



## THE ROLE OF INTERLEUKIN-10 PROMOTER POLYMORPHISM RS1800872 (-592 C>A) IN SUDANESE HEPATITIS B INFECTED PATIENTS

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### ABSTRACT

Hepatitis B virus (HBV) infection is a serious global public health problem. The outcomes of hepatitis infection is determining between viral factors and host genetic as Interleukin-10 (IL-10) which is a multifunctional cytokine participates in the susceptibility of many diseases., The polymorphisms in promoter region are more important than others, which regulate gene transcription. **Objectives:** Aimed to evaluate the correlation between HBV infection and polymorphisms (SNP) in IL-10 gene polymorphisms (-592C/A.) **Material and methods:-** Hepatitis B virus (HBV) infection In Sudan as in other developing countries there are increases, this study was performed on 46 cases of Hepatitis B and 48 healthy matched controls. DNA was extracted and PCR for genotyping of IL10 promoter polymorphism -592C/A was applied followed by restriction fragment length polymorphism (RFLP). In this study participants age ranged from 17 to 67 with Mean (SD) [34.1±15.1] years (male 85, female 10). **Results:** - Three genotypes were detected Using PCR-RFLP method CC for wild type, AC heterozygous and AA homozygous mutant. Wild type CC was more frequent among controls 72%. Allele C frequency was higher in controls group when compared to allele A which increased among patient's. All genotypes and allele frequency were in Hardy Weinberg Equilibrium (HWE) among cases. Single point analysis using the fisher test and chi square showed significant Correlation between -592 promoter polymorphisms and the disease with p. value 0.01. These results suggested that IL-10 592 C/A (rs 1800872) polymorphism appears to have some influences on the infection of HBV.

**KEYWORDS:** rs1800872, Promoter polymorphism, IL- 10, Hepatitis B virus.

### INTRODUCTION

Hepatitis B virus (HBV) infection is a serious global public health problem leads to acute, chronic hepatitis, liver cirrhosis and hepatocellular carcinoma.<sup>[1]</sup>

An estimated 500,000–1 million deaths are recognized to the infection each year.<sup>[2]</sup> The prevalence of hepatitis B virus vary greatly, it is endemic in China, Southeast Asia and most of sub-Saharan Africa. About one third of the population has been infected with HBV, 6% are chronic carriers and over 600000 people die each year from acute disease or chronic secondary to HBV infection.<sup>[3,4]</sup>

Chronic HBV patients are implicit to have majority of the Th2 response over that of Th1, resulting in an increase in the production of anti-inflammatory cytokines, Anti-inflammatory cytokines, such as IL-10. Interleukin (IL)-10 which act as key function in the regulation of immunity which act as the balance

between the cellular and humoral responses suppression of pro-inflammatory cytokine secretion.<sup>[5]</sup> Polymorphisms in some cytokine genes, particularly Th2 cytokine, influence persistence of HBV infection.<sup>[5,6,7]</sup> Several pro inflammatory cytokines such as Th1 cytokines (including IL-2 and IFN-g) and TNF-are believed to participate in elimination of HBV. quite the opposite, IL-10 and IL-4, Th2 cytokine, act as potent inhibitors of Th1 effectors mechanisms.<sup>[6,8]</sup> There are some evidences that the capacity for cytokine production in individuals has a major genetic component. Several polymorphic sites within the IL-10 gene promoter region including three bi- allelic polymorphisms at positions-1082, -819 and -592 from the transcription start site were identified.<sup>[5,9]</sup>

Studies of the effects of IL10 on HBV infection have also been uncertain According to a meta-analysis of approximately 1,500 chronically infected patients and 1,300 controls in the position 592A/C found that this

polymorphism was associated with a risk of developing chronic hepatitis.<sup>[10,11, 12]</sup>

The cytokines are responsible for the regulation of growth differentiation and activation of immune cells. The ability to produce cytokines by an individual is influenced by genetic components that have been attributed to molecular mechanisms, including variations in the transcription, translation and secretion pathways. The present study aimed to evaluate association between HBV infection and single nucleotide polymorphisms (SNP) in the promoter regions of the IL-10 gene (-592C/A.).

## MATERIAL AND METHODS

### Ethical consideration

Ethical clearance for the study was obtained from the Institute of Endemic Diseases, University of Khartoum. Written Informed consent was obtained from each participant.

### Study design

A case control study (pilot) was conducted at Khartoum teaching Hospital and National public health center, Blood bank.

### Samples collection

Ninety four samples were collected from National public health center, Blood bank and Khartoum Teaching Hospital from April to June 2014. The study population consisted of 48 HBs Ag positive and 46 healthy controls (all controls were HBs Ag negative). Written consent was obtained from all study subjects. Three ml of whole blood was collected in to EDTA tube and preceded to DNA extraction.

Genomic DNA was extracted using guanidine chloride method. Three ml of Blood was transferred to 15ml falcon tube, 10 ml red cell lysis buffer (RCLB) were added and centrifuged for 5 min- at 3000rpm. White blood cells at the bottom, 2ml of lysis buffer, 5µl proteinase K, 1mL guanidine chloride and 300 µl ammonium acetate were added and incubated over night at 37°C. After that 2ml pre chilled chloroform were added then centrifuged for 5 min at 3000 rpm, the upper layer transferred to new tube and 10ml cold absolute ethanol were added and kept at -20°C Cover night.

Then centrifugation for 15 min, tube inverted on tissue paper for 5 min. 4ml of 70% Ethanol were added to wash the pellet and then centrifuged for 15 min 3000rpm. The supernatant poured off and the pellet was left to dry. The pellet was re-suspended with 50 µl d H<sub>2</sub>O and stored at -4°C Cover night and measured by Nano drop to identify purity and quantity.

### Polymerase chain reaction (PCR) and restriction fragment length Polymorphism assay (RFLP) for detection of -592C/A polymorphism

(PCR-RFLP) was used to genotype the -592C/A (rs1800872) SNP. PCR was Performed using forward primer5: CCT AGG TCA CAG TGA CGT GG 3.

The reverse primer: 5 GGT GAG CAC TAC CTG ACT AGC 3.

The PCR reaction was carried out in total volume 25µL PCR reaction buffer2.0mMMgCl, 10 mMdNTPs, 1 unit Tag polymerase, 10 M of each primers and dd H<sub>2</sub>O. The PCR conditions were: Denaturation at 94°C for 2 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds; annealing at 58°C for 30 seconds; and extension at 72°C for 4 minutes 30 seconds. This was followed by final extension at72°C for 5 minutes, PCR product were run in 2% agarose gel, stained with bromide, visualized and photographed by gel documentation system. The product was digested by Rsa I restriction enzyme over night at 37°C. The digested products were run in 3% agarose gel, stained with bromide, visualized and photographed by gel documentation system.

### Statistical Analysis

Genotype frequencies of single nucleotide polymorphism (SNP) were compared using online statistic Fisher Probability. Logistic regression and chi square test was performed to evaluate whether there was a difference in the effect of SNP -592.

All statistical tests were 2- tailed. P values less than 0.05 were considered statistically significant. The analysis was performed by the SPSS statistical package for Social Sciences software version 20.

## RESULTS

### Demographic information

Of the 94 participants 85 were males and 9 were females, ages ranged from 17 to 67 with Mean age ± standard deviation (SD) was [34.1±15.1] years (figure 1).

### PCR result

All samples successfully gave a PCR positive result with a target band of 420bp length (figure 3).

### Genotyping of (-592) polymorphism

Three genotypes were detected for IL-10 promoter Polymorphisms CC wild type, AC heterozygous and AA homozygous mutant (figure 4). Wild type CC was more among controls 72% (figure 2). The homozygous AA mutant observed in 6(12.5%) of patients when compared to controls 4(8.7%) (table1). Allele C frequency was higher in control group compared to allele A which increased among patients (table 1).

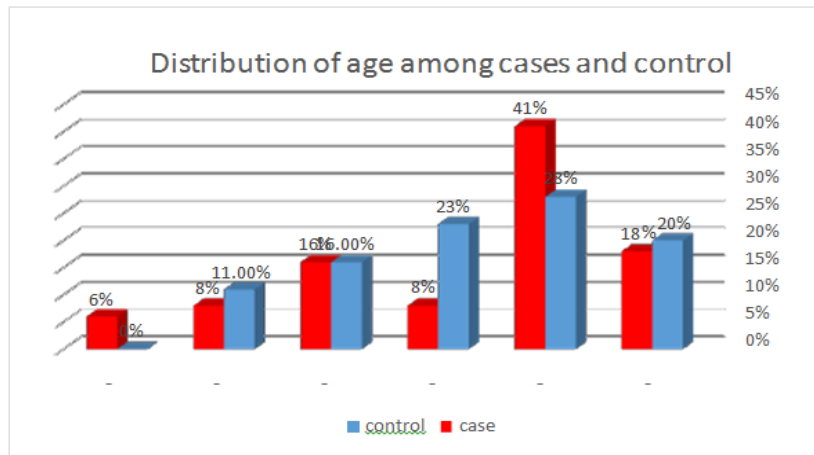


Figure (1) Distribution of age among cases & controls.

Table (1) -592 genotypes & alleles among patients & controls.

Genotype	All subject No=94	cases No=48	Controls No=46
AA	10(10.6%)	6(12.5%)	4(8.7%)
AC	31(33%)	22(46%)	9(19.5%)
CC	53(56.4)	20(42%)	33(71.8%)
A allele	0.27	0.18	0.09
C allele	0.72	0.32	0.4

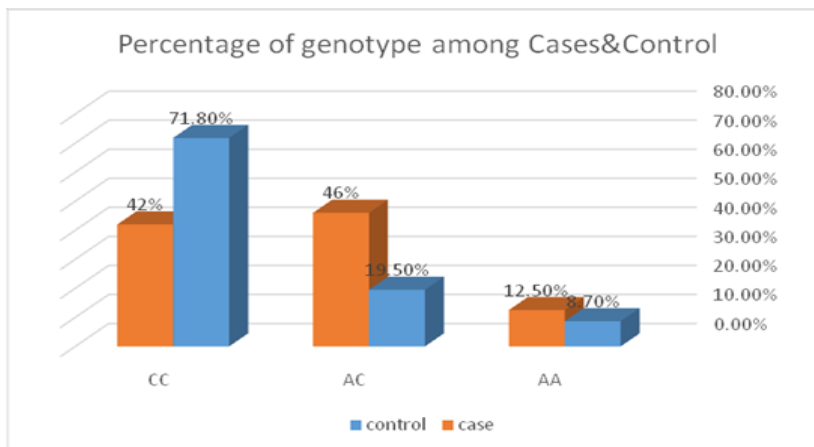


Fig: (2) Percentage of different genotypes among patients & control.

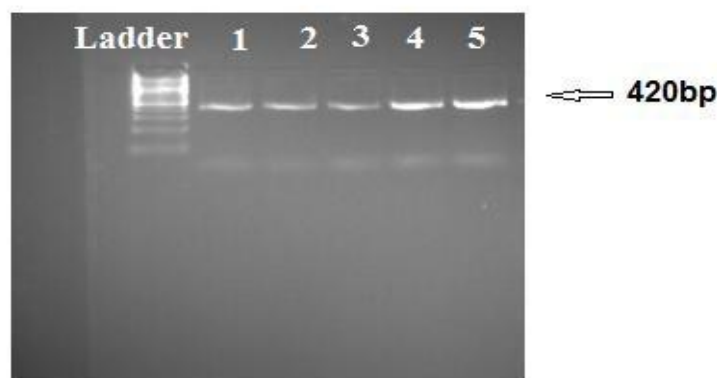
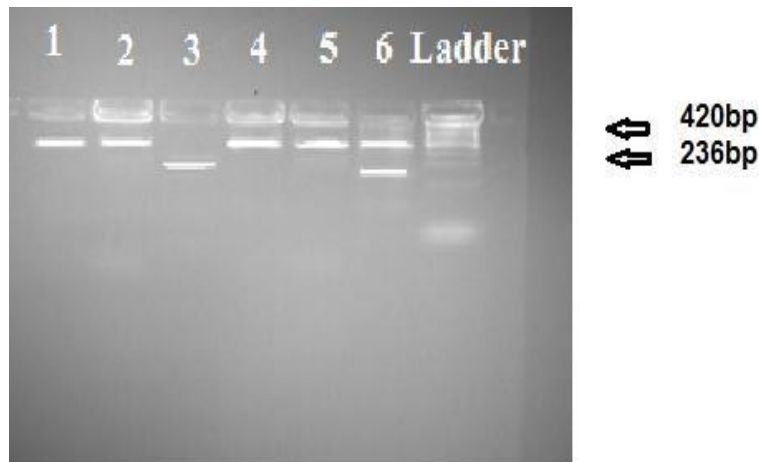


Figure: (3) PCR amplification the IL-10 promoter polymorphisms (-592): 1-5 represent patient samples, ladder (molecular marker) size 100 bp.



**Figure (4): RFLP results of IL-10 promoter polymorphisms (-592 polymorphism): lane 1, 2, 4, 5, wild type (420bp) lane 3, homozygous AA subject; lane 6 heterozygous AC subject. The ladder size is 100pb.**

## DISCUSSION

Interleukin 10 is an immune modulator molecule, which is act an important anti- inflammatory cytokine secreted from Th2 cells.<sup>[5]</sup> The levels of IL-10 production determine immune regulation and the balance between the inflammatory and humoral responses producing IgM, IgG and IgA.<sup>[13]</sup> There are contradictory reports about the exact effects of IL-10 promoter gene polymorphisms on the outcome of HBV infection<sup>[6]</sup> no data available in previous study among Sudanese, So this study conducted for the first time among Sudanese infected with HBV was undertaken in Khartoum and set out with the aim of assess the importance of polymorphism in promoter region to evaluate the correlation between HBV infection and single nucleotide polymorphisms (SNP) at the region (-592C/A.).

Our study revealed that the frequency of A allele was 2 fold higher (0.18) among cases when compared with controls (0.09). furthermore finding was that Genotypes frequency showed that heterozygous AC have higher percentage in cases (Carrier) (46%) than controls (17.5%) which is similar to meta-analysis by Yan Z *et al*<sup>[5]</sup> who suggested IL-10-592 A/C allele as risk factor for HBV in Japanese, also In Chinese population the frequency of the AA genotype at position -592 was significantly lower in HBV patients when compared to genotype AC Yan Z *et al*.<sup>[5]</sup> Also this finding in line with those obtained by Srivastava *et al*<sup>[14]</sup> who reported that genotype AC significantly higher in chronic HBV patients than in healthy controls and also corroborate the finding to African American in a study by Truelove *et al*<sup>[15]</sup> who reported that genotype AC is higher in chronic HBV patients than in healthy control.

This is similar to our results. Cheong *et al*.<sup>[11]</sup> reported that high producer genotypes of IL-10 (IL-10-592 C/C carriers had a better capacity to recover from HBV spontaneously.

The -592A/C Polymorphism was examined in Iranian study by Sofian *et al*<sup>[6]</sup> who reported that it has no association with Hepatitis B infections and controls, which is different from our data, that may due to the difference in ethnic groups or that Hepatitis susceptibility is influence by others polymorphism in the same locus like IL (19, IL 20).

In meta-analysis also showed IL 10 polymorphism -592, the carrier proposed to have high risk of persistence infection, No association to chronic HBV infection.

Fitness of Hardy– Weinberg Equilibrium (HWE) was tested using chi square ( $\chi^2$ ) test and control samples showed deviation with p-value = 0.007, the source of deviation may due to small samples size.

## CONCLUSION AND RECOMMENDATIONS

The present study confirms previous findings and contributes additional evidence that -592A/C associated to Hepatitis disease in Sudanese population, It is recommended that Further studies in a large samples size and ethnic diversity needed to identify the role of this polymorphism in susceptibility of Hepatitis B in Sudan.

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## REFERENCES

1. Conde, S. R., R. N. Feitosa, F. B. Freitas, R. B. Hermes, S. Demachki, M. T. Araujo, M. C. Soares, R. Ishak and A. C. Vallinoto (2013). Association of cytokine gene polymorphisms and serum concentrations with the outcome of chronic hepatitis B. *Cytokine*, 61(3): 940-944.

2. Yu, R., R. Fan and J. Hou (2014). Chronic hepatitis B virus infection: epidemiology, prevention and treatment in China. *Front Med*, 8(2): 135-144.
3. Chen, X., X. Chen, W. Chen, X. Ma, J. Huang and R. Chen (2014). Extended peginterferon alfa-2a (Pegasys) therapy in Chinese patients with HBeAg-negative chronic hepatitis B. *J Med Virol*, 86(10): 1705-1713.
4. Yousif, M., H. Mudawi, S. Bakhiet, D. Glebe and A. Kramvis (2013). Molecular characterization of hepatitis B virus in liver disease patients and asymptomatic carriers of the virus in Sudan. *BMC Infectious Diseases*, 13: 328-328.
5. Yan, Z., W. Tan, W. Zhao, Y. Dan, X. Wang, Q. Mao, Y. Wang and G. Deng (2009). Regulatory polymorphisms in the IL-10 gene promoter and HBV-related acute liver failure in the Chinese population *Viral Hepat*, 16(11): 775-783.
6. Sofian, M., E. Kalantar, A. Aghakhani, S. Hosseini, M. Banifazl, A. Eslamifar, A. jourabchi, A. A. Farazi and A. Ramezani (2013). No Correlation between Interleukin-10 Gene Promoter Polymorphisms and Hepatitis B Virus Infection Outcome. *Hepatitis Monthly*, 13(5): e8803.
7. Shin, H. D., B. L. Park, L. H. Kim, J. H. Jung, J. Y. Kim, J. H. Yoon, Y. J. Kim and H.-S. Lee (2003). Interleukin 10 haplotype associated with increased risk of hepatocellular carcinoma. *Human Molecular Genetics*, 12(8): 901-906.
8. Zhang, T. C., W. F. Zhang, Y. Q. Zhao, F. M. Pan, Y. F. Gao, H. Yuan, X. Q. Liu, X. D. Chen, C. W. Zeng, L. J. Liu and Y. Q. Chen (2014). Gene variation in IL10 and susceptibility to chronic hepatitis B. *J Infect*, 69(1): 75-80.
9. Qiu, X. Q., C. H. Bei, H. P. Yu, X. Y. Zeng and Q. A. Zhong (2011). Study on the relationship between single-nucleotide polymorphisms in IL-6, IL-10 genes and HBV-related hepatocellular carcinoma. *Zhonghua Liu Xing Bing Xue Za Zhi*, 32(5): 510-513.
10. Asadullah, K., W. Sterry and H. D. Volk (2003). Interleukin-10 therapy-review of a new approach. *Pharmacol Rev.*, 55(2): 241-269.
11. Wang, S., D. Huang, S. Sun, W. Ma and Q. Zhen (2011). Interleukin-10 promoter polymorphism predicts initial response of chronic hepatitis B to interferon alfa. *Virol, J*, 8: 28.
12. Sodsai, P., T. Surakiatchanukul, P. Kupatawintu, P. Tangkitvanich and N. Hirankarn (2013). Association of cytokine and cytokine receptor gene polymorphisms with the risk of chronic hepatitis B. *Asian Pac J Allergy Immunol*, 31(4): 277-285.
13. ZHU, Q.-R., Y.-L. GE, S.-Q. GU, H. YU, J.-S. WANG, X.-H. GU, L.-E. FEI and Z.-Q. DONG (2005). Relationship between cytokines gene polymorphism and susceptibility to hepatitis B virus intrauterine infection. *Chinese medical journal*, 118(19): 1604-1609.
14. Srivastava, M., A. Ranjan, J. K. Choudhary, M. K. Tripathi, S. Verma, V. K. Dixit, G. Nath and A. K. Jain (2014). Role of proinflammatory cytokines (interferon gamma) and anti-inflammatory cytokine (interleukin-10) gene polymorphisms in chronic hepatitis B infection: an Indian scenario. *J Interferon Cytokine Res.*, 34(7): 547-551.
15. Truelove, A. L., T. K. Oleksyk, S. Shrestha, C. L. Thio, J. J. Goedert, S. M. Donfield, G. D. Kirk, D. L. Thomas, S. J. O'Brien and M. W. Smith (2008). Evaluation of IL10, IL19 and IL20 gene polymorphisms and chronic hepatitis B infection outcome. *International journal of immunogenetics*, 35(3): 255-264.