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Genetic variants of Nuclear Factor-Kappa B were associated with different outcomes of Hepatitis C virus infection among Egyptian patients

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SUMMARY

Background: Hepatitis C virus (HCV) is a major risk factor for chronic hepatitis and hepatocellular carcinoma (HCC). Nuclear factor kappa B (NF-κB) is a transcription factor that functions in health and disease. Genetic variants of the NF-κB gene can affect its function and are associated with chronic inflammatory changes and malignant transformation. This case-control study is aimed to determine the possible association between NF-κB genetic variants and different outcomes of HCV infection among Egyptian patients.
Subjects and Methods: 295 subjects were recruited with allocation of participants in the representative group according to results of serological and molecular tests. Patients in the case group (group A) were further divided into three subgroups; subgroup I, mild chronic HCV, subgroup II, cirrhosis, and subgroup III, HCC subgroups (59 for each subgroup), group B included participants who experienced spontaneous viral clearance (n=59). All were compared to matched healthy control subjects, Group C (n=59). All participants were genotyped for NF-κB polymorphisms, rs11820062 by TaqMan assay and rs28362491 by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).
Results: Risk analysis indicated that subjects carrying the rs11820062 A genotype are more susceptible to HCV infection (OR: 3.1; 95%, CI= 1.4-6.9). Subjects carrying the rs28362491 insertion genotype are at more risk of progression to cirrhosis when compared to healthy -controls and patients with mild chronic HCV (OR:7.7; 95% CI=2.4-24.3 and OR:5.1, 95% CI= 1.7-15.7, respectively) and also are at more risk of developing HCC when compared to healthy controls (OR:2.6; 95% CI= 0.94-7.3).
Conclusion: Polymorphisms affecting NF-κB different genes would modulate HCV infection susceptibility and clinical disease progression among Egyptian patients.

Keywords: Nuclear factor kappa B, single nucleotide polymorphism, HCV, susceptibility, cirrhosis, hepatocellular carcinoma.

INTRODUCTION

Hepatitis C virus (HCV) belongs to the family *Flaviviridae* genus *Hepacivirus* and HCV infection is a potentially growing burden. Globally, 71 million persons are living with chronic HCV infection. Some areas have high rates of infection. In lower-middle income countries, serological evidence of past or present HCV infection is the highest [1].

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The national campaign for screening and treatment of HCV in Egypt revealed a prevalence rate of 4.6% for HCV anti-body positivity and 3.5% for HCV viremia [2]. Egyptian population have always been an important research target regarding HCV due the higher prevalence of anti-HCV compared to other industrialized countries, which is most probably a result of schistosomiasis mass-control campaigns using parenteral tartar emetic conducted from the 1950s up until 1982 [3]. However, the current rate of new case development is associated with different risk factors including medical or surgical procedures, blood transfusion or intravenous drug use [4].

Despite therapeutic advances achieved by direct acting antivirals (DAAs), HCV remains an issue of global interest probably due to the occurrence of resistance-associated variants (RAVs) and the possibility of reinfection, particularly in high-risk populations, and the persistence of unsafe injection practice in some regions [5, 6]. Furthermore, the contribution of worsening factors such as pre-existing inflammation, liver fibrosis, dysregulation of immune response of the host to HCV, the presence of epigenetic scar and genetic susceptibility cannot be ignored [7].

The pathologic effect of HCV is mediated through a complex interaction between the virus and the host immune responses; this is mediated by different activated transcription factors [8, 9].

Among the master transcription factors is the nuclear factor kappa B (NF-κB) which upon activation can trigger and control the transcription of several inflammatory mediators and adhesion molecules [10].

NF-κB is a family of five protein members; NF-κB-1 (p50/p105), NF- κB-2 (P52/p100), RelA (P65), RelB and cRel that all share a rel homology region or domain (RHR) responsible for DNA binding. Another domain, the activation domain, is shared by RelA, RelB and cRel only and is responsible for transcriptional activation [11]. After being activated, NF-κB members heterodimerize to get to the nucleus and exhibit their functional transcriptional activity [12]. Under resting condition, this heterodimerization is prevented by an inhibitor of NF-κB (IκB), upon activation, an enzyme called IκB kinase (IKK) eliminates this inhibitory effect [13].

Two activation pathways are known for NF-κB proteins. First, the canonical pathway is activated through proinflammatory receptors, including Il-1B, TNFα, lipopolysaccharide, and leads to formation of the active heterodimers of NF-κB1 with p65/RelA or with c-Rel. The non-canonical pathway is activated through receptors of tumor necrosis factor receptors (TNFRs), lymphotoxin-β (LTβR) and the BAFF (BAFFR), and leads to formation of the active heterodimer of NF-κB2/p52 and its partner RelB. In either pathways the active heterodimers translocate to the nucleus for further downstream target gene transcription regulation [14].

In normal cells, the activation of NF-κB is transient and tightly controlled. So, it is considered as a switcher or sensor for multicellular processes and other interacting signaling pathways. In the case of HCV infection, NF-κB is constitutively activated. The viral core protein activates NF-κB non-canonical pathway through activating TNFR1 death domain, the viral non-structural protein 5A (NS5A) induces reactive oxygen species leading to activation of the canonical pathway through the IκB [15, 16].

Correlation between NF-κB genetic polymorphisms and several inflammatory and malignant disorders has been studied extensively [17]. Furthermore, previous studies demonstrated that certain NF-κB genetic variants can be associated with increased HCV-susceptibility and further may influence the progression and outcomes of HCV infection [18-20]. However, scarce data are available among Egyptian patients who are characterized by different genetic and environmental backgrounds in addition to prevalence of HCV genotype 4. Therefore, this study aimed to explore the possible association between NF-κB genetic variants and different outcomes of HCV infection among Egyptian patients.

MATERIAL AND METHODS

This case-control study was conducted during the period from September 2019 to March 2022 in Departments of Medical Microbiology and Tropical Medicine of Zagazig University Hospitals, Egypt. The study followed the principles of the Helsinki Declaration and ethical approval was obtained from the Institutional Review Board: IRB number (ZU-IRB #5574/10-9-2019). A written consent was obtained from all participants. The study was conducted according to the international guidelines of

Strengthening the Reporting for Observational Studies in Epidemiology [21].

Two hundred and thirty-six subjects were enrolled to the case group. They were further divided into groups A and B. Group A was further subdivided into three sub-groups (group I; mild chronic HCV, group II; cirrhotic patients, and group III; HCC cases, each with 59 patients). Patients were allocated in any of the three subgroups depending on their virological markers (serological and molecular markers) to confirm diagnosis of HCV infection, ultrasonographic (US) data and/or triphasic computed tomography (triphasic CT) to confirm diagnosis of cirrhosis (subgroup II) or HCC (subgroup III), patients infected with HCV without developing cirrhosis or HCC were allocated in subgroup I (mild chronic HCV). Group B included 59 subjects with spontaneous viral clearance who were defined by their HCV seropositivity combined with negativity to HCV RNA. Fifty-nine healthy age and sex-matched controls were included in the healthy control group (Group C).

Sample size was estimated depending on the assumed prevalence of the studied polymorphisms through previous studies, using Epi Info 6 with test power of 80% and confidence interval of 95%. The calculated sample size was 295 participants [22]. Exclusion criteria included refusal, coinfection with hepatitis B virus (HBV) or human immunodeficiency virus (HIV), diagnosis of any metabolic, alcoholic, or autoimmune liver disease, and treatment with antiviral medications before or during the study period.

Peripheral blood samples (3 ml) were withdrawn on EDTA coated vacutainers. Extraction of peripheral blood mononuclear cells (PBMC) genomic DNA was performed according to the manufacturer's recommendations using QIAamp DNA Blood Mini Kit (Qiagen, Netherlands). DNA quantification and purity assessment were checked for by UV/Vis spectrophotometry method (A260/A280 measurement). Extracted DNA was kept sealed at -20°C for further genotyping analysis.

HCV genotyping

HCV genotyping was performed to ensure agreement of the studied genotypes with the expected homogenous distribution of HCV genotype 4 in Egypt as stated by epidemiological studies [23]. Genotyping was performed using the Murex HCV Serotyping 1-6 Assay ELISA (Abbott, Wiesbaden, Germany) to determine the type-specific antibodies of various HCV genotypes and confirm the epidemiological predominance of genotype 4.

NF-κB SNP studying

NF-κB SNPs were selected, based on the haplotype pattern, using Haploview software (version 4.2; Broad Institute, Cambridge, MA, USA) and based on the linkage disequilibrium (LD) data considering the potential regulatory effects of adjacent sequences, 2000bp upstream and downstream of NF-κB gene transcription initiation sites.

RelA rs11820062 genotyping

The RelA rs11820062 single nucleotide polymorphism (SNP) was genotyped by TaqMan real-time PCR assay using the 5´nuclease assay for amplifying and detecting specific SNP alleles in purified genomic DNA samples. According to the manufacturer's protocol the reaction mix was prepared by adding 12.5 µL of 2X TaqMan genotyping master mix (Thermofisher, USA) and 1.25 µL of 20X TaqMan genotyping assay mix (Thermofisher, USA) that contains TaqMan major binding groove (MGB) probes and sequence-specific forward and reverse primers to amplify the polymorphic sequence of interest. Probe-G: FAM-TCCCTCAGTTTTC-MGB, Probe-A: HEX-TCCCTCAATTTTC-MGB, forward primer sequence 5'CTTGACTCAGTTTCCCTCCACAC3' and reverse primer sequence 5'GAGGGAAAACGGGGTAAGGAATC3' [20].

PCR cycle started with an initial AmpliTaq gold enzyme activation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 s and annealing/extension at 60°C for 1 min. After PCR amplification, endpoint plate read was performed using the real-time PCR device (Applied Biosystems, USA) where the fluorescence measurements were used to determine which alleles are in each sample. HEX dye fluorescence only indicated homozygosity to allele 1 (GG), FAM dye fluorescence only indicated homozygosity to allele 2 (AA), while mixed fluorescence indicated heterozygosity (GA) (Figure 1).

NF-κB1 rs28362491 genotyping

The NF-κB1 -94 Ins/Del ATTG (rs28362491) polymorphism was genotyped by "polymerase chain reaction-restriction fragment length polymorphism" (PCR-RFLP). NF-κB1 region of 285 bp was

amplified using the PCR primer sets NF-κB1 forward: 5'TGGGCACAAGTCGTTTATGA-3' and reverse: 5'-CTGGAGCCGGTAGGGAAG-3' [22].
PCR reactions were performed in a total volume of 50 µL consisting of, 10 µL extracted DNA, 25 µL DreamTaq Green master mix (Thermofisher, USA), 2.5 µL of each of the forward and reverse primers (20 pmol/µL), and 10 µL of nuclease-free water.
PCR products were then propagated through the step of restriction digestion by Van91I (PflMI) (10 U/µL) (Thermofisher, USA) according to the manufacturer's recommendations, and examined by 2% agarose gel electrophoresis.
For the different genotypes of rs28362491 polymorphism, the Ins genotype (Ins), which includes an ATTG insertion, contains a specific PfIMI restriction site taking the form 5'...CCANNNN↓NTGG...3' and as a result, PfIMI performed its restriction digestion activity and yielded two fragments giving DNA bands at 45 bp and 240 bp. Deletion genotype (Del) includes only one ATTG and as a result, remains undigested giving the full-sized band of 285 bp (Figure 2).

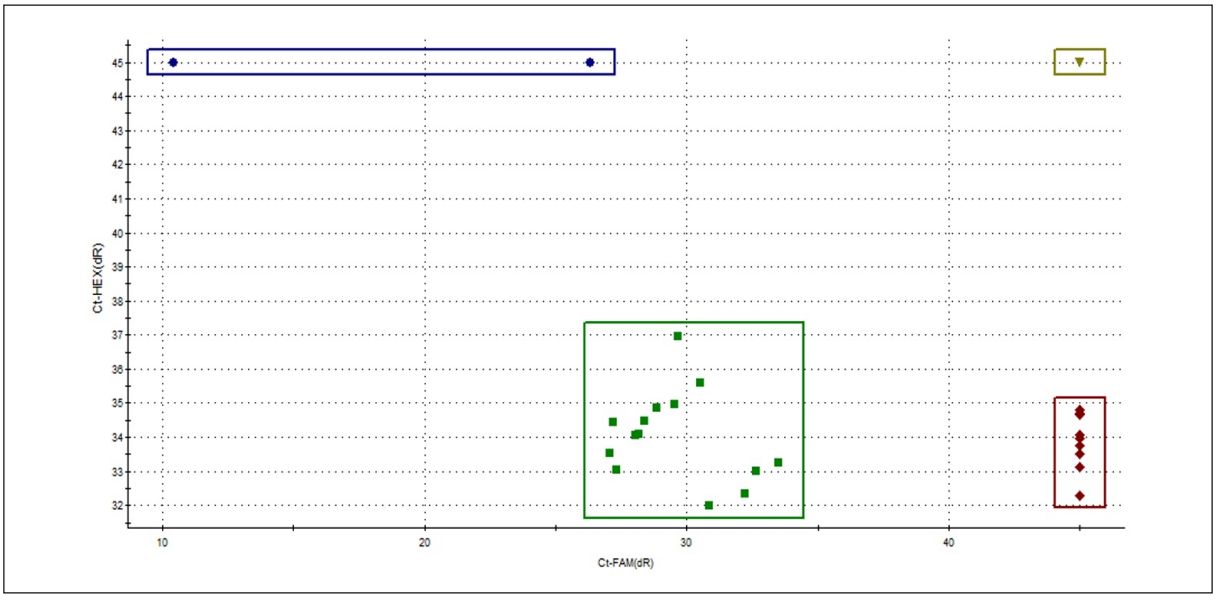

Figure 1 - Dual color scatter plot of RelA rs11820062 SNP:

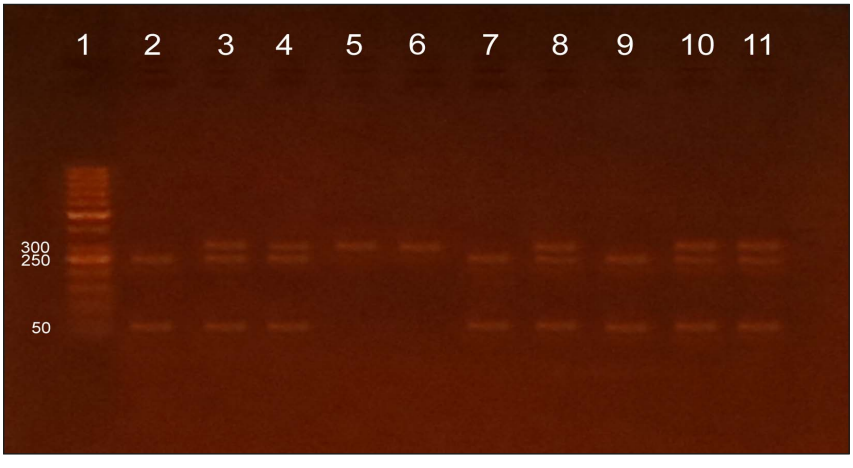

Figure 2
Gel electrophoresis of PCR-RFLP products demonstrating different NF-κB1 -94 Ins/Del ATTG genotypes of the studied participants Lane 1: 50-bp DNA Ladder; lanes 2,7 and 9: ATTG Ins/Ins homozygotes with one restriction site in each allele (two fragments 45bp, and 240bp); lanes 3,4,8,10 and 11: ATTG heterozygotes Ins/Del with one restriction site in one allele (three fragments 285bp, 45bp, and 240bp); lanes 5 and 6: ATTG homozygotes Del/Del with no restriction sites (one band 285bp).

Statistical analysis

Data were coded, validated and analyzed using the IBM SPSS 23.0 for windows (SPSS Inc., Chicago, IL, USA) and NCSS 11 for windows (NCSS LCC., Kaysville, UT, USA). Mean ± standard deviation (SD) was used to represent quantitative data. Frequency and percentage were used to represent qualitative data. P value <0.05 was considered significant, P value <0.001 was considered as highly significant, and P value >0.05 was considered insignificant.

RESULTS

The mean ages of mild chronic HCV, cirrhotic, HCC patients and spontaneous viral clearers were, 50.6, 51, 52.1 and 49.3 years respectively. Males represented 68.2%, while females represented 31.8% of the case group. Regarding the residence of the case group, 66.9% were from rural area while 33.1% were from urban areas with no significant difference with the controls (Table 1).

Laboratory investigations of the study groups indicated a highly statistically significant difference among them regarding the levels of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), serum total bilirubin, international normalized ratio (INR), serum albumin and serum creatinine levels (P value <0.001 for all variables). HCV viral load was detected among the three infected subgroups where it showed non-significant difference (Table 2).

The infected subgroups were compared regarding their US data. A highly significant difference was detected regarding liver and spleen sizes, presence of ascites and presence of focal lesions (P value < 0.001 for all variables). Child-Pugh and Model of End-stage Liver disease (MELD) scores, as prognostic systems, were calculated for cirrhotic and HCC patients. The results demonstrated statistically significant differences among them (P values 0.03 and 0.04 respectively for both scores) (Table 3).

To study the association between RelA rs11820062 polymorphism and infection susceptibility, study groups were compared regarding their RelA rs11820062 genotypes, considering the GG genotype the wild genotype, AA the homozygous mutant and GA the heterozygous mutant genotype. There was a statistically significant difference regarding the differential distribution of both AA and GA genotypes among the three main study groups (P value < 0.001 and 0.002 respectively for both genotypes). The genotype distribution in diseased groups was in accordance with expected values by Hardy-Weinberg equilibrium (P value=0.6). Risk association study revealed a higher risk of infection susceptibility among carriers of both genotypes when compared to healthy controls (OR:3.1; 95% CI:1.4-6.9 and OR:2.8; 95% CI: 1.5-5.4 respectively for both genotypes). P values remained significant after Bonferroni correction for multiple comparisons (Table 4).

Fluorescent signals obtained from real-time PCR wells. The horizontal axis represents the cycle threshold (Ct) of FAM dye while the vertical axis represents the Ct of HEX. HEX green signals indicate GG genotype, FAM red signals indicate AA genotype while mixed blue signals indicate heterozygosity GA.

The NF-κB1 -94 Ins/Del ATTG (rs28362491) genotypes were investigated in relation to the clinical progression of HCV infection and related liver diseases among the different study groups. The deletion genotype (Del) is considered the reference wild genotype, the Ins is the homozygous mutant, and the insertion/deletion (Ins/Del) is the heterozygous mutant. A statistically significant difference in genotypes distribution was detected among the three case subgroups, and the spontaneous viral clearance as with the healthy controls (P value < 0.001). The genotype distribution in diseased groups was in accordance with expected values by Hardy-Weinberg equilibrium (P value 0.7). Subjects carrying the Ins allele were at a higher risk of developing cirrhosis when compared to healthy controls and mild chronic HCV patients (OR: 13.8; 95% CI:4.1-47.3 and OR: 6.9; 95% CI: 2.16-22.3 respectively for both groups), and also

Table 1 - Sociodemographic data of study groups.

	Cases N= 236	*Controls N= 59*	*P*
Age Mean ± SD	50.78 ± 9.8	51.47 ± 18.5	0.3 #
Gender Male Female	 161 (68.2%) 75 (31.8%)	 43 (72.9 %) 16 (27.1%)	 0.5 [A]
Residence Rural Urban	 158 (66.9%) 78 (33.1%0	 39 (66.1%) 20 (33.9%)	 0.8 [A]

Notes: SD = standard deviation, # = student t test, [A] = fisher exact test.

Table 2 - Laboratory investigations of the study groups.

	Group A			*Group B*	*Group C*	*KW*	*P*
	Group I N=59	*Group II* N=59	*Group III* N=59	N=59	N=59	*Test*	
Serum ALT (U/L) *Median* *Range*	110 6 – 300	124.3 40 – 85	64.3 42 – 101	18.97 7 – 35	24.8 9 – 40	159.2	<0.001*
Serum AST (U/L) *Median* *Range*	102 4 – 291	82 45 – 131	80 56 – 136	15 5 – 31	20 8 – 35	185.5	<0.001*
Serum Bilirubin ((mg/dL) *Median* *Range*	1.2 0.9 – 1.5	2.2 0.9 – 5.4	2.9 1.1 – 25	1 0.9 – 1.2	1 0.9 - 1.2	202.3	<0.001*
INR *Median* *Range*	1 0.8 – 1.2	1.6 0.9 – 4.1	1.8 0.9 - 5	1 0.8 – 1.1	1 0.8 – 1.1	106.1	<0.001*
Serum Albumin (g/dL) *Mean±SD*	3.95±0.31	2.7±0.55	2.4±0.51	4.13±0.27	3.97±0.27	242.1#	<0.001*
Serum Creatinine (mg/dL) *Median* *Range*	1 0.7 – 1.4	0.81 0.8 – 4.1	0.95 0.7 – 4.2	1 0.7- 1.3	1 0.7 – 1.3	80.4	<0.001*
Viral load *IU/mL* *Mean±SD*	3.99×10 ± 3.09×10^6	3.89×10 ±3.07×10^6	4.13×106 ±3.1×106	NA	NA	0.197	0.9

Notes: * = significance, KW = Kruskal Wallis, ALT = Alanine transferase, AST = Aspartyl transferase, INR = International Normalized Ratio, # = F test (ANOVA)
Reference serum levels: ALT: 4-36 U/L, AST: 0-35 U/L, Bilirubin: 0.1-1.2 mg/dL, INR: 1.1 or below, Albumin: 3.4-5.4 g/dL, Creatinine: 0.6-1.2 mg/dL, Viral load: undetectable ≤ 12 IU/mL, low viral load: ≤ 8×105 IU/mL, high viral load: > 8×105 IU/mL.

Table 3 - Ultrasonographic and clinical data of infected subgroups.

	Group I N=59	*Group II* N=59	*Group III* N=59	*P*
Liver Normal size Enlarged Shrunken	16 (27.1%) 43 (72.9%) 0 (0.0%)	8 (13.6%) 21 (35.6%) 30 (50.8%)	19 (32.2%) 30 (50.8%) 10 (16.9%)	<0.001^A
Spleen Normal size Enlarged	39 (66.1%) 20 (33.9%)	5 (8.5%) 54 (91.5%)	6 (10.2%) 53 (89.8%)	<0.001^A
Ascites No Mild Moderate Severe	56 (94.9%) 3 (5.1%) 0 0	3 (5.1%) 26 44.1%) 17 (28.8%) 13 (22%)	7 (11.9%) 18 (30.5%) 20 (33.9%) 14 (23.7%)	<0.001^A
Focal lesions	0 (0.0%)	0 (0.0%)	59 (100%)	<0.001^B
CHILD score Mean ± SD	NA	9.79 ± 2.9	10.95 ± 2.9	0.03^B
MELD score Median Range	NA	15 6 – 40	20 7 – 40	0.04^C

Notes: ^{A} = Chi square test, ^{B} = t test, ^{C} = Mann-Whitney test, MELD = Model of End-stage Liver Disease, SD = standard deviation.

Table 4 - Differential rs11820062 genotype distribution among study groups and association with infection susceptibility.

Genotype	*Group A N=177*	*Group B N=59*	*Group C N=59*	*P[A]*	*Group A+B vs Group C*	*P#*
	N (%)	*N (%)*	*N (%)*		*OR (95% CI)*	
GG	38 (21.5%)	21 (35.6%)	29 (49.2%)	1	1	1
GA	86 (48.6%)	28 (47.5%)	20 (33.9%)	0.002*	2.8 (1.5-5.4)	0.002*
AA	53 (29.9%)	10 (16.9%)	10 (16.9%)	<0.001*	3.1 (1.4-6.9)	0.006*

Notes: OR = Odds Ratio, CI = confidence interval, [A] = Chi-square test of significance, # = P value for the difference between case groups (A+B) versus controls (C). Adjusted P value after Bonferroni correction for multiple comparisons: 0.016.

Table 5 - Differential rs28362491 genotype distribution among study groups and association with clinical progress of HCV-related liver disease.

Genotype	*Group A*			*Group B N=59*	*Group C N=59*	*P*
	Group I N=59	*Group II N=59*	*Group III N=59*			
	N (%)	*N (%)*	*N (%)*	*N (%)*	*N (%)*	
Del	20 (33.9%)	5 (8.4%)	10 (16.9%)	13 (22.0%)	24 (37.3%)	
Ins\Del	24 (40.7%)	28 (47.4%)	28 (47.4%)	34 (57.6%)	26 (44.1%)	<0.001*
Ins	15 (25.4%)	26 (44.2%)	21 (35.7%)	12 (20.3%)	9 (18.6%)	
	OR (95%) CI					
	Group II vs I	*Group III vs I*	*Group II vs C*	*Group III vs C*		
Del	1	1	1	1		
Ins\Del	4.7 (1.5-14.3)*	2.3 (0.9-4.9)	5.17 (1.7-15.5)*	2.58 (1-6.4)		
Ins	6.9 (2.16-22.3)*	2.8 (1.02-7.7)	13.8 (4.1-47.3)*	5.6 (1.9-16.4)*		

Notes: * = significance, OR = Odds ratio, CI = confidence interval, Del = deletion, Ins; insertion, vs; versus.
Adjusted P value after Bonferroni correction for multiple comparisons: 0.005.

were at a higher risk of developing HCV related HCC when compared to healthy controls (OR: 5.6; 95% CI:1.9-16.4). Subjects carrying the Ins/Del genotype were at a higher risk of progressing to cirrhosis when compared to healthy controls and mild chronic HCV patients too (OR: 5.17; 95% CI:1.7-15.5 and OR: 4.7; 96% CI:1.5-14.3 respectively for both groups). P values remained significant after Bonferroni correction for multiple comparisons (Table 5).

DISCUSSION

Chronic HCV related complications represent the fourth common cancer in Egypt [24]. The effect of sustained virologic response (SVR) achieved by DAAs on the incidence of HCC is still a matter of debate [25, 26]. Elucidating the mechanisms underlying susceptibility to HCV infection and its different outcomes could be of great importance to control HCV infection.

In this study there was no statistically significant difference between cases and controls regarding age. However, the exact patients age at the time of HCV acquisition which really matters in determining different outcomes was undetermined owing to the retrospective nature of the study and the nature of initial infection which passes asymptomatic in most cases [27-29].

Regarding the gender distribution among the case group, higher male ratio (68.2%) was recorded. This gender ratio complies with difference in host factors of susceptibility to HCV infection and was in accordance with other studies [30]. However, higher female ratios were reported by other studies [31,32]. Difference regarding residence among groups was non-significant and was in agreement with several studies that were conducted inside and outside Egypt [33, 34].

Laboratory data obtained by the current study represent the hallmarks of chronic liver disease and decompensation of different liver functions.

Patients with mild chronic disease, cirrhosis and HCC had mild elevation of aminotransferases with AST being more elevated in cases of liver cirrhosis and HCC [35-38]. Another hallmark of end stage liver disease is the moderate hyperbilirubinemia caused by liver cell failure [36]. Marked hyperbilirubinemia can occur in some HCC cases due to obstruction by the tumor mass itself or due to pressure by lymph nodes in porta hepatis [37] which was detected in some HCC cases of the current study. In the current study, insignificant differences in HCV viral load among the three infected groups were detected. The relation between HCV viral load and the progression of liver pathology is controversial. While some studies reported no obvious relation [39, 40] which is consistent with the current findings, other studies reported viral load as a predictive factor for liver pathology progression [41, 42].

Highly significant US differences were detected among the three infected subgroups of the current study. This could be due to different progression level in liver disease among the infected subgroups [42, 43]. The Child-Pugh and MELD scoring systems help to categorize cirrhotic patients into different survival/mortality categories [44], with a broader range of prognostic stratification of cirrhotic patients by including serum creatinine in the scoring parameters of MELD score. This makes the significant difference between cirrhotic and HCC groups of the current study regarding both scoring systems justifiable due to higher mean of bilirubin level among HCC patients caused by the possible obstructive nature of HCC.

Several host genetic polymorphisms have been reported to contribute to HCV susceptibility and infection outcomes [45]. NF-κB and its genetic variants have been studied as candidate contributors in the pathogenesis of many inflammatory and malignant disorders [17, 46, 47].

In the current study, the SNP affecting intron 1 of RelA gene (rs11820062) was studied to investigate its association with HCV susceptibility. As this SNP is located at a regulatory element's region, it can therefore affect RelA gene expression process, NF-κB pathway activation and accordingly the susceptibility to HCV infection [18]. The differential distribution of both AA mutant and GA heterozygous mutant genotypes showed a statistically significant difference among the study group. Moreover, subjects carrying both genotypes were more susceptible to HCV. This is in agreement with previous studies [20,48]. However, other studies reported a nonsignificant association between (rs11820062) and HCV susceptibility. In one of these studies, HCV infected cases exclusively belonged to genotype 1 and were further subdivided into subtypes 1a and 1b where HCV 1a core protein inhibits NF-κB, while HCV 1b core protein activates it. The final statistical analysis was performed on mixed subtypes. Therefore, the confounding effect of HCV subtype could not be excluded [49].

In the current study, the association between another SNP (rs28362491) and the progression of liver disease was also studied. Subjects carrying the Ins genotype had higher risk for developing cirrhosis when compared to healthy controls and to patients with mild chronic HCV disease, and also were at a higher risk of developing HCV related HCC when compared to healthy controls. Subjects carrying the Ins/Del genotype were at a higher risk of progressing to cirrhosis when compared to healthy controls and mild chronic HCV patients too. These results were consistent with other studies on HCV clinical progression [22], and on liver pathology in obese patients [50]. With HCV-related HCC, the Ins allele was reported to increase the levels of NF-κB expression favoring more sensitization of hepatocytes to carcinogenic stimuli and was further detected in a significantly higher ratio of HCC patients than healthy subjects with an increased risk of HCC development (Adjusted OR 2.23, 95% CI 1.32-3.77) [51] which supported the current finding. In a different study, a significant association was reported between the Del genotype and HCC development [52]. Interactions from non-genetic risk factors such as epigenetic factors [53] may account for this difference. The interactions of NF-κB signaling pathway with other crucial pathways including PI3K/AKT, MAPK, JAK-STAT,TGF-β, Wnt, Notch, Hedgehog, and TLR signaling may be targeted as potential future therapeutic interventions [54].

Potential clinical applications: The two genetic variants may serve as prognostic biomarkers for monitoring therapeutic efficacy of HCV infection. In addition, the interactions of NF-κB signaling pathway with other crucial pathways including PI3K/AKT, MAPK, JAK-STAT,TGF-β, Wnt, Notch, Hedgehog, and TLR signaling may be targeted as potential future therapeutic interventions [54].

Limitations: This study had some limitations. First, the small sample size included. Second, as all included infected subjects are Egyptians where the same HCV genotype is prevalent, the comparison between different HCV genotypes was not possible. Another limitation is the existence of a potential inevitable selection bias that was reduced by adjusting corrections.

CONCLUSIONS

Polymorphisms affecting NF-κB different genes can modulate HCV infection susceptibility and clinical disease progression among Egyptian patients. The rs11820062 located in RelA gene intron 1 has an association with HCV susceptibility and the rs28362491 located in the promoter region of NF-κB1 gene has an association with progress to advanced liver disease (cirrhosis and HCC). So it may be applied in prediction, prevention and future treatment strategies after confirmation by large randomized controlled studies.

Ethical approval
The study was approved by the Institutional Review Board (IRB), Faculty of Medicine, Zagazig University (approval #5574/10-9-2019).

Availability of data and materials
All data and materials related to the study are available.

Informaed consent
Informed consent was obtained from all individual participants included in the study. The participant has consented to the submission of this research to the journal.

Funding
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Conflict of interest
The authors have no competing interests to declare that are relevant to the content of this article.
This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Review Board (IRB), Faculty of Medicine, Zagazig University (approval #5574/10-9-2019).
All data and materials related to the study are available.

Authors' contribution
All authors contributed to the study conception and design. Conceptualization: Nahla Abd-Elhamid, Wafaa S. Metwally; Methodology: Alaa O. Abdel-Kareem, Ahmed Lotfy Shara; Formal analysis and investigation: Sahar A. El-Nimr, Ahmed Abouelkhair Badawy and Manal M. El Gerby; Writing - original draft preparation: Marian Asaad Gerges and Hassan Shora ; Writing - review and editing: [Wafaa S. Metwally], Supervision: [Wafaa S. Metwally].
All authors read and approved the final manuscript

REFERENCES

[1] World Health Organization. Updated recommendations on treatment of adolescents and children with chronic HCV infection, and HCV simplified service delivery and diagnostics. Geneva: World Health Organization; 2022. Licence: CC BY-NC-SA 3.0 IGO. 9789240052734-eng.pdf (who.int) accessed June 2024
[2] Waked I, Esmat G, Elsharkawy A, et al. Screening and treatment program to eliminate hepatitis C in Egypt. *New Engl. J. Med*. 2020; 382(12): 1166-1174. Doi: 10.1056/NEJMsr1912628.
[3] Habib M, Mohamed MK, Abdel-Aziz F, Magder LS, Abdel-Hamid M, Gamil F, et al. Hepatitis C virus infection in a community in the Nile Delta: risk factors for seropositivity. *Hepatol*ogy. 2001; 33(1): 248-53. Doi: 10.1053/jhep.2001.20797.
[4] Kandeel A, Fahim M, Abukamar S, et al. Evidence for the elimination of viral hepatitis B and C in Egypt: Results of a nationwide survey in 2022. *Liver Internat*. 2024; 44(4): 955-965. Doi: 10.1111/liv.15843. Epub 2024 Jan 30.
[5] Bailey JR, Barnes E, Cox AL. Approaches, progress, and challenges to hepatitis C vaccine development. *Gastroenterololy.* 2019; 156(2): 418-430. Doi: 10.1053/j.gastro.2018.08.060.
[6] Pepin J, Abou Chakra CN, Pepin E,Nault V, Valiquette . Evolution of the global burden of viral infections from unsafe medical injections, 2000–2010. *PloS one.* 2014 ;9(6): e99677. Doi: 10.1371/journal.pone.0099677
[7] Sarrazin C. Treatment failure with DAA therapy: Importance of resistance. *J. Hepatol*. 2021; 74(6): 1472-82. Doi: 10.1016/j.jhep.2021.03.004.
[8] Lupberger J, Croonenborghs T, Suarez AAR , et al. Combined analysis of metabolomes, proteomes, and transcriptomes of hepatitis C virus–infected cells and liver to identify pathways associated with disease development. *Gastroenterol.* 2019;157(2):537-551. e539. Doi: 10.1053/j.gastro.2019.04.003
[9] Streicher F, Jouvenet N. Stimulation of innate immunity by host and viral RNAs. *Trends Immunol.* 2019; 40(12): 1134-1148. Doi: 10.1016/j.it.2019.10.009

[10] Zheng C, Yin Q, Wu H. Structural studies of NF-κB signaling. *Cell res.* 2011; 21(1): 183-195. Doi: 10.1038/cr.2010.171
[11] Gilmore TD. Introduction to NF-κB: players, pathways, perspectives. *Oncog.* 2006; 25(51): 6680-6684. Doi: 10.1038/sj.onc.1209954
[12] Kieran M, Blank V, Logeat F, et al. The DNA binding subunit of NF-κB is identical to factor KBF1 and homologous to the rel oncogene product. *Cell* 1990; 62(5): 1007-1018. Doi: 10.1016/0092-8674(90)90275-j
[13] Kracht M, Müller-Ladner U, Schmitz ML. Mutual regulation of metabolic processes and proinflammatory NF-κB signaling. *J. Allergy Clin. Immunol.* 2020; 146(4): 694-705. Doi: 10.1016/j.jaci.2020.07.027
[14] Liu T, Zhang L, Joo D,Sun SC. NF-κB signaling in inflammation. *Signal Transduct. Target Ther*. 2017; 2: 17023-. Doi: 10.1038/sigtrans.2017.23
[15] Chen Y, He L, Peng Y, et al. The hepatitis C virus protein NS3 suppresses TNF-α–stimulated activation of NF-κB by targeting LUBAC. *Sci. signal.* 2015;8(403):ra118-ra118. Doi: 10.1126/scisignal.aab2159
[16] Waris G, Livolsi A, Imbert V, Peyron J-F, Siddiqui A. Hepatitis C virus NS5A and subgenomic replicon activate NF-κB via tyrosine phosphorylation of IκBα and its degradation by calpain protease. *J. Biol. Chem.* 2003; 278(42): 40778-40787. Doi: 10.1074/jbc.M303248200
[17] Park MH, Hong JT. Roles of NF-κB in cancer and inflammatory diseases and their therapeutic approaches. *Cells* 2016; 5(2): 15. Doi: 10.3390/cells5020015.
[18] Yue M, Tian T, Wang C, et al. Genetic mutations in NF-κB pathway genes were associated with the protection from hepatitis C virus infection among Chinese Han population. *Sci. Rep.* 2019; 9(1): 1-11. Doi: 10.1038/s41598-019-47058-y.
[19] Fan H-z, Huang P, Shao J-g, et al. Genetic variation on the NFKB1 genes associates with the outcomes of HCV infection among Chinese Han population. *Infect, Genet. Evolut.* 2018; 65: 210-215. Doi: 10.1016/j.meegid.2018.07.031.
[20] Tian T, Wang J, Huang P, et al. Genetic variations in NF-κB were associated with the susceptibility to hepatitis C virus infection among Chinese high-risk population. Sci. Rep. 2018; 8(1): 1-8. Doi: 10.1038/s41598-017-18463-y.
[21] Limaye D, Limaye V, Pitani RS, et al. Development of a Quantitative Scoring Method for Strobe Checklist. *Acta Pol Pharm*. 2018; 75(5): 1095-1106. Doi: 10.32383/appdr/84804.
[22] Fakhir F-Z, Lkhider M, Badre W, et al. The-94Ins/DelATTG polymorphism in NFκB1 promoter modulates chronic hepatitis C and liver disease progression. *Infect, Genet. Evolut.* 2016; 39: 141-146. Doi: 10.1016/j.meegid.2016.01.023.
[23] Gower E, Estes C, Blach S, Razavi-Shearer K, Razavi H. Global epidemiology and genotype distribution of the hepatitis C virus infection. *J. Hepatol*. 2014; 61(1): S45-57.
[24] Rashed WM, Kandeil MAM, Mahmoud MO, Ezzat S. Hepatocellular Carcinoma (HCC) in Egypt: A comprehensive overview. *J Egypt. Natl. Cancer Inst.* 2020; 32(1): 1-11. Doi: 10.1186/s43046-020-0016-x.
[25] Rinaldi L, Nevola R, Franci G, et al. Risk of hepatocellular carcinoma after HCV clearance by direct-acting antivirals treatment predictive factors and role of epigenetics. *Cancers.* 2020; 12(6): 1351. Doi: 10.3390/cancers12061351.
[26] Abe K, Wakabayashi H, Nakayama H, et al. Factors associated with hepatocellular carcinoma occurrence after HCV eradication in patients without cirrhosis or with compensated cirrhosis. *PloS one* 2020; 15(12): e0243473. Doi: 10.1371/journal.pone.0243473.
[27] Minola E, Prati D, Suter F, et al. Age at infection affects the long-term outcome of transfusion-associated chronic hepatitis C. Blood, *J. Am. Soc. Hematol.* 2002; 99(12): 4588-4591. Doi: 10.1182/blood-2001-12-0192.
[28] Yan Z, Wang Y. Viral and host factors associated with outcomes of hepatitis C virus infection. Mol. Med. *Rep*. 2017; 15(5): 2909-2924. Doi: 10.3892/mmr.2017.6351
[29] Grebely J, Prins M, Hellard M et al. Hepatitis C virus clearance, reinfection, and persistence, with insights from studies of injecting drug users: towards a vaccine. *Lancet infect. Dis.* 2012; 12(5): 408-414. Doi: 10.1016/S1473-3099(12)70010-5
[30] Abbas EAER, Barakat AB, Hassany M, Youssef SS. The role of BCL9 genetic variation as a biomarker for hepatitis C-related hepatocellular carcinoma in Egyptian patients. *J. Genet. Eng. Biotechnol*. 2022; 20(1): 1-9. Doi: 10.1186/s43141-021-00282-4
[31] Grebely J, Page K, Sacks-Davis R, et al. The effects of female sex, viral genotype, and IL28B genotype on spontaneous clearance of acute hepatitis C virus infection. *Hepatol.* 2014; 59(1): 109-120. Doi: 10.1002/hep.26639.
[32] Wang CC, Krantz E, Klarquist J, et al. Acute hepatitis C in a contemporary US cohort: modes of acquisition and factors influencing viral clearance. *J. Infect. Dis*. 2007; 196(10): 1474-1482. Doi: 10.1086/522608.
[33] El-Ghitany EM, Farghaly AG. Geospatial epidemiology of hepatitis C infection in Egypt 2017 by governorate. *Heliyon* 2019; 5(8): e02249. Doi: 10.1016/j.heliyon.2019.e02249.
[34] Njei B, Esserman D, Krishnan S, et al. Regional and rural-urban differences in the use of direct acting antiviral agents for hepatitis C virus: the veteran birth cohort. *Med. Care.* 2019; 57(4): 279. Doi: 10.1097/MLR.0000000000001071.
[35] Giannini E, Botta F, Testa E, et al. The 1-year and 3-month prognostic utility of the AST/ALT ratio and model for end-stage liver disease score in patients with viral liver cirrhosis. *Am. J. Gastroenterol*. 2002; 97(11): 2855-2860. Doi: 10.1111/j.1572-0241.2002.07053.x
[36] Giannini EG, Testa R, Savarino V. Liver enzyme alteration: a guide for clinicians. *Cmaj.* 2005; 172(3): 367-379. Doi: 10.1503/cmaj.1040752.

[37] Dimitroulis D, Damaskos C, Valsami S, et al. From diagnosis to treatment of hepatocellular carcinoma: An epidemic problem for both developed and developing world. *World J. Gastroenterol*. 2017; 23(29): 5282. Doi: 10.3748/wjg.v23.i29.5282.
[38] Ginès P, Solà E, Angeli P, Wong F, Nadim MK, Kamath PS. Hepatorenal syndrome. *Nat. Rev. Dis*. Primers 2018; 4(1): 1-17. Doi: 10.1038/s41572-018-0022-7.
[39] Lagging LM, Garcia CE, Westin J, et al. Comparison of serum hepatitis C virus RNA and core antigen concentrations and determination of whether levels are associated with liver histology or affected by specimen storage time. *J. Clin. Microbiol*. 2002; 40(11): 4224-4229. Doi: 10.1128/JCM.40.11.4224-4229.2002.
[40] Heller T, Seeff LB. Viral load as a predictor of progression of chronic hepatitis C? *Hepatology*, 2005; 42: 1261-1263. Doi: 10.1002/hep.20982.
[41] Gretch D, Corey L, Wilson J, et al. Assessment of hepatitis C virus RNA levels by quantitative competitive RNA polymerase chain reaction: high-titer viremia correlates with advanced stage of disease. *J Infect. Dis.* 1994; 169(6): 1219-1225. Doi: 10.1093/infdis/169.6.1219.
[42] Tassopoulos N, Papatheodoridis G, Katsoulidou A, et al. Factors associated with severity and disease progression in chronic hepatitis C. *Hepat Gastroenterol*.. 1998; 45(23): 1678-1683.
[43] Zhu J-Y, Leng X-S, Dong N, Qi G-Y, Du R-Y. Measurement of liver volume and its clinical significance in cirrhotic portal hypertensive patients. *World J. Gastroenterol*. 1999; 5(6): 525. Doi: 10.3748/wjg.v5.i6.525.
[44] Malinchoc M, Kamath PS, Gordon FD, Peine CJ, Rank J, Ter Borg PC. A model to predict poor survival in patients undergoing transjugular intrahepatic portosystemic shunts. *Hepatology*.. 2000; 31(4): 864-871. Doi: 10.1053/he.2000.5852.
[45] Urabe Y, Ochi H, Kato N, et al. A genome-wide association study of HCV-induced liver cirrhosis in the Japanese population identifies novel susceptibility loci at the MHC region. *J Hepatol*. 2013; 58(5): 875-882. Doi: 10.1016/j.jhep.2012.12.024.
[46] Suzairi MSiM, Tan SC, Aizat AAA et al. The functional– 94 insertion/deletion ATTG polymorphism in the promoter region of NFKB1 gene increases the risk of sporadic colorectal cancer. *Cancer Epidemiol*. 2013; 37(5): 634-638. Doi: 10.1016/j.canep.2013.05.007.
[47] Wang X, Peng H, Liang Y, et al. A functional insertion/deletion polymorphism in the promoter region of the NFKB1 gene increases the risk of papillary thyroid carcinoma. *Genet. Test. Mol. Biomark*. 2015; 19(3): 167-171. Doi: 10.1089/gtmb.2014.0271.
[48] Ghazalah MK, El-Masry S, Helmy I, Abd-Elkhalek E. Genetic study of I kappa B alpha gene promoter polymorphism associated with hepatitis C virus in Egyptian patients. *J. Biosci. Appl. Res*. 2022; 8(3): 154-162. Doi: 10.21608/jbaar.2022.255496.
[49] Ting T, Hao-zhi F, Peng H. Effects of HCV genotypes and V-Rel reticuloendotheliosis viral oncogene homolog A rs11820062 on outcome of HCV infected patients. *Chinese J Public. Health* 2018; 34(1): 88-91. Doi: 10.11847/zgggws1113560
[50] Yenmis G, Soydas T, Arkan H, Tasan E, Sultuybek GK. Genetic Variation in NFKB1 Gene Influences Liver Enzyme Levels in Morbidly Obese Women. *Arch. Iran. Med*. 2018; 21(1).
[51] Cheng C-W, Su J-L, Lin C-W, et al. Effects of NFKB1 and NFKBIA gene polymorphisms on hepatocellular carcinoma susceptibility and clinicopathological features. *PloS one*. 2013; 8(2): e56130. Doi: 10.1371/journal.pone.0056130.
[52] Gao J, Xu H-L, Gao S, et al. Genetic polymorphism of NFKB1 and NFKBIA genes and liver cancer risk: a nested case–control study in Shanghai, China. *BMJ open*. 2014; 4(2): e004427. Doi: 10.1136/bmjopen-2013-004427.
[53] Zhao P, Malik S, Xing S. Epigenetic mechanisms involved in HCV-induced hepatocellular carcinoma (HCC). *Front. Oncol*. 2021; 11: 677926. Doi: 10.3389/fonc.2021.677926
[54] Guo Q, Jin Y, Chen X, et al. NF-κB in biology and targeted therapy: new insights and translational implications. *Signal Transduct Target Ther.* 2024; 9(1): 53. Doi: 10.1038/s41392-024-01757-9.