INTERLEUKIN-37 GENE SINGLE NUCLEOTIDE POLYMORPHISMS AND PATIENT SUSCEPTIBILITY TO HEPATITIS B AND C INFECTION

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Abstract. The study was carried out in Anbar from September to December 2022 with the aim of identifying genetic variations in the IL-37 subunit gene among persons with chronic hepatitis. We investigated the correlation between the impact of IL-37 gene single nucleotide polymorphisms (SNPs) and hepatitis B and C virus infections. The four-arm primer approach was employed to identify single nucleotide polymorphisms (SNPs) in a cohort consisting of 90 patients and 100 healthy controls from the Iraqi population. Four single nucleotide polymorphisms (SNPs) of the IL-37 gene were determined through genotyping. Patients exhibited significantly higher frequencies of the rs2708967 G allele and GG genotype in comparison to controls. On the other hand, the rates of the A allele and AA genotype were comparatively lower, while the frequencies of the AG genotype were same. The patients demonstrated markedly elevated frequencies of the T allele and TT genotype compared to the controls. In addition, they exhibited markedly reduced frequencies of the C allele and CC genotype compared to the control group. The frequencies of CT genotypes, allele A, and AG and AA frequencies for the rs3811047 SNP did not show any significant differences in patients compared to controls. Our work indicates that mutations in the IL-37 gene are responsible for the genetic risk factors that contribute to the high occurrence of HBV and HCV infections in the Iraqi population.

Keywords: interleukin-37, polymorphisms, hepatitis B, hepatitis C.

List of Abbreviations

HBV – Hepatitis B Virus
HCV – Hepatitis C Virus
DNA – Deoxy Ribonucleic Acid
RNA – Ribo nucleic Acid
HCC – Hepatocellular carcinoma
IFN-γ – Interferon-gamma
IL – Interleukin
PCR – Polymerase Chain Reaction
EDTA – Ethylene Di Amen Tetra Acetic Acid

Introduction

Hepatocellular carcinoma is a consequence of persistent hepatitis B virus (HBV) infection, representing a significant public health concern. Liver cirrhosis, identified as the primary cause of approximately 887,000 fatalities related to the virus, underscores the severity of this health issue (WHO, 2017) Host immune responses have the most impact on whether or not HBV infection is eliminated. To demonstrate the chronicity of HBV infection, a complex interplay between HBV and a compromised immune system is needed (Li *et al*., 2015; Mohsen *et al*.,2019). After infection, HBV can be primarily eliminated by efficient antiviral. They have established that acute HBV infection causes Responses from CD4+ and CD8+ T cells (Peeridogaheh *et al*., 2018; Sandhu, 2017). IFN is a critical cytokine in removing the disease and managing infection with HBV. On the other hand, increased levels of inhibitory activity result from a decline in T-cell activation (Maini & Pallett, 2018). Recently, the length of the viral infection has been connected to a second cytokine called IL-35. It reduces inflammatory responses and anti-HBV immune and persistent HBV infection responses (Shao *et al*., 2017. Mohsen *et al*., 2020). A quite recently discovered IL-1 member family is the cytokine IL-37. Members of this family of structurally related, mostly pro-inflammatory cytokines (Van *et al*., 2013). IL-37, formerly known as IL-1F7, is a ligand for the IL-18R and IL-18 binding protein and is also classified as a negative immunological regulator. It is secreted as the cytoplasm and nucleus both need a precursor molecule (Abulkhir *et al*., 2016). We describe IL-37, IL-18 binding protein, IL-18 receptor ligand (for-

merly IL-1F7), and negative immune regulator. It becomes a precursor molecule and is released in the cytoplasm and nucleus. The single immunoglobin IL- and the two different components of the IL-37 receptor SIGIRR, a protein linked to the are1 receptor, and IL-18R, a chain of the IL-18 receptor. Due to intracellular switches brought on by IL-37/IL-37 receptor signaling, pro-inflammatory genes are down-regulated and reduced cytokine production (Cavalli & Dinarello, 2018). As a result, IL-37 inhibits inflammatory mediators in infectious diseases, autoimmune disorders, and cancers, giving it immunosuppressive solid qualities against innate and acquired immune responses (Allam *et al*., 2020). Due to IL-37's reduction of the Th17 response and elevation of regulatory T-cell activity, the Coxsackie virus B3-induced viral myocarditis was decreased (An *et al*., 2017; Ren *et al*., 2022). During Strongyloides stercoralis infection, activating parasite antigens and neutralizing IL-37 enhanced the frequencies of CD4+ and CD8+ T cell subsets (Anuradha *et al*., 2017). Significantly, SIGIRR reduces protective cytotoxic T cell-induced immunity in colorectal cancer patients with colitis due to IL-37-mediated CD8+ T cell malfunction (Wang *et al*., 2022).

Materials and Methods

Individuals

Between September and December 2022, a case-control study was conducted in Al-Anbar to ascertain the single nucleotide polymorphisms (SNPs) of the IL-37 subunit genes in ninety individuals with chronic HBV and HCV infections (mean age ± SD: 41.2 ± ± 13.7 years; 58 males and 32 females). The Specialized Centre for Gastroenterology and Hepatology in Al-Anbar served as the patient source. Identifying interleukin-37 genes associated with HBV and HCV was a component of the molecular diagnostic investigation. A control group comprised one hundred healthy blood donors (mean age ± SD: 43.2 ± 11.9 years; 62 men and 38 women). The research techniques were approved by the Iraqi Ministry of Health and the Department of Biological Ethics Committee at the University of Al-Anbar. Prior to their participation in the study, every subject gave written, informed consent. Using a fast blood genomic DNA extraction kit (Gene Aid, Korea), DNA was extracted from whole EDTA blood in accordance with the manufacturer's suggested standard protocol for HBV and HCV molecular detection. The PCR reaction mixture comprised three microliters of DNA, two microliters of sense primer, two microliters of antisense primer, and five microliters of master mix. The volume was increased to 13 microliters using nuclease-free water. Electrophoresis of the PCR products was conducted for 60 minutes at 5 V/cm² on a 2% agarose gel stained with diamond dye. The migrating PCR products were visualized using a 100 bp DNA ladder pattern and the gel documentation system (Naito *et al*., 2001).

Ethics committee approval and patient consent

1) Written informed consent was obtained from all the participants.

2) The studies conformed to the standards set by the latest revision of the Declaration of Helsinki.

3) The Institutional Review Board approved experimental studies that include humans or animals (Ministry of Higher Education and Scientific Research, Anbar University, Scientific Study Ethics Committee, No. 140, dated 9/9/2023). To protect patient identity and privacy, no explicit images of patients, healthy individuals, or parts of them were included in the study.

IL-37 subunit gene SNPs

According to their minor allele frequency (MAF 10%), four IL-37 SNPs encoding genes were chosen: rs2708967, rs2708971, rs3811047, and rs2466449. The SNPs were found using particular primers and an allele-specific PCR technique (Table 1). The primers were created using generous software (version 10.2.2) (Kearse *et al*., 2012). PCR amplification was done after DNA isolation. Three liters of DNA, one liter of each primer, five liters of Master Mix (Integrated DNA Technologies, Inc., USA), and 25 liters of PCR mixture made up the combination. And thirteen liters of nuclease-free

Table 1

IL37 gene SNP forward and reverse primers are included in

Gene	SNP	Primer	Tm (°C): Molecular Size (bp)	
IL-37	rs2708967	IF67 5'CTGGAAGCCACCTGATCTATCACAATTA3′ IR67 5'CCACACCTATTCAACACATTTCAATGC3′ OF67 5'TTCTCTGGGACATGGGGACTCC3′ OR67 5'TTCCTTTGGCCAATAATAAATAGCATCCT3′	62	A allele: 177 G allele: 224
	rs2708971	IF71 5'AAGGATGTGATCTTGGGTAAATGGC 3′ IR71 5'AGGTGGTGCAGTTTAGAGAGGATTGA 3′ OF71 5'GACCACAGTCTTCGGTGAAGTTTAAGAG 3′ OR71 5'AGGCCAGCACAATAGTCAACCACT 3′	63	C allele: 184 T allele: 130
	rs3811047	IF47 5'ACCAGGCCCAAGCCTCCACA3′ IR47 5'GCCTTACTTGTGTGAACAAAATTCATTGC3′ OF47 5'ACCCCTAAATCCTTGGAAAATCCGAA3′ OR47 5'AAGGAAAGACTTCAGCCCCATCCA 3′	63	A allele: 185 G allele: 130
	rs2466449	IF49 5'GAAGCCTCCGATGAGAAGAAG 3′ IR49 5'AGCCCAGCCTTAGAGCGT3′ OF49 5'TTTCAAATTTCCCTGGACAAATAA 3′ OR49 5'AGATGAACTGGATCACGGGTT 3′	58	G allele: 208 A allele: 245

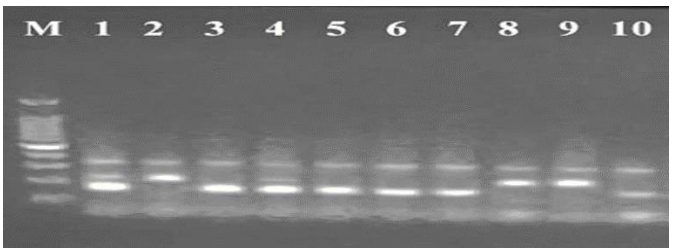

Fig. 1. DNA-PCR products for the SNP rs2708967(A/G) were electrophoresed on a 1.5% agarose gel at 5 V/cm2 for 60 minutes to show genotypes for ten samples (177 bp/224 bp/the product size of two outer primer 346 bp). These are samples 1 through 5: AG, GG, AA, AG, and AA. Type: AA Spec 7: AA is an example of sample 9 sample 10's GG in sample 8. DNA ladder of 100 bases, M

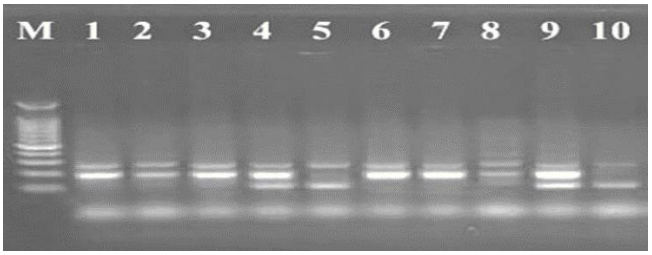

Fig. 2. Genotypes for ten samples are shown from a gel electrophoresis of DNA-PCR amplified products for the SNP rs2708971 (C/T) for 1.5% agarose (5 V/cm2 for 60 min) (184bp/130bp/the product size of two outer primer 263bp). Samples CC, CC, CC, CC, CT, and TT are used in the first four samples. Instance 6: CC CC Sample 7 8 sample CC, 9 sample CT DNA ladder (100 bp) for sample number 10: TT

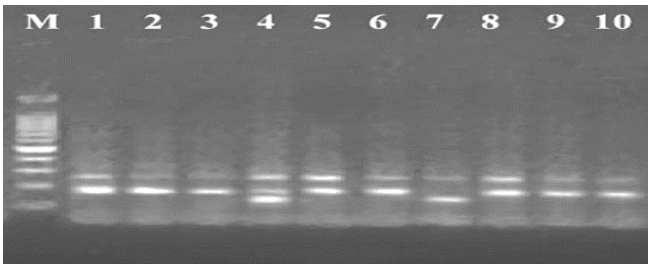

Fig. 3. Genotypes for ten samples are shown following 1.5% agarose gel electrophoresis of DNA-PCRamplified products (185 bp/130 bp/the product size of two outer primer 266 bp) for the SNP rs3811047 (A/G). Samples 1 and 2 both contain AA. 3rd sample: AA, Sample 4 is AG, while Sample 5 is AA. 6th example: AA exemplar 7:GG 8 AA sample, 9 AA Sample 10 is AA. M: 100-bp DNA ladder

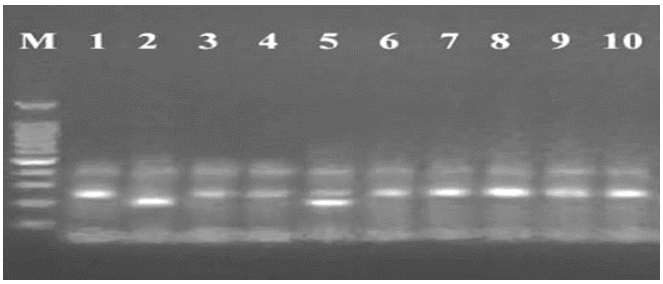

Fig. 4. Genotypes for ten samples are shown following 1.5% agarose gel electrophoresis of DNA-PCR amplified products (208 bp, 245 bp, and the product size of two outer primers, 414 bp) for the SNP rs2466449 (G/A). Samples 1 and 2 are AA, GG, and AA, respectively. Sample 4 is AA, while Sample 5 is AG. 6th example: AA Spec 7: AA8 AA sample, 9 AA Sample 10 is AA. M: 100-bp DNA ladder

water. The PCR procedure was done in the following ways: initial denaturation for five minutes at 94 °C; 40 cycles of denaturation for 35 seconds each at 94 °C; annealing for 35 seconds (the temperature is specified in Table 1); and extension for 35 seconds at 72 °C. A last extension lasting ten minutes at 72 °C came next. The PCR products were electrophoresed on a 1.5% agarose gel at a voltage of 5 volts per square centimeter for 60 minutes before being dyed with diamond dye; using a gel documentation system, the moving PCR products were then shown next to a 100 bp DNA ladder pattern (Fig. 1).

Statistical analysis

The sample size was calculated using G*Power 3.1.9.4. Odds ratios (OR), 95% confidence intervals (CI), and two-tailed Fisher's exact odds (p) were used to analyze the relationships between SNPs and diseases. Since there were so many comparisons, the p-value was altered using the Bonferroni correction. The corrected p (pc) value of 0.05 was regarded as significant. Two statistical software programs, SPSS (19.0) and Winpepi (version 11.65), were used to conduct this research (Faul *et al*., 2007).

Results

Sample size

Using the G*Power 3.1.9.4 program, the power of the present patient sample size for HBV and HCV was determined. An effect size of 0.3 and a capacity of 0.79 at = 0.05 were obtained from a sample of 80 patients, close to the minimal power deemed acceptable (0.80). But it's unquestionably better to have a higher power.

Table 2

Hepatitis B and C virus infection patients and controls were compared for genotype and allele frequencies of the IL37 gene SNPs

Gene	SNP	Allele/genotype	N (%)		OR (95% CI)	*p* (*pc*)
			Patients (*N*=)	Control (*N*=)		
	rs2708967	A	102 (0.378)	170 (0.567)	17.0	0.000
		G	78 (0.289)	30 (0.10)	21.33	0.000
		AA	36 (0.133)	75(0.25)	13.703	0.000
		GG	24 (0.089)	5 (0.0167)	12.448	0.000
		AG	30 (0.111)	20 (0.067)	2.00	0.157
	x^2		2.40	81.5		
	rs2708971	C	96 (0.356)	148 (0.493)	11.082	0.001
		T	84 (0.311)	52 (0.173)	7.529	0.006
		CC	33 (0.122)	64 (0.213)	9.907	0.002
		TT	27 (0.10)	16 (0.053)	8.321	0.004
		CT	30 (0.111)	20 (0.067)	2.00	0.157
	x^2		0.60	42.56		
	rs3811047	G	113 (0.377)	180 (0.60)	15.321	0.000
		A	67 (0.248)	20 (0.067)	25.391	0.000
		GG	43 (0.159)	85 (0.283)	13.781	0.000
		AA	20 (0.074)	5 (0.0167)	9.00	0.003
		AG	27 (0.10)	10 (0.033)	7.811	0.005
	x^2		0.55	55.06		
	rs2466449	A	104 (0.385)	165(0.55)	13.833	0.000
		G	76 (0.281)	15(0.05)	40.89	0.000
		AA	37(0.137)	80(0.267)	15.833	0.000
		GG	23(0.085)	5(0.0167)	11.571	0.001
		AG	30(0.111)	15(0.04)	41.88	0.000
	x^2		2.11	45.99		

IL37: Interlukin-37; OR: odd ratio; CI: Confidence interval; P: Tow-tailed Fisher exact

HBV and HCV diagnosis

Molecule-based analysis indicates that every patient displayed a band of 1063 bp during PCR amplification and gel electrophoresis.

IL-37 gene SNPs

Table 2 shows the frequencies of rs2708967. The OR for this difference was (95% CI: 21.33), and the frequency of rs2708967snp allele G was substantially greater in patients than in controls (0.289 vs. 0.10%; p0.000). On the other hand, patients had a significantly lower frequency of the A allele (0.378 vs. 0.567%; p 0.000). Patients showed a considerably higher GG genotype frequency (0.089 vs. 0.0167%; OR = (95% CI: 12.448; p 0.000) but a significantly lower AA genotype frequency (0.133 vs. 0.25%; p 0.000) than controls. However, the AG genotype frequencies between patients and controls showed no noticeable difference (p = = 0.157). For the rs2708971 snp, the OR was (95% CI: 7.529), and the frequency of the allele T was substantially higher in patients than in controls (0.311 vs. 0.173%; P 0.006). In patients, the frequency of the C allele was significantly lower (0.356 vs. 0.493%; P 0.001). Regarding genotype frequency, patients had substantially higher rates of the TT genotype than controls (0.10 vs. 0.053%; P 0.004; OR = (95% CI: 8.321)). Compared to controls, patients had considerably lower frequencies of the CC genotype (0.122 vs. 0.213%; P 0.002). The CT genotype frequencies did not change significantly (P = 0.157). In terms of the frequencies of the

rs3811047 SNP, patients' allele frequencies of A were significantly higher than controls' allele frequencies (0.248 vs. 0.067%; P 0.000), but controls' allele frequencies of G were lower (0.377 vs. 0.60%; P 0.000). Patients showed greater frequencies of the AG genotype in proportion to genotype frequency (0.10 vs. 0.033%; P = 0.005). OR = (95% CI: 7.811) Compared to controls, AA genotype rates were higher (0.074 vs. 0.0167%; P 0.003). However, the GG genotype was less common in patients (0.159 vs. 0.283%; P 0.000). Regarding the second rs2466449 SNP, patients' G allele frequencies were substantially higher than controls' (0.281; 0.05%; P 0.000) when compared. The A allele, on the other hand, decreased (0.385; 0.55%; P 0.000). As previously reported, patients showed higher frequencies of the GG genotype (0.085 vs. 0.0167%; P 0.001) and the AG genotype (0.111 vs. 0.04%; P 0.000). Although the AA genotype was less common in patients (0.137 vs. 0.267%; P 0.000) (Table 2).

Discussion

The initial findings of this study suggest a potential association between IL-37 (rs2708967, rs2708971, rs3811047, rs2466449) and susceptibility to HBV and HCV infections. This investigation aligns with a previous study (Mohsen *et al*., 2020), which proposed that IL-35 may influence susceptibility to chronic HBV due to significant single nucleotide polymorphisms (SNPs: rs582054, rs428253, and rs7254021) present in both genes. There is a positive correlation observed with HBV infection. Considering the haplotypes of alleles within each gene provides a more accurate prediction of susceptibility impact. The progression of liver disease, infection outcome, and natural course of HBV are intricately dependent on immunological factors. As indicated by (Li *et al*., 2016; Yang *et al*., 2015), genes encoding inflammatory mediators, including TNF-, TGF-, and IL-37, serve as potential indicators for forecasting the severity of an HBV-mediated illness. Moreover, increasing data suggests that selective forces have caused single nucleotide polymorphisms (SNPs) in the IL37 gene to remain present in the human population. This has led to conjecture about the possible roles of SNPs in immune response regulation and susceptibility to health hazards. [Kang and colleagues, 2015]. Nonetheless, there were notable differences in the genotypic and allelic frequencies of the rs2708967 SNP between the patient and control groups. While the frequencies of the A allele and AA genotype were noticeably lower ($p < 0.000$), the frequencies of the G allele and GG genotype were significantly greater ($p < 0.000$) in patients compared to controls. Between patients and controls, there were no discernible variations in AG genotype frequencies ($p = 0.157$).

In contrast, patients had considerably higher frequencies of the T allele and TT genotype than controls for the rs2708971 SNP ($p < 0.006$ and $p < 0.004$, respectively). On the other hand, patients had lower frequencies of the C allele and the CC genotype than controls ($p < 0.001$ and $p < 0.002$, respectively). There were no discernible changes in the AG genotype frequencies ($p = 0.157$). Regarding the rs3811047 SNP, patients demonstrated considerably higher frequencies of the A allele and AG genotype than controls ($p < 0.000$, $p < 0.005$, and $p < 0.003$, respectively). Conversely, the frequencies of the GG genotype and G allele were lower in patients than controls ($p < 0.000$ and $p < 0.000$, respectively). The rs3811047 SNP has been associated with an elevated risk of COVID-19 infection in the present study. Although the p-value did not reach significance, individuals with the GA genotype may be 2.02 times more prone to illness. This variant has garnered more attention in research compared to other SNPs. various inflammatory, autoimmune, and viral diseases have been correlated with this SNP, either conferring resistance or susceptibility (Al-Anzi *et al*., 2019; Allam *et al*., 2016.; Lin *et al*., 2018; Offenbacher *et al*., 2018; Ozguclu *et al*., 2019). SNPrs3811047, one of the IL-37 SNPs investigated in the Saudi population, has been linked to genetic susceptibility to autoimmune thyroid illness. Additionally, in the Chinese population, SNPrs3811047, among the IL-37 SNPs studied, has demonstrated a genetic predisposition to autoimmune thyroid illness (Yan, 2015). Notably, Chinese patients with Behcet's

disease exhibited significantly higher frequencies of the G allele and the GG genotype of the rs3811047 SNP compared to controls in inflammatory and autoimmune diseases within China (Tan *et al*., 2016) within China. These findings, however, were not confirmed in Turkish people, and rs3811047 did not seem to be connected to Behcet's disease (Ozguclu *et al*., 2019). In four genetic models (allele, recessive, dominant, and chimeric), a strong association was discovered between the rs3811047 variation and susceptibility to autoimmune disorders. and heterozygous), Several autoimmune conditions, including Behcet's disease, Vogt-Koyanagi-Harada syndrome, autoimmune thyroid disease, and ankylosing spondylitis, have been linked to Chinese patients, according to a meta-analysis of those cases (Lin *et al*., 2018). Interestingly, in people with ankylosing spondylitis, the GG genotype of rs3811047 impacted the expressions of C-reactive protein and erythrocyte sedimentation rate (inflammatory markers) (Chen *et al*., 2011). These results imply that rs3811047 could impact a person's susceptibility to infectious and inflammatory diseases. Regarding the second rs2466449 SNP, patients had considerably higher frequencies of the G allele and the AG and GG genotypes than controls (P 0.000, P 0.000, and P 0.001, respectively). At the same time, both those of the A allele and the AA genotype decreased (p 0.000; 0.000). According to the study's findings (Ahmed *et al*., 2022; Mohsen *et al*., 2022), there were no appreciable differences between COVID-19 patients and controls regarding rs2466449 snp, allele, or genotype frequencies. The importance PCR applied in different filed of medicine to detect disease related to pathogens or genes related to cancers such as, Detection genetic variations in breast cancer patients compared to those with other cancers (*Buniya et al.*, 2018; Hassoon *et al.*, 2017), and detection of SNP in medicine field (Bresam *et al*., 2023a; Bresam *et al*., 2023b)), use of the Newcastle disease virus as a modified vector in gene therapy (Rasoul *et al.*, 2022a; Rasoul *et al.*, 2023b). However, our work indicates that mutations in the IL-37 gene are responsible for the genetic risk factors that contribute to the high occurrence of HBV and HCV infections in the Iraqi population.

Conclusions

IL-37 subunit genes are suggested to influence the risk of hepatitis B and C infection, as they have shown positive associations with hepatitis virus infection. An exposure effect is also predicted when considering haplotypes between alleles of each gene.

Conflict of interest: the authors have declared that no conflict of interest exists.

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