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Single Nucleotide Polymorphisms in IL-1β and IL-6 genes and their Effects on Susceptibility to Typhoid Fever

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Abstract : Background: Typhoid fever (TF) caused by *Salmonella* bacteria represents a major public health problem in developing countries. Pro-inflammatory cytokines play a critical role in the resistance to this bacterium. Many single nucleotide polymorphisms (SNPs) in the promoter region of these cytokine genes can influence the transcription of the corresponding cytokine and subsequently the susceptibility to TF.
Aims: This case/control study aimed to assess the role of two SNPs (IL-1β -511C>T and IL-6-174G>C) in the susceptibility to TF.
Subjects and Methods: Blood samples were obtained from 62 patients with TF and 48 apparently healthy controls. DNA was extracted from these samples, and IL-1β and IL-6 genes were amplified with specific primers using PCR technique. PCR products were directly sequenced and the frequencies of IL-1β -511C>T and IL-6-174G>C were calculated in patients and controls.
Results: For the SNPIL-1β -511C>T, there were no significant differences in either genotype or allele frequencies between TF patients and controls. On the other hand, the homozygote mutant genotype (CC) and allele C of the SNPIL-6-174G>C were more frequent in controls compared to TF patients.
Conclusions: These results strongly suggested that the variant IL-6-174C has a protective role against the infection with TF.
Keywords: typhoid fever, single nucleotide polymorphism, IL-1β -511C>T, IL-6-174G>C.

Introduction

Typhoid feveris a major public health problem in the developing countries. It is estimated that about 27 million new cases of the disease occur annually, most of which in these countries[1,2]. Clinically, *Salmonella* species are categorized in two distinct categories; the typhoidal species (*S. enterica* serotype *typhi* and *S. enterica* serotype *paratyphi*) and non-typhoidal species which include many other serptypes of *S. enteric* especially *S. enterica* serotype*choleraesuis*[3]. As the bacteria are a facultative intracellular organism, it is reasonable to assume that both humoral and cell-mediated immunity (CMI) might have a role in the protection against the disease. However, clinical evidences indicated that CMI, especially cytokines of this immunity arm, play the major role in the host defense against infection with Salmonella[4]. Experimental infections have linked the stimulation ofperipheral blood mononuclear cells(PBMC) with *S. typhi* flagella with rapid synthesis of many proinflammmatory cytokines among which IL-1β and IL-6[5]. In fact, increased production of these cytokines to certain levels in patients with TF associates with better disease outcome and response to treatment[6].

Many single nucleotide polymorphisms (SNPs), especially those in the promoter region of the*IL-1β* and*IL-6* genes have been reported to be associated with the susceptibility to certain infectious diseases and

malignancies[7-10]. However, the association of these SNPs with the incidence of TF received little attention. Thus, this study aimed to assess the effect of IL-1β -511C>T and IL-6-174G>C on the susceptibility to TF among Iraqi patients.

Subjects and Methods

Study population and sample

This case-control study involved 62 outpatients diagnosed primarily as having TF in Al-ImamainAlKahumain Medical City / Baghdad / Iraq for the period from April 2014 to September 2014. The diagnostic criteria were either positive blood culture for S. *enterica* serotype *typhi*or*S. enterica* serotype *paratyphi* or a fourfold rise in antibody titer against theseserotypesData (age, sex, clinical symptoms, laboratory findings and the duration of the symptoms) were collected through direct interview with the patient, and by seeking his/her hospital record as well as previous medical reports. Other 48 age and sex-matched apparently healthy individuals who underwent minor surgeries in the same City were recruited to represent control group.

Three-milliliters (ml) of blood were taken from each participant in EDTA tubes and kept at -20°C until be used for DNA extraction.

DNA Extraction and Genotyping

DNA was extracted from blood samples using ready kit (Favor prep DNA extraction mini kit/ Favor Gene Biotechnologies/ Taiwan) according to the manufacturer's instructions. The primers used for amplification of *IL-1β* and *IL-6*genes are shown in table 1. Template DNA (10 µL) from each sample and primers (5 µL from each) were added to each master-mix tube (50 µL PCR master-mix (Bioneer/Korea). After mixing, the master-mix tubes were transferred to the thermocycler (Hybaid/ England) which was previously programmed with certain protocols according to gene to be amplified.

Table 1: Specific polymerase chain reaction primers and fragment length for IL-1β -511C>T and IL-6-174C>G SNPs.

Polymorphisms	Primers (**Primers (5'→3')**	Fragment length
rs16944	F: 5′-TGGCATTGATCTGGTTCATC-3′ R: 5′-GTTTAGGAATCTTCCCACTT-3′	304 bp
rs1800795	F: 5’-TGACTTCAGCTTTACTCTTTG-3 R: 5’-CTGATTGGAAACCTTATTAAG-3’	198 bp

For -511C>T (rs16944) polymorphism in IL-1β gene, cycling conditions were an initial denaturationfor 5 min at 95℃, followed by 35 cycles of 30s at 95℃; for 45s at 61℃ and for 30s at 72 ℃ followed by final elongation for 7 min at 72℃. The same condition except that for annealing (which was 58℃ for 30s) were applied for IL-6-174C>G (rs1800795) polymorphism in IL-6 gene. PCR products were directly sequenced by Macrogen Company (Korea). The resulted sequences were aligned with the reference sequence in National Center for Biotechnology Information (NCBI)using Chromas pro software.

Statistical Analysis

The distribution of the genotypes in patients and controls was compared with that expected from Hardy-Weinberg equilibrium (HWE) by the chi square (χ^2) test. Frequencies of genotypes and alleles were compared between TF patients and controls using binary logistic regression test. Odds ratios (OR) were calculated together with their 95% confidence intervals (95%CI). Significance level was taken at $P \leq 0.05$. Statistical tests were performed using the software SPSS 16.0 (SPSS Inc., Chicago, Illinois).

Results

The demographic and clinical characteristics of the patients are shown in Table 2.

Table (2): Demographic and clinical characteristics of the patients

Characteristics	Number of patients (%)
Mean age in years (SD)	62.12 (8.82)
Gender	
Male	39(62.91%)
Female	23(37.09%)
Clinical symptoms	
Fever	62 (100%)
Chills	21 (33.87%)
Diarrhea	44 (70.97)
Vomiting	29 (46.77%)
Abdominal pain	13 (20.97%)
Headache	22 (35.48%)
Constipation	2 (3.22%)
Anorexia	9 (14.51)
Laboratory findings	
TLC <4000/mm^3	20 (32.26%)
Hb<12g/dl	46 (74.19%)
Platelet count < 100000/mm^3	16 (25.81%)
Mean ESR (SD)	38.19 (13.08)
Mean duration of the symptoms	5.12 days

SD: standard deviation; TLC: total leukocyte count; Hb; hemoglobin; ESR: erythrocyte sedimentation rate

Allele frequencies in patients and controls were in accordance with Hardy Weinberg Equilibrium (HWE) for both SNPs.

IL-1β-511C>T Genotyping and Allele frequencies

This SNP appeared in three genotypes (CC, CT and TT) among TF patients and control (figure 1). Although the heterozygte and homozygote mutant genotypes appeared to have a protective role against TF, the differences were not significant (CTvis CC OR=0.559, 95% CI=0.254-1.234, *P*=0.15 and TT visCC OR= 0.923, CI=0.144-5.930, *P*= 0.923). At allelic level, T allele was more frequent (27.08%) among controls than TF patients (20.97%), however, the difference was also not significant (OR= 0.714, 95%CI=0.383-1.333, *P*=0.184) as shown in table (3).

Table 3: Genotypes and allele frequencies of IL-1β-511C>T and IL-6-174G>C polymorphisms in TB patients and controls

Variables	Cases N=62	Control N=48	*P*-value	OR(95%CI)
rs16944				
Genotypes				
CC	39(62.9%)	24 (50%)	0.350	1.0
CT	20(32.26%)	22(45.58%)	0.150	0.559(0.254-1.234)
TT	3(4.84 %)	2(4.17%)	0.923	0.923(0.144-5.930)
Alleles			0.184	
G	98 (79.03%)	70(72.92 %)		1.0
T	26(20.97 %)	26(27.08%)		0.714 (0.383-1.333)
rs1800795				
Genotype				
GG	45 (72.85%)	28(58.33%)	0.109	1.0
GC	15 (24.19%)	13 (27.09%)	0.460	0.718(0.298-1.731)
CC	2 (3.23%)	7(15.58%)	0.039	0.178(0.034-0.917)
Alleles		69(71.88%)	0.016	
G	105(84.68%)	27(28.12%)		1.0
C	19(15.32%)			0.462 (0.239-0.895)

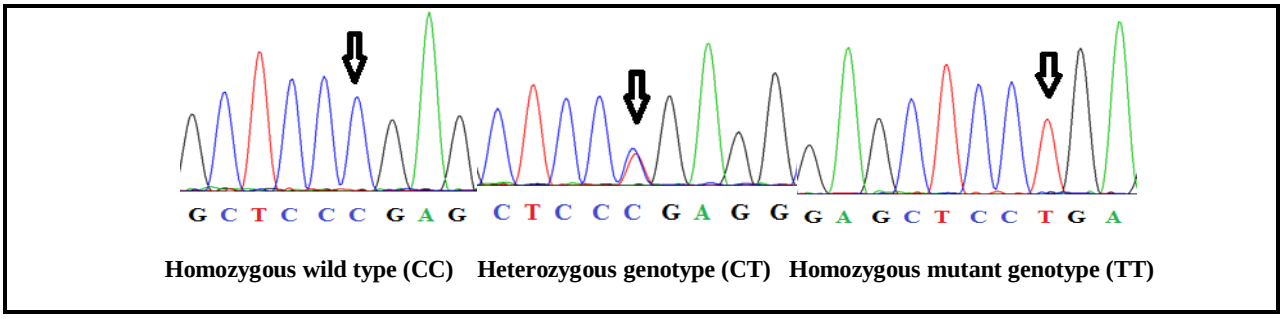

Figure 1: Different genotypes of the variant IL-1β-511C>T

IL-6-174G>C Genotyping and Allele frequencies

Like IL-1β-511C>T, the SNP IL-6-174G>C had three genotypes which were GG, CG and CC (figure 2). The homozygote mutant genotype (CC) was more prevalent among controls than TF patients (15.58% and 3.23% respectively) with significant difference (OR=0.178, 95%CI=0.034-0.917, *P*=0.039).

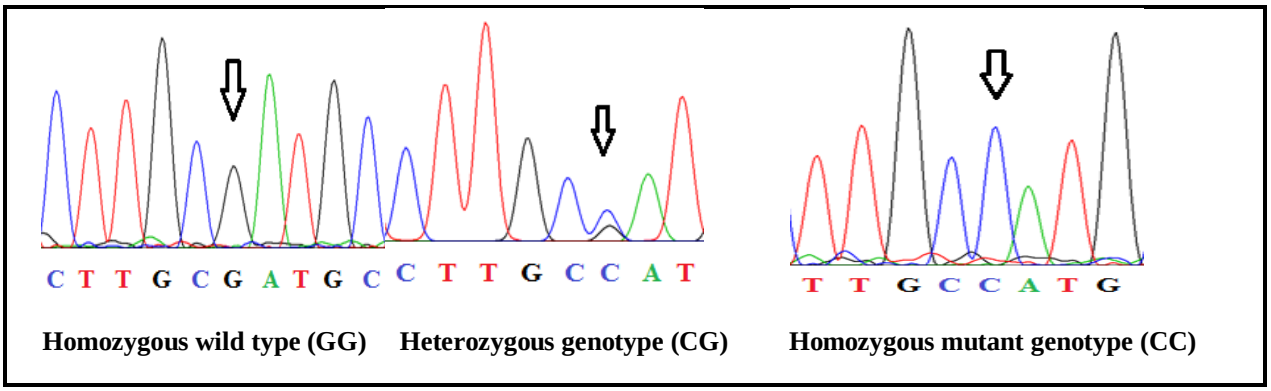

Figure 2: Different genotypes of the variant IL-6-174G>C

As indicated in table (3), Allele frequencies confirmed this result as the frequency of mutant allele (C) in TF patients was 15.32% compared to 28.12% in controls with significant difference (OR=0.462, 95%CI=0.239-0.895,*P*=0.016).

Discussion

The current study revealed that IL-6-174G>C but not IL-1β-511C>T has significant association with the incidence of TF with C allele of SNP IL-6-174G>C has a protective role against this disease. Studies regarding genetic components of TF are very few. Dunstan et al.[11]reported an association between A allele of the SNP TNF-α-308 (as one of the most important proinflammatory cytokines) and the susceptibility to TF among Vietnamese patients. However, Ali et al.[12] did not record any influence of this SNP on TF among Indonesian patients.

The SNPs IL-6-174G>C is located at 174bp upstream from the start site of transcription[13]and was found to influence IL-6 gene expression, with homozygous GG genotype has approximately twice higher levels of this cytokine than CC genotype[14,15]. This explains the protective role of C allele against TF as this allele associate with low or moderate expression of IL-6. The mechanism by which this polymorphism regulates this expression is not fully understood, but the change from a G to a C at position -174creates a potential binding site for the transcription factor NF-1. It has been demonstrated thatthis factor could be considered a repressor of gene expression[16].

Generally, moderate levels of proinflammatory cytokines are very important in inflammatory and immune response. Beyond these levels, the collateral damage of these cytokines overridestheir benefit. Stoycheva and Murdjeva[17] reported significant and conversely proportional correlation between IL-6 and Na^+

and Cl^- concentration. The authors referred this correlation to inflammatory effect of this cytokine accompanied with great loss of these elements in acute inflammatory response. Moreover, a critical step in the progression of TF is the ability of the causative agent to penetrate the gut epithelium. In this regard, IL-6 may encode proteins that are expressed in the epithelium and involved in the *S. typhi* entry or passage.

Both of IL-6 and IL-1β are proinflammatory cytokines. Furthermore the SNP IL-1β-511C>T is in the promoter region of IL-1β gene and different alleles of this polymorphism associate with different levels of IL-1β[18]. Although T allele, in the current study associated with some protection level, the difference was not significant. Then why the protective effect is significant for IL-6 and non-significant for IL-1β? This can be explained by the cross-talk between the two cytokines. Schindler et al.[19] compared cytokine production by PBMC as a main source of both cytokines. They found there was a significant correlation between IL-6 and IL-1 beta (r = 0.72), and IL-6 suppressed IL-1 beta and TNF production induced by LPS by 30% ($P< 0.01$). This suppression appears to be on the level of transcription.Hence IL-6 may provide negative feedback signal to regulate the production of other proinflammatory cytokines[20-22]. Thus even with presence of high expression IL-1β-induced allele, IL-6 could suppress this production.

Collectively, these data suggest that IL-6-174G>C but not IL-1β -511C>T associated with the susceptibility to TF with the variant IL-6-174C has a protective role against this disease. This provides an important tool in defining disease risk and targeting Salmonella eradication in high-risk individuals.

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