

The IL-10 Promoter Polymorphism at Position –592 is Correlated with Susceptibility to Occult HBV Infection

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Abstract—Occult hepatitis B infection (OBI) is characterized as a form of hepatitis in which detectable amounts of HBV-DNA can be monitored in the peripheral blood of patients whereas the hepatitis B surface antigen is undetectable. The main aim of this study was to investigate whether there is a relationship between OBI and single nucleotide polymorphisms in the –592 region of the IL-10 gene. In this study, the polymorphism at position –592 of the IL-10 promoter of 57 OBI cases was compared and correlated to that of 100 healthy controls by PCR-RFLP techniques. Our results showed that patient and control groups had significant differences regarding genotypes and alleles of the –592 polymorphism in the IL-10 gene. Based on our results, it can be concluded that the –592 polymorphism within the promoter of the IL-10 gene is associated with OBI.

KEY WORDS: occult hepatitis B infection; IL-10; polymorphism; HBsAg; HBV-DNA.

INTRODUCTION

Occult hepatitis B infection (OBI) is described as a clinical form of hepatitis B in which, despite the lack of detectable Hepatitis B surface antigen (HBsAg) in patient serum, those patients are positive for HBV-DNA in periphery blood [1]. This type of hepatitis imposes a considerable threat to blood transfusion services, and its detection remains a significant challenge for those agencies [2]. Despite the application of programs for appropriate screening of all donated blood and blood components for HBsAg, some cases of post-transfusion hepatitis B are reported worldwide [3]. The

majority of post transfusion hepatitis B infections are caused by OBI [4], which we previously reported in our investigations in Isfahan [5] and Kerman [4], the two main central provinces of Iran. The mechanisms responsible for progression of OBI are yet to be clarified; however, some investigators have suggested that genetic and immunological parameters may play a significant role in the resistance of some individuals and sensitivity of others [4, 6, 7]. The key roles of IL-10 as an inhibitory cytokine of autoimmune and inflammatory reactions [8] raise questions concerning the impacts of this cytokine in the pathogenesis of OBI. Elevated levels of IL-10 in OBI patients were previously reported by our research team [9]. Therefore, it can be suggested that IL-10 creates an inhibitory effect on the immune system of OBI patients, and they fail to completely clear the HBV infection. Our previous findings encouraged us to explore the reasons for overexpression of IL-10 in OBI patients, with a view that if we could understand the regulatory mechanisms that are disrupted in OBI patients, this may open opportunities to explore potential therapeutics. Several studies showed that the polymorphisms within the promoter of IL-10 gene (especially –592) can influence the expression of the cytokine [10]. Therefore, the aim of

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Table 1. The Table Shows the Sequence of the Primers Used in this Study as Well as the Appropriate Annealing Temperatures and Expected PCR Product Sizes

Genes	Primers	Annealing temperature	Product size (bp)
S gene (HBV)	F: TCGTGGTGGACTTCTCTC R: ACAGTGGGGGAAAGCCC	60°C	500
IL-10	IL-10 -592 F: 5'-GTAATATCTCTGTGCCTC-3' IL-10 -592 R: 5'-CATTCCAGAATACAATGG-3'	53°C	437

this study was to investigate the relation between OBI and the -592 polymorphism of IL-10 gene promoter.

Detection of Polymorphism

The -592 IL-10 gene polymorphism (within the gene promoter) was analyzed by the PCR-RFLP method as described in our previous study [11].

MATERIAL AND METHODS

Patients

Peripheral blood samples were collected from 57 OBI patients and 100 healthy controls. HBsAg-negative and HBV-DNA/anti-HBc-positive samples were considered as OBI, while, HBsAg/HBV-DNA-negative, and anti-HBc-positive samples were selected as healthy controls. Selection of OBI patients and healthy controls was described previously [4]. The study protocol was approved by the ethical committee of the Rafsanjan University of Medical Sciences.

Prior to sample collection, all participants of this study filled out and signed the informed consent form which was designed and based on the aims and objectives of the current study.

Genomic DNA Extraction

Peripheral blood was collected on EDTA, and genomic DNA was extracted using a commercial kit (Bioneer, Korea) following the manufacturer's recommended procedures. Extracted DNA was aliquoted (for each patient sample) and stored at -20°C for further use.

Statistical Analysis

Hardy-Weinberg equilibrium was assessed using the genotype data. Allele and genotype frequencies were calculated in patients and healthy controls by direct gene counting. Statistical analysis of the differences between groups was determined by the χ^2 test using EPI 2000 and SPSS software version 13. A *P* value of less than 0.05 was considered significant.

RESULTS

Evaluation of polymorphisms within the -592 region of the IL-10 gene by *Rsa*-1 restriction digestion showed that the prevalence of the C/C genotype was 31 (54.4%) in patients and 22 (22%) in controls, the frequency of the A/C genotype was 24 (42.1%) and 55 (55%) in patients and controls, respectively, and the values for the A/A genotype in the patient group was 2 (3.5%) and in controls was 23 (23%) (Table 1). Statistical analysis showed a significant difference between groups regarding these genotypes (*p*=0.04). The frequency of the C allele was 86 (75.44%) and 99 (49.5%) in patients and controls, respectively. Twenty-eight (24.56%) of the A alleles were seen in patients, but the frequency of this allele was 101 (50.5%) in controls. Statistical analysis showed that the difference in these genotypes were also significant (*p*=0.001) (Table 1).

Table 2. Frequency of Polymorphisms within the -592 Region of the IL-10 Gene in OBI Patients and Controls

Condition	Patients	Control	p value
C/C <i>n</i> (%)	31 (54.4%)	22 (22%)	<i>p</i> =0.001
A/C <i>n</i> (%)	24 (42.1%)	55 (55%)	
A/A <i>n</i> (%)	2 (3.5%)	23 (23%)	
Alleles			
C <i>n</i> (%)	86 (75.44%)	99 (49.5%)	<i>p</i> =0.001
A <i>n</i> (%)	28 (24.56%)	101 (50.5%)	

DISCUSSION

Increased serum levels of IL-10 subsequent to the viral infections is now well documented [9], and it has

IL-10 Polymorphism in Occult HBV Infection

111 also been reported that the expression level of IL-10 is
 112 related to the type of clinical presentation and stage of
 113 hepatitis B virus infection (Table 2). In addition, IL-10
 114 levels have also been associate with relevant liver
 115 disease [12]. In agreement with the evidence suggesting
 116 a potential correlation between IL-10 and disease status,
 117 our results showed that the frequency of evaluated
 118 alleles and genotypes were different between OBI
 119 patients and healthy controls. Therefore, based on our
 120 results, it can be concluded that these polymorphisms are
 121 associated with OBI. In a previous study, we reported the
 122 overexpressed circulating levels of IL-10 in OBI patients
 123 [9]; hence, when considering these data together, it
 124 might be concluded that the evaluated polymorphisms
 125 probably had an impact on IL-10 production. To the best
 126 of our knowledge, this is the first study to evaluate the
 127 IL-10 -592 polymorphisms in OBI patients. However,
 128 several studies have shown that IL-10 polymorphisms
 129 correlate with hepatitis B [13–15]. For instance, LU
 130 Yong-Liang reported that the polymorphisms at the -592
 131 position of the IL-10 gene were associated with HBV
 132 infection in an Asian ethnic group [13]. Interestingly,
 133 they did not find any relationship between HBV
 134 infection and any other polymorphisms found within
 135 the IL-10 promoter [13]. Some studies also demonstrated
 136 that the polymorphisms at the -592 position of IL-10 are
 137 associated with HBV infection [15, 16]. Interestingly, a
 138 meta-analysis showed that the frequency of A allele at the
 139 -592 position of IL-10 is more likely to be related to
 140 spontaneous HBV clearance [17]. In this study, we
 141 revealed that the frequency of A allele was decreased in
 142 the OBI patients; thus, it can be concluded that the
 143 evaluated IL-10 polymorphisms in OBI patients may lead
 144 to a weakened immune system which fails to clear HBV.
 145 Interestingly, our previous study showed that the serum
 146 levels of IL-12 were decreased in OBI patients [4].
 147 Additionally, we showed in that study that the poly-
 148 morphisms within IL-12 were not associated with IL-12
 149 serum levels [4]. Therefore, according to our current and
 150 previous findings, it may be concluded that low levels of
 151 inflammatory cytokines such as IL-12 are related to the
 152 inhibitory effects of IL-10.

153 The strength of our study is the high number of the
 154 evaluated blood donors. However, there is a lack of
 155 functional correlation between the presence of the
 156 polymorphism and its role in regulating IL-10 expres-
 157 sion. In the future, this could be resolved using a
 158 luciferase reporter assays in which reporter expression is
 159 studied under the control of the wild-type and diseased
 160 promoters. In addition, future studies should focus on

the expression levels of IL-10 mRNA in the immune
 cells of OBI patients *versus* healthy controls by real-time
 PCR. It would also be interesting to measure circulating
 serum levels of IL-10 in OBI patients carrying the -592
 polymorphism *versus* healthy controls.

Finally, due to the complexity of OBI, other aspects
 of the disease need to be examined. Therefore, our future
 studies will be focused on exploring polymorphisms and
 the expression levels of related cytokines and their
 receptors in OBI patients.

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REFERENCES

- Hollinger, F.B., and G. Sood. 2009. Occult hepatitis B virus infection: a covert operation. *Journal of Viral Hepatitis* 17: 1–15.
- Schmeltzer, P., and K.E. Sherman. 2010. Occult hepatitis B: clinical implications and treatment decisions. *Digestive Diseases and Sciences* 55: 3328–3335.
- Candotti, D., and J.P. Allain. 2009. Transfusion-transmitted hepatitis B virus infection. *Journal of Hepatology* 51: 798–809.
- Arababadi, M.K., A.A. Pourfathollah, A. Jafarzadeh, G. Hassanshahi, S. Daneshmandi, A. Shamsizadeh, and D. Kennedy. 2011. Non-association of IL-12 +1188 and IFN-gamma +874 polymorphisms with cytokines serum level in occult HBV infected patients. *Saudi Journal of Gastroenterology* 17: 30–35.
- Pourazar, A., M. Salehi, A. Jafarzadeh, M.K. Arababadi, F. Oreizi, and K. Shariatinezhad. 2005. Detection of HBV DNA in HBsAg Negative Normal Blood Donors. *Iranian Journal of Immunology* 2: 172–176.
- Zerbini, A., M. Pilli, C. Boni, P. Fiscaro, A. Penna, P. Di Vincenzo, T. Giuberti, A. Orlandini, G. Raffa, T. Pollicino, G. Raimondo, C. Ferrari, and G. Missale. 2008. The characteristics of the cell-mediated immune response identify different profiles of occult hepatitis B virus infection. *Gastroenterology* 134: 1470–1481.
- Demir, M., E. Serin, S. Gokturk, N.A. Ozturk, S. Kulaksizoglu, and U. Yilmaz. 2008. The prevalence of occult hepatitis B virus infection in type 2 diabetes mellitus patients. *European Journal of Gastroenterology and Hepatology* 20: 668–673.
- Sanjabi, S., L.A. Zenewicz, M. Kamanaka, and R.A. Flavell. 2009. Anti-inflammatory and pro-inflammatory roles of TGF-beta, IL-10, and IL-22 in immunity and autoimmunity. *Current Opinion in Pharmacology* 9: 447–453.
- Arababadi, M.K., A.A. Pourfathollah, A.A. Jafarzadeh, and G. Hassanshahi. 2010. Serum levels of Interleukin (IL)-10 and IL-17A in occult HBV infected south-east Iranian patients. *Hepatitis Monthly* 10: 31–35.
- Karjalainen, J., J. Hulkkonen, M.M. Nieminen, H. Huhtala, A. Aromaa, T. Klaukka, and M. Hurme. 2003. Interleukin-10 gene promoter region polymorphism is associated with eosinophil count

Q2

213 and circulating immunoglobulin E in adult asthma. *Clinical and*
 214 *Experimental Allergy* 33: 78–83.

215 11. Arababadi, M.K., M.R. Mirzaei, S.M.A. Sajadi, G. Hassanshahi, B.
 216 N. Ahmadabadi, V.A. Salehabadi, R. Derakhshan, and D. Kennedy.
 217 2011. Interleukin (IL)-10 gene polymorphisms is associated with type
 218 2 diabetes with and without nephropathy: a study of patients from the
 219 South-East region of Iran. *Inflammation*, in press.

220 12. le Song, H., V.Q. Binh, D.N. Duy, J.F. Kun, T.C. Bock, P.G.
 221 Kremsner, and A.J. Luty. 2003. Serum cytokine profiles associated
 222 with clinical presentation in Vietnamese infected with hepatitis B
 223 virus. *Journal of Clinical Virology* 28: 93–103.

224 13. Lu, Y.L., X. Wu, H.L. Huang, and L.C. Dai. 2010. Allele
 225 polymorphisms of interleukin-10 and hepatitis B, C virus infection.
 226 *Chinese Medical Journal (Engl)* 123: 1338–1344.

227 14. Gao, Q.J., D.W. Liu, S.Y. Zhang, M. Jia, L.M. Wang, L.H. Wu, S.Y.
 228 Wang, and L.X. Tong. 2009. Polymorphisms of some cytokines
 229 and chronic hepatitis B and C virus infection. *World Journal of*
 230 *Gastroenterology* 15: 5610–5619.

231 15. Wang, C.J., K.R. Shan, Y. He, T. Zhang, Y. Li, X.L. Qi, Y. Zhao, Y.
 232 Xiao, C.X. Wu, Z.Z. Guan, and X.L. Ren. 2008. Study on the
 233 association of IL-10 -592 polymorphism with susceptibility to
 234 hepatitis B viral infection in Han, Yi and Yao ethnic groups in
 235 Guizhou province. *Zhonghua Liu Xing Bing Xue Za Zhi* 29:
 236 444–448.

237 16. Miyazoe, S., K. Hamasaki, K. Nakata, Y. Kajiya, K. Kitajima, K.
 238 Nakao, M. Daikoku, H. Yatsushashi, M. Koga, M. Yano, and K.
 239 Eguchi. 2002. Influence of interleukin-10 gene promoter poly-
 240 morphisms on disease progression in patients chronically infected
 241 with hepatitis B virus. *American Journal of Gastroenterology* 97:
 242 2086–2092.

243 17. Zhang, T.C., F.M. Pan, L.Z. Zhang, Y.F. Gao, Z.H. Zhang, J. Gao,
 244 R. Ge, Y. Mei, B.B. Shen, Z.H. Duan, and X. Li. 2010. A meta-
 245 analysis of the relation of polymorphism at sites -1082 and -592
 246 of the IL-10 gene promoter with susceptibility and clearance to
 247 persistent hepatitis B virus infection in the Chinese population.
 248 *Infection* 39: 21–27.

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