The Prevalence of Toll-Like Receptor 3 (Rs13126816) Genotype: A Study on Iraqi Children

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Abstract
Background: Many SNPs in TLR3 gene have been identified and linked to many diseases in human. Objective: Study the prevalence of TLR3 rs13126816 gene polymorphism in an Iraqi population. Materials and methods: Amplification-refractory-mutation -system (ARMS-PCR) technique was used to identify rs13126816 genotypes in all 93 healthy children. Results: The genotypes of the targeted SNP were distributed as following: AA (45.1%), A/G (34.4%), GG (20.5%). Conclusion: The most prevalent genotype of the TLR3 SNP was the wild genotype and the mutant allele represented about one third of total count of alleles
Keywords: Toll-like receptors, TLR3, polymorphism, genotype, Iraqi children

1. Introduction

Toll-like receptors (TLR) are major parts of the innate immune system of human. They are present on human cells surfaces, there are 11 types of the human TLRs that have been discovered to date. TLR3 particularly is in endosomes and is specialized to target retroviral double-stranded RNA. Immune cells that express TLR3 include dendritic cells, macrophages, natural killer cells and mast cells. The expression of TLR3 is strongly induced in a variety of cells by (IFN-1), viral infections or exposure to dsRNA, signaling through TLR3 activates a cascade of molecules through a pathway that ends in expression of (IFN-β) and other pro-inflammatory cytokines (Sanclemente et al., 2014; Skevaki et al., 2015). Several genetic variations within TLR3 gene were linked to with high susceptibility or resistance to immunological or infectious diseases (Svensson et al., 2012; Biyani et al., 2015; Jiménez-Sousa et al., 2015; Bucciol et al., 2021; Wang et al., 2021). This study will be one of few that investigate TLR3 polymorphism in general and rs13126816 in an Iraqi population.

2. Subjects and Methods

93 healthy children were chosen from a hospital at the middle Euphrates area/Iraq for the study. Blood samples were drawn by venipuncture and collected using EDTA tubes. Amplification-refractory-mutation-system (ARMS-PCR) technique was used for detection of the TLR3 SNP. The period of samples collection was from November 2021to April 2022.
Genomic DNA from blood samples were extracted by using gSYAN DNA extraction kit (frozen Blood) GENEaid/ USA and done according to manufacturer instruction.
The extracted DNA form blood samples was checked using (Nanodrop spectrophotometer/ THERMO-USA), which measured DNA concentration (ng/µl) to check the DNA purity by reading absorbance at (260/280nm).
ARMS-PCR master mix was prepared using GoTaq® G2 Green Master Mix Kit and this master mix was used to execute two reactions for each sample (one for the wild type allele and the other for the mutant type allele) according to the manufacturer instructions. The mix consisted of 5µltof template DNA, 2µltof forward primer (for each reaction), 2µl of the common reverse, 12.5µltof G2 Green master mix, and 3.5µlt of PCR water.
Thermocycler conditions were 95°C/5 min pre-denaturation for a single cycle, 95°C/30 sec denaturation, 55°C/30 sec annealing/extension, 72°C/30 sec extension each for 35 cycles, and 72°C/5 min for final extension.
PCR master mix were transferred into Exispin vortex and centrifuged at 3000rpm for 3 minutes, then placed in PCR thermocycler (BioRad/USA).
The PCR product was electrophoresed on 2% agarose gel with ethidium bromide stain at 100 volt and 80 AM for 1 hour and was visualized using Ultraviolet transilluminator (ATTA/Korea).

3. Results

The binding between the extracted human DNA and the specific primers for TLR3 gene promoter site was successful and is demonstrated in figure 1, the product was detected by gel electrophoresis analysis by a DNA marker ladder (INtRON/Korea) and has a size of (520) base pair (bp) in both patients and control groups.

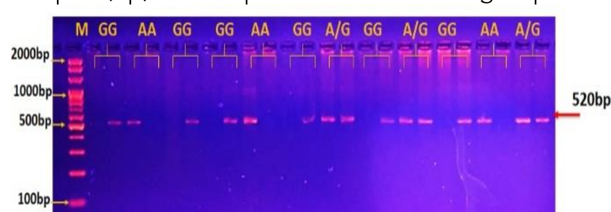

Figure 1; Agarose gel electrophoresis image that showed the ARMS-PCR product analysis of Toll-like receptor 3 TLR-3 gene (rs13126816) A/G gene polymorphism. Where M: marker (1500-100bp). The lane (AA) wild type homozygote was showed as A allele only. The lane (GG) mutant type homozygote was showed as G allele only, whereas the (A/G) heterozygote were showed as both A and G allele. The presence of A or G allele were observed at 520bp product size.

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The ARMS-PCR results analysis for the 93 individuals implicated in this study showed the AA genotype was seen in 42 (45.1%) of them, A/G genotype was seen in 32 (34.4%), and the GG genotype was seen in 19 (20.5%) (table 1). Allele frequency for the mutant allele was (37.7%) (table 2).

Table 1: Distribution of TRL3 rs13126816 genotypes			
TLR-3 genotype (rs13126816)	Male; n (%)	Female; n (%)	p-value
AA	27 (55.1%)	15 (34.1%)	0.113 C NS
A/G	13 (26.5%)	19 (43.1%)	
GG	9 (18.4%)	10 (22.8%)	
n: number of cases; C: chi-square test; NS: not significant			

Table 2: Allele frequency for both wild and mutant alleles.			
Allele	Male	Female	P- value
A	67 (61.12%)	49 (54.88%)	0.074 C NS
G	31 (36.88%)	39 (33.12%)	
n: number of cases; C: chi-square test; NS: not significant			

4. Discussion

The prevalence of the SNP in females were slightly higher than males, however, the correlation with gender was not significant (p=0.113). Regarding allele frequency, the mutant allele was closely distributed between genders, but again, the correlation with gender was not significant.

In a study performed in Brazil on type 1 diabetes mellitus, this SNP was found in (89.2 %) of the total study population (patients and control groups). The genotype (GG) was the most prevalent in both groups, followed by (A/G) and lastly, (AA). Males represented (47.5%) of the (AA) genotype, and (58.4%) of both (A/G) and (GG) combined (Assmann et al., 2014).

In another study in Poland that investigated multiple SNPs in TLR3 gene, the most seen genotype for rs13126816 was (GG) also. And the most distributed allele was the mutant allele (Fichna et al., 2016).

A study in China, several SNPs were implicated to study its correlation with enterovirus A17 hand, foot, and mouth disease in children. They found that the mutated genotypes (GG and A/G) were the most prevalent and the mutant allele is the most prevalent (Chen et al., 2021).

The conflict with the results of these studies and those of the current study could be attributed to multiple reasons. TLR3 SNPs are known to be associated to susceptibility to systemic autoimmune diseases and also viral infections (Fichna et al., 2016), and these studies were conducted to investigate the relationship between TLR3 polymorphism and a certain disease or condition, while the current study involved healthy children. Additionally, the different ethnicity and environment may also have their impact on the results.

5. Conclusion

This study found that the mutated allele (G) is present in nearly one third of the study population (Iraqi children) and the mutated genotypes were mostly seen in females. This is calling for further investigation by researchers as it may explain many viral infections and/or autoimmune diseases in the country. Although the study population was relatively small and within certain limit of age, this study may provide a necessary data for later research and studies.

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