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IL-18 SNP (rs 1946518) and Vulnerability to HBV Infection

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Abstract

Background. Despite development of vaccines and antiviral treatment, HBV continues to be significant health threaten globally and many risk factor associated with HBV chronic infection including fibrosis, cirrhosis and hepatocellular carcinoma. Pro-inflammatory cytokine as IL -18 has pivotal role in many infectious and inflammatory disease. Genetic variations of IL-18 may be having an impact to HBV chronic infection. **Aim.** Our investigation aimed to understanding role of IL-18 SNP (rs 1946518) and vulnerability to HBV chronic infection. **Methods.** A total of ninety blood samples were included in this study; thirty sample were collected from individuals infected with HBV and CKD; those patients with mean age 48.8 ± 13.57 and thirty CKD patients without HBV infection. Thirty healthy individuals were selected randomly to represent the control group with mean age 36 ± 10.16 for comparison purpose. Genomic DNA for both groups was extracted. **Results.** Our findings revealed increased level serum IL-18 in HBV and HDP patients were the mean concentration 350.23±185.17 pg / ml as compared to control group with less mean concentration 176.53±50.33 pg / ml. Genotyping and allele frequencies of (rs 1946518) showed non-significant variation between patients and control were ($P > 0.05$). Moreover. individuals with CA and AA mutation are more likely to be infected with HBV in comparison with individuals with wild type AA and there was significant correlation between IL-18-607 C/A SNP and its concentration. **Conclusion.** CHBV patients and HDP patients have higher level of IL-18 in comparison with healthy individuals with less level. IL-18 -607 C/A had an impact on the level of this pro-inflammatory cytokine. Also, individuals with CA and AA mutation at high risk to HBV and CKD infection than those individuals with CC genotype.

Keywords: HBV, HDP, CKD, HCC, IL-18 and SNPs.

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Introduction

Hepatitis B virus infection remains serious health issue and according to estimates, about 257 million of population infected with the virus. This liver inflammation caused by virus belongs to hepadnaviridae family (Hammood et al., 2022). Also, other factor can cause hepatitis like toxic substances (certain drugs, alcohol), other infections, autoimmune disease and bacteria. Infection with the hepatitis B virus may be acute, subclinical or progression to chronic hepatitis, which is determined by the host immunity (Hasson, 2024). Chronic HBV patients at risk of cirrhosis, liver failure and hepatocellular carcinoma HCC. Annually, about 877, 000 of mortality rate due to liver cirrhosis or/and HCC. According to community based- study in Iraq, the prevalence of HBsAg were 1.6%. HBV is tiny; partially double stranded DNA virus with genome length 3.2 Kb, including four genes HBsAg, X gene (transcriptional activator), HBcAg and HBV Pol/RT (Qassim et al., 2016). HBV is blood-borne virus that

transmitted via direct contact with infectious blood or other body fluids. Also, haemodialysis treatment poses risks of HBV transmission due to blood transfusion process. Interaction between the invading virus and host immune response cause impairing of innate and adaptive immunity causing persistent infection.

Hepatitis B chronicity divided into 4 phases including immune tolerant phase, immune active phase, immune control phase and lastly immune clearance. Compared to HBV chronic carriers, patients with acute HBV are more infectious than those chronic carriers. In addition to liver dysfunction, various consequences associated with HBV persistent infection including impaired of renal function due to the decrease renal blood supply causing renal dysfunction, renal illness maybe acute or progress to chronic renal failure (Massat et al., 2022). IL-18 is pro-inflammatory cytokine belongs to IL-1 superfamily, produced by activated immune cells such as macrophage, monocyte, immature dendritic cell and Kupffer cell (Ihim et al., 2022). IL-18 triggers cytotoxic and inflammatory immune cell response leading to autoimmunity. IL-18 is crucial modulator of T- cell activation and proliferation, linked with several types of inflammatory, autoimmune diseases and cancers. The gene of IL-18 located on chromosome 11q22.2–q 22.3, promoter region have three SNPs are 607 C/A (rs 1946518), 137 G/C (rs 187238) and 656 G/T (rs1946519). Those genetic variations that alter transcriptional activity of IL-18 which can effect on T-cell function, tigger an immunological dysfunction and eventually lead to development of chronic infection.

Materials and Methods

Ninety blood sample of (67male and 23 female) have been collected, then divided into thirty patients infected with HBV and CKD; thirty CKD patients without HBV infection and the remaining thirty individuals chosen randomly to represent the control group. The mean ages of patients and control were 48.8 ± 13.57 and 36 ± 10.16 respectively. All procedures were conducted after obtaining requisite ethics committee permission from Biology Department (University of Baghdad) with authorization reference number CSEC/1223/0135 in 16 December 2023 and acquire patients' permission.

Blood samples

Five ml of venous blood was drawn from both patients and control; three ml were placed into serum separator tube (gel tube). Following that, centrifuged at 3000 rpm for 10 minutes to aspirate the serum. The obtained serum used for detection of HBsAg and assessment of IL-18 serum level.

Serological Diagnosis of HBV

Enzyme Linked Immunosorbent Assay was used to detect presence of HBsAg by using (USCN / China) ELISA kit for both patients and control group and in compliance to the manufacturer's instructions.

DNA extraction

The two ml placed in EDTA tube for genomic DNA extraction by using DNA extraction kit (Geneaid human kit). DNA purity and concentration checked using Nanodrop (Thermo, USA), purity measured at 260/280 nm and the concentration

calculated (ng/ µL). DNA stained with Ethidium bromide confirmed no damage when running on 1% of agarose gel at 80 volts for half hour. IL-18 SNP (rs 1946518) using Allele Specific- Polymerase Chain Reaction (AS-PCR) IL-18 -607 C/A was investigated.

The PCR reaction for (rs 1946518) with final volume was 25µL, consisting of 1 µL of forward primer, 1 µL of reverse primer, 12.5 µL of master mix, 1.5 of DNA and 9 µL of nuclease free water. The PCR reaction preformed in (Rotor gene, U.S) Thermocycler and as displayed in table 2. One percent of agarose gel used for PCR product electrophoresis; length/size was measured in comparison with 100 bp of DNA ladder (Geneaid, Taiwan).

Table 1: Primer sets used in current investigation (Hasan and Naif, 2017).

ID SNP	Description	Sequence	Amplicon size (bp)
rs 946518	Two sequence specific forward primer	F1:GTTGCAGAAAGTGTAAAAATTATTAC	196
		F2:GTTGCAGAAAGTGTAAAAATTATTAA	
	Reverse	TAACCTCATTCAGGACTTCC	

Table 2: The PCR program used in IL-18 SNP (rs 1946518) amplification

Step	Temperature	Temperature and Duration	Cycle number
Initial denaturation	95 °C	5 minutes	1 cycle
Second denaturation	94 °C	30 second	40 cycles
Annealing	54 °C	60 second	
Extension	72 °C	30 minutes	
Final extension	72 °C	5 minutes	1 cycle

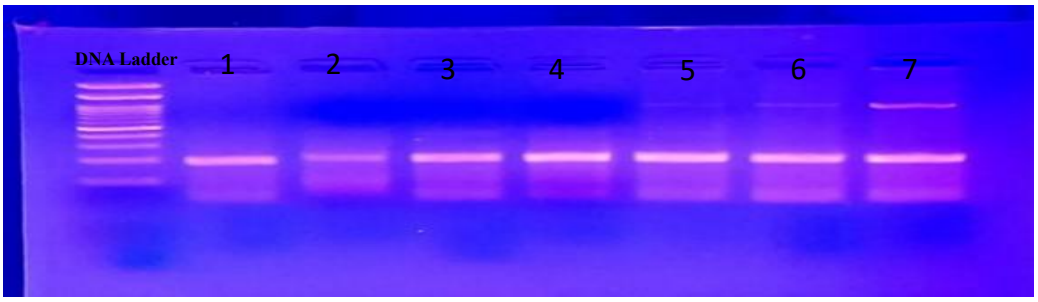

Figure 1: Agarose gel electrophoresis of 196 bp of IL-18 (rs 1946518) that was stained with ethidium bromide. Ladder lane: DNA ladder with 100 bp; lanes 1,2,3,4,5,6 and 7 were the tested samples.

The homozygous genotypes (CC or AA) were presented by single band on one line, while heterozygous genotype (CA) was presented by single band on two adjustment lines for each sample.

Statistical analysis

Analysis was conducted using R 4.4.1 to conduct all the statistical analysis used in this project. Hardy-Weinberg equilibrium (HWE) was evaluated using the exact 2x2 function from the exact2x2 package, ensuring accurate assessment of allele distribution within the population.

Pearson's chi-square test was applied to compare allele frequencies between groups, providing a robust measure of association. In cases where expected cell counts were too low for reliable chi-square results,

Fisher's exact test was employed to ensure statistical validity. p-values were calculated for each analysis to assess the significance of any observed differences, with a particular focus on understanding the genetic variation and its potential implications for group-specific outcomes. These methods allowed for a detailed evaluation of genetic patterns and their broader biological relevance (cox & Lewis, 1966).

Results

Measurement of Interleukin 18 serum level Our finding showed significantly higher increased of IL-18 serum level in patient groups were the mean concentration 350.23±185.17 pg / ml as compared to control group with less mean concentration 176.53±50.33 pg / ml as shown in table 3 ompatible with another study done by Zhou et al., were showed elevated level of serum IL-18 in HBV patients as compared with control group.

Table 3: Serum IL-18 level in patients and control

Group	IL-18 (pg/ml)
Patients	350.23±185.17
Control	176.53±50.33

Purity of DNA and concentration

DNA concentration determined using Nanodrop that measured absorbance at wavelength 260 nm. Purity calculated using general formula:

DNA purity (ng/ µL) = absorbance at 260 nm/ absorbance at 280 nm. IL-18-607 C/A Genetic polymorphism and allele frequencies. Two alleles were detected at

Interleukin-18 (rs 1946518) including the AA wild type allele and CC homozygous mutation. Samples were split into three groups (CC, AA and AC). The result in table (4) demonstrated that there were non-significant differences in (CC and CA and AA) genotyping between CHBV patients and CKD in comparison to control group were P > 0.05.

Table 4: Allele Frequencies and Genotype of rs 1946518 between CHBV and CKD compared to control group.

SNP	Study group	Genotype	Observed		Expected		*P* value
			N	%	N	%	
rs1946518	CHBV	C/C	8	27.58	9.9	34.36	0.322
		C/A	19	62.02	14	48.51	
		A/A	3	10.34	4.96	17.12	
	HDP	C/C	10	33.33	9.6	32.11	0.963
		C/A	14	46.66	14.73	49.11	
		A/A	6	20	5.6	18.78	
	Control	C/C	10	33.33	12.67	42.25	0.099
		C/A	19	63.33	13.65	45.5	
		A/A	1	3.33	3.67	12.25	

The study carried out by Yu and his group in 2019 showing significant association of IL-18 polymorphisms (rs 1946518) and (rs 187238) with susceptibility to HBV and HCV viral infection. Due to the effect of these variations on biological activity of IL-18 causing an immunological dysfunction, affecting on immune responses to viral infection and eventually confer vulnerability to viral hepatitis (Yu et al., 2019).While (Quan et al., 2019) investigated that this genetic variation effects on biological activity of IL-18, causing immunological dysfunction and chronic hepatocellular injury that eventually increase susceptibility to HCC. Accordance to study done by (Qader et al., 2021), it has been linked this polymorphism with many types of malignancies such as colorectal cancer CRC, which revealed that presence of heterozygous CA increase risk of colorectal cancer, whereas the homozygous CC genotype protect against this type of malignancy (Qader et al., 2021). Moreover, table 5 showed that comparison of CC, CA and AA allele frequencies and genotyping among HBV patients and healthy subjects.

Table 5: Allele analysis of IL-18 SNPs and their association with increased risk of contracting the HBV virus.

SNP	Genotypes and alleles	CHBV		Control		Odds ratio	95%CI	P value
		N	%	N	%			
rs1946518	C/C	8	27.58	10	33.33	3	0.32 to 28.7	0.34
	C/A	19	62.02	19	63.33	0.33	0.03 to 2.7	0.36
	A/A	3	10.34	1	3.33	0.26	0.02 to 2.06	0.29

The statical test (Table 6) revealed that individuals with CA and AA mutation are more likely to be infected with HBV in comparison with individuals with wild type AA.

Table 6: Allele analysis of IL-18 SNPs and their association with increased susceptibility to CKD.

SNP	Genotypes and alleles	HDP		Control		Odds ratio	95%CI	P value
		N	%	N	%			
rs1946518	C/C	10	33.33	10	33.33	6	0.80 to 56.25	0.097
	C/A	14	46.66	19	63.33	0.123	0.01 to 1.03	0.064
	A/A	6	20	1	3.33	0.167	0.02 to 1.33	0.125

Also, Chronic kidney disease susceptibility increased in individuals with CA and AA genotype more than those individuals with CC genotype were OR < 1. group. Moreover, individuals with CA and AA mutation are more vulnerable to HBV and CKD infection than those individuals with CC genotype The Pearson test showing significant correlation of IL-18 SNP (rs 1946518) and IL-18 level where it cause increasing in the level of this pro-inflammatory cytokine (Table 7).

Table 7: Correlation between SNPs and IL-18 concentration.

SNP	Study group	Genotype	number	Concentration	*P* value
rs1946518	CHBV	C/C	8	103	*P*<0.05
		C/A	19	198	
		A/A	3	206	
	HDP	C/C	10	217	*P=0.02*
		C/A	14	176	
		A/A	3	215	
	Control	C/C	10	105	P=0.59
		C/A	19	102	
		A/A	1	115	

Discussion

The present study demonstrated a significant elevation in serum IL-18 levels in patient groups compared with the control group, suggesting an important role of this cytokine in inflammatory and immune responses associated with disease conditions. These findings are consistent with the study conducted by Zhou *et al.,* which reported elevated serum IL-18 levels in patients infected with HBV compared with healthy controls. In addition, the current study investigated the IL-18 gene polymorphism (rs1946518) and its possible association with disease susceptibility. Although the genotype distribution did not show statistically significant differences between patient and control groups, individuals carrying CA and AA genotypes appeared to be more susceptible to HBV infection and CKD compared with those carrying the CC genotype. These results are partially supported by the study conducted by Yu et al. (2019), which reported a significant association between IL-18 polymorphisms (rs1946518 and rs187238) and susceptibility to HBV and HCV infections. The authors suggested that these polymorphisms may affect the biological activity of IL-18, leading to immune dysfunction and altered immune responses to viral infections. Similarly, Quan et al. (2019) demonstrated that IL-18 genetic variations may influence cytokine activity, contributing to chronic hepatocellular injury and increased susceptibility to hepatocellular carcinoma (HCC). Furthermore, Qader et al. (2021) reported that IL-18 polymorphisms are associated with several malignancies, including colorectal cancer (CRC). Their findings indicated that the heterozygous CA genotype may increase the risk of colorectal cancer, whereas the CC genotype may have a protective role against this malignancy.

Overall, the findings of the present study suggest that IL-18 gene polymorphism may influence IL-18 production and inflammatory responses, thereby potentially contributing to susceptibility to diseases such as HBV infection and chronic kidney disease.

Conclusions

CHBV patients and HDP patients have higher level of IL-18 in comparison with healthy individuals with less level. IL-18 -607 C/A had an impact on the level of this pro-inflammatory cytokine. Also, individuals with CA and AA mutation at high risk to HBV and CKD infection than those individuals with CC genotype.

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