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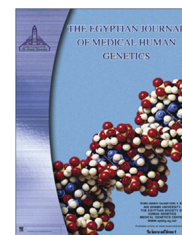

ORIGINAL ARTICLE

Association of Interleukin 27 gene polymorphism and risk of Hepatitis B viral infection in Egyptian population

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KEYWORDS

Hepatitis B virus;
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Abstract *Background:* According to the World Health Organization, Hepatitis B virus (HBV) is considered a major global public health problem. The genetic background may be a crucial etiologic factor in HBV infection and its complications. Interleukin-27 (IL-27) is a newly discovered cytokine encoded by 2 genes (EBI3 and p28). Mutations in the IL-27 gene may lead to altered cytokine production and/or activity and thus modulate individual's susceptibility to HVB infection.

Aim of the study: This work was designed to study the association of IL-27p28 (−964A/G, 2905T/G and 4730T/C) gene promoter single nucleotide polymorphism (SNP) with the risk of Hepatitis B virus (HBV) in Egyptians. To the best of our knowledge, this study is the first one that examines IL-27p28 promoter polymorphism in Egyptian patients.

Subjects and methods: One hundred and sixteen patients with HBV infection and 101 healthy controls were genotyped by using polymerase chain reaction/restriction fragment length polymorphism (PCR/RFLP) in Egyptian population.

Results: There were no significant differences in the genotype and allele frequencies of IL-27p28 gene polymorphisms between patients and controls. Furthermore, no association was found between the distributions of the haplotypes and HBV risk.

Conclusion: Our data suggested that polymorphisms in the IL-27 gene may not contribute to HBV susceptibility. Further studies with large sample size should be conducted to validate these results in Egyptian population.

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1. Introduction

Hepatitis is considered a major clinical problem, according to the World Health Organization [1]. Approximately 30% of world populations are estimated to carry detectable HBV antigens and about 5 million cases of acute Hepatitis B occur annually [2]. More than half a million of patients worldwide

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die annually as a result of HBV-related liver diseases [3,4]. A large proportion of these infected individuals do not clear the virus, which may progress to persistent infection with or without liver disease [5]. It is estimated that 50% of male carriers and 14% of female carriers will eventually die of the complications of cirrhosis and hepatocellular carcinoma [6]. Egypt is among the countries with intermediate endemicity of HBsAg [7,8]. Awadalla et al. [9] demonstrated that the prevalence rate of HBV among 1000 Egyptian healthy volunteers' blood donors was 5%. It was more frequent in young adults and in males than females.

An efficient cellular cytotoxic T-lymphocyte (CTL) immune response is a major contributor to HBV elimination, virus specific T cell appears to target a very few infected hepatocytes when chronicity developed [10]. Numerous studies have shown that cytokines are of critical importance for modulating the intensity and duration of the host immune responses against HBV infections [11,12].

IL-27 is a new member of the IL-6/IL-12 family that was described in 2002 [13], it is heterodimeric cytokine composed of the p28 subunit that was noncovalently linked to Epstein–Barr virus-induced gene 3 protein (EBI3) subunit [13,14]. IL-27 is produced and released early in response to various inflammatory stimuli by antigen-presenting cells. IL-27 plays an essential role in a link between innate and adaptive immunities in viral infection through binding with a heterodimeric receptor complex. It appears to act as early mediator of naive T-cell proliferation, and it is a potent inducer of interferon gamma (IFN-γ) production (particularly in synergy with IL-12) [15,16], which inhibit the viral replication and recruit specific and non-specific effector immune cells besides their role in the activation of adaptive immune response [17]. Matsui et al. [18] and Fakruddin et al. [19] demonstrated that IL-27 could be an inhibitor of HIV-1 replication in $CD4^+$ T cells and macrophages and also as a potent adjuvant for epitope-specific CTL induction against HCV infection.

Over the past few years, single nucleotide polymorphisms (SNPs) have been proposed as the next generation of markers for the identification of loci associated with diseases and its complications [20,21]. Polymorphisms in several cytokine genes, such as IFN-γ [22], tumor necrosis factor-a (TNF-α) [23], IL-10 [24], IL-18 [25], and IL-1B [26], are considered to be correlated with the severity of liver disease in patients with HBV infection. Human IL-27gene is located on chromosome 16p11 and consists of five exons [27]. Recently, the −964A/G, 2905T/G and 4730T/C SNPs have been identified. Although little is known about the SNPs of IL-27 gene in HBV development, there is an important A versus G transition at position −964 in the promoter region. It was reported to be associated with susceptibility to HBV [28]. In this study, we evaluated the effect of −964A/G, 2905T/G and 4730T/C SNPs on susceptibility of Egyptians to HBV infection (See Fig. 1).

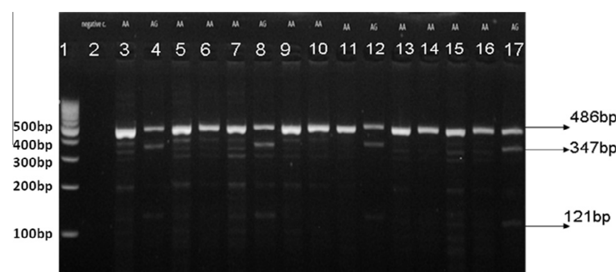

Figure 1 IL-27p28 (−964A/G) PCR product after digestion with Xho1 enzyme. Sample lanes (4, 8, 12 and 17) showed AG genotype whereas the 486 bp band was digested into three bands with sizes of 486, 347 and 121 bp. While sample lanes (3, 5, 6, 7, 9, 10, 11 and 13–16) show the AA genotype whereas the 486 bp band was not digested. Lane 1 shows 100 bp DNA. Lane 2 shows negative control.

2. Patients and methods

2.1. Patients and controls

One hundred and sixteen patients were enrolled in this study. HBV infected patients were recruited from the National Liver Institute, Menofiya University, Egypt. Samples included 98 males and 18 females with the mean age 42.71 ± 10.04 years (range from 22 to 70) and were clinically diagnosed and confirmed to be chronically infected with HBV by laboratory investigations. One hundred and one healthy controls (68 males and 33 females) with no history of previous liver disease (HBV and HCV infection are negative) were included in our study. Patients with HCV or other viral infections or any other liver diseases were excluded. Patients and controls were matched for age, ethnicity and residence. All investigations were performed in accordance with the Menofiya University, Health and Human Ethics Clearance Committee guidelines for Clinical Researches. Local ethics committee approved the study protocol and informed consents were obtained from all subjects. The work is carried out in accordance with The Code of the World Medical Association (Declaration of Helsinki) for experiments involving humans (See Fig. 2).

2.2. Virological assessment

All patients and controls were tested for Hepatitis B surface antigen (HBsAg) using commercially available kits (Sorin Biomedica, Milan, Italy). The confirmation of the presence of HBV-DNA in HBV-positive samples was tested by a standard polymerase chain reaction (PCR) (Roche Diagnostics Corp., Indianapolis, IN). HCV antibodies were tested for both groups using the enzyme-linked immunosorbent assay (ELISA)

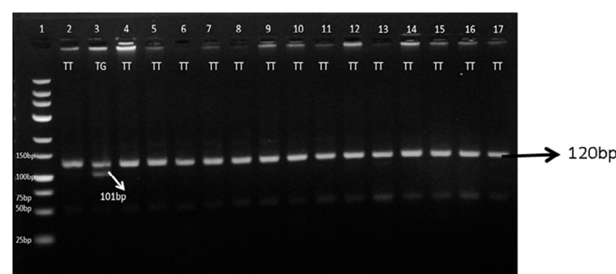

Figure 2 IL-27p28 (2905T/G) PCR product after digestion with BstuI (Bsh1236I) enzyme. Sample lane 3 showed TG genotype whereas the 120 bp band was digested into three bands with sizes of 120, 101 and 19 bp. while sample lanes (2, 4–17) show the TT genotype whereas the 120 bp band was not digested. Lane 1 shows 25 bp DNA ladder.

(Murex Biotech Ltd., Dartford, UK). Alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP) (BioMérieux S.A, Marcy l'Etoile, France), albumin (Human Gesellschaft Fur Biochemica Und Diagnostica mbH, Wiesbaden, Germany), and bilirubin (Roche Diagnostics Corp., Indianapolis, IN), were all measured according to their respective manufacturer's kit instructions. Similarly blood urea and creatinine (BioMérieux S.A) were measured for all cases following the manual structure of manufacturer's kit (See Fig. 3).

2.3. DNA isolation

Blood was collected by withdrawal of 5 ml venous blood from each individual involved in this study into sterile vacutainer tubes containing tri-potassium ethylenediaminetetraacetic acid (EDTA.K_3). Genomic DNA was extracted from whole blood-EDTA samples by the Wizard Genomic DNA Purification Kit (Promega Corporation, Madison, USA) according to manufacturer's instructions [29]. Extracted DNA was applied to 1% agarose gel to confirm the presence of DNA. The concentration of DNA in all samples was measured by using a Spectrophotometer (GE Healthcare Biosciences).

2.4. Genotyping of IL-27p28 SNPs

IL-27p28 SNPs were determined by PCR restriction fragment length polymorphism (PCR-RFLP) [30]. The digested PCR products were detected on 4% agarose gel electrophoresis.

2.4.1. Genotyping of IL-27p28 −964A/G SNP (rs153109)

The PCR primers were designed as previously described [28] forward primer: 5′-GGCTGTGCTGGAAGGGAGAC-3′ and reverse primer: 5′-ATATCTGGGACCAGGGTTAGG-3′. A 25 μl of PCR reaction mixture contained DreamTaq Green Master Mix 2× (Fermentas, Thermo Fisher Scientific Inc.), 10 pmol/l of each primer (Metabion, Martinsried, Germany) and 0.1 μg DNA. The PCR cycling was performed as follows; 1 cycle of 94 °C for 5 min, followed by 35 cycles of 94 °C for 1 min, 56 °C for 45 s, 72 °C for 1 min and a final extension step at 72 °C for 10 min. PCR reaction was performed in a Biometra thermal cycler (Biometra GmbH, Germany). The 468 bp PCR products were visualized on 2% agarose gel. PCR product (15 μl) was subjected to digestion with XhoI restriction enzyme (Fermentas), which yielded DNA fragments of 468 bp for A/A, 468/347/121 bp for A/G and 347/121 bp for G/G genotypes in comparison to 100 bp DNA ladder (Fermentas).

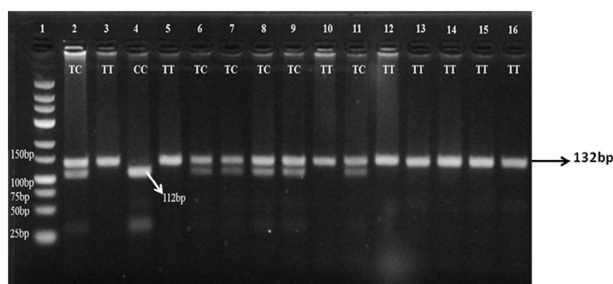

Figure 3 IL-27p28 (4730T/C) PCR product after digestion with FauI (SmuI) enzyme. Sample lanes (2, 6, 7, 8, 9 and 11) showed TC genotype whereas the 132 bp band was digested into three bands with sizes of 132, 112 and 20 bp. Sample lanes (3, 5, 10, 12–16) show the TT genotype whereas the 132 bp band was not digested. While lane 4 shows a mutant homozygous CC genotype. Lane 1 shows 25 bp DNA ladder.

2.4.2. Genotyping of IL-27p28 2905 T/G SNP (rs17855750)

The PCR primers were designed as described previously [31] forward primer: 5′-ATCTCGCCAGGAAGCTGCGC-3′ and reverse primer: 5′-CTGTTAGTGGGGGCCAGAAGGGA-3′. A 25 μl of PCR reaction mixture contained DreamTaq Green Master Mix 2× (Fermentas), 10 pmol/l of each primer (Metabion) and 0.1 μg DNA. The PCR cycling was performed as follows; 1cycle of 94 °C for 5 min, followed by 35 cycles of 94 °C for 45 s, 66 °C for 1 min, 72 °C for 1 min and a final extension step at 72 °C for 10 min. The 120 bp PCR products were visualized on 2% agarose gel. PCR product (15 μl) was subjected to digestion with BstuI (Bsh1236I) restriction enzyme (Fermentas), which yielded DNA fragments of 120 bp for T/T, 120/101/19 bp for T/G and 101/19 bp for G/G genotypes in comparison to 25 bp DNA ladder (Fermentas).

2.4.3. Genotyping of IL-27p28 4730T/C SNP (rs181206)

The PCR primers were designed as described previously [31] forward primer: 5′-GCTTCAGCCCTTCCATGCCC-3′ and reverse primer: 5′-TCTACCTGGAAGCGG AGGTGCC-3′. A 25 μl of PCR reaction mixture contained DreamTaq Green Master Mix 2× (Fermentas), 10 pmol/l of each primer (Metabion) and 0.1 μg DNA. The reaction was carried out at 1 cycle of 94 °C for 5 min, followed by 35 cycles at 94 °C for 45 s, annealing temperature 66 °C for 1 min and at 72 °C for 1 min. The final extension was completed at 72 °C for 10 min. The 132 bp PCR products were visualized on 2% agarose gel. PCR product (15 μl) was subjected to digestion with FauI (SmuI) restriction enzyme (Fermentas), which yielded DNA fragments of 132 bp for T/T, 132/112/20 bp for T/C and 112/20 bp for C/C genotypes in comparison to 25 bp DNA ladder (Fermentas).

2.5. Statistical analysis

The statistical analyses were performed by SPSS statistical package version 11 (SPSS, IBM Corporation, USA). Data were presented as means with the corresponding standard deviation (SD). Comparisons among patients and controls were performed by the independent *T*-test. Chi-square test was used to compare the frequency of variables in different groups. Odds ratios (OR) [with 95% confidence interval (CI)] were calculated to measure the relative risks in both control and HBV patients. Correlation between variables was determined using Spearman's correlation coefficient test. The haplotype frequencies were estimated with linkage disequilibrium coefficient (LD), D using SNPSTAT program (available at http://bioinfo.iconcologia.net/snpstats/start.htm) [32]. D was expressed as D' giving the value of D as a percentage of the maximum calculated value given the observed allele frequencies. Values of D' ranged between −1 and +1. A/D′/value of 1 denoted complete linkage disequilibrium whereas a value of 0 denoted complete linkage equilibrium.

3. Results

3.1. Clinical characteristics of the study population

All patients were positive for HBsAg, HBV-DNA, and negative for HCV antibodies. All controls were negative for HBsAg, HBV-DNA, and negative for HCV antibodies. There was positive correlation between the disease and male gender ($p < 0.001$). The biochemical characteristics of patients and controls enrolled in this study are shown in Table 1. Levels of liver enzymes showed significant increase in patients group in comparison to the control group ($p < 0.001$ for ALT, AST, GGT and ALP). Level of total bilirubin was significantly higher in HBV patients ($p < 0.001$) in comparison to the control group while albumin production significantly decreased ($p < 0.001$). Kidney functions were significantly elevated with the disease ($p < 0.01$ and $p < 0.001$ for urea and creatinine, respectively).

3.2. Association of IL27 gene polymorphisms with the risk of HBV infection

The genotypes distribution of IL-27p28 SNPs −964A/G and 4730T/C were in Hardy–Weinberg equilibrium while IL-27 SNP (2905 T/G) was out of the equilibrium in HBV patients (96.5%, 2.6%, 1% (observed) vs. 91.5%, 4.1%, 0.5% (predicted), for TT, TG, GG, respectively) ($p < 0.001$). The frequencies of the IL-27 −964G, 2905G and 4730C alleles among Egyptian patients were 24%, 2.2% and 15%, respectively, and that statistically was insignificant in comparison to those frequencies observed in controls (26%, 0.5% and 12%, respectively).

Table 2 shows that AA genotype of IL-27 −964A/G was higher than AG and GG genotypes in both groups. TT genotype of IL-27 2905T/G was dominant genotype between both groups, GG genotype was not detected in controls. The TT genotype of IL-27 (4730T/C) was higher than TC and CC genotypes in both groups. There was no significant difference in the distribution of all genotypes and alleles of IL-27 P28 SNPs by comparing HBV patients to controls.

3.3. Haplotype analysis

The possible four haplotype frequencies are shown in Table 3. Linkage disequilibrium (LD) was observed between allele A at locus −964 and allele T at locus 4730 [$D' = 0.5034$, $r^2 = 0.122$] and allele T at locus 2905 and allele T at locus 4730 [$D' = 0.9443$, $r^2 = -0.002$]. Major ATT haplotype accounted for 70.6% and 69.3% of these four haplotypes in both the cases and controls, respectively. Four haplotype frequencies of the IL-27 gene in chronic HBV patients were insignificantly different than that in healthy controls.

4. Discussion

To our knowledge, this is the first report to attempt an evaluation of the association between SNPs of IL-27p28 gene and HBV patients in Egyptian population. Despite the existence of effective vaccines for Hepatitis B virus from 1992 [33,34], the prevalence of HBsAg was still 2–7% in Egypt [7,8]. Patients group had increased in the number of males over the number of females which is in consistent with other studies [9,35] which suggest that HBV infection might be sex biased disease. There was a significant association between biochemical characteristics of patients in comparison to healthy controls, i.e. serum albumin, creatinine, ALT, AST, total Bilirubin, direct Bilirubin, ALP and GGT. Similarly, Bantel et al. [36], Kronenberger et al. [37] and Sumer et al. [38] found that levels of liver enzymes might be biased in HBV infection.

IL-27 is a pleiotropic cytokine that can do proinflammatory and anti-inflammatory functions. In $CD4^+$ and $CD8^+$ T cells, as well as in natural killer (NK) cells, IL-27 promotes the expression of the transcription factor T-bet and the cytokines IL-10 and IFN-γ [39]. It also limits the production of IL-17 by $CD4^+$ T cells and NK cells [40,41]. In response to IL-27, B cell subsets increase their proliferation and antibody produc-

Table 1 Biochemical characteristics of HBV patients and healthy controls.

Characteristics	Control $N = 101$ (M ± SD)	HBV $N = 116$ (M ± SD)	p-Value	Correlation with disease
Albumin (g/L)	4.27 ± 0.39	3.41 ± 0.58	$p < 0.001$	$r = -0.652$ $p < 0.001$
Creatinine (mg/dl)	0.88 ± 0.16	1.06 ± 0.21	$p < 0.001$	$r = 0.422$ $p < 0.001$
AST (IU/L)	21.16 ± 5.87	41.71 ± 18.50	$p < 0.001$	$r = 0.590$ $p < 0.001$
ALT (IU/L)	18.69 ± 5.08	44.03 ± 27.08	$p < 0.001$	$r = 0.534$ $p < 0.001$
Total Bilirubin (mg/dl)	0.66 ± 0.18	1.10 ± 0.46	$p < 0.001$	$r = 0.520$ $p < 0.001$
Direct Bilirubin (mg/dl)	0.10 ± 0.112	0.32 ± 0.39	$p < 0.001$	$r = 0.343$ $p < 0.001$
Urea (mg/dl)	29.05 ± 7.15	32.67 ± 9.31	$p < 0.01$	$r = 0.212$ $p < 0.01$
ALP (U/L)	100.79 ± 28.00	185.77 ± 100.88	$p < 0.001$	$r = 0.488$ $p < 0.001$
GGT (U/L)	26.17 ± 8.14	57.33 ± 23.68	$p < 0.001$	$r = 0.651$ $p < 0.001$

All data are presented as mean ± SD.

Table 2 The genotype and allele frequencies of IL-27 polymorphisms in HBV patients and controls.

Polymorphisms	Control (N = 101) N (%)	HBV (N = 116) N (%)	OR (95% CI)	p-Value
IL-27 −964A/G				
Alleles				
A	150 (74%)	177 (76%)	0.0896 (0.58–1.39)	NS
G	52 (26%)	55 (24%)	1.12 (0.72–1.7)	NS
IL-27 −964A/G				
Genotypes				
AA	61 (60.4%)	69 (59.5%)	0.963 (0.559–1.659)	NS
AG	28 (27.7%)	39 (33.6%)	1.32 (0.74–2.36)	NS
GG	12 (11.9%)	8 (6.9%)	0.55 (0.22–1.40)	NS
IL-27 2905 T/G				
Alleles				
T	201 (99.5)	221 (97.8)	4.55 (0.53–39.26)	NS
G	1 (.5)	5 (2.2)	0.22 (0.03–1.89)	NS
IL-27 2905 T/G				
Genotype				
TT	100 (99)	109 (96)	0.273 (0.030–2.470)	NS
TG	1 (1)	3 (3)	2.67 (0.27–26.12)	NS
GG	0 (0)	1 (1)	1.009 (0.99–1.03)	NS
IL-27 4730T/C				
Alleles				
T	177 (88%)	196 (85)	1.23 (0.71–2.14)	NS
C	25 (12%)	34 (15)	0.814 (0.47–1.42)	NS
IL-27 4730T/C				
Genotype				
TT	77(76.2)	85 (73.9)	0.88(0.48–1.64)	NS
T/C	23 (22.8)	26 (22.6)	3.60(0.39–32.78)	NS
CC	1 (1)	4 (3.5)	1.132 (0.610–2.103)	NS

Table 3 Haplotype frequencies of IL-27 gene in the patients with HBV and in controls.

IL-27 gene (−964/2905/4730) haplotypes	Control (N = 101) N (%)	HBV (N = 116) N (%)	OR (95% CI)	p-Value
ATT	70 (69.3)	82 (70.6)	1.00	NS
GTT	18 (17.8)	16 (13.7)	0.75 (0.45–1.26)	NS
GTC	8 (7.9)	12 (10.34)	1.39 (0.66–2.91)	NS
ATC	5 (4.9)	6 (5.17)	0.99 (0.43–2.28)	NS

tion [42,43]. In mast and eosinophil cells, it can promote proinflammatory responses by increasing expression of IL-1, TNF-α, and IL-6 [44]. However, in neutrophils, IL-27 limits cytokine secretion of IL-6 and IL-12p40 [45]. In human macrophages, IL-27 can inhibit their responsiveness to proinflammatory cytokines such as IL-1 and TNF-α by down-regulating expression of their cognate receptors [46].

Since IL-27 is important for Th1 promotion and persistent Th1 responses can lead to excessive cell-mediated immunity and uncontrolled tissue damage, it is reasonable to speculate that the incidence of fatal hepatitis in chronic Hepatitis B patients may be in part due to the high production of IL-27 [47]. Thus, the present study was designed to examine the effect of IL-27 SNPs on susceptibility to HBV infection. The present study observed insignificant change in genotypes and allele frequencies of IL-27p28 (−964A/G, 2905T/G, 4730T/C) polymorphisms with HBV infection. Similarly, Peng et al. [48] observed that there were no significant differences in the genotype and allele frequencies of IL-27 gene polymorphisms between chronic HBV patients and healthy controls. In contrast to our study, Wang et al. [28] observed that IL-27p28 (−964A/G) SNP was associated with the progression of chronic Hepatitis B in Chinese, so the mechanism of IL-27 with the disease may be affected with its polymorphism in HBV patient.

The major haplotype between both groups was ATT haplotype. Haplotype frequencies of the IL-27p28 gene were not associated with chronic HBV patients and this was consistent to the data presented by Peng et al. [48].

5. Conclusion

This study observed that there was no significant association between SNPs of IL-27p28 and the risk of HBV infection in Egyptian population. However, because of the relatively small sample size and strong LD of this study, further studies with large sample size are needed to validate these results and explore the effect of different environmental factors on these results, especially in Egyptian populations.

Conflict of interest

None declared.

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