The prevalence of TNF-α polymorphism in an Iraqi pediatric population

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Abstract:
Background: Many polymorphisms in the TNFα promoter gene had been reported. The G/A polymorphism at −308 has been found to be correlated with a 20%-40% increase in TNF-α production. Objective: To study the prevalence of TNF-α rs3093661 genotype in an Iraqi pediatric population. Materials and methods: Amplification-refractory-mutation -system (ARMS-PCR) technique was used to identify this genotype in all 96 subjects. Results: The genotypes of the targeted SNP were distributed as following: (rs3093661): 21 (21.9 %) AA genotypes, 19 (19.8 %) G/A genotype and 56 (58.3 %) GG genotype. Conclusion: The distribution of TNF-α (rs3093661) Polymorphism was detected by ARMS-PCR technique. At this locus there are three genotypes: GA, AA and GG. The wild type of homozygote genotype was showed only G allele amplification at 173bp product size. The mutant type of homozygote genotype was showed only A allele amplification at 173 bp product size. Whereas the heterozygote genotype was showed G and A alleles amplification at 173 bp product size respectively.
Key words: TNF alpha gene, polymorphism, genotype, Iraqi pediatric population

1. Introduction

Tumor necrosis factor alpha (TNFα), is well defined as acute-phase pro-inflammatory cytokine in a variety of disease states, including the host inflammatory response to infection (Mahallawi et al., 2018). The coding gene of this cytokine is positioned within the major histocompatibility complex calss III region in chromosome six in the short arm (El-Tahan et al; 2016). The genetic control of pro-inflammatory cytokine production has been widely explored. Among many reported polymorphisms in the TNFα promoter gene, the G/A polymorphism at −308 has been shown to correlate with a 20%-40% increase in TNFα production. and most significantly associated with susceptibility to infections (Saremi et al., 2021).

2. Material and Method

A cross sectional study was designed for 96 patients children. Samples will consist of blood samples for detection of genetic polymorphism. Blood samples were obtained by venous puncture. An amount of 1-2 ml of peripheral blood will be collected and transported using EDTA tubes. Freezing at 4°C for short time or −70 °C for long time will be the method for preservation of samples until tests are performed. Detection of TNF alpha gene genotypes will be carried out ARMS PCR. The period of samples collection was from October 2021 to 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 5µl of template DNA, 2µl of forward primer (for each reaction), 2µl of the common reverse, 12.5µl of G2 Green master mix, and 3.5µl 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.
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 Ultra violet transilluminator (ATTA/Korea).

3. Results

There are three genotypes: GA, AA and GG. The wild type homozygote genotype was showed only G allele amplification at 173bp product size. The mutant type homozygote genotype was showed only A allele amplification at 173 bp product size. Whereas the heterozygote genotype was showed G and A alleles amplification at 173 bp product size respectively electrophoresis image that showed the ARMS-PCR product analysis of TNFα gene (rs3093661)

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polymorphism (G/A) gene polymorphism. Where M: marker (1500-100bp). The lane (GG) wild type homozygote was showed as G allele only. The lane (AA) mutant type homozygote was showed as A allele only, whereas the (G/A) heterozygote were showed as both G and A allele. The presence of G or A allele were observed at 173bp product size..

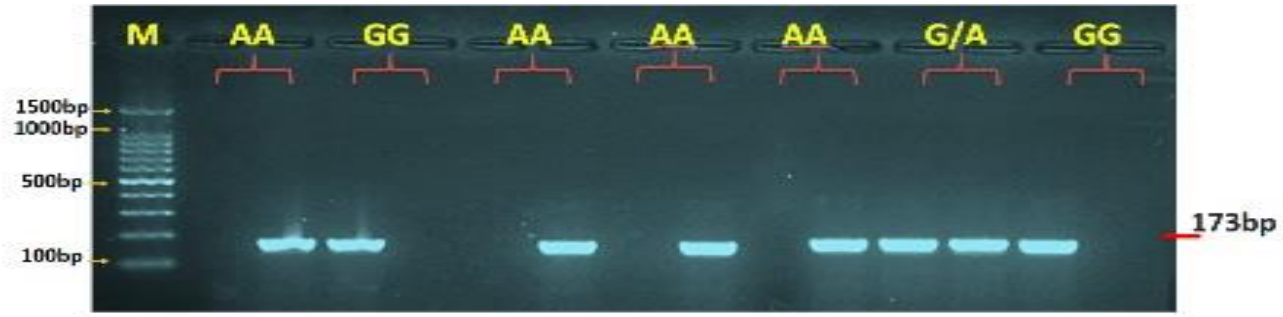

Figure-1: Agarose gel

The frequency distribution of children according to TNF-α (rs3093661) genotype is shown in table-1. In total, there were 21 (21.9 %) AA genotypes, 19 (19.8 %) G/A genotype and 56 (58.3 %) GG genotype.

Table-1: The frequency distribution of children according to TNF-α (rs3093661) genotype		
TNF-α (rs3093661) Genotype	n	%
AA	21	21.9
G/A	19	19.8
TNF- α: tumor necrosis factor-alpha; n: number of cases		

Table-2 Comparison of TNF-α (rs3093661) alleles numbers and percentage between children.	
Allele	n (%)
A	61 (63.5%)
G	35 (36.5%)

4. Discussion

In total, there were 21 (21.9 %) AA genotypes, 19 (19.8 %) G/A genotype and 56 (58.3 %) GG genotype. Tumor necrosis factor a (TNF-α) is the most important inducer of sPLA2 expression, and sPLA2 can, in turn, induce TNF-α expression in lung macrophages, leading to a dangerous positive feedback loop (Yang et al., 2014). TNF-α destroys pathogens by promoting chemotaxis and phagocytosis of the polymorph nuclear leukocytes, thereby playing an immune role. If a large number of pathogens invade the body, the reticuloendothelial cells of the whole body would become activated and thereby produce an excess amount of TNF-α, resulting in shock. (Alejo et al., 2019).
TNF is primarily produced by activated macrophages, NK cells, and T cells and it plays a key role in the initiation of the inflammatory response (Wallach, 2018). TNF normal function is to inhibit viral replication by inhibiting the synthesis of the viral proteins, and the production and infectivity of the viral particles, and also through killing of the virus-infected cells (Chen et al., 2019). Several single nucleotide polymorphisms (SNPs) within the TNF-α gene have been found. The SNP (rs3093661), SNP (rs1800610), SNP (rs3093662) and SNP (rs3093664) polymorphism are found in TNF-α gene and affect the serum concentration of TNF-α. These polymorphisms are differently distributed among human populations with different genotype frequencies in the same population.

5. Conclusion

These SNPs are located in the TNF-α gene promoter region and may affect the regulation of DNA transcription. The TNF-αgene is located in the major histocompatibility class III antigen region of the short arm of chromosome 6 (6p21.3) and is primarily produced by activated macrophages (Zhang et al., 2020). There were some limitations to this study that require further(rs3093661) genotype showed there was significant research and improvement. We did not estimate the difference in the observed counts and expected counts of TNF-α (rs3093661) genotypes.TNF-αlevels TNF-alpha

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