA concentration was measured on a nanodrop 1000 spectrophotometer (Thermo scientific-USA) at 260 and 280 nm. The mean concentration of the purified genomic DNA was 20.56 ng/uL. After extraction, IL-1gene

Table 1 Some clinical data of different studied groups.

	$\overline{X} \pm SD$	Range median				*P*-value
		Control group (n = 30)	Group A (n = 33)	Group B (n = 37)	Total patients (n = 70)	
Age (years)	$\overline{X}$	43.2	43.6	51.5	47.8	$P_2 = 0.002^{**}$
	±SD	10.1	11.9	10.4	11.7	$P_4 = 0.003^{**}$
Sex	Females	17 (56.7%)	15 (45.5%)	20 (54.1%)	35 (50%)	0.641
	Males	13 (43.3%)	18 (54.5%)	17 (45.9%)	35 (50%)	
Duration of dialysis (months)	Range	–	24–192	12–180	12–192	0.829
	Median	–	48	42	47.5	
Weight (kg)	$\overline{X}$	63.1	62.9	67.3	65.2	0.146
	±SD	6.9	10.2	12.6	11.7	
Height (m)	$\overline{X}$	164	160.7	161	160.9	0.301
	±SD	0.88	0.71	0.17	0.91	
BMI (kg/m^2)	$\overline{X}$	23.4	24.4	25.8	25.2	$P_2 = 0.003^{**}$
	±SD	0.4	3.9	3.9	3.9	$P_3 = 0.016^{*}$
SBP (mmHg)	$\overline{X}$	114	121.5	121.9	121.7	$P_1 = 0.047^{*}$
	±SD	6.7	16.9	16.5	16.6	$P_2 = 0.032^{*}$
						$P_3 = 0.018^{*}$
DBP (mmHg)	$\overline{X}$	74.8	78.8	78.6	78.7	$P_3 = 0.029^{*}$
	±SD	4.9	9.3	8.9	8.9	
MBP (mmHg)	$\overline{X}$	87.9	93	93.1	93	$P_1 = 0.044^{*}$
	±SD	4.9	11.5	11.1	11.3	$P_2 = 0.037^{*}$
						$P_3 = 0.019^{*}$
CIMT (mm)	Range	0.1–0.5	0.3–0.7	0.8–1.4	0.3–1.4	$P_1 = 0.000^{**}$
	Median	0.3	0.5	1.0	0.8	$P_2 = 0.000^{**}$
						$P_3 = 0.000^{**}$
						$P_4 = 0.000^{**}$

Group A: Patients with ESRD on maintenance haemodialysis without atherosclerosis.
Group B: Patients with ESRD on maintenance haemodialysis with atherosclerosis.
n: Number of subjects.
P_1: Statistical significance between the control group and group A.
P_2: Statistical significance between the control group and group B.
P_3: Statistical significance between the control group and total patient group.
P_4: Statistical significance between group A and group B.
F = Female , M = Male.
BMI = Body mass index; SBP = Systolic blood pressure; DBP = Diastolic blood pressure; MBP = Mean blood pressure; CIMT = Carotid intima media thickness.
* Significant at $P < 0.05$.
** Highly significant at $P < 0.01$.

promoter region was amplified using primers [17](1) [F5-TGG CAT TGA TCT GGT TCA TC-3 (forward), R5-GTT TAG GAA TCT TCC CAC TT-3 (reverse)] and IL-6 1gene promoter region was also amplified using primer [18](2) [F5-TTG TCA AGA CAT GCC AAA GTG-3 (forward), R5-TCA GAC ATC TCC AGT CCT ATA-3 (reverse)]. Amplifications were

Table 2 Some biochemical data of different studied groups.

	$\overline{X} \pm SD$	Range median				*P*-value
		Control group ($n = 30$)	Group A ($n = 33$)	Group B ($n = 37$)	Total patients ($n = 70$)	
FSG (mg/dl)	$\overline{X}$	81.5	106.2	154	131.5	$P_2 = 0.000^{**}$
	±SD	8.9	36.2	79.8	67.2	$P_3 = 0.000^{**}$
						$P_4 = 0.000^{**}$
Creatinine (mg/dl)	$\overline{X}$	0.8	10.3	9.2	9.7	$P_1 = 0.000^{**}$
	±SD	0.2	2.7	2.8	2.8	$P_2 = 0.000^{**}$
						$P_3 = 0.000^{**}$
Albumin (g/dl)	$\overline{XX}$	4.2	3.8	3.8	3.8	$P_1 = 0.000^{**}$
						$P_2 = 0.000^{**}$
						$P_3 = 0.000^{**}$
	±SD	0.4	0.4	0.3	0.4	
CRP (mg/L)	Range	1.0–4.7	1.0–22	1.1–23	1.0–23	$P_1 = 0.009^{**}$
	Median	2.5	2.8	4.2	3.5	$P_2 = 0.000^{**}$
						$P_3 = 0.000^{**}$
TG (mg/dl)	Range	68–149	71–389	60–399	60–399	$P_1 = 0.017^{*}$
	Median	106	140	183	161.5	$P_2 = 0.000^{**}$
						$P_3 = 0.000^{**}$
						$P_4 = 0.020^{*}$
TC (mg/dl)	Range	120–190	115–238	98–311	98–311	$P_2 = 0.004^{*}$
	Median	158	163	169	168	$P_3 = 0.022^{*}$
						$P_4 = 0.040^{*}$
HDL-C (mg/dl)	Range	41–67	19–53	16–53	16–53	$P_1 = 0.000^{**}$
	Median	55	30	32	31	$P_2 = 0.000^{**}$
						$P_3 = 0.000^{**}$
LDL-C (mg/dl)	Range	54–96	57–157	43–194	43–194	$P_1 = 0.002^{**}$
	Median	82	86.4	103	99	$P_2 = 0.000^{**}$
						$P_3 = 0.000^{**}$

Group A: Patients with ESRD on maintenance haemodialysis without atherosclerosis.
Group B: Patients with ESRD on maintenance haemodialysis with atherosclerosis.
n: Number of subjects.
P_1: Statistical significance between the control group and group A.
P_2: Statistical significance between the control group and group B.
P_3: Statistical significance between the control group and total patient group.
P_4: Statistical significance between group A and group B.
FSG = Fasting serum glucose CRP = C-reactive protein; TG = Triglycerides TC = Total cholesterol; HDL-C = High density lipoprotein fractions; LDL-C = Low density lipoprotein fractions
* Significant at $P < 0.05$.
** Highly Significant at $P < 0.01$.

Table 3 Genotype frequencies of IL-1B -511(C > T) polymorphism among the studied groups.

Genotype	*n*	Control group ($n = 30$)	Group A ($n = 33$)	Group B ($n = 37$)	Total patients ($n = 70$)
	%				
CC	*n*	12	12	10	22
	%	40	36.4	27	31.4
CT	*n*	15	20	21	41
	%	50	60.6	56.8	58.6
TT	*n*	3	1	6	7
	%	10	3	16.2	10
Chi-square *p*-value	4.662				
	0.188				

Group A: Patients with ESRD on maintenance haemodialysis without atherosclerosis.
Group B: Patients with ESRD on maintenance haemodialysis with atherosclerosis.
n: Number of subjects.
$P < 0.05$ is significant.

Table 4 IL-1B -511(C > T) allele frequency among the studied groups.

Allele	*n* %	Control group ($n = 30$)	Group A ($n = 33$)	Group B ($n = 37$)	Total patients ($n = 70$)
C	*n*	39	44	41	85
	%	65	66.7	55.4	60.7
T	*n*	21	22	33	55
	%	35	33.3	44.6	39.3
Chi-square	2.21				
P-value	0.332				

Group A: Patients with ESRD on maintenance haemodialysis without atherosclerosis.
Group B: Patients with ESRD on maintenance haemodialysis with atherosclerosis.
n: Number of subjects.
$P < 0.05$ is significant.

done through polymerase chain reaction (PCR) cycles (95 °C, 3 min) × 1; (95 °C, 30 s, 55 °C, 30 s, 72 °C, 60 s) × 37; (72 °C, 10 min) × 1, using DreamTaq™ Green PCR Master Mix (Fermentas – Thermo, USA). Restriction digestion of PCR products was done using FastDigest®AvaI enzyme (Fermentas – Thermo, USA) for IL-1B (-511C/T). The digestion products were subjected to 2.5% agarose gel electrophoresis and showed that allele C which did not contain the AvaI restriction enzyme site remained undigested as 304 bp fragments, whereas allele T yields 190 bp and 114 bp fragments (Fig. 1). As regards the detection of IL-6 (-174G/C) gene polymorphism, FastDigest® N1a III restriction enzyme (Fermentas – Thermo, USA) was used. The digestion products were subjected to 2.0% agarose gel electrophoresis and showed that allele G which did not contain the N1a III restriction enzyme site was digested to 233 bp and 54 bp fragments, whereas allele C yielded 122 bp, and 111 bp together with the 54 bp fragment (Fig. 2).

4. Statistical analysis [19]

Statistical assessment was carried out with Statistical Package for Social Sciences (SPSS) version 17.0 for Windows statistical software [20]. Quantitative variables were tested for normality using Kolmogorov–Smirnov test. Data revealed normal distribution was represented as mean ± standard deviation, and the data revealed deviation from normal distribution was represented as median and range.

Analysis of variance (ANOVA) or *F*-test is used for comparison between more than two means when the data are normally distributed. Kruskal–Wallis was used for testing equality of population medians among groups. Mann–Whitney (U) and Chi-square (X^2) tests were used. Allele and genotype frequencies among cases and controls were compared with Hardy–Weinberg predictions using X^2 analysis. *P* Value of less than 0.05 was considered statistically significant.

5. Results

The results of some clinical and biochemical data of the different studied groups are included in Tables 1 and 2. It could be noticed that there was no significant difference between any of the groups regarding sex and there were no significant differences between group A and group B regarding duration of dialysis. However age and CIMT, FSG, TG, TC were significantly higher in group B than group A. As regards CRP, it was significantly higher in total patient group than the control group ($p = 0.000^*$).

In group B, 32 (86.5%) patients had ECG changes of ischaemic heart diseases, 5 (13.5%) patients had cerebrovascular insufficiency, 3 (8.1%) patients had peripheral vascular insufficiency and 11 (29.7%) patients had atherosclerotic plaques. None of the observed genotype frequencies deviated from the Hardy–Weinberg equilibrium. There was no significant difference in IL-1B (-511) genotypes and its allele frequency distributions among the studied groups ($X^2 = 4.662$, $P = 0.188$) (Table 3) ($X^2 = 2.21$, $P = 0.332$) (Table 4), respectively.

In the present study, no significant correlation was found between IL-1B (-511C > T) variants and the different studied parameters (Table 5).

Also there was no significant difference in IL-6 (-174) genotypes and its allele frequency distributions among the studied groups ($X^2 = 2.689$, $P = 0.611$) (Table 6) ($X^2 = 2.01$, $p = 0.365$) (Table 7), respectively.

No significant correlation was found between IL-6 (-174G/C) variants and the different studied parameters (Table 8).

6. Discussion

Chronic kidney disease is at least 3–4 times more frequent in Africa than in developed countries [21], and in Egypt, the prevalence of dialysis patients is presumed to be increasing [22]. Many risk factors and metabolic alterations observed in the uraemic milieu may contribute to the excessive risk of atherosclerosis in dialysis patients [23]. Many epidemiological studies have investigated the association between atherosclerosis and inflammatory cytokine gene polymorphisms. However, it remains to be seen whether or not the genetic factors for these inflammation-related mediators underlie atherogenesis [24]. We studied the possible association between interleukin-1 beta (-511C/T), and interleukin-6 (-174G/C) gene polymorphisms and atherosclerosis in end stage renal disease Egyptian patients on maintenance haemodialysis.

In the present study no significant difference was observed in the genotype distribution of IL-1B (-511) among the studied groups. In addition, no significant difference was observed in the frequency of the IL-1B (-511) alleles among the studied groups. These were in agreement with Arman et al. [15] who did not find any association in either allele frequency or

Table 5 Comparison of the measured variables between different genotypes of IL-1B -511(C > T) polymorphism.

Variables	Genotypes	*F*	Sig.
Weight	CC	0.997	0.373
	CT		
	TT		
Height	CC	1.169	0.315
	CT		
	TT		
BMI	CC	0.848	0.431
	CT		
	TT		
SBP	CC	0.827	0.440
	CT		
	TT		
DBP	CC	0.177	0.838
	CT		
	TT		
MBP	CC	0.450	0.639
	CT		
	TT		
CIMT	CC	2.071	0.132
	CT		
	TT		
FSG	CC	0.622	0.539
	CT		
	TT		
Creatinine	CC	0.139	0.870
	CT		
	TT		
Albumin	CC	0.339	0.713
	CT		
	TT		
CRP	CC	1.237	0.295
	CT		
	TT		
TG	CC	.196	0.822
	CT		
	TT		
TC	CC	0.214	0.808
	CT		
	TT		
HDL-C	CC	0.173	0.841
	CT		
	TT		
LDL-C	CC	0.304	0.738
	CT		
	TT		

BMI = Body mass index; SBP = Systolic blood pressure; DBP = Diastolic blood pressure; MBP = Mean blood pressure; CIMT = Carotid intima media thickness; FSG = Fasting serum glucose; CRP = C-reactive protein; TG = Triglycerides; TC = Total cholesterol; HDL-C = High density lipoprotein fractions.LDL-C = Low density lipoprotein fractions.
$P < 0.05$ is significant.

genotype distribution of IL-1B polymorphisms between a single vessel disease atherosclerosis group and the control group or multiple vessel disease group and controls or coronary artery disease and the control groups in the Turkish population. He concluded that those polymorphisms may modulate some of processes for the development of CAD. Zee et al. [25], in their study on seven gene polymorphisms within the interleukin-1 gene cluster among American men who subsequently developed athero-thrombotic events found that IL-1B -511C/T polymorphism was not associated with risk of athero-thrombotic disorders, and after further adjustment for traditional cardiovascular risk factors they yielded similar null findings.

The current work was also consistent with previous findings of Li et al. [26] who concluded the same results in Chinese population. In addition, Ye et al. [27] in their meta-analysis which enhances the statistical power and draws a more reliable conclusion compared to a single study indicated that there was no association between IL-1B -511C/T polymorphism and atherosclerosis and ischaemic stroke risk. Interestingly, when subgroup analyses for countries were performed, the results indicated that T allele was associated with increased risk of atherosclerosis and ischaemic stroke for Polish and associated with a trend of increased risk in Chinese population so they concluded the same results but inferred that IL-1B -511C/T polymorphism might be moderately associated with increased risk of atherosclerosis and ischaemic stroke [28]. In contrast, the data presented by Iacoviello et al. [29] indicated that the IL-1B (-511C/C) genotype was associated with myocardial infarction and ischaemic stroke at young age and increased the response of mononuclear cells to inflammatory stimulation. Also Soylu et al. [30] found that IL-1B allele C (-511) polymorphism in unstable angina pectoris patients was significantly different from that of control subjects in the Turkish population. In another controversial study, Zhang et al. [31] found that the distribution of IL-1B -511C/T polymorphism between ACS in the Chinese patients and controls was significantly different and CT and TT genotype carriers were at increasing risk of ACS with more than double ratio to CC genotype. He concluded that the polymorphism at position -511C/T in IL-1B is associated with the severity of coronary heart disease (CHD), and the DNA variation at this position may affect the secretion of IL-1B, and aggravate the reaction of inflammation and dyslipidemia. Also Oda et al. [24] found that -511T of IL-1beta was a significant risk factor for atherogenesis in the subclavian and intracranial arteries. In contrast, conventional risk factors for atherogenesis, such as hypertension and diabetes mellitus, conferred independent risks for almost all arteries. He concluded that functional SNPs in IL-1beta gene may play a role in atherogenesis, although their influences are less pronounced than those of conventional risk factors and appear to be limited to specific arteries in the elderly Japanese.

In the present study, no significant relation was found between IL-1B (-511C > T) variants and the different studied parameters. Another study in patients with end stage renal disease reported that males carrying IL-1B (-511T/T) showed a higher body mass index [32]. In addition, patients with hypertensive CHD carrying IL-1B -511C/T or T/T showed reduced CRP levels compared to those carrying IL-1B -511C/C [33].

Atherosclerosis is a multifactorial process determined by several genes, environmental factors and their interaction. It is characterized by chronic inflammation on the inner layer of the arterial wall, and several cytokines are related to the arterial wall inflammatory process [34]. It has been shown that IL-6 has strong predictivity on cardiovascular mortality than CRP in haemodialysis patients [35,36], and that IL-6 (G-174C) gene polymorphism modifies IL-6 production in non-renal and in renal patients [37,38].

Table 6 Genotype frequencies of IL-6 -174(G > C) polymorphism among the studied groups.

Genotype	*n* %	Control group ($n = 30$)	Group A ($n = 33$)	Group B ($n = 37$)	Total patients ($n = 70$)
GG	*n*	21	23	31	54
	%	70.0	69.7	83.8	77.1
GC	*n*	8	9	5	14
	%	26.7	27.3	13.5	20
CC	*n*	1	1	1	2
	%	3.3	3.0	2.7	2.9
Chi-square *p*-value	2.689 0.611				

Group A: Patients with ESRD on maintenance haemodialysis without atherosclerosis.
Group B: Patients with ESRD on maintenance haemodialysis with atherosclerosis.
n: Number of subjects.
$P < 0.05$ is significant.

Table 7 IL-6 -174(G > C) allele frequency among the studied groups.

Allele	*n* %	Control group ($n = 30$)	Group A ($n = 33$)	Group B ($n = 37$)	Total patients ($n = 70$)
G	*n*	50	55	67	122
	%	83.3	83.3	90.5	87.1
C	*n*	10	11	7	18
	%	16.7	16.7	9.5	12.9
Chi-square *p*-value	2.01 0.365				

Group A: Patients with ESRD on maintenance haemodialysis without atherosclerosis.
Group B: Patients with ESRD on maintenance haemodialysis with atherosclerosis.
n: Number of subjects.
$P < 0.05$ is significant.

In the present study, the genotype distribution and allele frequency for IL-6 (-174G/C) were compared in all subjects. No significant difference was observed in the genotype distribution of IL-6 (-174G/C) among the studied groups. In addition, no significant difference was observed in the frequency of the IL-6 (-174) alleles among the studied groups. Also, no significant relation was found between IL-6 (-174G/C) variants and the different studied parameters. These results were in agreement with Sekuri et al. [39] who found that IL-6 -174G/C polymorphism is not associated with the risk of premature CAD, and does not contribute to cardiovascular risk stratification in the Turkish population. Jang et al. [40] found no association between IL-6 -174G/C polymorphism and atherosclerosis in Korean men, and Banerjee et al. [41] found the same results in Indian population. Also López-Mejías et al. [42] confirmed the lack of association between IL-6 -174G/C gene polymorphism and CVD in Spanish patients. Our results were also comparable to those reported by Ghazouani et al. [43] who found that the distribution of -174G > C genotypes was similar between CAD patients and control subjects in Tunisians. Moreover, compared to GG genotype carriers, -174C allele carriage did not increase the CAD relative risk, which remained non significant after adjusting for traditional risk factors for CAD (age, smoking, hypertension, diabetes and obesity). In addition, Ye et al. [25] did not find any sufficient evidence to support an association between IL-6 -147G/C polymorphism and atherosclerosis.

The Northwick Park Heart Study, a prospective study of middle-aged healthy men, reported that the risk imparted by the -174G/C genotype was higher than that imparted by the -174C/C genotype, also they demonstrated a weak but significant association of the -174G > C variant with increased atherosclerosis risk and these effects remained statistically significant after adjusting for classical risk factors including blood pressure [44]. A Greek study demonstrated enrichment of the -174C allele in patients with myocardial infarction suggesting that the -174G/C variant may be involved in the pathogenesis of atherosclerosis [45]. Also Berg et al. [46] found that individuals who were homozygous for the C-allele had a higher risk for atherosclerosis both in univariate and multivariate analyses in Norwegian population.

Given the well-established role of inflammatory mechanisms in atherosclerosis pathogenesis, coupled with the role of IL-6 as a pro-inflammatory cytokine, it is tempting to speculate that induction of inflammation mediated by altered IL-1B and IL-6 levels regulated by specific IL-1B and IL-6 gene variants may modulate atherosclerosis development and progression especially in dialysis condition. However from the findings of the present work, it could be concluded that IL-1B (-511C/T) and IL-6 (-174G/C) genetic variations are unlikely to play an important role in the genetic predisposition to atherosclerosis. Conflicting results may be due to various reasons such as demographic features of subjects and different life styles; also sample size plays a crucial role. This situation

Table 8 Comparison of the measured variables between different genotypes of IL-6 -174(G > C) polymorphism.

Variables	Genotypes	*F*	Sig.
Weight	GG	0.264	0.769
	GC		
	CC		
Height	GG	0.972	0.382
	GC		
	CC		
BMI	GG	1.467	0.236
	GC		
	CC		
SBP	GG	0.085	0.919
	GC		
	CC		
DBP	GG	1.154	0.320
	GC		
	CC		
MBP	GG	0.515	0.599
	GC		
	CC		
CIMT	GG	0.773	0.465
	GC		
	CC		
FSG	GG	0.171	0.843
	GC		
	CC		
Creatinine	GG	0.188	0.829
	GC		
	CC		
Albumin	GG	0.218	0.804
	GC		
	CC		
CRP	GG	1.897	0.155
	GC		
	CC		
TG	GG	1.656	0.196
	GC		
	CC		
TC	GG	1.957	0.147
	GC		
	CC		
HDL-C	GG	0.672	0.513
	GC		
	CC		
LDL-C	GG	2.074	0.131
	GC		
	CC		

BMI = Body mass index; SBP = Systolic blood pressure; DBP = Diastolic blood pressure; MBP = Mean blood pressure; CIMT = Carotid intima media thickness; FSG = Fasting serum glucose; CRP = C-reactive protein; TG = Triglycerides; TC = Total cholesterol; HDL-C = High density lipoprotein fractions; LDL-C = Low density lipoprotein fractions.
$P < 0.05$ is significant.

encourages more and more attempts to be made to further assess the associations of these polymorphisms with the disease.

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