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Susceptibility of Interleukin-1 Beta SNP:-511C/Ton the Physiological Incidence Ofjuvenile Idiopathic Arthritisin Iraqi Children

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ABSTRACT

Background and objective: The condition characterized as mixed of idiopathic inflammatory arthritis that appears in children younger than 16 years of age has been called Juvenile idiopathic arthritis (JIA). The association between IL-1B gene single nucleotide polymorphism in the promoter region (SNP: -511C/T) with the incidence of JIA in Iraqi children samples depending on genders and ages was investigated in this study. Methods: levels of IL-1B were estimated by ELISA and genetic analysis was done by PCR-RFLP for investigation of SNP: -511C/T in patients and control groups. Results: The results of the present study suggesting highly significant differences in IL-1B levels (pg/ml) between JIA and the control group (p-value< 0.05). The results suggesting allele frequency and genotyping analysis that statistically significant differences by odds ratio and p-value in CC and TT between JIA and control groups but not significant of CT genotypes(OR=1.899, CI 95%=1.34-2.33). IL-1B levels (pg/ml) were investigated in all subtypes of JIA depending on genotypes of IL-1B gene in the patients group and the results were significant in O and S subtypes of JIA (p-value< 0.05). Conclusion: IL-1B SNP:-511C/T appears ahigh-risk factor for physiological incidence of JIA in Iraqi Children.

KEYWORDSJuvenile idiopathic arthritis, IL-1, SNP: -511C/T, children

INTRODUCTION

The last works of the literature suggested that the condition characterized as mixed of idiopathic inflammatory arthritis that appears in children younger than 16 years of age was called Juvenile idiopathic arthritis (JIA) and the period is six weeks or more than this period[1,2].The main cause of JIA incidence was unusual invulnerable reactions set off by the connections between ecological variables in a hereditarily helpless individual is theoretical [3]. JIA was classify according to presentation of subtypes in first six month of incidence by the organization that called ILAR[4]. The main three subtypes of JIA are systematic, Oligoarticular, and Psoriatic[5].Patients with Systemic-beginning JIA are in danger of a conceivably perilous inconvenience called macrophage initiation disorder. Rheumatoid factor (RF) is commonly negative in fundamental JIA [6]. Oligoarticular JIA is the more important and widely recognized JIA subtype, and happens when there are more than to 4 joints required during the initial a half year of illness. Two subclasses of oligoastrocytomasjoint inflammation exist: tireless oligoarthritis, where close to only tetra joints are influenced all through the entire sickness course; and broadened oligoarthritis, where multiple joints are influenced after the initial a half year of infection [7]. The psoriatic arthritis subtype of joint inflammation has been analyzed by the mix of joint pain and psoriasis or, joint inflammation and at any rate 2 of the accompanying: Dactylitis, nail-pitting, or Psoriasis in a first-degree relative. Psoriatic joint inflammation is normally unbalanced in its example of joint contribution

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and can include both enormous and little joints [8].Interleukin 1 beta (IL-1B) in any case called leukocytic pyrogen, leukocytic endogenous authority, mononuclear cell factor, lymphocyte sanctioning element, and various names, is a cytokine protein that in individuals is encoded by the IL-1B quality [9]. IL-1B is an individual from the interleukin 1 group of cytokines and this cytokine is delivered by initiated macrophages as a supportive of protein, which is proteolytically handled to its dynamic structure by caspase 1. This cytokine is a significant middle person of the provocative reaction and is associated with an assortment of cell exercises, including cell expansion, separation, and apoptosis [10].Two hereditary polymorphism of IL-1B quality (- 511 C/T SNP of advertiser locale of IL-1B quality, and the +3953 C/T SNP of IL-1B quality exon 5) can change the structure and capacity of protein without influence the polypeptide chains by change the arrangements of the amino acids [11]. The aim of this study to investigation of IL-1B gene polymorphism (SNP: -511C/T) on its levels in Iraqi children with JIA.

MATERIAL AND METHODS

Study group:

This study included 60 subjects, thirteen patients with JIA were complete diagnosis and physiological subdivided into three groups systemic (S), polyarticular (P), and oligoarticular (O). The rang of ages were (4-16 years) for patients group, control group also included 30 children matched with age and gender as showing in table 1:

Table (1): Physiological characterization of study groups

Parameters	**JIA patients N=30 (%)**	**CON N=30**	**p-value**
Age	9±7.2	8±8.1	0.122
Gender M/F	13/17	15/15	0.231
JIA subtypes			
S	8(27)	-	-
P	10(33)	-	-
O	12(40)	-	-
Age at onset of JIA, mean±SD (years)	7.3±2	-	-
4-8	17(57)		
8.1-12	7(23)		
12.1-16	6(20)		

Age (years) mean±SD

Abbreviations: S: systematic, P: Polyarticular, O: Oligoarticular, CON: control,

Measurement of IL-1B:

ELISA technique was used to assessment of IL-1B levels (pg/ml) following the manufacture instructions. The standard curve of this parameter is showing in figure 1:

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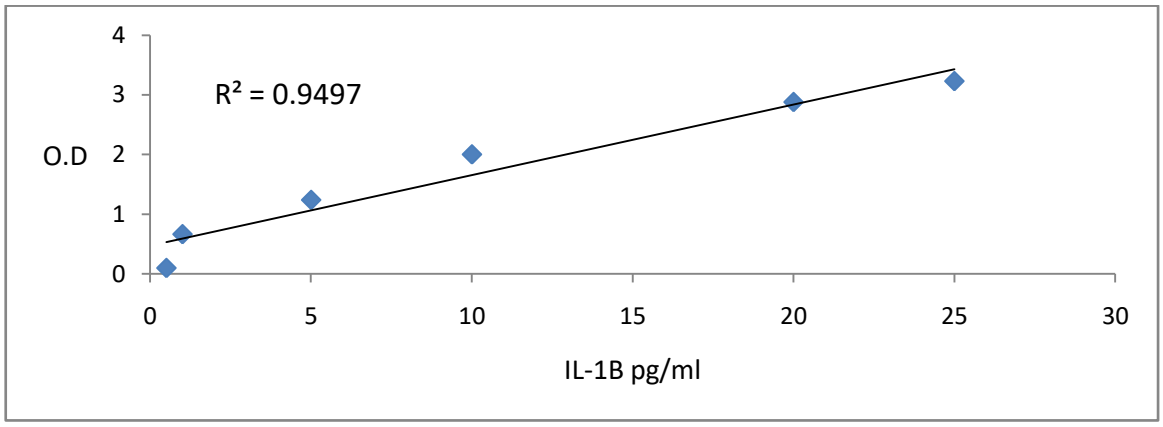

Figure (1): IL-1B standard curve of ELISA kit

IL-1B gene analysis:

The Genomic DNA was extracted from blood storage in EDTA tubes by using genomic DNA kit(Geneaid Biotechnology Ltd., Taiwan) that providing an efficient method for purifying of total DNA from whole and frozen blood. PCR-RFLP was performed by used unique primers for analysis of C/T IL-1B genotyping and restriction enzyme (RE) [12], as listed in table 2.

Table (2): primers and RE of IL-1B gene that used in genotyping analysis

SNP	Primer sequence(5'→3')	Amplicon length	*AvaI* RE bands
-511C/T	F: TGGCATTGATCTGGTTCATC R:GTTTAGGAATCTTCCCACTT	304 bp	TT: 190,114 bp TC: 304,190,114bp CC: 304 bp

The PCR weredone in average total volume of 25 µl of reaction mixture with Taqman polymerase and carried by the thermocycler (Biorad) and subjected to denaturation at 94 Cº for five min, following by 30 cycles of 94.5 Cº for 30 sec, 58.6 Cº for 40 sec and the final PCR extension phase at 72 Cº for 5 min. The final PCR amplicon of 304 bp was electrophoresis by agarose gel 1.5% and photo documentation the products. PCR ampliconwas digested by restriction endonuclease that called,*Aval*in water bath at 37 Cº for about 48 hours and then analyzed by 6% of the polyacrlamide gel electrophoresis (PAGE).After PAGE analysis documentation, the alleles coded as 304 bp for homozygote of T allele (TT); 190 and 114 bp for homozygote of C allele (CC); 304 , 190 and 114 bp for heterozygote of (CT) alleles.

Statistical Analysis:

The statistical analysis was applied by Microsoft Excel 2013 and SPSS version 22. The statistical values are expressed as the mean ± SD. The one way t-test was used for estimating the P-value and considered <0.05 to be statistical significant that results from compassion of study groups.

RESULTS

The gel electrophoresis and PCR product (304 bp) of -511 C/T of IL-1B gene is showing in figure 2:

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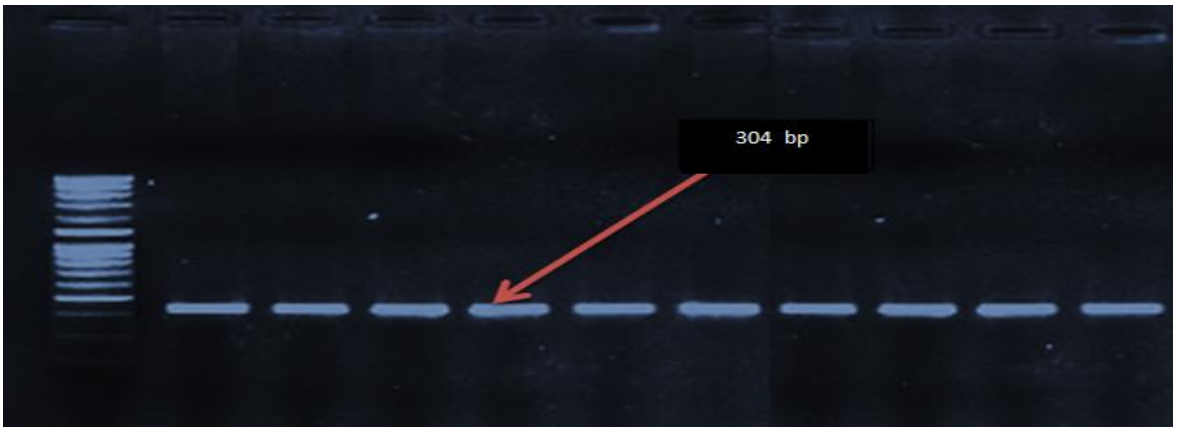

Figure (2): PCR (305 bp) product of IL-1B gene

The gel electrophoresis after digestion with *AvaI* for samples of patients with JIA and control groups is showing in figure 3:

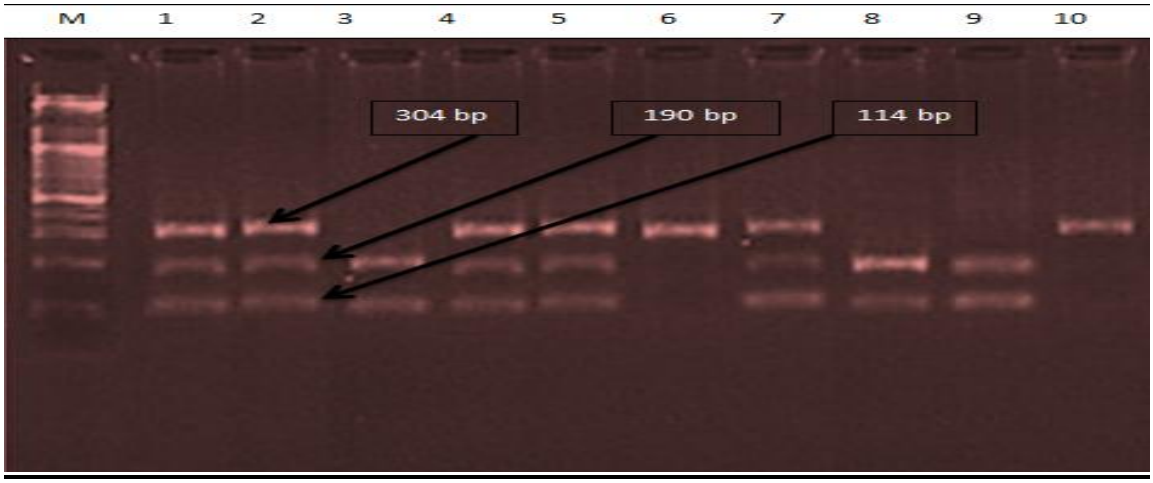

Figure (3): PCR-RFLP and PAGE of -511 C/T promoter of IL-1B gene showing three genotypes CC, CT, and TT in differentbands length(304, 114, and 190 bp).

The results of present study suggesting highly significant differences in IL-1B levels(pg/ml) between JIA and control group (p-value< 0.05), as listing in table 3:

Table (3): levels of IL-1B in study groups

Groups	IL-1B (pg/ml) mean± SD	P-value
JIA n=30	11.19±1.4	0.0000
Control n=30	6.68±1.3	

The results suggesting allele frequency and genotyping analysis that statistical significant differences by odd ratio and p-value in CC and TT between JIA and control groups but not significant of CT genotypes, as listed in table4:

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Table (4): Comparison of three genotypes prevalence in JIA and control groups

Genotypes	JIA	control	OR	CI 95%**	P-value
CC	15 (50%)	13 (43%)	2.449	1.97-3.74	S
CT	10 (33%)	9 (30%)	0.765	0.78-1.92	NS
TT	5 (17%)	8 (27%)	1.345	0.83-1.97	S
C allele	66%	58%	1.899	1.34-2.33	S
T allele	68%	42%			
Total	30(100%)	30(100%)	-	-	

IL-1B levels (pg/ml) were investigated in all subtypes of JIA depending on genotypes of above gene in patients group, as listed in the table 5:

Table (5): levels (pg/ml) of IL-1B in S,P, and O subtypes of JIA in patients group

Genotype JIA subtype	CC	CT	TT	P-value
S (n=8)	11.58±1.7	12.19±1.1	9.13±1.9	S
P (n=10)	12.87±1.3	13.76±1.8	13.11±2.3	NS
O (n=12)	11.72±1.4	13.29±1.6	10.08±2.1	S

DISCUSSION

The present work aim to investigation of -511 C/T SNP of IL-1B and its role on prevalence of different subtypes of JIA disease in Iraqi children. One of the well-known the most important commonchronic arthritis in younger children worldwide is called JIA and the children withJIA can experience delayed and restricted growth[13]. *Prahalad et al., 2008* were reported thatan intricate collaboration between singular quality defenselessness, cytokines enactment, and different natural triggers and this might be lead to a resistant irregularity that accordingly brings about articular and foundational indications of JIA[14].*Dinarello et al 1996*., suggested that IL-1B is the best characterized and most studied of the IL-1 family members and theIL-1B is an intense supportive of incendiary cytokine that is vital for have safeguards reactions to disease and injury [15-16]. With respect to gene polymorphism -511 C/T of IL-1B, allele and genotype frequenciesshowed significant variations between JIA patients and controls. Many previous investigationshave examined the association between -511 C/T of IL-1B genepolymorphisms JIA in Iraqi children.To the best of our knowledge this the first study concerning the association of -511 C/T IL-1B gene polymorphism in Iraqi JIA children.*Al-Mayouf., 2018* was reported that JIA is one of childhood chronic inflammatory arthritis and it addresses a phenotypically heterogeneous gathering of joint pain, along these lines; they have comparative incendiary articular changes. JIA is pivotal to perceive joint inflammation, which is a clinical finding showed as firmness [17].Cytokines have been ensnared in the turn of events and propagation of fiery reaction in JIA infection[18].*Charo et al.,2006* were founds that during active disease, cytokine

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concentrations in plasmaof patients with JIA increased 2 to 35-fold[19]. The results of present study suggesting highly significant differences in IL-1B levels (pg/ml) between JIA and control group (p-value< 0.05). The results suggesting allele frequency and genotyping analysis that statistical significant differences by odd ratio and p-value in CC and TT between JIA and control groups but not significant of CT genotypes(OR=1.899, CI 95%=1.34-2.33). By using the PCR-RFLP technique for-511 C/T IL-1Bgene polymorphism, the present study had foundthat the percentage of homozygous genotype CC in JIA patients were (50%) which was more than control group (43%) as shown in Table (4).Thus, it was noted that there was significant difference(P<0.051) between them and heterozygote genotype(CT) which was more frequent (33%) in total JIA than control group (30%).In addition, the mean SD of IL-1B levels were (12.87±1.3,13.76±1.8, and13.11±2.3) in P subtype of JIA in CC, CT, and TT genotypes, respectively and this results non-significant in compare to other subtypes S and O, as shown in Table (5).In other words, an children with a CT genotype have less risk to incidence of JIA compare to other genotypes those may be more risk factor for diseasein Iraqi children.

The present work was initiated to explore that IL-1B levels (pg/ml) were investigated in all subtypes of JIA depending on genotypes of IL-1B gene in patients group and the results were significant in O and S subtypes of JIA (p-value< 0.05) and this results in same line with other study on RA in Iraqi population that work on other gene and suggested of C174G polymorphism in promoter of IL6 gene with increasing in GH and TNFA levels consider a risk factor for incidence of psoriasis in different ages and genders[20].

CONCLUSION

Interleukin-1 beta SNP:-511C/T is suggested by this work as a high-risk factor for physiological incidence of Juvenile idiopathic arthritis (JIA) in Iraqi Children.

CONFLICTOF INTERST

Nil

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Non

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