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Relationship of IL-6 gene polymorphisms and IL-6 expression level with the burn-induced sepsis susceptibility in Al Diwaniyah

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Abstract:-

Sepsis is one of the most important causes of burn death and sepsis in the hospital after the injury. The present study aimed to investigate the correlation between single nucleotide polymorphism in IL-6 with serum level of IL-6 and MIP-1α expression in the development of burn patients with sepsis and burn patients without sepsis. Enzyme linked immunosorbent assay (ELISA) technique was used to detect serum level of IL-6 and MIP-1α in burn patient with sepsis and burn patient without sepsis. Genomic DNA was extracted from blood for molecular assay to study the correlation between IL-6 -174 G→C , TNF–α -308 G →A gene polymorphism in burn patient with sepsis . Genotyping done by usingTetra Amplification Refractory Mutation System – Polymerase Chain Reaction (T-ARMS-PCR) technique. Immunological assay results of this study showed that the serum level and median (IQR) of IL-6 and MIP-1α were significantly higher in burn patient with sepsis group than in burn patient without sepsis group they were 443.77 pg/ml (471.62) versus 299 pg/ml (295.3) at (P< 0.001) and 148.43 pg/ml (141.9) versus 140.4 pg/ml (144.49) respectively**.** The present results showed that the IL-6 serum level was significantly higher in the female patients with sepsis compared to the burn patient without sepsis (P =0.001) . The present study showed that IL-6 was correlated between IL-6 serum and IL-6 genotype in burn patient with sepsis and burn patient without sepsis. Heterozygous GC genotype was significantly higher in correlated between IL-6 serum and IL-6 genotype in burn patient with sepsis and burn patient without sepsis groups (P < 0.001). Also, Homozygous GG genotype was significantly higher in correlated between IL-6 serum and IL-6 genotype in burn patient with sepsis and burn patient without sepsis group (P < 0.002). While, Homozygous CC genotype was not significantly in correlated between IL-6 serum and IL-6 genotype in burn patient with sepsis and burn patient without sepsis control group (P > 0.05) .

Key words: - burn, sepsis, IL-6 gene, IL-6level and Single nucleotide polymorphisms (SNPs)

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INTRODUCTION

Burn injury remains an important cause of morbidity and mortality worldwide. Infection complications, including sepsis, septic shock and sepsis –related organ failure, are common among patients with moderate to severe burn injuries. Sepsis is a life-threatening organ-dysfunction condition caused by a dysregulated response to an infectious condition that can cause complications in patients with major trauma. Burns are one of the most destructive forms of trauma; despite the improvements in medical care, infections remain an important cause of burn injury-related mortality and morbidity, and complicated sepsis predisposes patients to diverse complications such as organ failure, lengthening of hospital stays, and increased costs. Accurate diagnosis and early treatment of sepsis may have a beneficial impact on clinical outcome of burn – injured patients (1) .

In sepsis, the immune response that is initiated by an invading pathogen fails to return to homeostasis, thus culminating in a pathological syndrome that is characterized by sustained excessive inflammation and immune suppression (2) . The increase of cytokine production both in humans and in experimental models seems to play important roles in the pathophysiology of sepsis and septic shock after burn injury (3). Pro-inflammatory interleukin IL- 6 is increased early after burn and secreted by monocytes, endothelial cells and fibroblasts, and is able to stimulate B and T lymphocytes and induce fever. Some studies have indicated that IL-6 may play a key role in the inflammatory response to microbial invasion and also, it is one of the most important members which may be associated with sepsis risk and outcome. (4, 5).

Single nucleotide polymorphisms (SNPs) , variations in a nucleotide at a specific chromosome location, have been linked to sepsis susceptibility and differences in prognosis (6) . Among these diverse cytokines, IL-6 has attracted considerable attention. The human IL-6 gene was mapped to chromosome 7p21 region and contains several single nucleotide polymorphisms. The G to C polymorphism at position-174 of the IL-6 gene (rs1800795) is associated with an adverse outcome in a number of inflammatory diseases, but its association with sepsis remains unclear (7, 8).

MATERIAL AND MOTHED

This is a case-control study in which 40 burn patients with sepsis and 30 burn patient without sepsis, with age ranging from 15 to 55 years, who were observation in Specialized Centre for burns of Al – Diwaniyah , during the period from March 2019 to the end of November 2019. Sera and whole blood were collected from each participant , noting that the sera was used to determine IL-6 and MIP-1α concentrations for all specimens, while whole blood was used to extract DNA .

Levels of IL-6 and MIP-1α were determined using a human ELISA kit (Elabscinece, USA) in compliance with the protocol of the manufacturer.

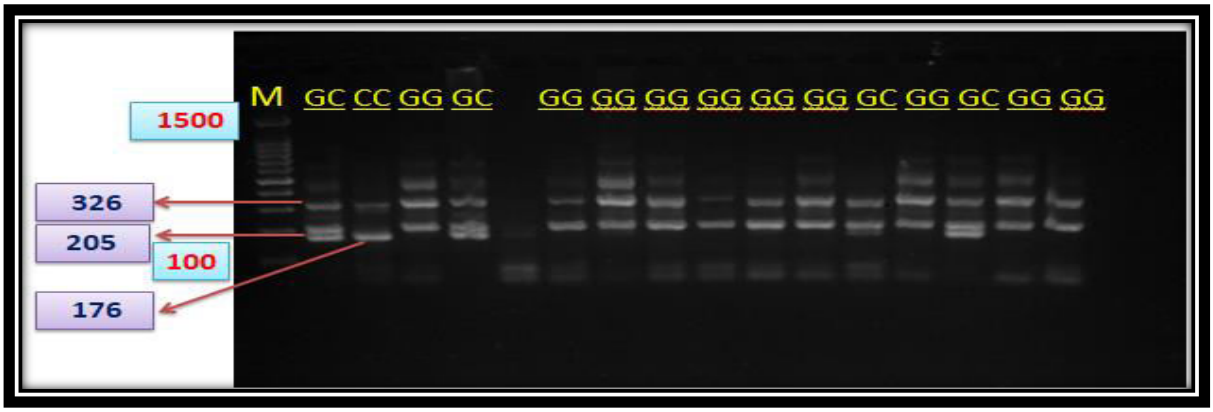
DNA extraction was used to detect SNPs in IL-6 (IL-6 -174 G→C) with the use of Tetra amplification refractory mutation system polymorphism PCR (T-ARMS – PCR) technique , which is a rapid , easy and sensitive method .T-ARMS – PCR was performed by Thermocycler (Analytic Jena – Germany) ,gene polymorphism primers were designed by (9) and these primers were provided from (Bioneer company, Korea) .

The ARMS-PCR master mix of IL-6 gene polymorphism was done one reaction for each samples by using AccuPower^{TM} PCR PreMix (Bioneer , Korea) were employed . five µl DNA , 1µl inner primers , 1µl outer primers (10 pmol) and 11µl PCR . After that, these ARMS-PCR master mix reaction components that mentioned above, placed in standard PCR tubes containing the PCR PreMix as lyophilized materials containing all other components needed to PCR reaction such as (Taq DNA polymerase , dNTPs , Tris . HCl pH: 9.0 , KCl , $MgCl_2$, stabilizer , and loading dye) . Then all PCR tubes transferred into Exispin vortex centrifuged at 3000rpm for 3 minutes. Then placed in PCR Thermocycler (Mygene . Korea).The ARMS-PCR was carried out using Thermocycler conditions with Initial denaturation at 95°C for 5 minutes. Then 35cycle including : temperature of 95°C for 30 seconds for denaturation , temperature 57◦C for 30 seconds for the connection of primers, 72ºC for 40 seconds for extension and 72ºC for 5 minutes for final extension.

The PCR products were analyzed by electrophoresis on 2% agarose gel containing 3µL of ethidium bromide stain . Then bands were seen by UV ray (ATTA , S/Korea) figure 1.

The difference between frequency of genotypes and alleles in burn patient with sepsis and burn patient without sepsis groups was determined by chi-square test using SPSS V.24 program was used in statistical analysis of the data software . p<0/05 was considered significant. Logistic regression test was used to estimate the odds ratio (OR) and confidence interval 95% (CI).

Figure 1:- Agarose gel electrophoresis image that show the T. ARMS-PCR product analysis of IL-6 gene (-174 G→C) in patient sample. T. ARMS-PCR product was analysis by 2% agarose gel. Where M: marker (100bp – 1500bp) , lane GG wild type homozygote was show at two bands (326 bp control band and 205 bp G allele) , lane CC mutant type homozygote was show at two band (326 bp control band and 176 bp C allele) , and lane GC heterozygote was show at three band digested at (326 bp control band , 205 bp G allele and 176 bp C allele)



RESULTS

The Distribution of burn patients with sepsis and burn patient without sepsis subjects according to age is shown in table 1 . There was somewhat even distribution of patients with sepsis according to age with higher percentages being seen in 24-34 years and >35years, 19 (47.5%) and 13 (32.5 %), respectively. Burn patient without sepsis subjects were selected in order to ensure comparable frequency distribution with that of burn patient with sepsis group. Indeed , mean age of study group was higher than that of Burn patient without sepsis

group , 32.1 ± 9.62 years versus 27.53 ± 9.03 years , respectively ; however, the difference was not significant from statistical perspective ($P = 0.051$) .

Table 1:-Distribution of burn patients with sepsis and burn patient without sepsis subjects according to age

		Age groups			Total	Chi-Square Sig.
		15-24 year	24-34 year	>35 year		
Study groups	burn patients with sepsis	8	19	13	40	4.618 0.099
		20.0%	47.5%	32.5%	100.0%	
	burn patient without sepsis	13	9	8	30	
		43.3%	30.0%	26.7%	100.0%	
Total		21	28	21	70	
		30.0%	40.0%	30.0%	100.0%	

The data of this study showed that the serum level and median (IQR) of IL-6 and MIP-1α were significantly higher in burn patients with sepsis group than in burn patient without sepsis group they were 443.77 pg/ml (471.62) versus 299 pg/ml (295.3) and 148.43 pg/ml (141.9) versus 140.4 pg/ml (144.49) respectively, as shown in table (2).

Table 2: Serum median of IL-6 and MIP-α in control and patients' groups.

Study groups	burn patients with sepsis n=40			burn patient without sepsis n=30			
	Mean (SD)	Median (IQR)	Range	Mean (SD)	Median (IQR)	Range	p-value
Age (Year)	**32.1** (9.62)	31	37	27.53 (9.03)	**25**	30	0.051
IL-6 (pg/ml)	**443.77** (42.63)	471.62	137.9	299 (120.11)	**295.3**	360.87	<0.001
MIP -α (g/ml)	**148.43** (34.63)	141.9	136.12	140.4 (36.93)	**144.49**	124.73	0.354

N = number of cases , (SD)=Std. Deviation, IQR= Inter-Quartile Range

IL-6 genotype frequency distribution in burn patients with sepsis and burn patient without sepsis groups is shown in table 3 . The genotypes relative frequency in burn patient with sepsis were as follow : GG (50 %) , GC (35%) and CC (15%) ; while in burn without sepsis subjects : GG (66.7%) , GC (26.7%) and CC (6.7%). G allele is higher frequency (67.5%) in burn patient with sepsis than C allele (32.5%)

There was no significant difference in the IL-6 allele frequency distribution between control and patients groups (P = 0.103), as shown in table 4.

Table 3: IL-6 genotype frequency distribution in burn patients with sepsis and burn patient without sepsis groups

IL-6 SNP	Study groups		Total	P-value	B	OR	95% CI for OR	
	burn patients with sepsis N=40	burn patient without sepsis N=30					Lower Bound	Upper Bound
CC	6 15.0%	2 6.7%	8 11.4%	0.210	1.099	3.000	0.539	16.689
GC	14 35.0%	8 26.7%	22 31.4%	0.304	0.560	1.750	0.602	5.087
GG	20 50.0%	20 66.7%	40 57.1%	Reference Category				
P-value	0.074	0.058						

B = Probability, OR= Odds Ratio, CI= Confidence Interval

Table 4 : IL-6 allele frequency distribution in burn patients with sepsis and burn patient without sepsis groups

IL-6	study groups		Total	P-value	OR	95% CI for OR	
	burn patients with sepsis	burn patient without sepsis					
C	26 32.5%	12 20.0%	38 27.1%	0.103	1.926	0.877	4.230
G	54 67.5%	48 80.0%	102 72.9%				
Total	80 100.0%	60 100.0%	140 100.0%				
P-value	0.002	0.001					
RR 95% CI	1.292 0.97-1.71	0.671 0.40-1.11					

RR=Relative Risk, Reference Category G

Correlation between IL-6 serum and IL-6 Genotype in burn patient with sepsis and control burn patient without sepsis

The present study showed that IL-6 was correlated between IL-6 serum and IL-6 genotype in burn patient with sepsis and burn patient without sepsis is shown in table 5 .

Table 5: Correlation between IL-6 serum and IL-6 Genotype in burn patient with sepsis and control (burn patient without sepsis).

IL-6 SNP		IL-6 (pg/ml)		p-value
		Patient	Control	
CC	N	6	2	
	Mean	451.33	362.94	0.867
	Std. Deviation	40.31	160.86	
	Median	472.07	362.94	
	Range	102.17	227.49	
GC	N	14.00	8.00	
	Mean	436.67	268.53	<0.001
	Std. Deviation	48.51	102.84	
	Median	472.07	295.30	
	Range	129.70	286.31	
GG	N	20.00	20.00	
	Mean	446.46	304.80	<0.002
	Std. Deviation	40.38	126.11	
	Median	470.80	292.17	
	Range	137.90	360.87	

DISCUSSION

Severe burn injury is generally associated with systemic inflammation or systemic infection, leading to sepsis. It is characterized by extensive release of pro-inflammatory cytokines such as IL-6 and TNF-α and suppression of anti-inflammatory mediators (10, 11).The present results showed that the IL-6 serum level higher in burn patients with sepsis compared to the burn patient without sepsis group this in due to the fact that the IL-6 mediates inflammatory responses, and during sepsis, the plasma levels of this cytokine are increased. Higher levels of IL-6 in patients with sepsis were observed compared to without sepsis subjects, suggesting that this cytokine may possess a role in the pathogenesis of sepsis in burned patients (4) .

Some studies have indicated that IL-6 may play a key role in the inflammatory response to microbial invasion. Previous studies revealed that high IL-6 level was associated with increased severe sepsis mortality and risk (12, 13) . In addition, IL-6 has been investigated among other infection markers to improve the diagnostics of suspected neonatal infection (14). In the other hand, IL-6 play role in diagnostic of sepsis (15) .

It has been documented that IL-6 production is elevated in patients with sepsis (16,17) , indicating that IL-6 is associated with the development of sepsis (17). Further studies indicated that the IL-6 level in patients with shock is higher than that in patients without shock, and in those who died from severe sepsis (18) , suggesting

that IL-6 is the key cytokine in the pathophysiology of severe sepsis . In addition, an increased level of IL-6 was found to be associated with the highest risk of death in patients with sepsis (19). Among the milieu of cytokines induced during sepsis, plasma IL-6 has the best correlation with mortality rate (20).

Macrophage inflammatory protein-1 alpha (MIP-1α / CCL3) is a chemotactic chemokine secreted by macrophages. It performs various biological functions, such as recruiting inflammatory cells, wound healing , inhibition of stem cells, and maintaining effector immune response . It activates bone resorption cells and directly induces bone destruction. Cells that secrete MIP-1α/CCL3 are increased at sites of inflammation and bone resorption. MIP-1α/CCL3 plays an important role in the pathogenesis of various inflammatory diseases and conditions that exhibit bone resorption, such as periodontitis, multiple myeloma, Sjögren syndrome, and rheumatoid arthritis (21).

The present study show MIP-1α was no significant between burn patient with sepsis and burn patient without sepsis because MIP-α play role in crucial for immune responses towards infection and inflammation (22). While, Serum MIP-1α for burn patient with sepsis higher than burn patient without sepsis 148.43 ± 34.63 versus 140.4 ± 36.93, respectively. MIP-1α was increased in burnt mice with susceptibility to sepsis (3).

Genetic variation within the regulatory part of the IL-6 gene may affect the incidence and outcome of sepsis (7) .Our data showed that the IL-6 GG genotypes are associated with burn patient with sepsis and without sepsis. However, no significant in the IL-6 allele frequency distribution between control and patients groups (P = 0.103). In another study has shown that IL-6 -174 GG genotype was more frequent in the septic group (43%) than in the control group (38.5%), the difference was not significant and none of the variant genotypes or allele was associated with sepsis, compared to the controls (23) .

A study found that *IL-6*-174 G allele was associated with early-onset sepsis in Saudi infants (24) . However , Mao Z *et al* and Feng B *et al*. thought it was *IL-6*-174 C allele rather than G allele that contributed to the risk of sepsis induced by pneumonia (25,26) . When it came to the mortality of septic patients , Lorente *et al* discovered better survival of septic patients with CC genotype (27). Jimenez-Sousa *et al*. found the possible association between *IL-6*-174 CC genotype and a higher septic shock-related mortality in patients who underwent major surgery (28). These evidences suggest that IL-6 may be an appealing candidate gene for sepsis (29).

The present study showed that IL-6 was correlated between IL-6 serum and IL-6 genotype in burn patient with sepsis and burn patient without sepsis is shown in table 6 .Heterozygous GC genotype was significantly higher in correlated between IL-6 serum and IL-6 genotype in burn patient with sepsis and burn patient without sepsisgroups (P < 0.001). Also, Homozygous GG genotype was significantly higher in correlated betweenIL-6 serum and IL-6 genotype in burn patient with sepsis and burn patient without sepsis group (P<0.002).In anotherstudy, the presence of sepsis appears to be influenced by the circulating levels of IL-6 and the genotype at -174 IL-6 gene sequence .septic patients with-174 G genotypes actually showed higher serum IL-6 levels compared to non-septic patients (30). The Schlüter study also identified that the IL-6 -174 G/G genotype was correlated with an improved sepsis outcome (31).Tischendorf has shown that a significantly lower

level of IL-6 -174 G / G genotypes has been associated with higher mortality in septic patients compared to surviving septic patients , which suggests that C allele transport puts individuals at risk (32).

Homozygous CC genotype was not significantly in correlated between IL-6 serum and IL-6 genotype in burn patient with sepsis and burn patient without sepsis group (P > 0.05).Müller- Steinhardt found that the allele IL-6 -174C was associated with increased IL-6 secretion, while the allele IL-6 -174G was associated with low levels of IL-6 (33).

CONCLUSION

The present study showed that heterozygous GC genotype was significantly higher in correlated between IL-6 serum and IL-6 genotype in burn patient with sepsis and burn patient without sepsis groups (P < 0.001). Also , homozygous GG genotype was significantly higher in correlated between IL-6 serum and IL-6 genotype in burn patient with sepsis and burn patient without sepsis groups (P < 0.002) .

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