Archives of Microbiology & Immunology

Volume 1, Issue 1 Research Article

The polymorphism of Interleukin 4 in Chronic Hepatitis B Virus Infection in Hemodialysis Patients at Khartoum State, Sudan

Yousif N[1], Khair O[2], Abdel Rahim M El Hussein[2], Isam M Elkhidir[3] and Khalid A Enan[2*]

[1]Department of Microbiology, Faculty of Medical Laboratories, Al Neelain University, Khartoum, Sudan

[2]Department of Virology Central Laboratory - The Ministry of Higher Education and Scientific Research, Khartoum, Sudan

[3]Department of Microbiology and Parasitology, University of Khartoum, Khartoum, Sudan

[*]**Corresponding Author:** Khalid A Enan, Department of Virology Central Laboratory - The Ministry of Higher Education and Scientific Research, Khartoum, Sudan, Tel: +249912651103; E-mail: khalid.enan@gmail.com

Received: 23 March 2017; **Accepted:** 03 April 2017; **Published:** 07 April 2017

Abstract

Background: Interleukin-4 is best known as an important mediator and modular of immune and inflammatory responses to several diseases conditions such as hepatitis B, which is a significant health concern worldwide.

Objectives: To estimate the frequency of different genotypes in IL 4 polymorphism gene -590 C/T in chronic Hepatitis B Virus patients and the control group. To correlate the different genotype of IL4 gene polymorphism with age, gender and location in Khartoum state.

Method: IL4-590C/T polymorphism was examined in 56 patients with chronic hepatitis B and 30 non-hepatitis B infected controls, using the polymerase chain reaction –restriction length polymorphism method.

Result: Our results showed a significant difference between the C/C, T/C, and T/T genotypes and the C and T alleles of the -590 region of IL-4 in chronic hepatitis B patients compared with the controls.

Conclusion: Results of this study indicate that the functional gene polymorphisms of IL-4 may play an important part in the development of chronic hepatitis B.

Keywords: HBV; IL4; Khartoum; Sudan

1. Introduction

Hepatitis B virus (HBV) is a member of the Hepadnaviridae family of viruses, and has a double-stranded circular DNA and a DNA polymerase enzyme. It has two major proteins: hepatitis B surface antigen (HBs Ag), and hepatitis B core antigen, an inner protein. A third protein is hepatitis B e antigen (HBe Ag) [1], The virion envelope contains three protein species designated as small (S), medium (M) and large (L).

The M and L proteins are longer versions of the S Protein and each envelope protein has one or more glycosylation sites [2]. Chronic HBV infection is defined as the presence of HBsAg in serum for at least 6 months or the presence of HBsAg and the absence of anti-HBC immunoglobulin M (IgM). The risk of developing chronic infection varies inversely with age and is highest (up to 90%) for infants infected in the perinatal period [3]. HBV affects more than 200 millions people around the world causing an estimated 600000 deaths per year. Hepatits B remains as one of the major causes of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma [4] HBV is present in blood and bodily secretions. The virus is most commonly spread through sexual contact, but it may also spread from mother to child at birth, through contaminated needles and transfusion (extremely rare). The incubation period of HBV infection averages 75 days (range 1-6 months) [5]. The cellular and humoral immune responses to HBV infection are complex.

Most research in the field suggests that HBV is not directly damaging to infected liver cells and that the cellular immune response to viral proteins correlates with the severity of clinical disease and viral clearance [6]. Cytokine are small proteins secreted by immune system cells and other cells responsible for immune response. These soluble proteins play their role by binding specific cell receptors that either induce or inhibit cytokine regulatory genes during viral infection [7].

Interleukin-4 (IL-4), mainly produced by activated T helper 2 (Th2) cells, function major roles as a mediator and modulator of immune and inflammatory responses [8]. It is also a survival and growth factor for lymphocytes. Although it was recognized as a B cell differentiation and stimulatory factor (3), It also has a crucial role in regulating T cell differentiation during the immune response [9]. The IL-4 gene in humans is situated on chromosome 5q31, within 25 Kbp of the proximal portion [10].

The polymorphism -590C/T (rs2243250) in the IL-4 gene promoter region is the most commonly reported variation of this gene [11]. In this region, the T allele of the 590C/T polymorphism of the IL-4 promoter gene was correlated with increased IL-4 gene promoter activity [12]. IL-4 gene polymorphisms may have a role in hepatitis-related HCC as -590C/T and 233C/T which are linked with increased risk for HBV related HCC and IL-4 -590C/T which has been described to be linked with HBV progression to cirrhosis and HCC [13].

The present study was conducted to estimate the frequency of different genotype in IL 4 polymorphism gene in CHB Virus infection and also aimed to correlate the different genotype of IL-4 gene polymorphism with age, gender and location in Sudan.

2. Materials and Methods

Ethical approval for this study was obtained from Sudan Ministry of Health. Only patients agreeing to participate were recruited in this study and informed written consent was obtained regarding the data and the collection of blood samples. The demographic data such as name, age, gender, period and place of dialysis, history of jaundice, date of infection with CHBV and the results for various previous investigations were collected using a structured questionnaire.

3. Sample Collection

Whole blood samples were collected from Hemodialysis patients who are chronically infected with HBV during the period of September 2016 to February 2017. Fifty six samples were collected from hemodialysis patients from different hospitals at Khartoum state (Ibin Sina hospital, Omdurman teaching hospital, Al-now hospital, Al-Amal hospital). In addition, 30 control samples were collected from healthy (Non HBV infected hemodialysis) donors in Khartoum.

A volume of 5 ml of whole blood was collected from each patient through venipunctures technique using Ethylenediaminetetraacetic acid (EDTA) container.

3.1 DNA extraction

Genomic DNA was extracted By using the standard saturated sodium chloride method from 300 µl whole blood.

3.2 PCR amplification

Conventional PCR (Thechni England) was performed to detect genomic 589C/T in IL-4 by amplification of this region. The reaction was performed in a total was performed in a total Volume of 20 µl in the first PCR reaction, containing 13 µL of water and 5 µl of DNA mixed with 2 µl of each primer Forward: 5'AACACCTAAACTTGGG AGGA3' Reverse: 5'CTGTCATGGAAAAGCTGATCT3' (Sang on Biotech Company (Shanghai, China). 5 µL of 2 mM dNTP mix, 2 µL of 25 mM $MgCl_2$, 2.5 U Tag DNA Polymerase (Promega Corporation, Madison, WI, USA), The amplification was conducted using 35 cycles of PCR reaction (denaturation at 95°C for 1 Minute, annealing at 58 – 54 ^{0}C (touchdown) for 1 minute and extension at 72°C for 45 seconds).

The amplicons were resolved and screened using a 2% agarose gel electrophoresis method. Only 56 bands with the best optimum resolution were selected for Restriction fragment length polymorphism (RLFP).

3.3 Genotyping

A total of 5 µl of PCR product in 8 µl of sterile water was added to 1 µl of NE Buffer and 0.5 µl of BSA, 0.5µL BsmFI (500 unit) (sib enzyme Russia) to give a final volume of 15 µl. The restriction digests mixture was then incubated at 37°C for one hour. The reaction was stopped by the addition of 5 µl to 2.5% agarose gel loading buffer (5xTBE, 0.5 mol/l EDTA, 10% (v/v) glycerol, 0.05% (w/v) ethidium bromide.

3.4 Statistical Analysis

Variances in both groups were calculated, the frequencies of different variables in both test and controls were calculated and cross tabulation of genotypes and alleles. Binary logistic regression analysis was used **Ypred = a + b1X1 + B2X2 … + BnXn**. IL4 polymorphism pred= a+ b1 x age + b2 x gender + b3 x central*east + b4 x central * west + b5 HBV infection. Odds ratios (OR) 80% Confidence Intervals (CI) were used to estimate the association between individual polymorphisms a age, gender, locality and chronic HBV. P-values of less than 0.05 were considered statistically significant. All statistical analyses were performed using the SPSS 16.0 software.

4. Results

In this research the case group consisted of 56 chronic HBV patients, including 40 male and 16 female individuals, and the control group consisted of 30 healthy individuals with 23 males and 7 females. The results of the RFLP assay are given in Figure 1. Table 1 summarizes the frequencies between ages, gender and locality. The cross tabulation of genotype and allelic frequencies of IL-4 polymorphism gene variation of the patient and control groups are presented in Table 2. The frequencies of genotypes in the case group were 7.1%, 73.2%, and 19.6% for, CC, CT and TT genotypes, respectively, while in the control group these frequencies were 2%, 3.3%, and76.7%. However, after A binary logistic regression analysis was conducted to predict IL4 polymorphism from five predictors based on the equation. IL4 polymorphism pred = 5.979+ -0.02 age+ 0.346 gender + -0.346 central and east + 0.262 central and west + -4.147 HBVinfection (Table 3). The model was statistically significant (p = 0.000). HBV infection had the highest statistically significant contribution of -4.247 with a P-value of 0.000. Age, gender and locality 1&2 were contributing less for -0.02, 0.346, 0.346 and -4.147 respectively, with a P-value of 0.347, 0.631, 0.638 and 0.835 respectively, which was statistically insignificant. They are significantly found in the CT genotype between chronic hepatitis B and gene polymorphism (OR: 0.014, P = 0.000).

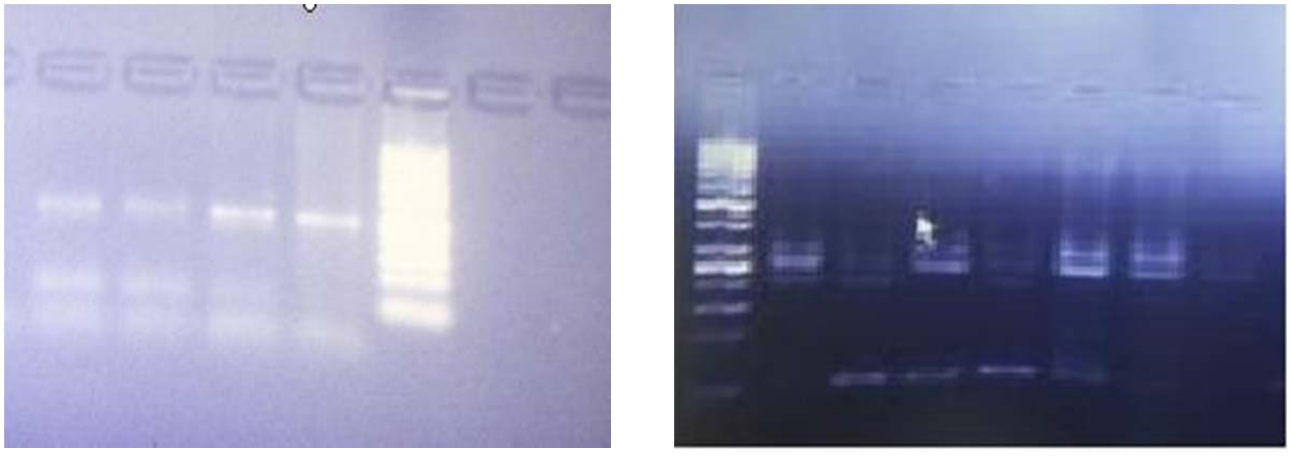

Figure 1: PCR-RFLP assay for analyzing the IL4-590C/T polymorphism of the IL4 gene. PCR product was digested by restriction enzyme and visualized under agarose gel.

Variable	Number	%
Gender (n=86)		
Control (n=30)		
Female	7	23.3
Male	23	76.7
Patient (n=56)		
Female	16	28.6
Male	40	71.4
Age in years (n=86)		
Control (n=30)		
Mean (Sd)	33.7 (12.3)	
Range (Min-Max)	18-62	
Patient (n=56)		
Mean (Sd)	36.7 (13.9)	
Range (Min-Max)	14-70	
locality (n=86)		
Control (n=30)		
Central	24	80
West	6	20
Patient (n=56)		
West	42	75
Central	10	17.9
East	4	7.1

Table 1: Study participants (patients and controls) distribution by gender, age and Locality (n =86).

Genotype	Control	% control	Patient	%patients	Total
CC	6	20.0	4	7.1	10
CT	1	3.3	41	73.2	42
TT	23	76.7	11	19.6	34
Total	30	100.0	56	100.0	86

Table 2: Distribution of the genotype and alleles in controls and HBV patients (n=86).

Variable	B	Wald(Chi squre)	Df	p-value	Odd ratio
Age	-0.02	0.885	1	0.347	0.98
Gender(1)	0.346	0.231	1	0.631	1.413
Locality	-	0.317	2	0.853	-

locality(1)	-0.356	0.221	1	0.638	0.7
locality(2)	0.262	0.043	1	0.835	1.299
HBVinfection	-4.247	13.438	1	0.000	0.014
Constant	5.979	14.292	1	0.000	394.92

Table 3: Logistic regression Predicting IL4 polymorphism from age, gender, locality and HBV infection (n=86).

5. Discussion

From the model a IL4 polymorphism (CC& TT was considered as a normal alleles of IL4 gene, while CT was considered as abnormal allele) was negatively affected with HBV infection. Exploring the IL-4 590C/T polymorphism and its susceptibility to liver disease including HBV infection, HCV infection, liver cirrhosis, etc., significant associations between the IL-4 590C/Tpolymorphism and increased chronic hepatitis B was found in Sudanese populations (Ahmed et al 2016 unpublished data), Cytokines play a critical role in immune and inflammatory responses. However, cytokine coding genes are highly polymorphic, which means some of these polymorphisms can affect the expression of cytokines. Single nucleotide polymorphisms (SNPs) are the most frequent types of genetic variations. In this regard, SNPs within cytokine genes can affect the gravity and progression of immune-mediated and chronic inflammatory diseases [14].

Polymorphisms in the regulatory regions of the cytokine genes may influence their expression [15]. IL-4 is synthesized mainly in Th2 lymphocytes and of induces the expression MHC particles class I and II on the lymphocytes, which facilitates recognition of the viral antigens. IL-4 lso stimulates the cytotoxicity and phagocytosis of monocytes and macrophages.

This may provoke the incidence of the auto immunological processes found in HCV infection [16]. Dendritic cells, as a group of antigen-presenting cells, can build a bridge between pathogens and the T-cell system. This mechanism also has been described for viral diseases such as Hepatitis B infection [17, 18]. The natural outcome of HBV infection varies dramatically among individuals. Infection is usually self-limited in the majority of cases, while a minority of subjects develops persistent infections [19, 20]. The HBV infected individuals who carry the low-activity genotypes of major Th1 cytokines and/or the high-activity genotypes of major Th2 cytokines may be at extraordinarily high risk for HBV [21]. However, Some of the previous data revealed that there was no relation or no significant differences were observed regarding the IL-4 2590C/T and 233C/T polymorphisms genotypes, alleles, or haplotypes between the patient groups and the healthy controls [8]. While other studies suggest that polymorphisms in some cytokine genes influence persistent HBV and HCV infection [22]. Roli Saxena, Indo Verma reported that the significant positive association between IL-4,IL-2, IL-12B [23]. The difference in genotype and alleles distribution might be due to difference in study design, sample size and different selection criteria adopted for

patients and controls in particular clinical manifestation, ethnicity and environmental risk factors may also be possible confounders.

IL-4 polymorphism haplotypes demonstrated by some previous experimental data to be associated with respiratory syncytial virus [24], multiple sclerosis [25], oral cancer [26], and systemic lupus erythematosus (SLE) [27], suggesting that certain polymorphisms could affect the regulation of this cytokine [8]. In this study, the results indicated that the CT genotype of the (rs2243250) could significantly be correlated with HB chronic infection in our patients. To our knowledge this represents the first study performed to correlate the polymorphisms in the -590 region of IL-4 gene with chronic hepatitis B among Sudanese patient. It is recommended that cytokines be used as markers for diagnosis in Sudan, especially in the high risk groups such as hemodialysis patients.

6. Conclusion

Results varied considerably between different populations studied This may be due to differences in sample size, the ethnicity of the study population, the disease stage, and even the genotyping method, and it is difficult to conclude definite associations based on the available data. Moreover, a thorough understanding of host virus interactions, which may or may not end in chronic hepatitis B infection, is still in progress.

7. References

1. Kudesia G, Wreghitt. Clinical and Diagnostic Virology. 1st ed. United States of America: Cambridge United Press 2009.
2. Carter JB, Saunders VA. Virology principles and application. 1st ed. England: John Wiley & Sons Ltd 2007.
3. Zuckerman JA, Banatvala EJ, et al. Principles and Practice of Clinical Virology. 6th ed. United Kingdom: John Wiley & Sons Ltd. 2009.
4. Tunçbilek S. Relationship between cytokine gene polymorphisms and chronic hepatitis B virus infection. World journal of gastroentology 20 (2014): 6226-6235.
5. Haaheim LR, Pattison JR, et al. Practical Guide to Clinical Virology 2nd ed. England: John Wiley & Sons Ltd. 2002.
6. Zheng Z. Human genes involved in hepatitis B virus infection. World journal of gastroentology 20 (2014): 7696-7706.
7. Fallah P, Ferri C, et al. Cytokines and hepatitis c –related disorders. Clinical and developmental immunology 2012: 10.
8. Lu Y, Wu Z, et al. Role of IL-4 Gene Polymorphisms in HBV-Related Hepatocellular Carcinoma in a Chinese Population. Plos One 9 (2014): 10.
9. Zamorano J, Rivas MD, et al. Interleukin-4: A multifunctional cytokine. Revision 22 (2003): 215-224.
10. Wu Z, Qin W, et al. Association Between IL-4 Polymorphisms and Risk of Liver Disease. Medicine 94 (2015): 35.
11. Wang T, Tian L, et al. Interleukin-4-590 >T polymorphism is not associated with the susceptibility of gastric cancer and updated Meta –analysis .Annals of Medicine and Surgery 9 (2016): 1-5.

12. Jun TY, Uklee K, et al. Polymrphism of interleukin 4 promoter and receptor gene for schizophrenia in the Korean population. Psychlatry and clinical neurosciences 57 (2003): 283-288.
13. Dondeti MF, El-Maadawy EA, et al. Hepatitis-related hepatocellular carcinoma: Insights into cytokine gene polymorphisms. World journal of gastroenterology 22 (2016): 6800-6816.
14. Yucesoy B, Johnson VJ, et al. Eytokine polymorphism and relationship to disease. Cytokines in human health. New York city: humana press (2007): 113-132.
15. Gao QJ, Wuliu D, et al. Polymorphism of some cytokines and chronic hepatitis B and C virus infection. World journal of gastroenterology 44 (2009): 5610-5619.
16. Lapinski TW. The level of IL-1B, IL-4 and IL-6 in the serum and liver tissue of chronic HCV-infection patintes. Journal of Leukocyte Biology 49(2001): 311-316.
17. Jun MC, Pape GR. Immunology of hepatitis B infection. The Lancet infectious diseases 2 (2002): 43-50.
18. Shokouhi S, Rakhshan M, et al. Effect of hepatitis B –surface and hepatitis B core antigen from hepatitis BV genotypes B and con invetro apoptosis. Hepat mon 7 (2007): 217-221.
19. Alter MJ, Margolis HS, et al. The natural history of community-acquired hepatitis C in the United States. The Sentinel Counties Chronic non-A, non-B Hepatitis Study Team. N Engl J Med 327 (1992): 1899-1905.
20. Seeff LB. The natural history of chronic hepatitis C virus infection. Clin Liver Dis 1 (1997): 587-602.
21. Wun J, Meng MZ, et al. Toll-like receptor mediated control of hepatitis B virus replication by non parenchymal liver cells in mice. Hepatology 46 (2007): 177.
22. Kim HJ, Chung JH, et al. Polymorphisms of Interferon Gamma Gene and Risk of Hepatocellular Carcinoma in Korean Patients with Chronic. Hepato- Gastroenterology 60 (2013): 1117.
23. Saxena R, Chawla YK, et al. Effect of IL-12B, IL-2,TGF-β1, and IL-4 polymorphism and expression on hepatitis B c progression. Interferon and cytokine. Journal of Interferon & Cytokine Research 34 (2014): 117-128.
24. Puthothu B, Krueger M, et al. Association between severe respiratory syncytial virus infection and IL13/IL4 haplotypes. J Infect Dis 193 (2006): 438-441.
25. Suppiah V, Goris A, et al. Polymorphisms in the interleukin-4 and IL-4 receptor genes and multiple sclerosis: a study in Spanish-Basque, Northern Irish and Belgian populations. Int J Immunogenet 32 (2005): 383-388.
26. Tsai MH, Chen WC, et al. Interleukin-4 gene, but not the interleukin-1 beta gene polymorphism, is associated with oral cancer. J Clin Lab Anal 19 (2005): 93-98.
27. Wu MC, Huang CM, et al. Polymorphisms of the interleukin-4 gene in Chinese patients with systemic lupus erythematosus in Taiwan. Lupus 12 (2003): 21-25.