RESEARCH ARTICLE

Association of nuclear factor-kappa B1 (NF-κB1) Ins/Del gene polymorphism with Hepatitis C virus outcomes

Shaimaa Rahem Al-Salihy[1], Refif Sabeeh Al-Shawk[2], Safaa Abdul-Karim Al-Waysi[3]; Maarib Nazih Rasheed[4]

Abstract

Objective: To explore the association of nuclear factor-kappa B1 polymorphism in the promotor area of the gene with hepatitis C virus infection outcomes.

Method: The case-control study was conducted at the Hepatology and Gastroenterology Teaching Hospital, Baghdad, Iraq, from Dec 1, 2020, to Aug 30, 2021, and comprised individuals ages 20-68 years. Group 1 had patients with persistent hepatitis C virus infection, group 2 had subjects with spontaneous hepatitis C virus clearance, group 3 had subjects treated with direct-acting antiviral drugs, and group 4 had healthy controls. Venous blood was collected for polymorphism genetic analysis of nuclear factor-kappa B1 insertion/deletion ATTG (Adenine-Thymine-Thymine-Guanine) at rs28362491 using a high-resolution melting technique. Data was analysed using SPSS 27.

Results: Of the 88 subjects, there were 22(25%) in each of the 4 groups. Overall, there were 55(62.5%) females and 33(37.5%) males, and 40(45.45%) were aged 20-39 years while 48(54.54%) were aged 40-68 years (p>0.05). The Ins allele of rs28362491 was significantly more frequent in the patients than in controls (p=0.0053). The carriage of rs28362491 insertion/insertion and insertion/deletion genotypes, compared to wild-type homozygous deletion/deletion, had a significantly higher risk of developing hepatitis C virus infection (p=0.0013). No association was found between rs28362491 and spontaneous hepatitis C virus clearance (p>0.05).

Conclusion: The insertion allele of rs28362491 was found to be associated with increased susceptibility to developing hepatitis C virus infection.

Key Words: Antiviral, Gastroenterology, Hepaciviral, Thymine, Alleles, Homozygote, Hepatitis C, Chronic, Adenine, Guanine.

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Introduction

The hepatitis C virus (HCV) represents a major health issue worldwide. It causes progressive liver diseases ranging from chronic inflammation to fibrosis, cirrhosis, and even hepatocellular carcinoma (HCC) .[1] Globally, it is estimated that about 71 million people are living with chronic hepatitis C (CHC), with approximately 1.75 million newly-emerged HCV infections .[2] Nearly 35% of patients with an acute HCV infection will clear the virus spontaneously, meaning without any treatment, within 6 months of infection, but little is known about the factors that lead to diversified HCV infection outcomes.[2,3] Thus, factors that are related to the occurrence and clearance of HCV infection need to be reconnoitred.

Nuclear factor κ-light-chain enhancer of activated B cells (NF-κB) is a family of eminent stimulated transcription factors that regulate an enormous number of genes involved in inflammation, cell growth, and apoptosis.[4] NF-κB consists of 5 subunits; NF-κB1(p50), NF-κB2 (p52), NF-Kb3 (RELA) or (p65), RELB, and c-Rel. Unlike the other subunits, NF-κB1 and NF-κB2 are synthesised as precursors p105 and p100 that are proteolytically cleaved to deoxyribonucleic acid (DNA)-binding proteins p50 and p52, respectively.[5] NF-κB subunits, which can form different types of homodimers and heterodimers, remain sequestered in the cytoplasm of unstimulated cells by binding to the inhibitor of κB proteins (IκB). The NF-kB signalling pathway, which is triggered by several stimuli, is responsible for the phosphorylation and degradation of IκB, allowing NF-B dimers to be translocated into the nucleus.[6] Improper activation of NF-κB can mediate inflammation and tumorigenesis.[4]

Polymorphisms within genes coding for the NF-κB pathway have been proposed to influence different diseases and cancers. Of these, functional polymorphisms in NF-κB1 promotor-94 insertion/deletion (Ins/Del) ATTG (Adenine-Thymine-Thymine-Guanine) rs28362491 is shown to be associated with coronary artery disease (CAD)[7,8], atherosclerosis [9], Hashimoto thyroiditis[10] and

[1]Department of Microbiology, University of Diyala, Baqubah, Iraq. [2]Department of Microbiology, Mustansiriyah University, Baghdad, Iraq.[3]Hepatology and Gastroenterology Teaching Hospital, Baghdad, Iraq. [4]Institute of Genetic Engineering and Biotechnology, University of Baghdad, Iraq

Correspondence: Shaimaa Rahem Al-Salihy **Email:** sh.r802011@gmail.com

HCC.[11]

The current study was planned to explore the association between rs28362491 polymorphism and HCV infection occurrence and outcome.

Patients and Methods

The case-control study was conducted at the Hepatology and Gastroenterology Teaching Hospital, Baghdad, Iraq, from Dec 1, 2020, to Aug 30, 2021. After approval from the ethics review committee of Mustansiriyah University, Baghdad, the sample was raised using a convenience sampling technique. Those included were patients of either gender aged 20-69 years who were exposed to HCV, who were divided into 3 groups; chronic HCV patients in group 1, those who had spontaneously recovered HCV infection, based on negative HCV polymerase chain reaction (PCR), in group 2, and HCV patients who showed sustained antiviral response, based on a viraemia 12 weeks after the completion of HCV antiviral therapy, in group 3. Healthy controls matched for age and gender formed group 4.

Patients with any other cause of liver disease, autoimmune or metabolic disorder, co-infection with hepatitis B virus (HBV) and/or human immunodeficiency virus (HIV), liver steatosis, malignancies, and current alcohol abuse were excluded.

After taking verbal consent from all the participants, 5ml of blood was drawn by venepuncture from each subject; 2ml of which was poured into an ethylenediaminetetraacetic acid (EDTA) tube and stored at -20° C for DNA extraction and viral load estimation. The remaining blood was left for serum separation that was used to detect anti-HCV antibodies.

Qualitative detection of anti-HCV antibodies was done using fourth-generation enzyme-linked immunosorbent assay (ELISA)) kit (bio-ELISA HCV 4th generation Fortress diagnostics, United Kingdom), according to the manufacturer's instructions. HCV ribonucleic acid (RNA) analysis was measured using quantitative PCR assay for those who gave a positive result.

DNA extracted from peripheral blood mononuclear cells was used to investigate the polymorphism of NF-κB1-94 ATTG Ins/Del at rs28-362491 in the promoter region.

Genomic DNA was isolated from the whole blood sample, according to the protocol of Gene aid Extraction, using a kit (Presto Mini gDNA Kit, Taiwan). Quantas Fluorometer

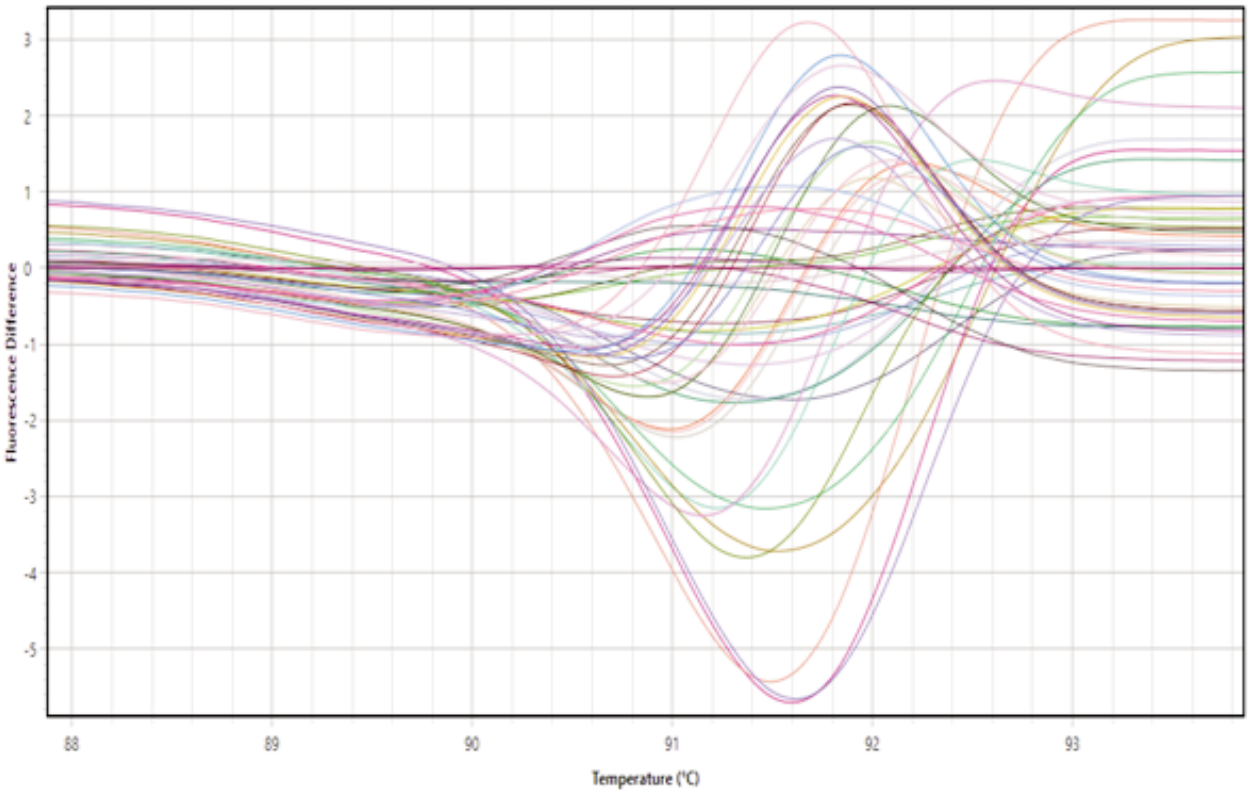

Figure: High-resolution melting curve (HRM) analysis for the detection of nuclear factor-kappa B1 (NF-kB1) rs28362491 genotype.

was used to detect the concentration of extracted complementary DNA (cDNA), and highly-concentrated DNA samples were subsequently diluted with PCR-grade water to a final concentration of 10ng/L.

The primer sequences used for polymorphism were Forward (5`-CATGACTCTATCAGCGGCACT-3`) and Reverse (5`- GGCTCTGGCTTCCTAGCAG-3`). These primers were in a lyophilised form (Microgen Company, South Korea). The reaction mixture consisted of 5 GoTaq qPCR Master Mix, 0.5µL of each primer, and 1ng of genomic DNA completed with nuclease-free water to a total volume of 10µL.

PCR amplifications were performed with Mic qPCR (Thermo Cycler, Biomolecular System, Australia) with initial denaturation at 95°C for 5 min followed by 40 cycles of denaturation at 95°C for 20 sec.; annealing at 60°C for 20 sec.; extension at 72°C for 20 sec.; and one cycle of 95°C for 15 sec. Afterwards, high-resolution melting (HRM) step was used, from 45°C for 60-sec Melt to 50°C and then to 95°C at 0.3°C/sec. Post-PCR analysis of HRM curves (HRMCs) (Figure) was used to determine variations in -94 ATTG Ins/Del of the rs28362491 gene. The genetic association parameters were analysed using SNP Stats software.[12]

Data was analysed using SPSS 27. Data was presented as mean + standard deviation and frequencies and percentages, as appropriate. Quantitative data was compared using student t-test and analysis of variance (ANOVA). Pearson's chi-square test was used to compare qualitative data with the application of Yat's correction or Fisher exact test. Genotype frequencies and allele distribution of rs28362491 were assessed using Hardy-Weinberg equilibrium.

Phenotypic odds ratio (OR) was calculated using medcalc statistical software, so that P<0.05 was considered statistically significant.

Results

Of the 88 subjects, there were 22(25%) in each of the 4 groups. Overall, there were 55(62.5%) females and 33(37.5%) males, and 40(45.45%) were aged 20-39 years while 48(54.54%) were aged 40-68 years (p>0.05) (Table 1).

Table-1: Study groups according to age and gender

Variables	G1 No. (%)	G2 No. (%)	G3 No. (%)	Control No. (%)	P value
Age (years)					
20-39	9 (40.9%)	9(40.9%)	11 (50%)	11 (50%)	0.336
40-68	13 (59.1%)	13 (59.1%)	11 (50%)	11 (50%)	
Range	20-68	20-60	20-68	23-60	
Mean + SD	38.77 ± 13.26	39.18 ± 13.22	45.64 ± 14.23	41.45 ±11.05	
Gender					
Male	8 (36.3%)	8 (36.3%)	8 (36.3%)	9 (40.9%)	0.986
Female	14 (63.7%)	14 (63.7%)	14 (63.7%)	13 (59.1%)	

SD: Standard deviation.

The genotype frequencies and allele distribution of rs28362491 were in accordance with the Hardy-Weinberg equilibrium expectation in the control group.

The Ins allele of rs28362491 was significantly more frequent in the patients than controls (OR: 2.776; 95% confidence interval [CI]: 1.334-5.774; p=0.0053). On the contrary, the Del allele represented a protective factor against developing infection (OR: 0.360; 95% CI: 0.173-0.749) (Table 2).

Table-2: Association of allele frequency for rs28362491 polymorphisms with susceptibility to hepatitis C virus (HCV) infection.

Allele	(G1+G2+G3)/control					
	Control		Patients		OR	P- value
	No	Freq.	No.	Freq.	(95% CI)	
Del	31	0.70	61	0.46	0.360 (0.173-0.749)	0.0053
Ins	13	0.30	71	0.54	2.776 (1.334-5.774)	

Ins: Insertion, Del: Deletion, OR: Odds ratio, CI: confidence interval.

Logistic regression analysis showed that the carriage of rs28362491 Ins/Ins and Ins/Del genotypes, in comparison to wild-type (WT) homozygote Del/Del, had a significantly higher risk of developing HCV infection (OR: 6.92; 95% CI: 2.10-22.86; p=0.0013). Corresponding to the effect of

Table-3: Genotype association of nuclear factor-kappa B1-94 (NF-κB1-94) polymorphisms with hepatitis C virus (HCV) infection.

Genotype	G1+G2+G3/control				G1+G3/G2			
	Control	Patients	OR (95% CI)	P- value	Control	Patients	OR (95% CI)	P- value
Del/Del	9 (40.9%)	6 (9.1%)	1.00 (reference)	-	0 (0%)	6 (13.6%)	1.00 (reference)	--
Ins/Del-Ins/Ins	13 (59.1%)	60 (90.9%)	6.92 (2.10-22.86)	0.0013	22 (100%)	38 (86.4%)	0.132 (0.007-2.448)	0.174

*OSCE: Objective Structured Clinical Examination.
*HPE: Health Professions Education.

rs28362491 polymorphism in the spontaneous clearance of the virus, no association was observed between rs28362491 genotypes and spontaneous clearance of HCV (OR: 0.132; 95% CI: =0.007-2.448; p=0.174). All the patients showed a sustained viral response (SVR) which precluded statistical analysis, suggesting that the response to therapy had not been influenced by rs28362491 polymorphism (Table 3).

Discussion

Considering the importance of NF-κB in the activation of the immune system and its essential role in liver function and pathophysiology, one may ask as to what extent polymorphisms of this gene may have contributed to variable HCV infection outcomes. The rs28362491 polymorphism in the NF-κB1 promoter was identified to be associated with various diseases in different populations[13,14], as it has a regulatory effect on the NF-κB1 gene and could potentially influence gene transcription, in addition to the level and function of NF-κB protein.[15]

The current study investigated the impact of rs28362491 polymorphism on HCV infection susceptibility and outcomes. The genetic model utilised to analyse the association between rs28362491 polymorphism and HCV susceptibility was the dominant model.

There was no association between rs28362491 polymorphism and spontaneous HCV clearance. The explanation for this might be a partial lack of polymorphism genotypes in spontaneous clearance participants, as well as the insufficient number of subjects in the study.

On the other hand, the Ins allele of rs28362491 was significantly associated with increased susceptibility to HCV infection, and the carrier of rs28362491 Ins/Ins and Ins/Del genotypes, compared to Del/Del, had a 6 times higher risk of developing HCV infection. Insertion of 4 base pairs in the NF-κB1 promotor region results in significantly higher promoter activity and increased p105/p50 NF-κB protein production, thereby increasing nuclear protein binding affinity.[15] It has been demonstrated that the p50 homodimer plays a crucial role as an anti-inflammatory transcription factor by suppressing the expression of pro-inflammatory genes and promoting the expression of anti-inflammatory genes.[16] For this significance, it is not prodigious that this NF-kB complex activity is firmly regulated, hence, several post-transcriptional modifications (PTMs) have been found that have the capacity to affect either the processing of p105 or the activity of p50.

The association of the Ins allele with increased susceptibility to HCV infection is assumed to be due to the fact that p50/50 protein inhibits pro-inflammatory genes by competing with activating NF-κB dimers and preventing them from binding to κB sites on the promoters of target genes[17], allowing the virus to establish the infection. Later, PTMs and viral-dependent NF-kB activation work in concert to activate NF-kB P65/50 heterodimer, which, in turn, promotes the production of pro-inflammatory cytokines, chemokines, and matrix metalloproteinases, which can finally result in chronicity, fibrosis, cirrhosis and the development of liver cancer.

The current findings seem consistent with a recent study[18] in Morocco which showed the association of Ins/Ins genotype with an increased risk of developing advanced liver disease. In addition, previous reports indicated that the Ins allele increased the incidence of HCC in a Taiwanese sample .[11] Another study linked the Ins allele with HBV-related HCC.[19]

Surprisingly, Gao et al.[20] found a positive association between Ins/Del and Del/Del genotypes with a higher risk of liver cancer (OR: 1.54; 95% CI: 1.04-2.28) in a sample of Shanghai population In China.

This disparity in findings might be related to the complicated transcriptional and post-transcriptional modifications of the NF-kB1 gene.

The current study has several limitations. It was challenging to determine the subjects' exact age and period of initial HCV infection that may have had an influence on the subjects' immune responses and HCV infection outcomes. Besides, there was a lack of HCV genotypes among individuals with spontaneous clearance.

Limitation: The sample size was small, and the fact that the sample size was not calculated could have affected the power of the study.

Conclusion

NF-kB1-94 Ins/Del ATTG polymorphism was associated with increased but not spontaneous clearance of HCV infection.

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Conflict of Interest: None.

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