Genetic polymorphisms in interleukin-1beta were associated with typhoid fever in Babylon city in Iraq.

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Abstract

A total of 50 clinical samples were taken from the blood of typhoid fever patients who visited Al-Hillah Surgical Teaching Hospital and private laboratories in the AL-Hillah/Babylon region between February and August 2021. This research included 50 people who looked to be healthy and disease-free. Individuals with genotype GA were significantly represented among the patients with typhoid fever: 33 (66%), P-value = 0.0030, as compared with healthy control subjects, 18 (36%), and had an increased risk of developing typhoid fever infection. The IL-1beta (G) allele was less frequent among patients (51%, n=51) than control, and the (A) allele was more frequent among patients than control (49%, n=49). The results showed that there was a significant association between GA genotype and typhoid fever under most inheritance models. The A allele represents a risk factor for patients with typhoid fever and the inheritance of codominant, dominant, and overdominant models as risk factors. The GA genotype has an odd ratio of 4.07 (1.54–10.79) in the model of inheritance. Codominant and dominant models have a related odd ratio of 3.04 (1.21–7.60) and the overdominant model has a related odd ratio of 3.45 (1.52–7.85). A highly interesting Nucleic Acid Polymorphism (SNP) was detected in this study in the investigated samples, in which guanine was replaced with adenine at position 122, namely G122A for the IL-1beta gene.

Keywords: Interleukin-1beta, Typhoid fever, Babylon, Infection

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Introduction

Typhoid fever, which causes diarrhea, enteric fever, and septicemia, is a serious public health concern in many low- and middle-income countries. The availability of antibiotic treatment, as well as better water quality and sanitation, are long-term remedies to this problem, and vaccination in high-risk regions is a viable control method [1]. Typhoid fever is a potentially lethal illness of the gastrointestinal tract and circulatory system caused by harmful microorganisms. *Salmonella enterica* serotype typhi is a gram-negative, non-capsulated, rod-shaped, facultative anaerobe of the *enterobacteriaceae* family with flagella, somatic, and outer coat antigens that only lives in humans because it is an infectious illness spread orally through person-to-person contact, contaminated food, or contaminated water [2,3]. IL-1beta protects against infections by inducing a number of reactions, including the fast recruitment of neutrophils to inflammatory areas, activation of endothelium adhesion molecules, generation of cytokines and chemokines, and the development of the febrile response. IL-1beta is a pro-inflammatory and pyrogenic cytokine produced by a variety of cells, most notably innate immune cells like monocytes, macrophages, and dendritic cells [4]. Single nucleotide polymorphisms in genes that control DNA mismatch repair, cell cycle regulation, metabolism, and immunity are linked to a higher risk of cancer [5]. Cytokine polymorphisms in cytokines change the activity of interleukins and may modify cytokine function, resulting in cytokine expression disorders [6].

Materials and Methods

Blood samples

Two ml of blood were drawn and stored at -20 ℃ in an Ethylene Di Tetra Acetic Acid (EDTA) tube for DNA extraction for molecular analysis.

Primers of the IL-1beta gene

Primers of IL-1beta gene were designated in this study as shown in table (1).

Primer name	Primer sequence 5′ to 3′	Annealing temp	Product size
ZF27	F : 5′-TGTGCCTCGAA GAGGTTTGG-3′	60.25 ℃	368 bp
	R : 5′-GTGTCTTCCACT TTGTCCCAC-3′	59.05 ℃	
ZF29	F : 5′-GGTGCTCCCTG TTGGATCTT-3′	59.67 ℃	369 bp
	R : 5′-TTACAGGTCAGT GGAGACAC-3′	59.40 ℃	

Table1: *Primers of the interleukin-1beta gene.*

DNA extraction

Genomic DNA from blood samples was extracted by using the genomic DNA kit extraction kit protocol (frozen blood), favorgen, Taiwan, and done according to company instructions.

DNA concentration

The extracted blood genomic DNA was checked by using a nanodrop spectrophotometer, which measured DNA concentration and checked the DNA purity by reading the absorbance.

Results and Discussions

DNA purity and concentration

The extraction of DNA from samples of whole blood was successfully extracted and the concentration of DNA was ranged between (20–195 µg/ml) and the purity of DNA was ranged between (1.7–2.0) as shown in figure (1).

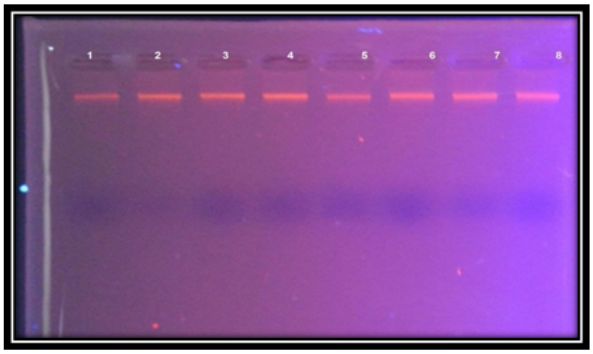

Figure 1: *Electrophoresis of DNA extracted from blood samples, 1% agarose gel, 5 volts/cm for 1 hour.*

PCR of IL-1beta gene

All the 50 confirmed positive cases and the 50 control subjects were submitted to PCR for the detection of IL-1beta by using specific primers as shown in figures (2) and (3).

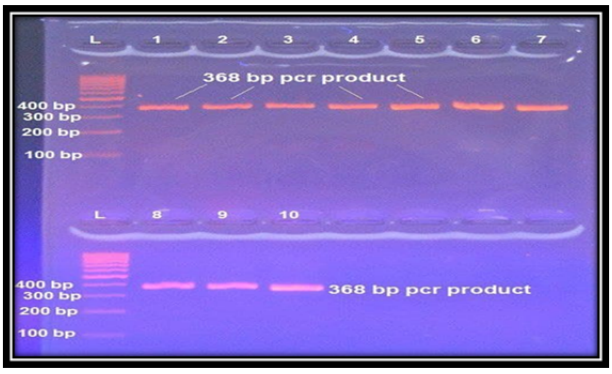

Figure 2: *Agarose gel electrophoresis of IL-1β1.*

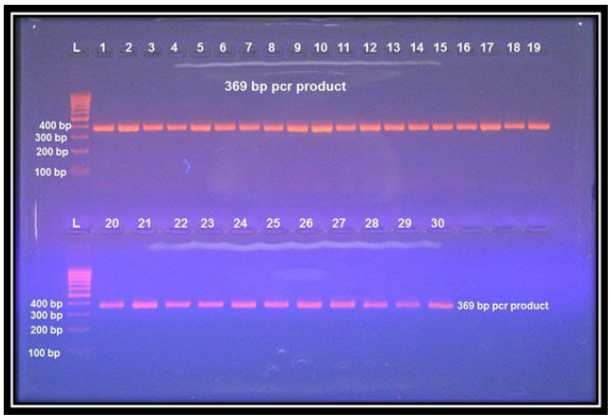

Figure 3: *Agarose gel electrophoresis of IL-1β2.*

Detection of IL-1β genotyping by SSCP-PCR

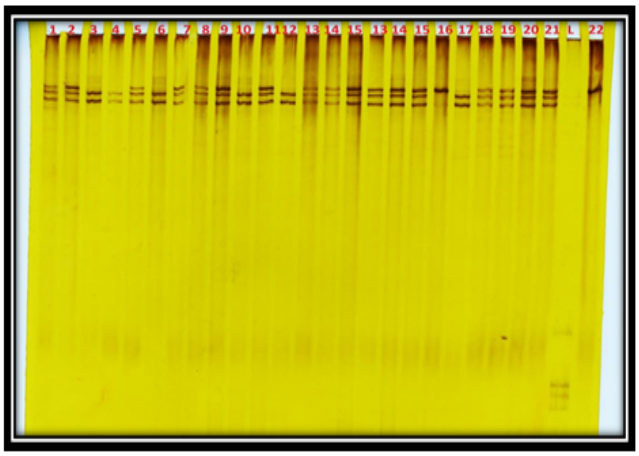

Figure 4: *Silver stained polyacrylamide gel of PCR-SSCP for rs1143629 genotyping.*

Lane L DNA ladder 100 bp lanes 1,2,5,7,8,9,11,13,14,15,18,19,20, and 21 A pattern lanes 3,4,6,10,12, and 17 B pattern lanes 16, and 22 C pattern lanes. The DNA sequence analysis reveals that patterns A, B, and C represent the GA, AA, and GG genotypes of rs1143629, respectively.

Association of rs1143629 with genotypes and allele frequency

According to the results, individuals with genotype GA were significantly represented among the patients with typhoid fever: 33 (66%), P-value=0.0030, as compared with healthy control subjects, 18 (36%), and had an increased risk of developing typhoid fever infection. The IL-1β (G) allele was less frequent among patients (51%, n=51) than control, and the (A) allele was more frequent among patients than control (49%, n=49). As shown in table (2).

Genotype variation	Healthy (n=50)	Patient (n=50)	P-value	Odd ratio (C.I 95%)
GG	20 (40%)	9 (18%)	0.0067	0.754 (0.431-1.317)
GA	18 (36%)	33 (66%)		
AA	12 (24%)	8 (16%)	0.32	1.327 (0.759-2.318)
Allele Frequency				
Allele type	Healthy (n=50)		Patient (n=50)	
G	58 (58%)		51 (51%)	
A	42 (42%)		49 (49%)	

Table 2: *Genotypes and allele frequency for rs1143629 in typhoid fever patients and controls.*

The data has been further examined for each genotype correlated with typhoid fever under different inheritance models. The results showed that there was a significant association between GA genotype and typhoid fever under most inheritance models. The A allele represents a risk factor for patients with typhoid fever and the inheritance of codominant, dominant, and overdominant models as risk factors. The GA genotype has an odd ratio of 4.07 (1.54–10.79) in the model of inheritance. Table (3) shows that the codominant, dominant model has a related odd ratio of 3.04 (1.21-7.60) and the overdominant model has a related odd ratio of 3.45 (1.52-7.85).

Model	Genotype	Control	Case	OR (95% CI)	P-value
Codominant	G/G	20 (40%)	9 (18%)	1	0.0084
	G/A	18 (36%)	33 (66%)	4.07 (1.54-10.79)	
	A/A	12 (24%)	8 (16%)	1.48 (0.45-4.88)	
Dominant	G/G	20 (40%)	9 (18%)	1	0.014
	G/A-A/A	30 (60%)	41 (82%)	3.04 (1.21-7.60)	
Recessive	G/G-G/A	38 (76%)	42 (84%)	1	0.32
	A/A	12 (24%)	8 (16%)	0.60 (0.22-1.63)	
Over dominant	G/G-A/A	32 (64%)	17 (34%)	1	0.0025
	G/A	18 (36%)	33 (66%)	3.45 (1.52-7.85)	

Table 3: *Association of rs1143629 genotypes under different models of inheritance.*

Sequencing analysis

The genotypes observed in this study were validated by sequencing data. The 6 samples were sequenced using PCR-sequences to determine the nucleotide alterations responsible for the findings of the alignment analysis. They were compared with the relevant reference DNA sequences.

Amplicon	Reference locus sequences (5′ - 3′)	Length
DNA sequences within the IL-1β gene	*GGTGCTCCCTGTTGG ATCTTGAGGCCTAACC TCTAGCCCAGCAGAGT CAGCTAAAATCTGAGC TCTCCCTCTCCCTCCA AGCCACACTTTGCAAA GGGATTCCTTGTATTG TGGGCTTGGAGTCTTT TCTCCCCATTTGCCTC TGCAGGAAGCCCTTG CAACAACACATCTGGA TAGCCTCCAGGTCCCA AGGCTGGAGGGACTT GTAATGGGAAAGTAGT CTTTAAATCAGATTTAC TTGGCACCCTGTTTGC CACTGAAAGAGGCAAT TTAGGGGAAAAATCTG GTCTCCAAGCACAGAT AACACTCTACTCTTGA AAGAGGAGACCTGCT CATGTTACTGGTCTCA GCGTCTCCACTGACCT GTAA**	369 bp

Table 4: *The position and length of the 369 bp PCR amplicons used to amplify a portion of the intron-2 sequences within the IL-1β gene located within chromosome 2 (Gen Bank acc. no. NG_008851.1).*

**refers to the reverse primer sequences (placed in a reverse complement direction)*

***refers to the forward primer sequences (placed in a forward direction)*

The gray-colored sequences refer to the positions of the reverse and forward primers, respectively, as placed in the negative strand. The red-highlighted nucleotide refers to the targeted SNP. The alignment results of the 369 bp samples revealed the presence of only one variation in some of the analyzed samples in comparison with the referring reference DNA sequences. A highly interesting Nucleic Acid Polymorphism (SNP) was detected in this study in the investigated samples, in which guanine was replaced with adenine at position 122, namely G122A.

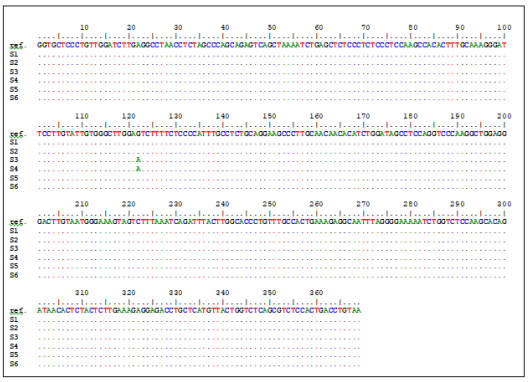

Figure 5: *DNA sequences alignment of 6 genotyped samples with their corresponding reference sequences of the 369 bp amplicons of the coding sequences of the IL-1β gene.*

The sequencing chromatograms of the identified variation, as well as its detailed annotations, were documented, and the chromatogram of this sequence was shown according to its position in the PCR amplicon.

However, this SNP was detected in heterozygous G/A status in both S1 and S2, homozygous A/A status in both S3 and S4, and homozygous G/G status in S5 and S6.

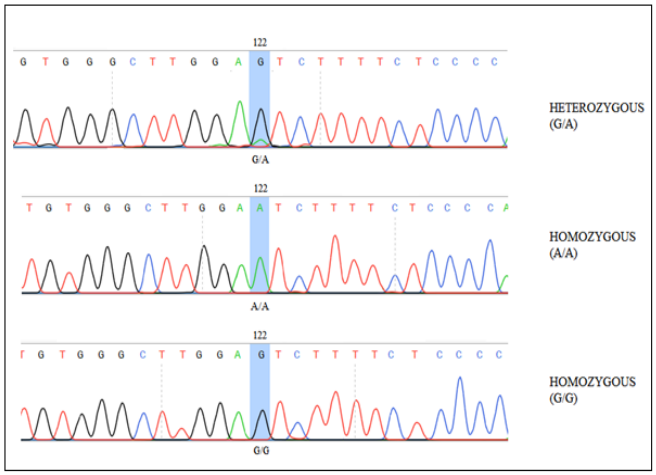

Figure 6: *The pattern of the detected G122A SNP within the DNA chromatogram of the targeted 369 bp amplicons of the IL-1β gene.*

The identified SNP was highlighted according to its position in the PCR amplicons. In the highlighted polymorphic locus, S1/S2, S3/S4, and S5/S6 samples exhibited the G/A, A/A, and G/G states, respectively.

To elucidate the position of the targeted SNP concerning their deposited SNP database of the sequenced 369 bp fragment, the corresponding position of the IL-1β gene was retrieved from the dbSNP server.

To find out the nature of this SNP, a graphical representation was performed concerning the IL-1β dbSNP database on chromosome 2 (Gen Bank Account. No. NC_000002.12).

By reviewing the dbSNP engine, it was found that this detected SNP was previously known as it was deposited as rs1143629. However, this SNP was located in the intron-2 sequences of the targeted IL-1β gene.

A relatively high frequency of the deposited rs1143629 SNP in the dbSNP database was seen, which was estimated to be 0.39 for allele G according to the gnomAD database and 0.40 for the same allele according to the TOPMEd database.

However, this SNP has been reported in many publications regarding the potential effect of the IL-1β locus on many variations associated with immunological and metabolic issues.

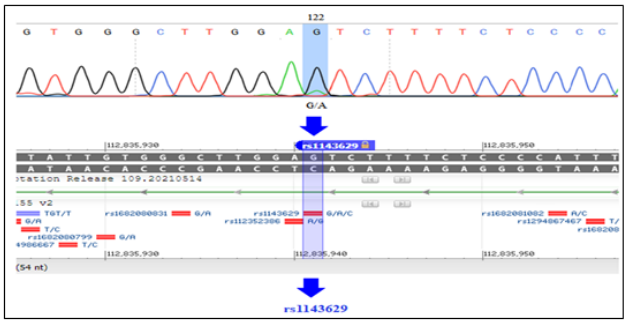

Figure 7: *The SNP's novelty checking of IL1B genetic single nucleotide polymorphisms using the dbSNP server.*

The identified G122A SNP was marked with a blue color. The Gen Bank account. no. NC_000002.12 was used in the positioning of the highlighted substitution SNP. The position of the targeted sequences was found in the negative strand. To summarize the results obtained from the sequenced 369 bp fragments, the detailed position of the observed variation was described in the NCBI reference sequences.

No.	Zygosity status	Position in the PCR fragment	Position in the reference genome	SNP type	Variant summary
S1, S2	Heterozygous (G/A)	122	112835941	Intron variant	rs1143629
S3, S4	Homozygous (A/A)	122	112835941	Intron variant	rs1143629
S5, S6	Homozygous (G/G)	122	112835941	Intron variant	rs1143629

Table 4: *The pattern of the observed SNP in the 369 bp amplicons designed to amplify a portion of the intron-2 sequences within the IL-1β gene in comparison with the NCBI referring sequences (Gen Bank acc. no. NC_000002.12).*

Interleukin-1β is a proinflammatory cytokine produced by monocytes and macrophages that plays an important role in the innate immune response to infection [7]. A recent study with IL-1β rs1143629 in HCV patients found that the AA genotype was significantly more common and that the polymorphisms had no significant association with response to interferon treatment in Egyptian Chronic Hepatitis C patients [8]. Rs1143629 has been linked to malaria, which is consistent with our findings. It was discovered that the rs1143629 G allele was more common in patients than in controls, and that the G/G and A/G genotypes confer an increased risk of developing juvenile systemic lupus erythematous compared to the main genotype A/A [9]. Other studies conclude that polymorphisms in proinflammatory genes such as IL-1β do not contribute to typhoid fever susceptibility and, in light of previous findings, show that the polymorphism is more likely connected to the severity of an existing disease than to vulnerability in general [10]. These findings differ from those of recent Korean research in which SNPs in the IL-1β rs1143629 gene were not linked to an increased incidence of acute pancreatitis [11]. In

those with dilated cardiomyopathy, the IL-1β rs1143629 G/G genotypes work together to increase the risk of atrial fibrillation [12]. The GG genotype in IL-1 rs1143629 was more frequently associated with dental caries and gingivitis [13]. A single-nucleotide variation in interleukin-1β was related to significant levels of child distress [14].

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