The Association of single nucleotide polymorphism of IL4 with serum level of anti-Hepatitis B Antibodies

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Abstract

Hepatitis is a liver infection and inflammation that can be acute or chronic with a diverse etiology, including viruses, bacteria, medicines, and hazardous chemicals.Hepatitis B is the most common causes of chronic and acute hepatitis disorders worldwide. A cross-sectional study was carried out in Salahaldeen governorate from 15th of December 2021 to 25th of Augest2022.The study included 90 hepatitis patients with hepatitis B ranging in age from18 to 61 years.These individuals were hospitalized to general Sammarra hospital. The control group, which was matched to the patients analyzed, consisted of 30 people who appeared to be healthy.5ml of blood was taken from each patient in this study for the purpose of ELISA testing to check HBs Ag,antibodies to HBs and molecular test to IL4 by pcr.

The result showed that all patient with hebatitis B had HBsAg and the number of people with HBV in males more than female the frequency distribution of HBsAg did not differ significantly between males (60 %) and females (40 %) .The study included investigation the polymorphism of IL4 genes for patients which infected with HBV and healthy control,and it included (C-589T (rs2243250) , T + 2979G (rs2227284). For T + 2979G (rs2227284) it was found that there was a significant increase in p-value (p 0.05) for the genotype(GG,GT,TT) when comparing patients with control.This indicates that the genotype GG causes the risk of developing hepatitis B and the frequency of the T allele for healthy is the highest compared to patients .For C-589T (rs2243250) it was found that there was homzygous mutant genotype (TT) was more frequent in HBVpatients than controls (20% vs. 16.67%), the difference was not signifcant. Likewise, there was no signifcant difference between patients and controls in allele frequency. Furthermore, neith er dominant nor recessive inheretance model had a signifcant difference between the two group. On the other hand, the homozygous genotype (CC) was more common among patients than control (43.33% vs 40 %) with no significant difference. The mean serum level of IL-4 in patients carrying TT genotype of rs2227284 was 92.24±71.25 pg/ml which significantly higher than those carrying GG genotype (50.96±13.6 pg/ml) or those carrying GT genotype (47.74±9.6 pg/ml). Although, patients carrying the TT genotype also had higher level of anti- HBs IgG level than either those carrying GT or GG genotypes, the differences were not significant.For the rs2243250, the different genotypes associated with comparable levels of IL-4 and anti-HBs IgG with no significant differences.Incontrol group Persons carrying the TT genotype had also higher level of IL-4 and anti-HBs IgG (92.15±18.69 pg/ml and 16.21±5.72 U/L, respectively) than those carrying GG genotype (81.55±16.61 pg/ml and 12.48±4.54 pg/ml, respectively) or those carrying GT genotype (70.05±7.4 pg/ml and14.26±6.12 U/L, respectively); however, the differences were not significant.

I- INTRODUCTION

Hepatitis B virus (HBV) infection is one of the primary causes of liver disease.Despite the availability of a very effective recombinant vaccination for more than 30 years 1 ,the number of people infected with human hepatitis B virus (HBV) continues to rise 2 .Around 292 million persons worldwide were chronic HBV carriers , putting them at high risk of developing liver cirrhosis and hepatocellular cancer (HCC)3,4 .HBV infection caused approximately 890,000 fatalities in 2015, and the trend is increasing 5 .According to the WHO, only about 11% of those infected have been diagnosed with hepatitis B (HB) and are thus aware that they are infected; less than 2% of chronically infected patients have access to cutting-edge antiviral treatment 5 .Because of this factual under-diagnosis and under-treatment, residents of low-income countries suffer the most from this plague 6 .IL-4 plays critical roles in both humoral and cell-mediated immunity 7 .It has been proposed that variables influencing IL-4 expression and function may result in a reduced cell-mediated immune response, making the host susceptible to infections and inflammation-related illnesses8.Previous research on IL-4 gene polymorphisms and our new findings support this idea9,10.The human IL-4 gene is found on chromosome 5q31, around 25 kbp from the proximal end11 .The IL-4 gene is made up of four exons , with exon 2 being the smallest 48 bp, encoding 16 aa) and exon 3 being the longest.The full-length IL-4 protein, which is encoded by all four exons, is the version that is often referred to as just IL-4 and is by far the most structurally and functionally investigated isoform.Direct sequencing of the transcript provided the first definitive demonstration of alternative exon 2 exclusion from IL-4 mRNA in humans in 1996.

Polymorphism in the IL-4 gene may modify its expression and downstream signaling, influencing vaccination reactions.So far,more than 50 IL-4 single nucleotide polymorphisms (SNPs) have been identified, and multiple epidemiological studies have shown that IL-4 gene polymorphisms are linked to a variety of disorders. Numerous studies have revealed that IL-4 gene polymorphisms play critical roles in hepatitis B vaccination response and HBV infection risk12,13, 14,15

Over 2 billion people worldwide are affected with liver disease, and any changes that affect the expression and function of IL-4 can decrease immune responses and increase vulnerability to infections and inflammation16,17.

Single nucleotide polymorphism (SNPs) is a change with only one nitrogen base that leads to achange in the gentic DNA sequence.This change usually occurs in a pair of nucleotides in that location and is important in identification of genetic variation that occurs in the protein coding region 18.The SNP provides analysis of this changes in DNA sequences to understand the genetic basis of disease 19 .Wholer 20 noted SNPs in organic materials,Nyman 21 indicated that SNPs with different stability may spontaneously switched from the unstable form to the constant form agiven temperature .Vignal 22 classified the methods for detecting SNP heterogeneity in to two groups ,the first by hybridization technique and the second by other techniques for separating the product of the specific allel such as restriction enzyme,primer extension ,oligonucleotide ligation assay and DNA chain building exonuclease detection .Gene polymorphisms can occur in any region of the genome 23

II- MATERIAL AND METHODS

A cross sectional study was carried out in Salahaldeen governorate from 15th of Desember 2021 to 25th of Augest2022. The study included collection of 100 samples , 60 patient who are infected with HBS and 40

healthy people as a control group ranging in age from 18to 60 years , as the samples were collected from auditors of the samarra hospital . 5ml of blood was taken from each patient in this study via vein puncture using disposable syringes.Blood samples were collected in two tubes, one of which contained the anticoagulant EDTA.Which kept in deep freez at -20 c for molecular test of IL4.The second component of the sample was 3 ml, which was deposited in plane tubes, let to clot for 30 minutes at 37 °C, then centrifuged for 15 minutes at 3000 rpm. Sera from the second tube were aspirated and transferred to Eppendorf tubes, which were then maintained at -20°C 24,25 .for later serological testing for HBs Ag ,HBs IGg antibody using the ELISA technique.An interview was carried out with these patients using questionnaire form designed by the investigator and consisted of the following variables. age, sex, occupation, …. etc (Appendix I).

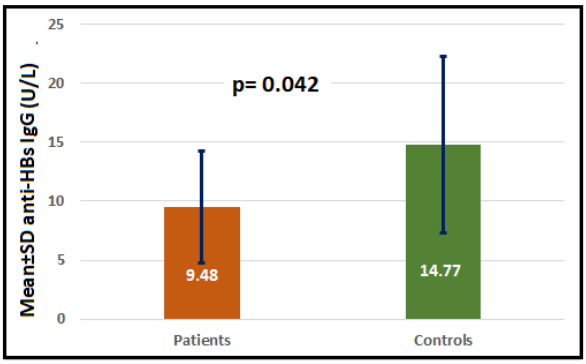
III-RESULT AND DISCUSSION

Immunological parameters

Serum Level of Anti-HBs IgG

The study included 100 person ,60 HBV patient and 40 healthy control by using serum sample and measured level of anti –HBs IgG by using ELISA technique,We used the work kit that was provided by a company(Sunlong ,China),and the work method recommended by the manufacture,which approved on the method used was followed by26 .Patients with HBV showed lower serum level of anti-HBs IgG than controls (9.48±4.1 U/L versus 14.77±7.22 U/L)with a significant difference (figure 4-2).

Figure 4-2: Mean serum level of anti-HBs IgG in HBV patients and controls



This study shows significant decrease in Anti-HBs IgG level in patients with HBV group in comparison with control group during the study period.These results agree with the study of Tzu-Hsin Tsai et al 27 .who reveal significant decrease in anti HBs IgG in HBV patient .These results differed with the study of Lina Wang et al 28.Which exhibited a significantly higher concentration of IgG in HBV patients compared with control.

Molecular Assay

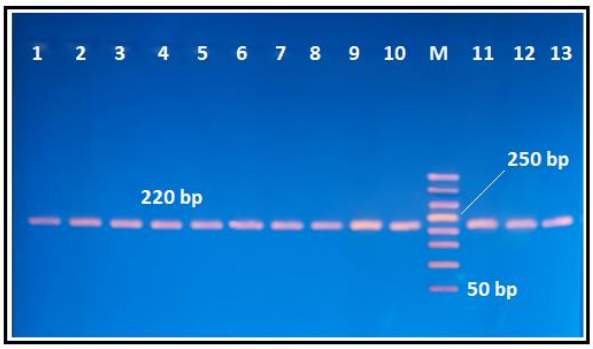
We extracted DNA from whole blood by using working kit that was provided by a company(Geneaid,Taiwan),and the work method recommended by the manufacture ,which was approved on the method used was followed by 29 ,and then detection of IL4 SNP.Two SNPs in IL-4 gene were investigated in the present study for their association with HBV.Restriction fragment length polymorphism was used for genotyping. The different genotypes in all included SNPs were in a good a ccordance with HWE in both group.

rs2227284

We used primer for detection of IL 4 SNP T +2979G (rs 2227284) which fragment length 220bp by using RFLP-PCR that illustrate in paragraph (3.5.2) .Gel electophoresis of the PCR product of rs2227284 polymorphism is

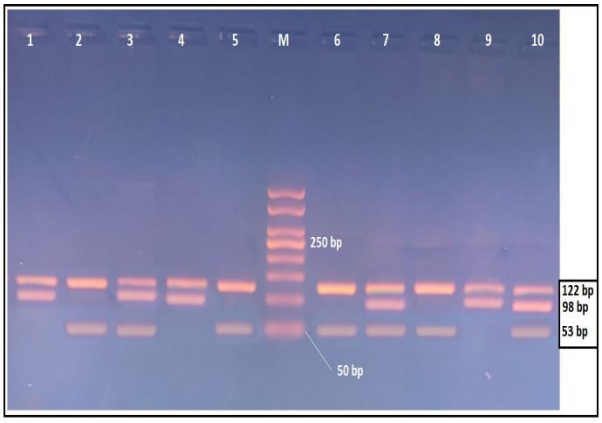
shown in figure 4-3. The fragment length was 220bp.

Figure 1: Gel electrophoresis of T + 2979G (rs2227284) gene plymorphism amplified with specific pair of primers using conventional PCR. The PCR product was stained with ethidium bromide.The fragment length was 220bp



Digestion with AluI restriction enzyme revealed three genotypes: GG, GT and TT as shown in figure 2.

Figure 2: Genotyping of T + 2979G (rs2227284) gene plymorphism after digestion with AluI and stained with ethidium bromide. Lanes 1,4 and 9: GG genotype; lanes 2,5,6 and 8: TT genotype, lanes 3,7 and 10: GT genotype; M: DNA 50 bp DNA maker



The homozygous mutant genotype (GG) was far more frequent in patients (60%) than controls (36.67%) with a significant difference. On the other hand, the heterozygous genotype (GT) was more common among controls than patients (40% vs 33.33%) with a significant difference (OR= 0.18, 95%CI= 0.04-0.71, p=0.015). This polymorphism seems to have both dominant and recessive inheritance. At allelic level, the mutant allele (T) was more frequent in controls than patients (23.33% vs. 6.67 %) with a highly significant difference (OR= 0.4, 95%CI=0.21-0.77, p= 0.006) as shown in table 1.This result disagree with the study of Eun et al 30 which show no association between this SNP and anti Hbs antibodies.

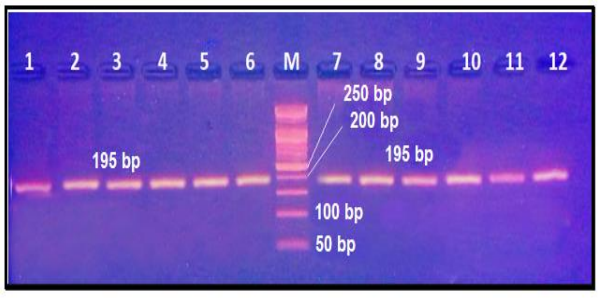
The frequency of different genotypes and allele of rs2227284 in HBV patients and controls

Rs*2227284*	Patients (n=60)	Controls (n=30)	*P*-value	OR(95%CI)
Genotypes				
GG	36(60%)	11(36.67%)	**0.043**	**1.0**
GT	20(33.33%)	12(40%)	**0.015**	**0.18(0.04-0.71)**
TT	4(6.67%)	7(23.33%)	0.140	0.34(0.08-1.42)
HWE	0.597	0.309		
Dominant models				
GG+GT	56(93.33%)	23(76.67%)	**0.031**	**1.0**
TT	4(6.67%)	7(23.33%)		**0.23(0.06-0.88)**
Recessive model				
GG	36(60%)	11(36.67%)	**0.039**	**1.0**
GT+TT	26(43.33%)	19(63.33%)		**0.39(0.16-0.95)**
Alleles				
G	92(76.67%)	34(56.67%)	**0.006**	**1.0**
T	28(23.33%)	26(43.33%)		**0.4(0.21-0.77)**

rs2243250

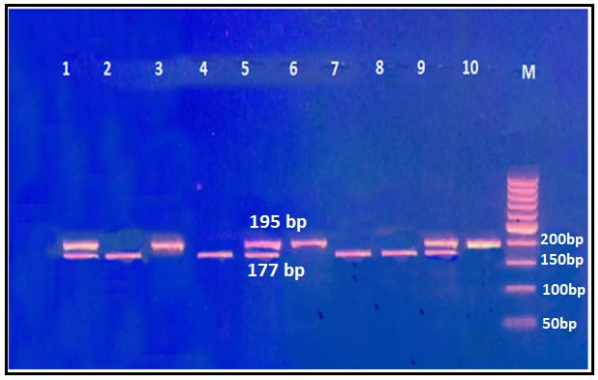
We used primer for detection of IL 4 SNP C-589T (rs 2243250) which fragment length 195 bp by using RFLP-PCR that illustrate in paragraph (3.5.1).Gel electophoresis of the PCR product of rs2227284 polymorphism is shown in figure 4-5. The fragment length was 195bp

Figure 3: Gel electrophoresis of C-589T (rs2243250) gene plymorphism amplified with specific pair of primers using conventional PCR. The PCR product was stained with ethidium bromide.The fragment length was 195bp.



Digestion with AvaII restriction enzyme revealed three genotypes: CC, CT and TT as shown in figure 4.

Figure 4: Genotyping of C-589T (rs2243250) gene plymorphism after digestion with AvaII and stained with ethidium bromide. Lanes 1,5 and 9: CT genotype; lanes 2,4,7 and 8: CC genotype, lanes 3,6 and 10: TT genotype; M: DNA 50 bp DNA maker



Although the homzygous mutant genotype (TT) was more frequent in HBVpatients than controls (20% vs. 16.67%), the difference was not signifcant. Likewise, there was no signifcant difference between patients and controls in allele frequency. Furthermore, neith er dominant nor recessive inheretance model had a signifcant difference between the two groups (Table 2). On the other hand, the homozygous genotype (CC) was more common among patients than control (43.33% vs 40 %) with no significant difference. Cui et

al 15found no link between the 590C/T polymorphism and HBV infection susceptibility.Zheng et al (30) on the other hand, proposed that the -590C > T polymorphism may increase the incidence of HBV and HCV infections.These results agree with the study of Zhitong et al 31 which found that the CT and CC genotype frequencies have been considerably higher in patients with chronic hepatitis B virus (HBV) infection and aberrant alanine aminotransferase levels A meta-regression analysis of the data for the -590C/T SNP revealed that ethnicity, genotyping method, type of liver disease, source of control, and quality score were not effective modifiers in the TTCT versus CC and CT versus CC models,but quality score may be an effective modifier in the T versus C model (P=0.041).These results differed with the study of Yu lu et al 32 .Significant differences was observed in male in CC genotype in co-dominant genetic model CC versus TT+CT. CC genotype in males was associated with a significantly increased risk of CHB compared with TT genotype . In female ,similar trend towards an increase risk effect of CC genotype in chronic hepatitis B was also observed,but differenses wasnot significant.These results are agree with the study from that in JPT (Japanese in Tokyo) and CEU (Utah residents with northen and western European ancestry) populations ,the frequencies of genotype TT and allele T in JPT and CEU populations are lower ,and the rate of CC genotype and C allele are higher in control.The possible factors leading to such controversial results may include the relatively small sample sizes used in previous studies and errors in the data reported.

Table 2: The frequency of different genotypes and allele of rs2243250 in HBV patients and controls

Rs*2243250*	**Patients (n=60)**	**Controls (n=30)**	***P*-value**	**OR(95%CI)**
Genotypes				
CC	26(43.33%)	12(40%)	0.820	1.0
CT	22(36.67%)	13(43.33%)	0.872	1.11(0.32-3.86)
TT	12(20%)	5(16.67%)	0.583	1.42(0.41-4.94)
HWE	0.082	0.647		
Dominant models				
CC+CT	48(80%)	25(83.33%)	0.704	1.0
TT	12(20%)	5(16.67%)		1.25(0.4-3.95)
Recessive model				
CC	26(43.33%)	12(40%)	0.763	1.0
CT+TT	34(56.67%)	18(60%)		0.87(0.36-2.13)
Alleles				
C	74(61.67%)	37(61.67%)	1.0	1.0
T	46(38.33%)	23(16.67%)		1.0(0.53-1.89)

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