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ORIGINAL ARTICLE

Association between Interleukin-6 Promoter Polymorphism (-174 G/C), Serum Interleukin-6 Levels and severity of sepsis

[1]Mysa S. Mostafa*, [2]Ahmed W. Mahedy, [1]Amany K. Shahat
[1]Department of Medical Microbiology and Immunology, Faculty of Medicine, Benha University
[2]Department of Internal Medicine, Faculty of Medicine, Benha University

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Corresponding Author:
Mysa S. Mostafa
Department of Medical Microbiology & Immunology, Faculty of Medicine, Benha University.
Tel: 01100434205
mysasaid@yahoo.com

ABSTRACT

Background: *Sepsis is a complicated clinical syndrome resulting from inflammatory reactions to bacteria or their products. IL-6 is a potent inflammatory cytokine produced by many cells, such as leucocytes, monocytes, endothelial cells, and fibroblasts. IL-6 plays an important role in the immune response and inflammation reactions regulation.* ***Objective****: the present study aimed to detect if IL-6 serum level and polymorphism correlate with severity of sepsis.* ***Methodology:*** *The study includes 70 subjects, 50 patients (Case) were chosen with at least 2 SIRS criteria and 20 healthy who were age matched. The patients were categorized into systemic inflammatory response syndrome (SIRS), sepsis, severe sepsis, septic shock and multi-organ dysfunction syndrome (MODS) according to ACCP/SCCM. Bactec Culture on automated blood culture system (Bact/ALERT 3D) was used as a gold standard method to prove sepsis . Serum IL-6 was measured by Enzyme linked immunosorbent assay (ELISA). Genotyping of the IL-6–174 G/C promoter polymorphism was performed using restriction fragment length polymorphism (RFLP) analysis.* ***Results:*** *Our study reported a statistically high significant difference of the IL-6 values between the case and control groups where $p<0.001$ and there was increasing values of serum IL-6 parallel with the developing stages of sepsis. Also, the IL-6 values in the bacteriological culture positive and negative groups showed differences of statistically significant ratios ($p<0.05$).* ***Conclusion****: Our findings reported a significant association between IL-6 promoter polymorphism (-174 G/C), levels of IL6 and severity of sepsis .*

INTRODUCTION

Sepsis is a complicated clinical syndrome resulting from inflammatory reactions to bacteria or their products. Despite the improvement in antibiotic therapy and life support, the global rate of death resulting from sepsis reaches up to 30–60% .[1]

The causes of sepsis include severe infections of lung, abdomen, blood, and urethra. Among them, pulmonary infection accounts for about 64% of all cases of septicemia.[2]

Multi-organ dysfunction syndrome is the commonest and serious complication secondary to sepsis, one of the important factors of sepsis death in the world.[3]

Therefore, predictive markers to identify high-risk populations are urgently needed for early diagnosis and prevention of this case. Cytokines are involved in immune response modulation of host, while changes occuring in cytokine levels also help in sepsis development. [4]

The variation in gene expression and associated differences in responses to sepsis might facilitate producing new genetically diagnostic and therapeutic interventions, which might improve outcome in patients with sepsis susceptibility.[5]

The change of immune function is considered as a key factor in the deveolpment of sepsis.[6] IL-6 is a potent inflammatory cytokine produced by many cells, such as leucocytes, myocytes, endothelial cells, and fibroblasts .[7]

IL-6 plays an important role in the immune response and inflammation reactions regulation. Increasing levels of IL6 associated with the increase in risk and death rate of severe sepsis. [8]

The gene of IL-6, located on 7p21, contains four introns and five exons. There are several polymorphisms identified in the promoter region of IL-6 gene. Among these, the common -174G/C polymorphism (rs1800795) which contains a DNA-binding site for nuclear factor IL-6, which is a transcription factor that can bind to estradiol receptor complexes regulating gene expression of IL6.[9]

As the most common genetic variation, single nucleotide polymorphism (SNP) has attracted more and more attentions in the research on sepsis. According to previous studies, it has been obvious that single nucleotide polymorphism can predict the risk and prognosis of sepsis.[10]

The study aimed to detect if IL-6 serum level and polymorphism correlate with sepsis severity.

METHODOLOGY

This case control study was conducted at Microbiology and Immunology Department, Faculty of Medicine, Benha University in collaboration with Intensive Care Unit in the period from May 2021 to December 2021. The study protocol was approved by the Local Ethics Committee of the Faculty of Medicine, Benha University.

The study included 70 subjects. 50 patients (Case) were chosen with at least 2 SIRS criteria and 20 age matched healthy persons. The age of them was above 18 years and all subjects under study were exposed to full medical history and examination

All patients had been selected from the ICU Unit . All patients were classified into SIRS, sepsis, severe sepsis, septic shock and MODS according to ACCP/SCCM definition.

Bactec Culture on automated blood culture system (Bact/ALERT 3D) was used to prove sepsis .

Serum IL-6 was measured by Enzyme linked immunosorbent assay (ELISA).

Genotyping of the IL-6–174 G/C promoter polymorphism: The detection of IL-6 promoter polymorphism (-174 G/C) (rs1800795) was done in the Department of Medical Microbiology and Immunology. We performed genotyping by polymerase chain reactions (PCR) and restriction fragment length polymorphism (RFLP) analysis. We prepared DNA from 3 mL of peripheral blood using treatment with proteinase K, extraction of phenol-chloroform extraction and precipitation of ethanol.

We used approximately 100 ng DNA as the template in PCR using the following primers:
5´-TTGTCAAGACATGCCAAAGTG-3´ and
5´-TCAGACATCTCCAGTCCTATA-3´, and the temperature profile: 94˚C-52˚C-72˚C , 30 s each, for 30 cycles. We restricted the amplified DNA with endonuclease NIa III (CATG) for two hours at 37˚C. The resulting DNA fragments were separated by gel electrophoresis in 2% agarose gel and visualized under ultraviolet light. In the absence of a NlaIII restriction site, a fragment of 300 bp was detected (G allele), whereas fragments of 169 and 131 bp corresponded to the C allele.

Statistical Analysis:

We recorded categorical variables as frequencies and percentages, and we compared them between groups by chi-square test. To determine the association of IL-6 promoter polymorphism (-174 G/C) with sepsis severity. In addition, we also carried out a multivariate logistic regression analysis was used to determine the association of levels of serum IL6 with sepsis severity . The clinical impact for the predictor variables was calculated by odds ratio (OR) and 95% confidence intervals (CI). We plotted sepsis severity of patient groups with CC, GC and GG genotypes using the Kaplan-Meier method and we compared them using log-rank test. We tested the Hardy -Weinberg equilibrium in the genotypes of our series using the chi-square test. P-values of less than 0.05 were considered statistically significant. NCSS 2000 and SPSS 17.0 were used for statistical analyses.

RESULTS

The study included 70 subjects. Where 50 patients (Case) were chosen with at least 2 SIRS criteria and 20 age matched healthy control.

Patients were categorized into SIRS, sepsis, severe sepsis, septic shock and MODS according to ACCP/SCCM guideline. 9 patients (12.9 %) had SIRS, 13 patients (18.6 %) had sepsis , 11 patients (15.7%) had sever sepsis, 9 patients (12.9 %) had septic shock , and 8 patients (11.4%) had MODS as shown in table 1 and figure 1.

Table 1: Distribution of study group and stages of sepsis:

Cases	**SIRS**	9	12.9%
	sepsis	13	18.6%
	Sever sepsis	11	15.7%
	Septic shock	9	12.9%
	MODS	8	11.4%
control		20	28.6%
Total		70	100%

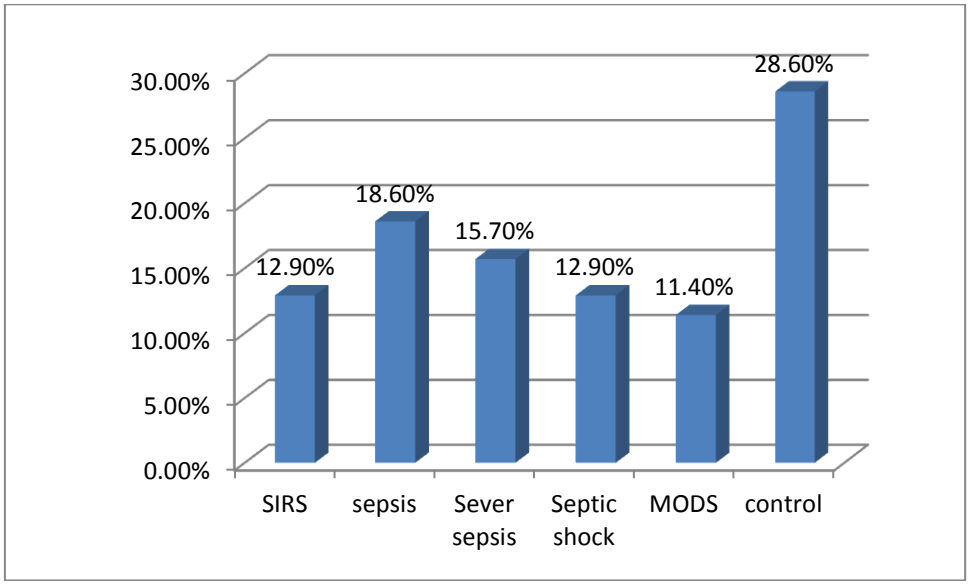

Fig. 1: Distribution of study group and stages of sepsis.

Results of bacteriological culture are explained in figure 2 , it revealed that 64.3% (45 subject) of study group were culture positive , and 35.7% (25 subject) were culture negative .

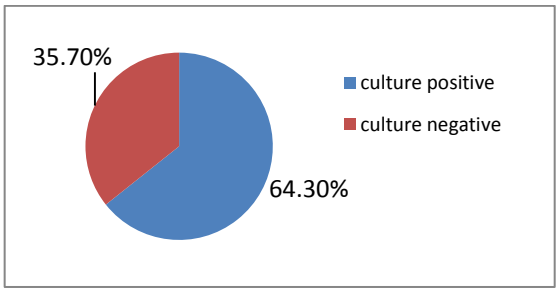

Fig. 2: Results of bacteriological culture

Our study reported a statistically highly significant difference between the IL-6 values in case and control subjects where p <0.001 as shown in table 2.

Table 2: Significance of serum IL-6 values in patient and control.

IL6 level pg/ml	STUDY GROUP	Mean	P value
	Case	200.55± 373	< 0.001
	Control	5.77±8.55	

By measurement of serum IL6 level in cases with different stages of sepsis , we recorded an increasing trend of serum IL-6 level with the developing stages of sepsis as shown in table 3 .

Table 3: Relation of IL-6 level with developing stages of sepsis:

IL6 level pg/ml	STUDY GROUP	Mean
	Control	5.77±8.55
	SIRS	100.04±111.56
	Sepsis	170.01±349.56
	Sever sepsis	333.19±727.33
	Septic shock	1150±1630.67
	MODS	2500 ±52.35

Also , a statistically significant difference was found in the IL-6 level between culture positive and negative groups ($p < 0.05$) as shown in table 4 .

Table 4: Significance of serum IL-6 level in culture positive and negative groups :

IL6 level (pg/ml)	Culture	Mean	P
	+ve	312.5±	0.002
	-ve	102.2±	

Our findings reported a significant association between IL-6 promoter polymorphism (-174 G/C) and severity of sepsis as shown in table 5. Thus, CC homozygous might be a favorable genotype in septic patients with low severity and low risk of early mortality.

Table 5: Distribution of IL-6 gene (rs1800795) genotypes within studied groups:

IL-6 gene Geno-types		Control n= 20		SIRS n= 9		Sepsis n=13		Sever sepsis n= 11		Septic shock N=9		MODS n=8		P
		n	%	n	%	n	%	n	%	n	%	n	%	
	GG	6	30%	1	11.1%	5	38.5%	4	36.4%	5	55.6%	5	62.5%	0.07
	GC	5	25%	2	22.2%	6	46.1%	5	45.4%	3	33.3%	3	37.5%	0.23
	CC	9	45%	6	66.7%	2	15.4%	2	18.2%	1	11.1%	-	0%	0.01

DISCUSSION

Sepsis is a major cause of mortality and resource consumption. Interleukin (IL-6) is one of pro-inflammatory cytokines responsible for immune regulation in systemic response . Higher circulating IL-6 levels were shown in non-surviving rather than surviving septic patients.[11]

This study revealed a highly significant difference between the IL-6 values in case and control subjects ($p<0.001$). This is in agreement with Moniruzzaman et al.[12]

The present study showed that serum level of IL 6 increased in parallel with the developing stages of sepsis that was in agreement with Uusitalo-Seppälä et al.[13] who proved that PCT and IL-6 are superior to CRP in detecting patients complaining of severe sepsis and that , PCT and IL-6 are considered independent predictors for sepsis diagnosis.

Our study found a statistically significant difference in the values of IL-6 in the bacteriological culture positive and negative groups ($p < 0.05$), a result that comes in accordance with Moniruzzaman et al.[14] who reported that IL-6, IL-8 and PCT values are statistically significant to predict bacterial infection and also agreed with GAO et al.[15]who studied PCT, CRP and IL-6 as markers for early diagnosis of bacterial infection in patients with septicopyemia.

Regarding IL-6 promoter polymorphism (-174 G/C) we found a significant association between this polymorphism and the severity of sepsis , that CC homozygous were significantly associated with low severity and low risk for MODS and mortality.

Also Lorente et al.[16], a multicenter, prospective and observation study carried out with 263 patients complaining of severe sepsis from six Intensive Care Units of Spain to show if there is an association between IL-6 promoter polymorphism (-174 G/C), IL-6 serum levels and 30-day mortality in a large series of adult septic patients, recorded a relation between IL-6 levels and severity and early mortality in septic patients.

Our results of decreasing early death rate in severe septic patients with CC homozygous are matched with that of Balding et al.[17] who studied 183 children diagnosed as bacteremia and reported also a decreased early mortality in CC homozygous patients.

Also, Lorente et al.[16] reported a low 30-day mortality rate and serum IL6 in patients with genotypes CC followed by GC and finally GG patients.

In addition, another two studies Zidan et al.[18], Schluteret al. [19] reported higher early mortality in GG patients than patients of other genotypes which come in accordance with our results .

This result did not matched with Feng et al.[20] conducted a study on 277 Chinese patients complaining of severe pneumonia-induced sepsis , found no association between IL-6 promoter polymorphism (-174 G/C) and mortality.

Also our finding of lower mortality in CC homozygous severe septic patients contradict those of Martin-Loecheset al.[21] who found that patients with GG genotype had a decreasd rate of early death.

The discrepancy between study findings could be due to differences in sample size, age (adult, pediatric, neonatal), type of infection (sepsis, CAP, meningococcemia, pneumococcal infection) and severity of infection.

CONCLUSION

From our study we can conclude a significant association between IL-6 promoter polymorphism (-174 G/C), levels of IL6 and severity of sepsis. Also we conclude that CC homozygous might be a favorable genotype in patients complaining of sepsis with low severity and low risk of early mortality.

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as author. To the best of my knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted.

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