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ORIGINAL ARTICLE

RELATIONSHIP BETWEEN THE HEPATITIS B VIRUS INFECTION AND THE *TNF-α-308*(G/A) PROMOTER GENE POLYMORPHISM IN IRAQI PATIENTS

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ABSTRACT

Background: Tumor necrosis factor (TNF-α) is a key host factor that regulates immune responses and inflammation during infections The objective of this study was to know the connotation between *TNF-α-308*(G/A) polymorphism and HBV infection in Iraqi patients. https://admission.hed.gkp.pk/page.php?college_id=205&page_id=2501

Materials & Methods: A total of 100 HBV-infected patients and 40 healthy controls were genotyped. Genotype frequencies and allele distributions were analyzed and compared between patients and controls.

Results: The G/G genotype was more prevalent among patients, while the A/A genotype was absent in HBV cases but present in controls. Patients with higher occurrence of the G allele (84%) than controls (42.5%), whereas controls had a higher occurrence of the A allele (57.5%). Statistical analysis revealed a significant difference in genotype and allele distributions, with a X^2 value of 62.27 ($p < 0.0001$).

Conclusions: The TNF-α-308(G/A) polymorphism has been associated to an increased risk of HBV infection, with the G/G genotype increasing the risk of infection and the A allele providing potential resistance.

KEY WORDS: TNF-α; polymorphism; hepatitis B virus; Iraqi population; genetic susceptibility.

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INTRODUCTION

One of major public health concern is hepatitis B virus (HBV) infection, resulting in chronic liver disease, cirrhosis, and hepatocellular cancer. The outcome of HBV infection varies between individuals due to differences in viral, environmental, and host genetic factors. TNF-α is a key host factor that regulates immune responses and inflammation during infections.[1,2] *TNF-α* gene polymorphisms, particularly the -308(G/A) variant in the promoter region, can influence the production of TNF-α, potentially affecting the immune response to HBV.[3] It is currently unknown what mechanisms lead to the complete disappearance of an acute HBV infection or its development into chronicity.[4-6] Additionally, evidence indicates that activated effector cells of the innate and adoptive immune systems can release antiviral cytokines like interferon gamma and tumor necrosis factor-a (TNF-a) in close proximity to their targets, thereby inducing the replication in the liver and noncytolytic inhibition of HBV expression.[7] In vitro, TNF-a inhibits the HBV core promoter's transcriptional activity. A small proportion of infected hepatocytes in chimpanzees with acute HBV infection and a transgenic mouse model were killed by direct contact with cytotoxic cells. Most infected cells seem to be inhibited and HBV eliminated by cytokines that are not specific to antigens.[8] The objective of this study was to know the connotation between *TNF-α-308*(G/A) polymorphism and HBV infection in Iraqi patients.

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MATERIALS AND METHODS

This observational study included 100 HBV-infected patients and 40 healthy controls; all enlisted from outpatient internal medicine clinic in Samara General Hospital. HBV infection was confirmed based on positive HBsAg tests and detectable HBV DNA. The control group consisted of healthy individuals with no history of HBV infection table (1).

Table (1): Patients and controls features

Characters	Patient role	Control role
Number	100	40
Mean age (year)	33	29.25
Male or Female	56 / 44	20 / 20

TNF-α-308(G/A) Polymorphism Genotyping

Genomic DNA was isolated from whole blood according to method[9], then the DNA concentration was estimated using the Nanodrop device, and the polymorphism of the *TNF* -α -308(G/A) using the Tetra-Primer ARMS-PCR technique, which includes the use of four specialized primers designed for this purpose Table(2) as mentioned in [10] and using the (GoTaq Master Green) kit prepared by the American company Promega and according to the attached instructions.

Optimization experiments were performed to determine the ideal primer concentrations and template DNA amounts to achieve the most efficient and accurate amplification results (12.5 microliters) of the basic reaction mixture were placed in each tube, then the template DNA was added at a concentration of (100ng) and the four primers at a concentration of 3 picomoles/ microliter, after that, 25 microliters of sterile distilled water were added to the reaction volume. The tubes were carefully placed in the thermocycler to complete the amplification reaction after the components of the reaction had been thoroughly mixed. According to the program, the DNA strand was first denaturized for 10 minutes at 95°C. This was followed by 35 amplification cycles, each of which included denaturation of the template for 94°C, primer binding to the template DNA for 63°C, elongation for 72°C, and final elongation for 10 minutes at 72°C. After staining with ethidium bromide for 30 to 45 minutes under UV light, the amplification products were run on a 2% agarose gel with DNA ladder for 90 minutes (5 V/cm). The gel was then photographed using the Gel Documentation System. [11]

Using the SPSS software (version 20), the gene type was shown as frequency, and the significance of the group differences was assessed using the Chi square test. Significance is indicated by a P-value of less than 0.01.

RESULTS

A total of 100 HBV-infected patients and 40 healthy controls were genotyped. Genotype frequencies and allele distributions were analyzed and compared between patients and controls. The G/G genotype was more prevalent among patients, while the A/A genotype was absent in HBV cases but present in controls. Patients with higher occurrence of the G allele (84%) than controls (42.5%), whereas controls had a higher occurrence of the A allele (57.5%). Statistical analysis revealed a significant difference in genotype and allele distributions, with a X^2 value of 62.27 ($p < 0.0001$). After transferring the products of the Tetra-Primer ARMS-PCR technique on a 2% agarose gel, the bands resulting from the replication appeared for all samples, represented by the largest main band (323 base pairs) which should appear, the band with a size of 224 base pairs representing the G allele, and the band with a size of 154 base pairs representing the A allele. In the case of the appearance of the three bands, this means that the sample is heterozygous with the genetic makeup (GA), and in the case of the appearance of the main band and the band for the A allele (154) only, the sample is homozygous of the mutant type and with the genetic makeup (AA), but in the case of the appearance of the main band and the band for the G allele (224), the sample is homozygous of the normal type and with the genetic makeup (GG) as in Figure (1) table (3,4).

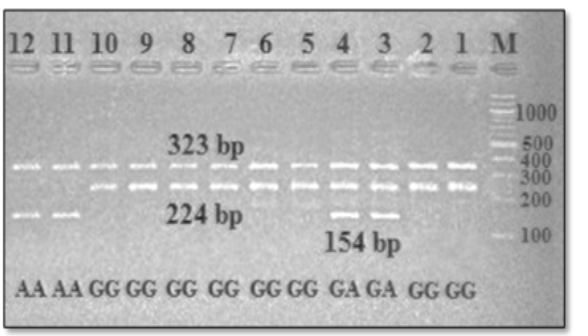

Table 2: Primers used in this study

No.	Primer	Sequence	Tm.	Band size
1	Forward primer (G allele)	5'TGGAGGCAATAGGTTTTGAGGGGCAGGA3'	63 °C	224
2	Reverse primer (A allele)	5'TAGGACCCTGGAGGCTGAACCCCGTACC3'	63 °C	154
3	Forward primer (5'–3')	5'ACCCAAACACACGCCTCAGGACTCAACA3'	63 °C	323
4	Reverse primer (5'–3')	5'AGTTGGGGACACGCAAGCATGAAGGATA3'	63 °C	323

Figure 1: Electrophoresis of the products of the Tetra-Primer ARMS-PCR technique for the polymorphism of the tumor necrosis factor-alpha gene promoter at the site (G-308A) on agarose gel (2%). Bands: 323 main band, 224 allele G, 154 allele A. Lines (10,9,8,7,6,5,2,1) genotype GG, lines (4,3) genotype GA, lines (12,11) genotype AA

Table (3) :The frequency of genotypes in controls and hepatitis B virus patients.

Gene type	Patients (%)	Control (%)	X^2	P value
G/G	84 (84%)	9 (22.5%)	62.27	0.0001
G/A	16(16%)	15(37.5%)		
A/A	0	16(40%)		
Total	100 (100%)	40(100%)		

The G/G genotype was highly prevalent among HBV patients (84%) compared to controls (22.5%). In contrast, the A/A genotype was absent in HBV patients but observed in 40% of the controls. The G/A genotype was more frequent in controls (37.5%) than in patients (16%). Chi-square analysis ($\chi 2 = 62.27$) with a p-value < 0.0001 indicates a highly significant difference in genotype frequencies between patients and controls.

Table (4):Comparing the frequency of an allele in patients to controls

Allele	Patients number (%)	Control number (%)	X^2	p-value
G	84 (84%)	17 (42%)	30.22	0.0001
A	16(16%)	23 (57.5%)		
Total	100 (100%)	40 (100%)		

The G allele was more prevalent among patients (84%) than in controls (41.25%), suggesting that it is associated with a higher risk of HBV infection. The A allele was significantly more frequent in controls (58.75%) compared to patients (16%), indicating a protective role against HBV infection.

DISCUSSION

The study's findings show a strong correlation between Iraqi patients' HBV infection and the TNF-α-308(G/A) polymorphism. The G/G genotype and G allele were prevalent among patients, suggesting that these variants may contribute to increased susceptibility to HBV. controls had a higher frequency of the A allele, indicating a potential protective effect against the virus.

These findings align with previous studies that associate the G allele with reduced TNF-α expression, impairing the immune response and allowing viral persistence. In contrast, the A allele has been linked to higher TNF-α production, which may enhance antiviral immunity and promote viral clearance, reducing the likelihood of chronic HBV infection.

The presence of the A/A genotype only in controls further supports the notion that this genotype offers protection against HBV. Similarly, the increased frequency of the G/A genotype in controls suggests that heterozygosity may confer some degree of resistance to infection, possibly through a balanced immune response. Studies conducted by others [12-14] revealed that a significant correlation existed between the G allele of the TNF-α-308(G/A) polymorphism and a higher risk of chronic HBV infection. These studies suggests that individuals carrying the G allele, especially the G/G genotype, are more likely to develop chronic HBV compared to those with the A allele, supporting the higher prevalence of the G allele in HBV patients in current results. Hohler et al.[15] reported conflicting results, where the TNF-α-308(G/A) polymorphism and HBV chronicity did not significantly correlate. In their study, the G allele was not observed at higher frequencies in HBV patients compared to controls, indicating that genetic susceptibility might vary across different populations.

CONCLUSION

This study provides evidence that the TNF-α -308(G/A) polymorphism plays a role in assessing Iraqi patients' susceptibility to HBV infection. The G allele and A higher risk has been associated to the G/G genotype, while the A allele was linked to protection against HBV. Genetic screening for this polymorphism could help identify individuals at higher risk of infection and guide personalized preventive strategies.

Ethical Approval and Consent: The Samara General Hospital Ethics Committee gave its approval to the study, and before to the collection of the samples, each participant provided written informed consent.

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CONFLICT OF INTEREST
Authors declare no conflict of interest.
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AUTHORS' CONTRIBUTION

The following authors have made substantial contributions to the manuscript as under:

Conception or Design:	GAA
Acquisition, Analysis or Interpretation of Data:	GAA
Manuscript Writing & Approval:	GAA

All the authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.