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PROINFLAMMATORY CYTOKINE GENES POLYMORPHISM IN PATIENTS WITH ACUTE CORONARY SYNDROME

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Abstract

Acute coronary syndrome describes the continuum of myocardial ischemia that ranges from unstable angina at one end of the spectrum to non-ST segment elevation myocardial infarction at the other end. **Aim:** The present study was conducted to evaluate the role TNF-α C-863A and IL6-C174G genes polymorphism in acute coronary syndrome, **Methods:** We have investigated single nucleotide polymorphisms of TNF-αC-863A and IL6- C174G genes in 88 subjects. Sixty eight were acute coronary syndrome patients while others were apparently healthy individuals used as controls, then the serum level of TNF-α and IL-6 was detected by ELISA technique. **Results:** The frequencies of TNF-α C-863A(35.29vs.20.00%) genotypes and A allele (52.94 vs.27.50%) were higher in patients with acute coronary syndrome than control group, while IL-6 C174G (20.59 vs 0.00%) genotypes and C allele(35.29 vs12.50%) were significantly higher in patient than control groups and associated with higher mean serum concentration of TNF-α (528.752±27.83) versus (81.05±4.512) and IL-6 (207.292±12.2) versus (16.84±1.235), in acute coronary syndrome patients than apparently healthy subjects. **Conclusion**: AA genotype and A allele for TNF- α –C-863A polymorphism and CC genotype with C allele for IL-6174C polymorphism are mainly expressed among acute coronary syndrome patients and susceptibility with disease might be prospected.

Key words: acute coronary syndrome, TNF-α, IL-6, Allele, RFLP

Introduction:

Acute coronary syndrome describes the continuum of myocardial ischemia that ranges from unstable angina at one end of the spectrum to non-STsegment elevation myocardial infarction at the other end [1] .It caused by one of two events: rupture of the fibrous cap of an atheromatous plaque or endothelial (intimal) erosion of the cap, both of which lead to the subsequent development of a thrombus and decreased myocardial perfusion [2][3].Acute coronary syndrome are classified in to two type, unstable angina which is identified when the typical anginal pain becomes more sever and more frequent ,comes on with less exertion or occurs at rest[4], and acute myocardial infarction is the medical term for an event commonly known as a heart attack. It happens when blood stops flowing properly to part of the heart and the heart muscle is injured due to not receiving enough oxygen[5]. Mortality in patients with AMI has been observed to increase for each 30 minutes [6]. In 2003 centers for Disease Control and prevention study of adults showed that the prevalence of respondents with 2 or more cardio vascular disease risk factors correlated with increased age, lower levels of education and lower level of income [7].Pro-inflammatory factors play a major role in the development of CAD, and elevated levels are used as diagnostic markers and risk factors [8][9]. Interleukin- 6,one of the pro-inflammatory cytokines, has recently become a focus of interest in CAD development and is produced by an array of immune cells including activated macrophages, lymphocytes and endothelial cells [8][10].

The role of IL-6 in the pathogenesis of CAD through the combination of autocrine, paracrine and endocrine mechanisms at which anautocrine and paracrine activation of monocytes by IL-6 in the vessel wall contribute to the deposition of fibrinogen. IL-6 decreases lipoprotein lipase activity and monomeric LPL levels in plasma, which increases macrophage uptake ,as IL-6 with both pro- and anti-inflammatory mediators affecting both B-cell immunoglobulin production and T-cell cytotoxic activity, and this indicates a possible role for IL-6 in progression of CAD. The local inflammatory response is accompanied by a systemic response known as the acute- phase response.IL-6 appear to be released mainly from vulnerable plaque or necrotic myocardium during the acute phase of MI[11].

TNF-α is one of the primary pro-inflammatory cytokines, mainly produced and secreted by inflammatory cells (i.e. monocytes and macrophages). Evidence shows that TNF-α is a key contributor in the development, progression, and complications of atherosclerosis.

TNF-$\alpha$is involved in reduced expression of endothelial nitric oxide synthase (eNOS) and thus impaired nitric oxide (NO) production leading to endothelial dysfunction. It has a profound effect on lipid metabolism and has been implicated in insulin resistance which produces changes in lipid and glucose associated with the cardiovascular disease risk[12]. In this study, therefore, the genotypes of the IL-6 and TNF-α SNPs were identified in subjects with ACS to determine the correlation between the genotypes/allelotypes and cytokine serum concentration.

MATERIALS AND METHODS

The patients group*:*

The current study comprised of 68 patients with ACS (19 females and 49 males) age ranged between 31-81years Iraqi patient. The clinical examination and diagnosis were performed by physician specialized in Al-Diwaniyah Teaching Hospital in period from November 2013 to April 2014. ACS was diagnosed on the basis of clinical history, physical examination with electrocardiography, chest radiography, echocardiography and coronary angiography.

The control group:

A control group included 20 (14 males and 6 females) age ranged between 20-60 years subjects who had no history or clinical evidence of cardiac diseases or any chronic disease.

Genotyping: The genotypes of the TNF-α C-863A and IL6- C 174G were determined by PCR–restriction fragment length polymorphism (RFLP) Table(1). The PCR products were purified using aAccu PowerTM PCR PreMix (Bioneer),then the PCR products were visualized in an ethidium-bromide-stained 1.5% agarose gel using aUV Transilluminator. Following which they were digested with the NlaIII restriction enzymes for IL-6 & BbsI restriction enzymes for TNF-α . The digested PCR products were visualized in an ethidium bromide-stained 2.5% agarose gel using a UV Transilluminator.

Table 1: The primer sets and restriction enzymes used for the PCR–RFLP analyses

Gene Variations	Restriction enzymes	Primers used for PCR analysis
TNF-α −863C>A	BbsI	F:5-GGC TCTGAGGAATGGGTT AC-3 R: 5-CTA CAT GGC CCT GTC TTC GTT ACG-3
IL-6 -174G>C	NlaIII	F: 5-GCGATG GAG TCA GAG GAA AC-3 R: 5-ATCTTT GTT GGA GGG TGA GG-3

Serum cytokine assay*:* Serum concentrations of TNF- α and IL-6 were measure by using ELISA Kit(USBiologicalBio Assay) following the manufacturer's instructions.

Statistical analysis: The Hardy–Weinberg equilibrium (HWE) assumption was assessed for both the patient and control groups by comparingthe observed numbers of each genotype with those expected under the HWE for the estimated allele frequency. Data were presented, summarized and analyzed using two software programs. These were Statistical Package for Social Science (SPSS) version 16 and Microsoft Office Excel 2007.Logistic regression analysis was used to estimate the odds ratios (OR) and 95% confidence intervals (CI) for the association between the genotypes, alleles or haplotypes and the risk of ACS. The results are presented as the mean values ± 1standard deviation (SD), and a P value of ≤0.05 was considered to indicate statistical significance.

RESULTS

Table (2):shows The demographic profiles of both ACS patient and control groups In which there was male more infected than female**.**

Table 2: Demographic characteristics of acute coronary syndrome patients and controls

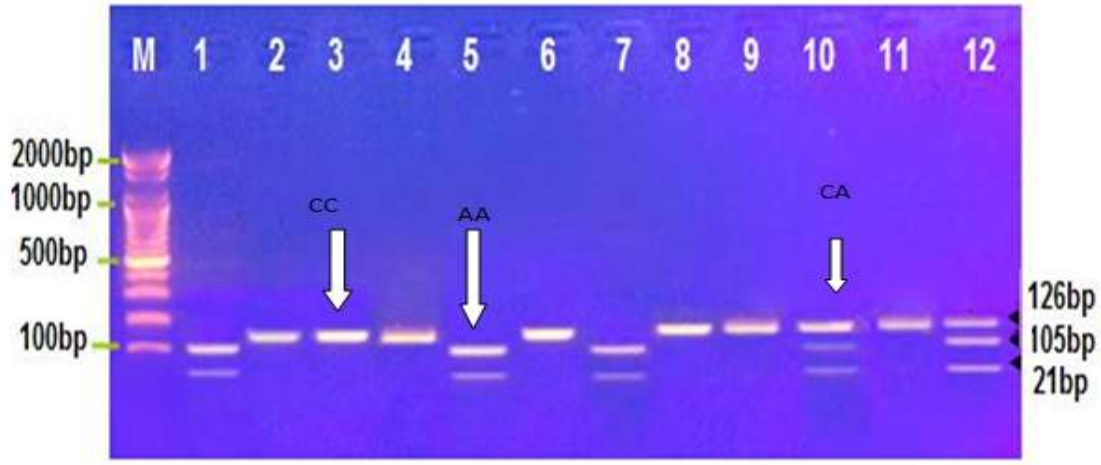
Parameters	Patients =68(mean ± SE)	Controls = 20 (mean ± S	P value
Age	59.29±1.36	32.70±2.80	<0.001
Male/female	2.58:1	2.33:1	0.858

Table (3)*:*shows The presence of hypertension, smoking, hypercholesterolemia, diabetes mellitus, and family history are an important contributory factor in ACS shown in which there the major risk factor was hypertension.

Table3: Frequency distribution of patient group according to risk factors.

Risk factor	**No.**	**%**
Diabetes mellitus	23	33.82
Hypercholesterolemia	11	16.18
Hypertension	41	60.29
Smoking	34	50.00
Family history	2	2.94

Distribution of TNF-α* C863A *and* C174G*Genotypes and Alleles in Patient and Control Group: Distribution of TNF-α C308A and IL-6 G174C polymorphism was detected by PCR-RFLP Technique, at this locus there're three genotype; for TNF-α C308A; CC, CA and AA with band sizes (126bp), (105,21,126 bp) and (105,21bp) respectively, figure (1),and for IL-6 G174C; at this locus there're three genotype; CC, GC and allele GG with band sizes(171/122/86bp),(208/171/122/86bp) and(208/171bp) respectively, figure (2).



Figure(1): Ethidium bromide-stained agarose gel of PCR – RFLP amplified 126bp of TNF-αgene for study groups. Lane (M): DNA molecular size marker (KAPA Universal Ladder), Lane 1,5,and 7: genotype AA (105,21), Lane 10 and 12: Hetrozygot CA (126.105and 21), Lane 2,3,4,6,8,9and11 CC genotype (126bp).

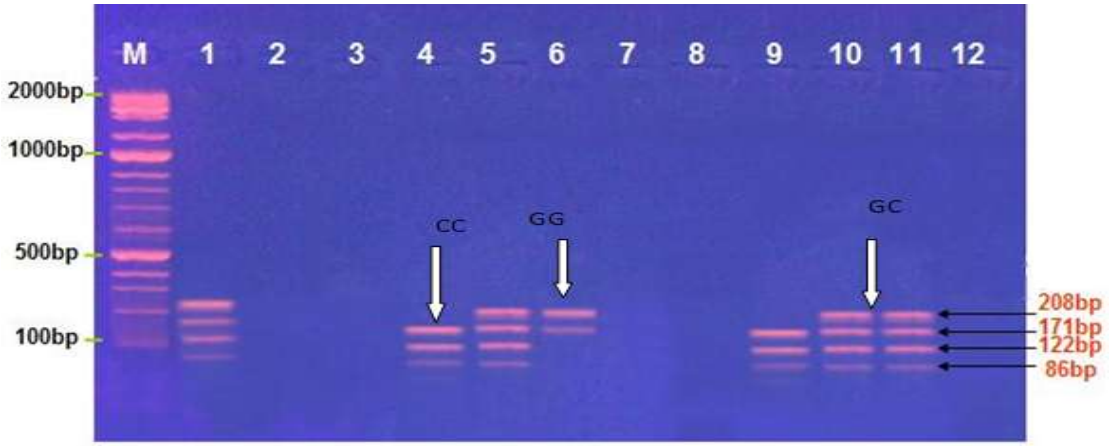


Figure (2): Ethidium bromide-stained agarose gel of PCR – RFLP amplified of IL- 6 gene for study groups. Lane (M): DNA molecular size marker (KAPA Universal Ladder), Lane: 1,5,10 and 11 genotype GC (208,171,122 and 86), Lane : 6 GG genotype (208,171bp), Lane: 4 and 9, CC genotype (171,122,86 bp).

Table (4):shows The frequency distribution of genotypes and alleles of TNF-α C308A and IL-6 in patient and control groups .

Table 4: Distribution of TNF-αC863A and – IL-6G174C genotypes and alleles in patient and control group

Genotype	Patient's N = 68 (%)	Controls N = 20 (%)	P value	OR (95% CI)	EF	PF
TNF- αC863A						
AA	24(35.29)	4 (20.00)	0.277	2.182 (7.268-0.655)	0.464	
CA	24(35.29)	3(15.00)	0.103	3.091 (11.621-0.822)	0.601	
CC	20(29.41)	13(65.00)	0.007	0.224 (0.645-0.078)	---	0.677
A	72(52.94)	11(27.11)	0.006	2.966 (6.415-1.371)	0.575	
C	64(47.06)	29(72.50)	0.006	0.337 (0.729-0.156)	---	0.575
IL-6G174C						
GG	34(50.00)	15(75.00)	0.072	0.333 (1.020-0.109)	---	0.581
GC	20(29.41)	5(25.00)	0.785	1.250 (3.903-0.400)	0.160	---
CC	14(20.59)	0(0.00)	0.033	10.908 (191.350-0.622	0.878	-----
G	88(64.71)	35(87.50)	0.006	0.262 (0.713-0.090)	-----	0.668
C	48(35.99)	5(12.50)	0.006	3.818 (10.388-1.403	0.688	----

Table (5):shows Mean serum concentration of tumor necrosis factor–α (528.752±27.83) versus (81.05±4.512)and IL-6 (207.292±12.2) versus (16.84±1.235) was significantly higher in the patient group in comparison to control group, with a p-value of (<0.001).

Table 5:Serum concentration of TNF-α and IL-6 in patients and control group

Cytokine	Control	Patients	
	Mean	Mean	P-value
TNF-α	81.05±4.512	528.752±27.83	0.001
IL-6	16.84±1.235	207.292±12.2	0.001

DISCUSSION

In this study the distribution of genotypes (CC,CA and AA) in patients and controls does not differ significantly from the expected Hardy-Weinberg equilibrium, these result agree with resultof Gul et al., 2013 which found at position-863of TNF-*α*gene, the variant genotype (CA + AA) was more prevalent in the patient group (70.32%) in comparison with control subjects (35.16%) this nucleotide variation from C>A at −863 of TNF-*α*gene may result in enhanced levels of the inflammatory cytokine in circulation that could affect the pathophysiology of CHD[12], in our study the homozygous mutant genotype (A/A) is uncommon in the control population (20%) but has an increased frequency in patients with CAS (35.29 %). This genotype confers an odds ratio (OR) of 2.182. While the heterozygous genotype C/A is found in 15% of the control subjects and35.29% of the patients and confers an OR of 3.091, in contrast the wild-type homozygous genotype (C/C) had a higher frequency in the control subjects (65%) compared with CAS patients (29.41%) (OR) of 0.224, Moreover, the AA and CA genotype has obviously suggests an etiology for CAS, as both had Etiologic Fraction (EF) of 0.464 and 0.601 respectively, In contrast, the CC genotype had rather preventive role as it had Protective Fraction (PF) of 0.677, with the possibility of C allele may be protective, whereas the A allele may increase susceptibility to ACS. In contrast to these findings, some studies reported a non-significant difference in the TNF-α−863 C/A genotype frequency between patients with CHD, myocardial infarction, and cardiomyopathy versus healthy controls[13][14][15].In this study the frequencies of IL-6 G174C genotypes were GG(50%), GC(29.41%) &CC(20.59%) in patients and GG(75%), GC (25%) in controls. The CC genotype was absent in healthy subjects. Our results revealed that the homozygote IL6 (C/C)genotype is uncommon in control group (0.00%) ,but it has an increased frequency in patients with CAS (20.59 %). This genotype confers an odds ratio (OR) of 10.908. While the heterozygous genotype G / C is found in 29.41 % of the patients and in 25.00 of the control subjects and confers an OR of 1.250,

in contrast the homozygous genotype (G/G) had a higher frequency in the control subjects (75.00%) compared with ACS patients (50,00%) (OR) of 0.333.Moreover, the GG and GC genotype has obviously suggests an etiology for ACS. This result was consistent with the results of a study conducted by Ghazouani and colleagues which revealed that there is increased frequency of minor allele C in ACS patients compared with healthy control. But they did not find any significant association of minor allele C with coronary artery disease in Tunisian patients [16].

Serum cytokine*:* In agreement with current results, Luo and associates reported that serum levels of IL-6 was significantly higher in ACS patients than in healthy control patients, Serum levels of IL-6 may have some diagnostic value for ACS, and can be useful marker reflecting disease stability. Therefore IL-6 may be used as biomarkers for evaluating inflammatory response and severity of coronary heart disease in patients with ACS [17].In the present study, there was a significant increased inserumconcentrationof TNF-α in all patient with ACS as compared to control group,these results are in agreement with those obtained by Mehdi etal., [18] who concluded that the serum TNF-αlevel is significantly increased in a 25 patients with AMI in which the mean TNF-α level was 614.32 pg/ml, and 4.80 pg/ml in control group. TNF-α may be produced in early stages of AMI as a results of initiation of an inflammatory process resulting in tissue damage and myocardial necrosis. Therefore TNF-αmay be as the tool to predict the AMI in high risk subjects, and consider as a risk factor for the disease[19].TNF-α seems to be an important cytokine during AMI. It is mainly secreted by resident, intra-myocardial macrophages and the myocardium itself [20].

CONCLUSION:

This study showed that Acute coronary syndrome increases in the age more than 45 years ,men more infected than women .There is significantly higher Concentration of IL-6and TNF-α in ACS patients in comparison to control group. This provides strong evidence that pro-inflammatory cytokines play a major role in the pathogenesis of ACS. The AA and CA genotype of TNF-α has obviously suggests an etiology for ACS and the CC genotype frequency of IL6 were higher in ACS patients.

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