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Genetic polymorphisms in interleukin-17A 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. Genotypes and allele frequency for rs1974226 showed individuals with two T alleles (homozygous for the TT) were significantly represented among the patients with typhoid fever: 14 (28%), P-value = 0.0061, as compared with healthy control subjects, 6 (12%), and had an increased risk of developing typhoid fever infection. The IL-17A (C) allele was less frequent among patients (62%, n =62) than control, and the (T) allele was more frequent among patients than control (38%, n =38). Genotypes and allele frequency for rs1974226 showed individuals with genotype TT were significantly represented among the patients with typhoid fever: 14 (28%), P-value = 0.0061, as compared with healthy control subjects, 6 (12%), and had an increased risk of developing typhoid fever infection. The data has been further examined for each genotype correlated with typhoid fever under different inheritance models. The results showed that there was no significant association between any genotype and typhoid fever under all studies of inheritance models. A highly interesting nucleic acid polymorphism (SNP) was detected in this study in the investigated samples, in which cytosine was replaced with thymine at position 226, namely C226T for the IL-17 gene.

***Keywords*---**interleukin-17A, typhoid fever, Babylon, infection.

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]. Interleukin 17 (IL-17) is an inflammatory cytokine and it plays a protective role against infection [4]. IL-17A is produced by Th17 CD4+ T cells and other leukocytes such as natural killer cells, lymphoid tissue inducer-like cells, and neutrophils [5]. Single nucleotide polymorphisms in genes that control DNA mismatch repair, cell cycle regulation, metabolism, and immunity are linked to a higher risk of cancer [6]. Cytokine polymorphisms in cytokines change the activity of interleukins and may modify cytokine function, resulting in cytokine expression disorders [7].

Material and Methods

Blood Samples

Two ml of blood were drawn and stored at -20oC in an ethylene di tetra acetic acid (EDTA) tube for DNA extraction for molecular analysis.

Primers of the IL-117A gene

Primers of IL-17A gene were designated in this study as shown in table (1).

Table (1): Primers of the interleukin-117A gene

Primer name	Primer sequence 5′ to 3′	Annealing temp	Product size
ZF25	F: 5′-GACCTCATTGGGGGCGGAAA-3′	62.49 Cº	301 bp
	R : 5′-CCATAGTCAGAACCCAGCGTTT-3′	60.62 Cº	
ZF26	F : 5′-GGGGAAAATGAAACCCTCCCC-3′	60.90 Cº	328 bp
	R : 5′-GGGGCGAAAATGGTTACGATG-3′	59.94 Cº	

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 Discussion

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).

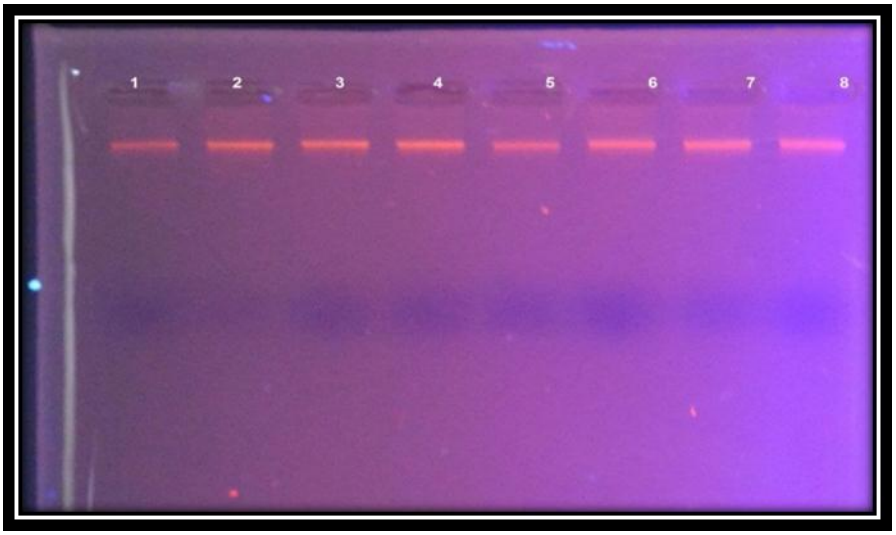

Figure (1): Electrophoresis of DNA extracted from blood samples, 1% agarose gel, 5 volts/cm for 1 hr.

PCR of IL-17A gene

All the 50 confirmed positive cases and the 50 control subjects were submitted to PCR for the detection of IL-17A by using specific primers as shown in figures (2) and (3).

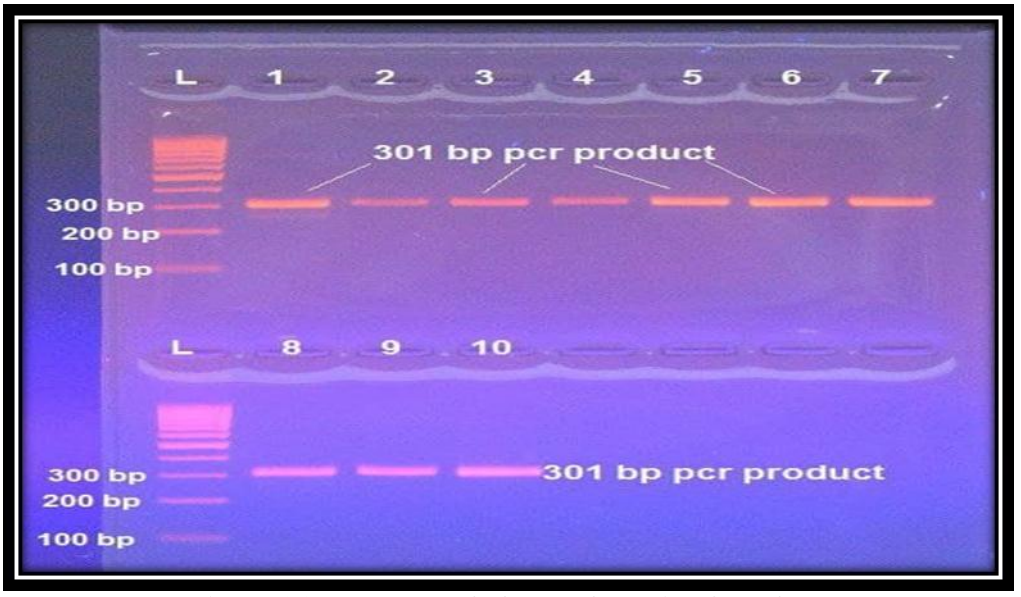

Figure (2): Agarose gel electrophoresis of IL-17A1

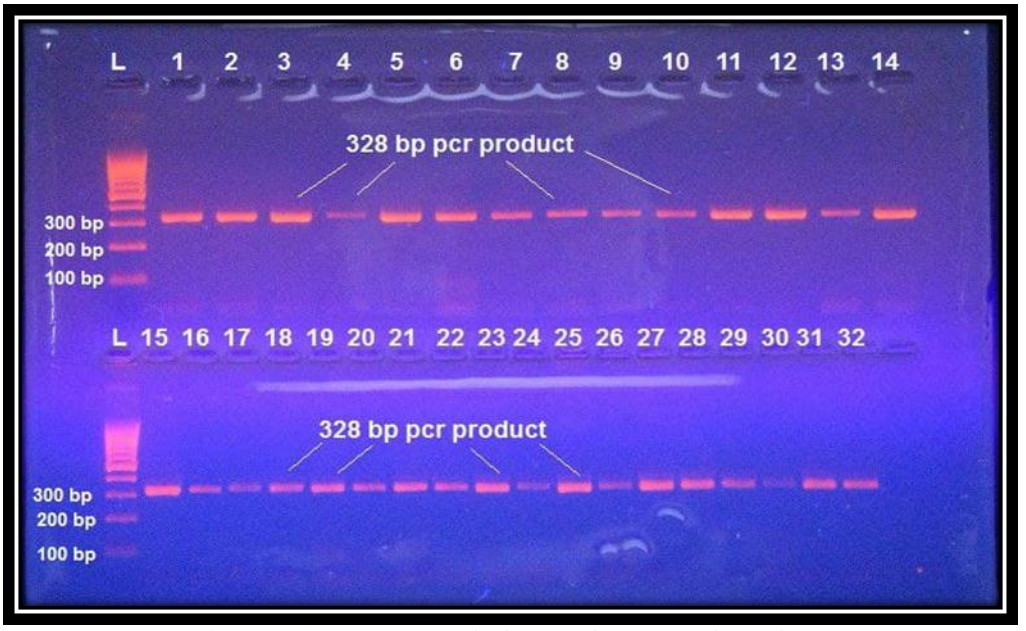

Figure (3): Agarose gel electrophoresis of IL-17A2

Detection of IL-17A Genotyping by SSCP-PCR

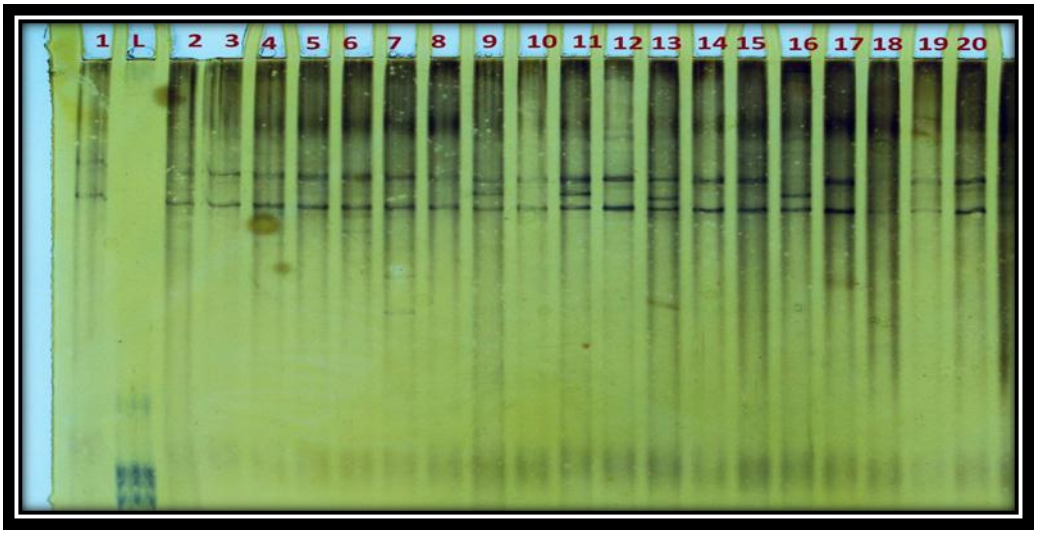

Figure (4): Silver stained polyacrylamide gel of PCR-SSCP for rs1974226 genotyping

Lane L DNA ladder, 100 bp, 9, 11, and 13 in an A pattern. Lane 16 has a C pattern, while the other lanes have a B pattern. The DNA sequence analysis reveals that pattern A, B, and C represent the CT, CC, and TT genotypes of rs1974226, respectively.

Association of rs1974226 with Genotypes and Allele Frequency

According to the results, individuals with two T alleles (homozygous for the TT) were significantly represented among the patients with typhoid fever: 14 (28%), P-value=0.0061, as compared with healthy control subjects, 6 (12%), and had an increased risk of developing typhoid fever infection. The IL-17A (C) allele was less frequent among patients (62%, n=62) than control, and the (T) allele was more frequent among patients than control (38%, n =38), table (2).

Table (2): Genotypes and allele frequency for rs1974226 in typhoid fever patients and controls

Genotype variation	**Healthy (n=50)**	**Patient (n=50)**	**P-value**	**Odd ratio (C.I 95%)**
CC	28 (56%)	26 (52%)	0.67	0.735 (0.350-1.150)
CT	16 (32%)	10 (20%)		
TT	6 (12%)	14 (28%)		
			0.0061	1.576 (0.870-2.856)
Allele Frequency				
Allele type	**Healthy (n=50)**		**Patient (n=50)**	
C	72 (72%)		28 (28%)	

T	62 (62%)	38 (38%)

The data has been further examined for each genotype correlated with typhoid fever under different inheritance models. The results showed that there was no significant association between any genotype and typhoid fever under all studies of inheritance models as shown in table (3).

Table (3): Association of rs1974226 genotypes under different models of Inheritance

Model	Genotype	Control	Case	OR (C.I 95%)	P-value
Codominant	C/C	28 (56%)	26 (52%)	1.00	0.092
	C/T	16 (32%)	10 (20%)	0.67 (0.26-1.75)	
	T/T	6 (12%)	14 (28%)	2.51 (0.84-7.51)	
Dominant	C/C	28 (56%)	26 (52%)	1.00	0.69
	C/T-T/T	22 (44%)	24 (48%)	1.17 (0.53-2.58)	
Recessive	C/C-C/T	44 (88%)	36 (72%)	1.00	0.043
	T/T	6 (12%)	14 (28%)	2.85 (1.00-8.17)	
Over dominant	C/C-T/T	34 (68%)	40 (80%)	1.00	0.17
	C/T	16 (32%)	10 (20%)	0.53 (0.21-1.32)	

The findings appear to support the link between the T/T genotype and susceptibility to typhoid fever. Th17 cells express IL-17A and IL-17F, which are involved in coordinating local tissue inflammation [8,9]. Polymorphisms in IL-17 cytokines alter interleukin activity and may alter cytokine function, resulting in dysregulation of IL-17 expression [7]. Because IL-17 is thought to be an important pro-inflammatory factor, many studies of IL-17 SNPs and susceptibility have focused on inflammation-related diseases [10]. A few studies have looked into the role of IL-17 SNPs in breast cancer susceptibility [11], and studies of gastric cancer have mostly focused on rs2275913 but have yielded contradictory results [12, 13]. Other studies have found that the IL-17 polymorphism plays an important role in many autoimmune diseases [14], when found that the IL-17 rs2275913 and rs3819024 variant alleles were associated with a lower risk of rheumatoid arthritis, while the IL-17 rs3819025 and rs8193036 variant alleles were associated with an increased risk of rheumatoid arthritis. The rs1974226 SNP was linked to an increased vulnerability to gram-positive infections. In two sepsis cohorts, patients with the rs1974226 GG genotype were more vulnerable to gram-positive infection than patients with the AG/AA genotype, and in the subgroup with lung infection, the IL17A rs1974226 G allele was related with greater 28 day mortality of severe sepsis [15]. Previous research indicated that the T allele of rs1974226 in IL17A was related to asthma, and that other genetic variations in the IL17 pathway genes were connected with both protection and

risk for asthma development with IgE levels[16]. According to Ahmed Ali et al. (2018), no significant association was found between IL-17 rs1974226 genotypes and related serum cytokine levels, implying that elevated serum IL-17 may increase the susceptibility to septic complications in polytrauma patients and thus could be a useful biomarker for trauma patient management [17]. There is mounting evidence that the IL-17 gene single nucleotide polymorphism (SNP) rs1974226 is linked to gram-positive bacterial resistance and susceptibility to infectious and noninfectious illnesses [18].

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