Human Immunology 80 (2019) 208–214

Contents lists available at ScienceDirect

Human Immunology

journal homepage: www.elsevier.com/locate/humimm

Interleukin-6 promotor gene polymorphisms and susceptibility to chronic hepatitis B virus in Egyptians

Eman A. El-Maadawy[a], Roba M. Talaat[a,*], Maha M. Ahmed[b], Soha Z. El-Shenawy[c]

[a] Molecular Biology Department, Genetic Engineering and Biotechnology Research Institute (GEBRI), University of Sadat City, Egypt
[b] Hepatology Department, National Liver Institute (NLI), Menoufiya University, Shebeen El-Kom, Menoufiya, Egypt
[c] Biochemistry Department, National Liver Institute (NLI), Menoufiya University, Shebeen El-Kom, Menoufiya, Egypt

ARTICLE INFO

Keywords:
HBV
IL-6
Polymorphism
Egypt

ABSTRACT

Aim: To investigate the association between IL-6 polymorphisms (−174G/C, −572G/C and −597G/A) and susceptibility to chronic hepatitis B virus (CHB) infection.
Method: Total 108 subjects with CHB infection and 102 healthy controls were enrolled in this study. IL-6 (−174G/C) was genotyped using Mutagenically separated Polymerase Chain Reaction (MS-PCR) while sequence specific primers-PCR (SSP-PCR) was used for studying −572G/C and −597G/A. IL-6 plasma level was measured using Enzyme-linked immunosorbent assay (ELISA).
Results: A significant increase ($P < 0.01$, $P < 0.01$, $P < 0.001$) in −174GG, −572GC and −597GA; respectively in the CHB group compared to control group, while −572GG genotype was significantly decreased ($P < 0.01$) in CHB patients. A significant increase ($p < 0.01$, $p < 0.01$) in −174 G and −597A alleles was observed in the CHB patient group; respectively. GGA haplotype is significantly increased ($P < 0.05$) while GCA haplotype is significantly decreased ($P < 0.001$) in the patient group. A moderate linkage disequilibrium (LD) ($D' = 0.719$, $r^2 = 0.474$; $P < 0.001$) between IL-6 (−572G/C and −597G/A) was observed. A significant reduction ($P < 0.01$) in IL-6 plasma level in CHB patients compared to healthy controls (22.28 ± 1.93 versus 32.08 ± 2.41), which was negatively correlated ($r = -0.216$; $P < 0.01$) with HBV infection.
Conclusions: This study pointed to the potential role of IL-6 (−174G/C, −572 G/C and −597G/A) gene polymorphisms in the susceptibility to HBV infection. Our results allow for only preliminary conclusions due to relatively small sample size. There is a need for further larger scale studies to fully examine the possible relationship between these cytokine gene polymorphisms and the development of CHB.

1. Introduction

Hepatitis B virus (HBV) is one of the most common causes of liver diseases [1–3]. Globally, over 350 million individuals are chronically infected with HBV, which continues to be a significant healthcare encumbrance in the world, particularly in developing countries where HBV infection is endemic [4–6]. In Egypt, the estimated national prevalence of HBV infection is about 1.7% among those aged 15–59 [7]. HBV infection causes a broad spectrum of clinical manifestations, varied from an asymptomatic carrier status to severe and chronic active hepatitis (CHB) [6,8]. The chronic phase of the disease will be developed into liver cirrhosis in 20–30% of patients, and only 5% further progress to hepatocellular carcinoma (HCC), which can lead to liver end-stage in 15%–40% of patients worldwide [2,5]. In a recent study by Abd-Elsalam et al. [9] HCC resulted from HBV infection is about 3.26% of 1440 Egyptian infected patients.

The outcome of HBV infection is influenced by some factors, including viral, environmental and host-related factors (such as host immune and genetic factors) [2,5,10–13]. HBV does not have a direct effect on the hepatocytes, but the viral antigens in infected hepatocytes targeted by the cellular immune response and consequently, cause the liver damage [14]. Studies have pointed out that genes of the immune response are supposed to be a useful genetic component in CHB infection [15].

Cytokines play an essential role in the regulation of the immune response and protective effect against viral infections [16]. Polymorphisms in the cytokine genes determine the capability of cytokine production among various individuals [17,18]. Recent studies have evaluated the influence of genetic polymorphisms in the cytokine overall expression and secretion by cells of the immune system [10,19].

* Corresponding author at: Molecular Immunology, Molecular Biology Department, Genetic Engineering and Biotechnology Research Institute (GEBRI), University of Sadat City (USC), Egypt.
E-mail address: roba.talaat@gebri.usc.edu.eg (R.M. Talaat).

https://doi.org/10.1016/j.humimm.2018.12.009
Received 2 May 2018; Received in revised form 6 December 2018; Accepted 19 December 2018
Available online 27 December 2018

Our previous studies [20–22] stressed on the role of cytokine gene polymorphism including TGF-β1 (T29C), IL-10 −1082G/G and TNF-α (−863C/A, −308G/A, −376G/A, and +489G/A) as host genetic factors in the susceptibility to HBV infection in Egyptians.

Human interleukin 6 (IL-6) a key anti-inflammatory and pro-inflammatory cytokine about 21–28 kDa, produced by a different type of cells, mainly T-lymphocytes, macrophages and monocytes [23]. It plays significant roles in the organization of the immune response, inflammation, and hematopoiesis [24,25]. IL-6 interferes with the regulation of the immune response to HBV infection [5]. It has a pivotal function in the regulation of the biological responses of hepatocytes [26]. Derived from activated monocytes, IL-6 plays a vital role in promoting lymphocytes responses that are important for effective viral control [27]. Given the role of IL-6 in balancing the differentiation of pro- and anti-inflammatory cells, several studies refer to its ability in facilitating HBV infection both in vivo and in vitro [26,28,29].

The gene encoded for IL-6 mapped to chromosome 7 (7p21), composed of 5 coding exons and one non-coding [30,31]. IL-6 is a highly polymorphic gene, various single nucleotide polymorphisms (SNPs) recognized within the IL-6 gene, that may be responsible for its variable protein expression, including three essential variations upstream of its coding sequence involving: −174G/C (rs1800795), −572G/C (rs1800796) and −597G/A (rs1800797). Prior studies indicated a collaborative effect of these SNPs on the regulation of IL6 transcription and expression [2,32–34]. Previously documented data on the impact of IL-6 gene polymorphism in Egyptians suffering from autoimmune disease, cancer or diabetes [32–34]. Although some studies on HBV have been performed elsewhere, no survey on IL-6 gene polymorphism in HBV patients has been carried out on Egyptians.

Accordingly and in light of the vital role of IL-6 in HBV infection, the present study is performed to investigate the association between these 3 SNPs of the IL-6 gene and genetic susceptibility to HBV infection in the Egyptian population. The influence of these polymorphisms on IL-6 secretion profile will be evaluated in the same HBV patients and control groups to determine whether the change in the level of IL-6 associated with or independent of the genetic polymorphism. Understanding the IL-6 genetic predisposition in the Egyptian population might affect the immunological status of patients, the modulation of disease progression, and individual responses to therapy.

2. Materials and methods

2.1. Subjects

One hundred and eight patients with CHB infection recruited from the National Liver Institute, Menoufiya University, Egypt were enrolled in this study. All investigations were performed in accordance with the Menoufiya University. This study was approved by the Health and Human Ethical Clearance Committee guidelines for Clinical Researches, and following recruitment, the subjects gave informed consent for genetic analysis. All patients with CHB infection fulfilled the diagnostic criteria: positive for hepatitis B surface antigen (HBsAg) for 6 months or more along with serum HBV DNA with more than 105copies/ml. HBV genotyping was done by Restriction Fragment Length Polymorphism (RFLP) technique according to [35], and our patient's group are all HBV subtype D. Patients with HCV or other viral infections or any liver diseases such as HIV, NAFLD, alcoholism, severe fibrosis were excluded from the study. The control group consisted of 102 subjects chosen from healthy people who had no history of the previous liver disease, routine liver function tests, and no HBV and HCV infection or other conspicuous diseases. Viral assessment and biochemical data were performed as previously described in our previous study [20].

2.2. DNA isolation

Five ml venous blood from each involved in this study were collected on ethylene-diamine-tetra-acetic acid (EDTA) sterile vacutainer tubes. Genomic DNA was extracted from whole blood samples using the Wizard® Genomic DNA Purification Kit (Promega Co. WI, USA) according to the manufacturer's instructions.

Table 1
Primers used to detect polymorphisms of IL-6 in CHB patients and controls.

Primer	Product size
IL-6 (−174) G/C (rs1800795)	
Forward G: 5′-GCACTTTTCCCCCTAGTTGTGTCTTACG-3′	121 bp
Forward C: 5′GACGACCTAAGCTTTACTTTTCCCCCTAGTTGTGTCTTGAC-3′	136 bp
Reverse: 5′-ATAAATCTTTGTTGGAGGGTGAGG-3′	
IL-6 (−572) G/C (rs1800796)	
Forward G: 5′-GGCCAGGCAGTTCTACAACAGCCG-3′	325 bp
Forward C: 5′-GGCCAGGCAGTTCTACAACAGCCC-3	
Reverse: 5′-ATTAGTGACTCAGCACTTTGG-3′	
IL-6 (−597) G/A (rs1800797)	
Forward G: 5′-AAGTAACTGCACGAAATTTGAGGG-3′	473 bp
Forward A: 5′-AAGTAACTGCACGAAATTTGAGGA-3′	
Reverse: 5′-TGTGCAATGTGACGTCCTTTA-3′	

2.3. Genotyping

IL-6 (−174G/C) SNP was analyzed using Mutagenically separated PCR (MS-PCR) using three primer mixtures as previously described [36]. The reaction was done in one tube with 25 ml final reaction volume. PCR mixtures consisted of DreamTaq Green PCR Master Mix (2X) (Fermentas), 10 pmoles of each forward primer, 20 pmoles of reverse primer and 40 ng of DNA. The size of PCR products was visualized on 4% agarose gel and estimated in comparison to 25 bp DNA ladder (Fermentas Life Science, Thermo Fisher Scientific Inc., MA, USA).

IL-6 (−572G/C and −597G/A) SNPs were analyzed by the polymerase chain reaction sequence-specific primer method (PCR-SSP). Primer sequences used were designed by using primosnp 3.4 (http://www.changbioscience.com/primo/ primosnp.html) and checked using primer blast (http://www.ncbi.nlm.nih.gov/tools/primer-blast) (Table 1). For each SNP, the reaction was done in two tubes, one for each allele, with 25 µl final reaction volume. The PCR mixtures consisted of the DreamTaq Green Master Mix, 2x (Fermentas), 10 pmoles of each allele-specific primer, 10 pmoles of reverse primer, and 100 ng of DNA. After amplification, the PCR resulted in an amplicon of 325 bp for −527G/C and 473 bp for −597G/A. The PCR products were visualized on 2% agarose gel and estimated in comparison to 100 bp DNA ladder (Fermentas). All PCR reactions were performed in 2720 thermal cycler (Applied Biosystems). As MS-PCR or SSP-PCR is easy to result in a false-positive result, 50% of samples were repeated, and we selected 10% of samples to be sequenced randomly to control the quality of genotyping and to validate our results.

2.4. Measurement of plasma IL-6

Blood samples were collected from HBV patients and healthy controls; plasma was separated by centrifugation at 1500 rpm for 10 min, aliquotted and stored at −80 °C for IL-6 secretion analysis. IL-6 concentrations were measured using a commercial enzyme-linked immunosorbent assay (ELISA) kit (limit: 9.38–600 pg/ml) according to the manufacturer's instructions (R&D System, Inc., Minneapolis, MN). The intensity of the developed color was measured by reading optical absorbance at 450 nm using a microplate reader (SunriseTM, Tecan Group Ltd. Ma¨nnedorf, Switzerland). The ELISA reader-controlling software (Softmax) processed the digital data of raw absorbance values into a standard curve, from which the IL-6 concentration of samples can be derived, and expressed as pg/ml. IL-6,

2.5. Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Science (SPSS) version 21 (IBM Corporation, USA). Comparisons between both groups were performed by independent, and the results were presented as the mean ± standard deviation. Categorical variables were presented as frequencies (%). Chi-squared (X^2) tests were performed to compare allele, genotype and haplotype distribution. The odds ratio (OR) and 95% confidence intervals (CI) were calculated to measure the relative risks in both control and HBV patients. The correlation between variables was determined using Spearman's correlation test. The online tool SNPstats (http://bioinfo.iconcologia.net/SNPstats) was used to perform Haplotype reconstruction from population genotype data and Linkage Disequilibrium (LD) parameters (D′ and r^2) and to identify a departure from the Hardy-Weinberg equilibrium (HWE). Comparative analysis of plasma IL-6 levels among participants with different genotypes/haplotypes was carried out by One-way analysis of variance (ANOVA). All P values were two-tailed, and P values that remained below 0.05 after correction for the number of variables (P-corrected) were considered significant (Bonferroni correction).

3. Results

3.1. Patients' characteristics

In CHB group, males are overnumbered females (84 male and 24 female) with a mean age of 38.86 ± 12.08 years. Control group represented by 63 male and 39 female with mean age of 36.48 ± 14.95.

3.2. Association between IL-6 polymorphisms and HBV infection

Genotype and allelic frequencies of IL-6 (−174G/C, −572G/C and −597G/A) in CHB patients and healthy controls are presented in Table 2 and Fig. 1. Analysis of the 3 SNPs revealed that there were significant changes in the distribution of IL-6 (−174G/C, −572 G/C and −597 G/A) genotypes between patients and healthy controls. The frequency of IL-6 (−174G/C) genotypes were within Hardy-Weinberg equilibrium (HWE) in controls [75, 26, 1 (observed) versus 75.9, 24.1,1.9 (predicted)] and were out of HWE in CHB patients [99, 7, 2 (observed) versus 97.2, 10.4, 0.28 (predicted); $P < 0.05$]. On the other hand, the values predicted by assumption of the HWE were different than those observed for IL-6 (−572G/C), in CHB patients [14, 92, 2 (observed) versus 33.3, 53.3, 21.3 (predicted); $P < 0.001$] and control group [31, 69, 2 (observed) versus 42, 46.8, 13 (predicted); $P < 0.001$]. Moreover, the genotype frequencies of IL-6 (−597G/A) did not meet the equilibrium criteria in patients group [1, 106, 1 (observed) versus 27, 54, 27 (predicted); $P < 0.001$] and [32, 69, 1 (observed) versus 43.3, 46.2, 12.3 (predicted); $P < 0.001$] in controls.

Analysis of IL-6 (−174G/C) SNP showed that the most prevalent genotype in both groups is the GG genotype. A frequency of −174GG genotype increased significantly ($P < 0.01$) in CHB group, while −174GC genotype was significantly elevated ($P < 0.001$) in controls. G allele was in high frequency in both groups. A significant increase ($P < 0.01$) in −174G allele in patients parallel to the significant increase ($P < 0.01$ (in C allele in controls. A positive correlation was found between CHB and the presence of GG genotype ($r = 0.241$, $P < 0.001$), while a negative correlation was detected by the appearance of GC genotype and the C allele ($r = -0.261$, $P < 0.001$; $r = -0.241$, $P < 0.001$; respectively). GG genotype and G allele might be considered as a risk factor [OR = 3.96; CI: 1.75–8.91), OR = 2.96; CI: 1.43–6.12; respectively] for CHB infection, while GC genotype and C allele [OR = 0.203; CI: 0.08–0.49, OR = 0.33; CI: 0.163–0.697; respectively] might be considered as protective factors for the disease.

Genotyping of IL-6 (−572G/C) SNP showed an increase in GC genotypes in both groups. A significant reduction ($P < 0.01$) in −572GG genotype was observed in the patient group, while the GC genotype showed a significant increase ($P < 0.01$) in the infected group compared to controls. A direct positive correlation was found between CHB and the presence of GC genotype and C allele ($r = 0.187$, $P < 0.01$; $r = 0.212$, $P < 0.01$; respectively). GC genotype (OR = 2.51; CI: 1.27–4.94) might be considered as a risk factor for HBV infection. On the other side, an inverse correlation was found between

Table 2
Genotype distribution and allelic frequency of IL-6 (−174 G/C, −572 G/C and −597 G/A) in controls and CHB patients.

Cytokine gene	Control group (N = 102)	CHB group (N = 108)	P-value/P-corrected	OR (95% CI)
IL-6 (−174 G/C) (rs1800795)				
Genotype Frequency (N, %)				
G/G	75 (73.5%)	99 (91.7%)	**P = 0.001/Pc = 0.003**	3.96 (1.75–8.91)
G/C	26 (25.5%)	7 (6.5%)	**P = 0.000/Pc = 0.000**	0.203 (0.08–0.49)
C/C	1 (1.0%)	2 (1.9%)	P = 1.000/pc = 3.00	1.90 (0.17–21.3)
GC/CC	27 (26.5%)	9 (8.3%)	**P = 0.001/Pc = 0.003**	0.25 (0.11–0.56)
Allele Frequency (N, %)				
G	176 (87.0%)	205 (95.0%)	**P = 0.003/Pc = 0.006**	2.96 (1.43–6.12)
C	28 (13.0%)	11 (5.0%)	**P = 0.003/Pc = 0.006**	0.33 (0.163–0.697)
IL-6 (−572) G/C (rs1800796)				
Genotype Frequency (N, %)				
G/G	31 (30.4%)	14 (13.0%)	**P = 0.002/Pc = 0.006**	0.34 (0.16–0.68)
G/C	69 (67.6%)	92 (85.2%)	**P = 0.008/Pc = 0.024**	2.51 (1.27–4.94)
C/C	2 (2.0%)	2 (1.9%)	P = 1.00	0.94 (0.13–6.82)
GC/CC	71 (69.6%)	94 (87.0%)	**P = 0.002/Pc = 0.006**	2.93 (1.45–5.91)
Allele Frequency (N, %)				
G	131 (64.0%)	120 (56.0%)	P = 0.07/pc = 0.014	0.696 (0.470–1.03)
C	73 (36.0%)	96 (44.0%)	P = 0.07/pc = 0.014	1.435 (0.969–2.12)
IL-6 (−597 G/A) (rs1800797)				
Genotype Frequency (N, %)				
G/G	32 (31.4%)	1 (0.9%)	**P = 0.000/Pc = 0.000**	0.02 (0.02–1.46)
G/A	69 (67.6%)	106 (98.1%)	**P = 0.000/Pc = 0.000**	25.3 (5.80–109.0)
A/A	1 (1.0%)	1 (0.9%)	P = 1.00/pc = 3.00	0.94 (0.05–15.2)
GA/AA	70 (68.6%)	107 (99.1%)	**P = 0.000/Pc = 0.000**	51.1 (6.84–382.8)
Allele Frequency (N, %)				
G	133 (65.0%)	108(50.0%)	**P = 0.001/Pc = 0.002**	0.53 (0.36–0.79)
A	71 (35.0%)	108 (50.0%)	**P = 0.001/Pc = 0.002**	1.87 (1.26–2.77)

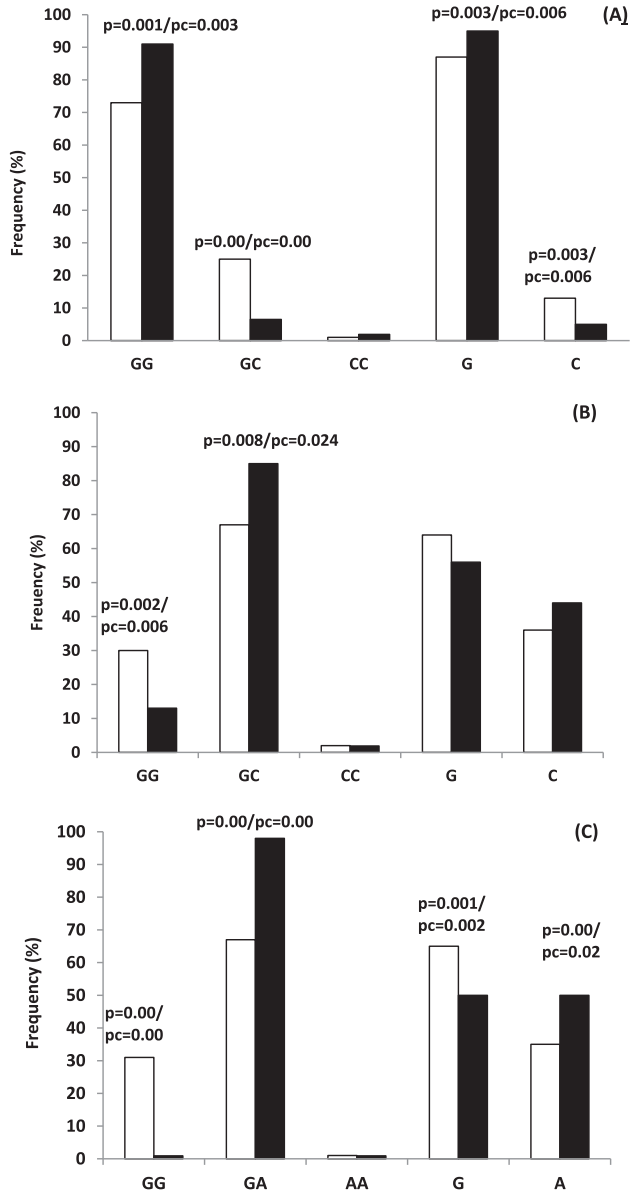

Fig. 1. Genotype distribution and allelic frequency of IL-6–174 G/C (rs1800795) (A), −572G/C (rs1800796) (B) and −597 G/A (rs1800797) (C) gene polymorphism in controls (□) and HBV (■) patients.

chronic infection and GG genotype (r = −0.212, P < 0.01), so it might be considered as a protective factor (OR = 0.34; CI: 0.16–0.68) from the infection.

Analysis of IL-6 (−597G/A) SNP revealed that GA genotype was the most frequent genotype in both groups. −597GG genotype decreased significantly (P < 0.001) in the control group, while −597GA genotype increased significantly (P < 0.001) in the CHB group. A significant elevation (p < 0.01) in the A allele was observed in the patient group combined with a significant reduction (p < 0.01) in G allele compared with healthy controls. GA genotype and A allele are positively correlated with the disease (r = 0.409, P < 0.001; r = 0.410, P < 0.001; respectively). On the other hand, the GG genotype is negatively correlated with the disease (r = −0.426, P < 0.001).

The frequency of IL-6 (−174G/C, −572 G/C and −597 G/A) haplotypes in HBV patients and healthy controls is shown in Table 3. Eight haplotypes were emerged (GGG, GCA, GGA, GCG, CGG, CCA, CCG, and CGA) from the estimates of haplotype frequencies. The GGG was the most frequent one in controls and, the GGA was the most frequent in patients while CGG, CCA, CCG, and CGA haplotypes were rare in both groups. A significant (P < 0.05) increase in GGA haplotype and a highly significantly (P < 0.001) decrease in GCA haplotype were detected in CHB patients compared to controls. LD pattern between the 3 SNPs showed (D′ = 0.275, r2 = 0.011; P < 0.05) between IL-6 (−174G/C) and (−572G/C),and (D′ = 0.719, r2 = 0.474; P < 0.001 between IL-6 (−572G/C) and (−597G/A). The latest correlation pointed to a moderate LD between IL-6 (−572G/C and −597G/A) and a strong linkage equilibrium between IL-6 (−174G/C and −597 G/A).

3.3. Differential expression of IL-6 in patients and controls according to polymorphisms and haplotypes

A significant decrease (P < 0.01) in IL-6 in CHB patients compared to healthy controls (22.28 ± 1.93 versus 32.08 ± 2.41) was observed with an inverse correlation (r = −0.216; P < 0.01) between IL-6 secretion level and HBV infection.

As shown in (Fig. 2), the mean plasma concentration of IL-6 (−174 G/C) was decreased significantly (P < 0.001, and P < 0.001) in CHB patients with GG genotype and G allele; respectively. In IL-6 (−572G/C), GC genotype, G and C alleles showed a significant (P < 0.01, P < 0.01 and P < 0.01; respectively) decrease in IL-6 levels in patients compared with controls. The same results were observed in IL-6 (−597 G/A) where IL-6 was diminished in patients with GA genotype, G and A alleles (P < 0.01, P < 0.01 and P < 0.01; respectively). According to the estimated haplotypes, the GGG and GCA haplotypes showed significant (P < 0.001 and P < 0.01; respectively) reduction in IL-6 levels in HBV patients versus controls (Table 4).

4. Discussion

Several reports have implicated CHB infection as a significant risk factor in the causation of HCC in humans [37,38]. Egypt has been classified as an area of intermediate HBV prevalence comprising nearly 6.7% of the population [39]. IL-6, a well-recognized cytokine as a critical regulator of inflammatory mechanisms, has been documented to play a vital role in HBV infection mediating chronic inflammation [11,38]. Studies on the cytokine gene polymorphisms point out to its role in the evolution of CHB infection [10], and as IL-6 might influence the development of chronic infection, SNPs of IL-6 gene can be selected as a useful marker for association study. Therefore, we conducted this study to better clarify the association between IL-6 (−174G/C, −572G/C, −597G/A) promoter and the risk of HBV infection.

All positions were out of HWE except those observed for IL-6 (−174 G/C) in the control group. Apprehension was directed toward a departure from HWE reported in our study which might be due to chance or infringement of one or more HWE assumptions. Results from around 10% of previous genetic case-control studies have shown formally statistically significant deviations from the HWE expected frequencies [40,41]. The power of available statistical tests to detect HWE violation is limited in such relatively small sample sizes study

Our data revealed that the G allele and the G/G genotype at position −174 had higher distribution among Egyptian CHB patients in comparison to controls. The −174GC genotype and C allele were significantly reduced in the patient group showing a negative correlation with the infection. Our results are consistent with the results of Fabris et al. [42] which reported a relationship between IL6 −174G/C polymorphism and HBV infection. In addition to the study of Attar et al. [14] which pointed out that the IL-6 −174GC genotype and G allele were strongly associated with susceptibility to HBV infection. Some other studies on different other populations such as; Zhai et al. [43] in Chinese, Ben-Ari et al. [19] from Israel, Park, et al. [44] in Koreans, Ribeiro et al. [10] in a Brazilian, Migita et al. [45] in a Japanese population showed no genetic association between IL-6 (−174G/C) SNP and the susceptibility to CHB infection. The same results were reported

Table 3
Haplotype frequencies of the IL-6 (−174G/C, −572 G/C and −597 G/A) haplotypes in control and CHB patients.

Haplotype	Control group (N = 102)	CHB group (N = 108)	P value/p-corrected	OR (95% CI)
GGG	50 (48.5%)	8 (7.6%)	–	1.00
GCA	18 (17.8%)	2 (1.1%)	**P = 0.0002/pc = 0.0006**	0.03 (0.00—0.17)
GGA	10 (9.9%)	50 (45.8%)	**P = 0.012**/pc = 0.36	0.08 (0.01–0.56)
GCG	11 (10.6%)	44 (40.2%)	P = 0.07/pc = 0.21	0.67 (0.09–5.01)
CGG	6 (5.4%)	0 (0%)		
CCA	7 (6.8%)	0 (0%)		
CCG	0 (0%)	2 (2%)		
CGA	0 (0%)	2 (2%)		

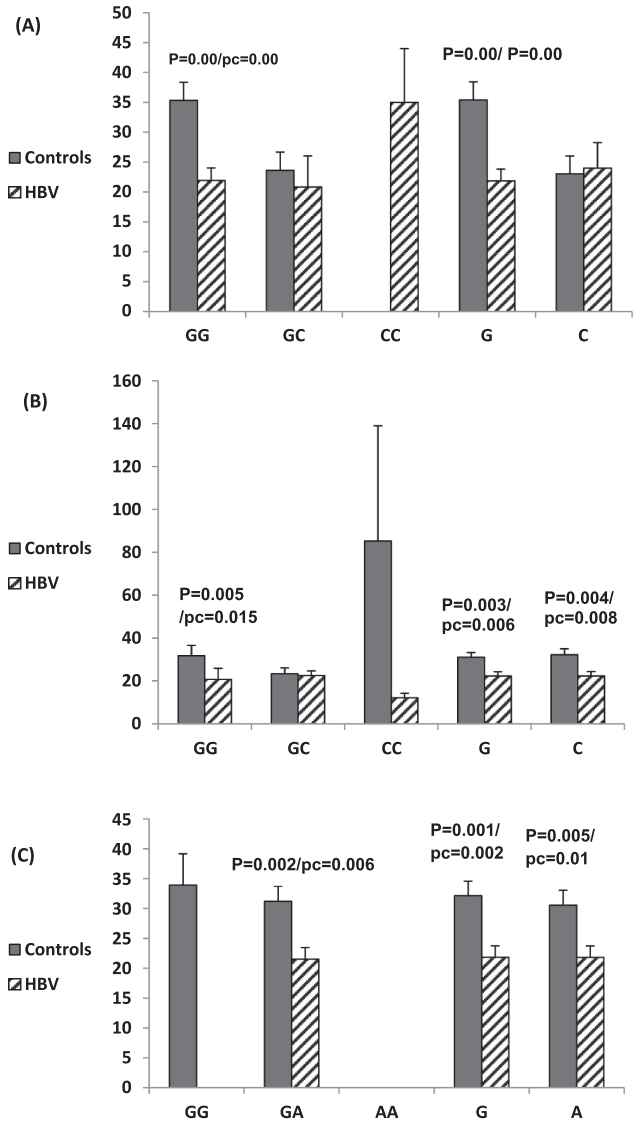

Fig. 2. Mean plasma concentrations of IL-6 (pg/dl) according to IL-6 (−74) G/C (rs1800795) (A), IL-6 (−572 G/C) (rs1800796) (B) and IL-6 (−597 G/A) (rs1800797) (C) in controls and HBV patients. Data are presented as mean ± SEM.

in a *meta*-analysisof Chang et al. [2] who did not find any association of IL-6 (−174G/C) and HBV risk.

The current study illuminated that IL-6 −572GC genotype had higher distribution in CHB patients (85.2%) compared to controls. In accordance with us, the results of Saxena et al. [38] pointed out that the GC genotype was distributed in 49.02% of CHB patients. On the other hand, −572CC genotype distribution is higher in two separate Taiwanese studies (65% and 59%, respectively) [6,45]. The results of our

Table 4
Mean plasma concentrations of IL-6 according to IL-6 Haplotype in controls and CHB patients.

Haplotype	Control group (N = 102) (Mean ± SE)	CHB group (N = 108) (Mean ± SE)	P-value/p-corrected
GGG	33.34 ± 2.49	21.76 ± 1.98	**P = 0.000/pc = 0.00**
GCA	35.13 ± 3.73	21.78 ± 2.24	**P = 0.002/pc = 0.006**
GGA	28.06 ± 4.53	22.84 ± 5.30	P = 0.468/pc = 1.404
GCG	34.56 ± 7.04	26.11 ± 13.99	P = 0.626/pc = 2.334
CGG	22.80 ± 6.08	35.00 ± 9.00	P = 0.317/pc = 0.951
CCA	24.45 ± 4.56	23.99 ± 4.72	P = 0.949/pc = 2.847
CCG	19.95 ± 0.85	–	–
CGA	13.20 ± 5.89	–	–

IL-6 data is expressed as pg/ml.

study demonstrated that (−572G/C) SNP in the IL-6 gene was associated with an increased risk of CHB infection in heterozygous and C allele. In accordance with these findings, several studies demonstrated that the IL-6 −572G/C might be associated with the risk of HBV infection [2,13,38,46]. On the other hand, other studies could not characterize definitive pieces of evidence supporting the involvement of the IL6-572 G/C SNP andCHB infection [12,44].

Our study sheds light on the IL-6 (−597G/A) role in the susceptibility to CHB infection. IL-6 −597GA genotype had higher distribution in CHB patients than controls, with a rarity in the A allele in both groups. A few studies examined the association of the IL-6 (−597G/A) SNP with HBV infection. In general, our data showed that the IL-6 −597G/A might play a part in the susceptibility to CHB in Egyptians as −597GA genotype, and A allele are increased concerning other genotypes and G allele. Consistent with our results Chang et al. [2] meta-analysis demonstrated that IL-6 (−597 G/A) SNP might play a role in CHB susceptibility. In contrary to ours, the study of Saxena et al. [39] demonstrated a predominance of −597GG genotype in CHB and control subjects. Likewise, the study of Park et al. [44] pointed to an extremely rare in −597GA genotype in the Korean population. Inconsistent with our results, results of Park et al. [44] demonstrated that −597G/A SNP is unlikely to be contributing significantly to CHB susceptibility. The different results of IL-6 promoter SNPs (−174 G/C, −572G/C and −597 G/A) between studies might be due to the different genetic background of ethnic diversity.

We demonstrated a significant reduction in the IL-6 level in CHB patients compared to controls. This result is in disagreement with some other studies that noticed a high level of IL-6 in CHB patients [12,38,47,48]. The reduction of the IL-6 level documented in our patient group was an obscure result for us. In accordance with our data, aprevious study on Egyptian HBV infected patients reported a reduction of the IL-6 level and overexpression of sIL-6R in HCC and liver diseased groups [49]. It appeared that IL-6 could play decisive roles in the induction of immune-tolerance against HBV antigens, and its activity is probably involved in determining of HBV outcomes. Considering 10^5copies/ml viral load and supporting to our results, several studies referred to the participation of IL-6 immune response controlling viral

infection through blocks of HBV replication by a moderate reduction of several viral transcripts as IL-4 and TGF-β1 [50]. In this context, Talaat et al. [20] demonstrated low levels of TGF-β1 in HBV infected Egyptian patients. This antiviral effect of IL-6 on HBV replication is mediated by a reduction of transcription factors [51] and by the redistribution of STAT3 binding from the viral cccDNA to IL-6 cellular target genes [52]. Many studies have shown that IL-6 serum levels are increased in HBV patients with severe, acute infections than patients with chronic infection [27]. They speculate that; in patients with HBV-related diseases, the IL-6 blockade could raise treatment efficacy.

In the analysis of SNPs with IL-6 expression level, we noticed a significant reduction in CHB patients with −174GG genotype and G allele compared to healthy subjects. No significant change in IL-6 secretion level between CHB patients with GG and GC genotypes. The study of Ben-Ari et al. [19] found no difference in the genetic ability to produce IL-6, between the HBV group and the controls, only −174CC genotype is associated with low levels of IL-6 while, −174 (GG and GC) genotypes correlated with the ability to produce high levels of IL-6.

In the case of IL-6 (−572G/C), we noticed a low production of IL-6 CC compared to GC and GG genotypes in the patient group although it did not reach statistical significance. Two studies indicated that possessing an IL-6 −572GG genotype associated with the reduction in IL-6 levels while the −572 CC genotype is associated with high levels of IL-6 [13,42]. In relation to IL-6 −597G/A SNP with IL-6 plasma levels, our results could not compare this SNP with production levels due to the low distribution of −597 GG and CC genotypes also, G and C allele have the same value. The study of Saxena et al.[53] showed no significant difference in IL-6 levels in any of the genotypes of IL-6 −597G/A.

5. Conclusion

The presented results support the view that IL-6 (−174G/C, −572G/C and −597G/A) gene polymorphisms are associated with susceptibility to HBV infection in Egyptians. IL-6 −174GG genotype, −572GC genotype and C allele in addition to −597GA genotype and A allele might be considered as risk factors for CHB infection. Our data could provide valuable clues for understanding the mechanism under laying viral infection. Although, this work allows for only preliminary conclusions due to relatively small sample size and should be confirmed in a bigger size population to validate these findings, and to fully elucidate the possible relationship between cytokine gene polymorphisms–interplay with other genetic and immunological factors participating in the CHB.

Conflict of interest

The authors declare no conflicts of interest.

Acknowledgment

This work was funded by National Liver Institute (NLI); Menoufiya University, Egypt.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.humimm.2018.12.009.

References

[1] X.S. Liang, C.Z. Li, Y. Zhou, W. Yin, Y.Y. Liu, W.H. Fan, Changes in circulating Foxp3+ regulatory T cells and interleukin-17-producing T helper cells during HBV-related acute-on-chronic liver failure, World J. Gastroenterol. 20 (26) (2014) 8558.
[2] L. Chang, T. Lan, L. Wu, C. Li, Y. Yuan, Z. Liu, The association between three IL-6 polymorphisms and HBV-related liver diseases: a meta-analysis, Int J. Clin. Exp. Med. 8 (2015) 17036–17045 PMID: 26770294.
[3] S.Y.C. Lin, H. Toyoda, T. Kumada, H.-F. Liu, Molecular evolution and phylodynamics of acute hepatitis B virus in Japan, PLoS One 11 (2016), https://doi.org/10.1371/journal.pone.0157103 e0157103 PMID: 27280441.
[4] A. Alexopoulou, P. Karayiannis, HBeAg negative variants and their role in the natural history of chronic hepatitis B virus infection, World J. Gastroenterol. 20 (2014) 7644–7652, https://doi.org/10.3748/wjg. v20.i24.7644 PMID: 24976702.
[5] S. Tunçbilek, Relationship between cytokine gene polymorphisms and chronic hepatitis B virus infection, World J. Gastroenterol. 20 (2014) 6226–6235, https://doi.org/10.3748/wjg.v20.i20.6226 PMID: 24876743.
[6] C.H. Chen, C.M. Lee, S.N. Lu, C.S. Changchien, H.L. Eng, C.M. Huang, J.H. Wang, C.H. Hung, T.H. Hu, Clinical significance of hepatitis B virus (HBV) genotypes and precore and core promoter mutations affecting HBV e antigen expression in Taiwan, J. Clin. Microbiol. 43 (2005) 6000–6006, https://doi.org/10.1128/JCM.43.12.6000-6006.2005 PMID: 16333089.
[7] A. Schweitzer, J. Horn, T.T. Mikolajczyk, G. Krause, J.J. Ott, Estimations of worldwide prevalence of chronic hepatitis B virus infection: a systematic review of data published between 1965 and 2013, Lancet 386 (10003) (2015) 1546–1555.
[8] M.M. Ledesma, O. Galdame, B. Bouzas, L. Tadey, B. Livellara, S. Giuliano, M. Viaut, S. Paz, H. Fainboim, A. Gadano, R. Campos, D. Flichman, Characterization of the basal core promoter and precore regions in anti-HBe-positive inactive carriers of hepatitis B virus, Int. J. Infect. Dis. 15 (2011) e314–e320, https://doi.org/10.1016/j.ijid.2010.12.009 PMID: 21367634.
[9] S. Abd-Elsalam, N. Elwan, H. Soliman, D. Ziada, W. Elkhalawany, M. Salama, N. Hawash, M. Arafa, R. Badawi, W.M. Shehata, H.S. Khalil, N. Elmashad, Epidemiology of liver cancer in Nile delta over a decade: a single-center study, South Asian J. Cancer 7 (1) (2018) 24–26, https://doi.org/10.4103/sajc.sajc_82_17 PMID: 21367634.
[10] C.S. Ribeiro, J.E. Visentainer, R.A. Moliterno, Association of cytokine genetic polymorphism with hepatitis B infection evolution in adult patients, Mem. Inst. Oswaldo Cruz 102 (2007) 435–440 PMID: 17612762.
[11] X.M. Zhao, Y.F. Gao, Q. Zhou, F.M. Pan, X. Li, Relationship between interleukin-6 polymorphism and susceptibility to chronic hepatitis B virus infection, World J. Gastroenterol. 19 (2013) 6888–6893, https://doi.org/10.3748/wjg.v19.i40.6888 PMID: 24187466.
[12] S. Tang, Z. Liu, Y. Zhang, Y. He, D. Pan, Y. Liu, Q. Liu, Z. Zhang, Y. Yuan, Rather than Rs1800796 polymorphism, expression of interleukin-6 is associated with disease progression of chronic HBV infection in a Chinese Han population, Dis. Markers 35 (2013) 799–805, https://doi.org/10.1155/2013/508023 PMID: 24371367.
[13] X. Zhang, H.G. Ding, Key role of hepatitis B virus mutation in chronic hepatitis B development to hepatocellular carcinoma, World J. Hepatol. 7 (2015) 1282–1286, https://doi.org/10.4254/wjh.v7.i9.1282.
[14] M. Attar, S.S. Azar, M. Shahbazi, Interleukin-6-174 promoter polymorphism and susceptibility to hepatitis b virus infection as a risk factor for hepatocellular carcinoma in Iran, Asian Pac. J. Cancer Prev. 17 (2016) 2395–2399 PMID: 27268603.
[15] P. Kummee, P. Tangkijvanich, Y. Poovorawan, N. Hirankarn, Association of HLA-DRB1*13 and TNF-alpha gene polymorphisms with clearance of chronic hepatitis B infection and risk of hepatocellular carcinoma in Thai population, J. Viral. Hepat. 14 (2007) 841–848, https://doi.org/10.1111/j.1365-2893.2007.00880.x PMID: 18070287.
[16] M.J. Koziel, Cytokines in viral hepatitis, Semin. Liver Dis. 19 (1999) 157–169, https://doi.org/10.1055/s-2007-1007107 PMID: 10422198.
[17] M. Rapicetta, C. Ferrari, M. Levrero, Viral determinants and host immune responses in the pathogenesis of HBV infection, J. Med. Virol. 67 (2002) 454–457, https://doi.org/10.1002/jmv.10096 PMID: 12116045.
[18] Y. Lu, J.G. Bao, Y. Deng, C.Z. Rong, Y.Q. Liu, X.L. Huang, L.Y. Song, S. Li, X. Qin, Role of IL-18 gene promoter polymorphisms, serum il-18 levels, and risk of hepatitis b virus-related liver disease in the guangxi zhuang population: a retrospective case-control study, Asian Pac. J. Cancer Prev. 16 (2015) 6019–6026 PMID: 26320490.
[19] Z. Ben-Ari, E. Mor, O. Papo, B. Kfir, J. Sulkes, A.R. Tambur, R. Tur-Kaspa, T. Klein, Cytokine gene polymorphisms in patients infected with hepatitis B virus, Am. J. Gastroenterol. 98 (2003) 144–150, https://doi.org/10.1111/j.1572-0241.2003.07179.x PMID: 12526950.
[20] R.M. Talaat, M.F. Dondeti, S.Z. El-Shenawy, O.A. Khamiss, Transforming growth factor– β 1 gene polymorphism (T29C) in Egyptian patients with hepatitis B virus infection: a preliminary study, Hepat. Res. Treat. 2013 (2013), https://doi.org/10.1155/2013/293274.
[21] R.M. Talaat, M.F. Dondeti, S.Z. El-Shenawy, O.A. Khamiss, Association between IL-10 gene promoter polymorphism and hepatitis B viral infection in an Egyptian population, Biochem. Genet. 52 (2014) 387–402, https://doi.org/10.1007/s10528-014-9655-8 PMID: 24838671.
[22] R.M. Talaat, M.S. Abdelkhalek, E.A. El-Maadawy, W.S. Abdel-Mageed, S.Z. El-Shenawy, M.A. Osman, Association of TNF-Alpha gene polymorphisms and susceptibility to hepatitis B virus infection in Egyptians, Hum. Immunol. 78 (2017) 739–746, https://doi.org/10.1016/j.humimm.2017.10.006.
[23] J.R. Prins, N. Gomez-Lopez, S.A. Robertson, Interleukin-6 in pregnancy and gestational disorders, J. Reprod Immunol. 95 (2012) 1–14, https://doi.org/10.1016/j.jri.2012.05.004 PMID: 22819759.
[24] K. Ishihara, T. Hirano, IL-6 in autoimmune disease and chronic inflammatory proliferative disease, Cytokine Growth Factor Rev. 13 (2002) 357–368, https://doi.org/10.1016/S1359-6101(000027-8) PMID: 12220549.
[25] N. Nishimoto, T. Kishimoto, Interleukin 6: From bench to bedside, Nat. Clin. Pract. Rheumatol. 2 (2006) 619–626, https://doi.org/10.1038/ncpheum0338 PMID: 17075601.
[26] C.J. Pan, H.L. Wu, S.F. Kuo, J.H. Kao, T.C. Tseng, C.H. Liu, P.J. Chen, C.J. Liu, D.S. Chen, Serum interleukin 6 level correlates with outcomes of acute exacerbation

of chronic hepatitis, B. Hepatol. Int. 6 (2012) 591–597, https://doi.org/10.1007/s12072-011-9299-2 PMID: 21769441.
[27] T. Lan, L. Chang, L. Wu, Y.F. Yuan, IL-6 plays a crucial role in HBV infection, J. Clin. Transl. Hepatol. 3 (4) (2015) 271–276, https://doi.org/10.14218/JCTH.2015.00024 Epub 2015 Dec 15.
[28] E. Galun, O. Nahor, A. Eid, O. Jurim, S. Rose-John, H.E. Blum, O. Nussbaum, E. Ilan, N. Daudi, D. Shouval, Y. Reisner, S. Dagan, Human interleukin-6 facilitates hepatitis B virus infection in vitro and in vivo, Virology 270 (2000) 299–309, https://doi.org/10.1006/viro.2000.0210 PMID: 10792989.
[29] W.E. Naugler, T. Sakurai, S. Kim, S. Maeda, K. Kim, A.M. Elsharkawy, M. Karin, Gender disparity in liver cancer due to sex differences in MyD88-dependent IL-6 production, Science, 317 (2007) 121–124, https://doi.org/10.1126/science.1140485 PMID: 17615358.
[30] W.E. Naugler, M. Karin, The wolf in sheep's clothing: the role of interleukin-6 in immunity, inflammation and cancer, Trends Mol. Med. 14 (2008) 109–119, https://doi.org/10.1016/j.molmed.2007.12.007 PMID: 18261959.
[31] K.J.H. Lim, O.A. Odukoya, T.C. Li, I.D. Cooke, Cytokines and immuno-endocrine factors in recurrent miscarriage, Hum. Reprod Update 2 (1996) 469–481 PMID: 9111182.
[32] K. Amr, R. El-Awady, H. Raslan, Assessment of the -174G/C (rs1800795) and -572G/C (rs1800796) Interleukin 6 Gene Polymorphisms in Egyptian Patients with Rheumatoid Arthritis, Open Access Maced. J. Med. Sci. 15;4(4) (2016) 574–577.
[33] R.M. Talaat, A.M. Abdel-Aziz, E.A. El-Maadawy, N. Abdel-Bary, CD38 and interleukin 6 gene polymorphism in egyptians with diffuse large B-cell lymphoma (DLBCL), Immunol. Invest. 44 (3) (2015) 265–278, https://doi.org/10.3109/08820139.2014.989328.
[34] A. Settin, A. Ismail, M.A. El-Magd, R. El-Baz, A. Kazamel, Gene polymorphisms of TNF-alpha-308 (G/A), IL-10(-1082) (G/A), IL-6(-174) (G/C) and IL-1Ra (VNTR) in Egyptian cases with type 1 diabetes mellitus, Autoimmunity 42 (1) (2009) 50–55, https://doi.org/10.1080/08916930802292510.].
[35] M. Lindh, C. Hannoun, A.P. Dhillon, G. Norkrans, P. Horal, Core promoter mutations and genotypes in relation to viral replication and liver damage in East Asian hepatitis B virus carriers, J. Infect. Dis. 179 (4) (1999) 775–782.
[36] R.M. Talaat, A.M. Abdel-Aziz, E.A. El-Maadawy, N. Abdel-Bary, CD38 and interleukin 6 gene polymorphism in Egyptians with diffuse large B-cell lymphoma (DLBCL), Immunol. Invest. 44 (2015) 265–278, https://doi.org/10.3109/08820139.2014.989328 PMID: 25564959.
[37] M.C. Kew, Interaction between hepatitis B and C viruses in hepatocellularcarcinogenesis, J. Viral Hepat. 13 (2006) 145–149, https://doi.org/10.1111/j.1365-2893.2005.00686.x PMID: 16475989.
[38] R. Saxena, Y.K. Chawla, I. Verma, J. Kaur, IL-6 (-572/-597) polymorphism and expression in HBV disease chronicity in an Indian population, Am. J. Hum. Biol. 26 (2014) 549–555, https://doi.org/10.1002/ajhb.22562 PMID: 24841049.
[39] H.S. Te, D.M. Jensen, Epidemiology of hepatitis B and C viruses: a global overview, Clin. Liver Dis. 14 (2010) 1–21, https://doi.org/10.1016/j.cld.2009.11.009 vii PMID: 20123436.
[40] Z. Bardoczy, B. Gyorffy, I. Kocsis, B. Vásárhelyi, Re-calculated Hardy-Weinberg values in papers published in Atherosclerosis between 1995 and 2003, Atherosclerosis 173 (2004) 141–143.
[41] G. Salanti, G. Amountza, E.E. Ntzani, J.P. Ioannidis, Hardy-Weinberg equilibrium in genetic association studies: an empirical evaluation of reporting, deviations, and power, Eur. J. Hum. Genet. 13 (2005) 840–848.
[42] C. Fabris, P. Toniutto, D. Bitetto, G. Fattovich, E. Falleti, E. Fontanini, A. Cussigh, R. Minisini, G. Occhino, M. Pirisi, Gene polymorphism at the interleukin 6–174 G > C locus affects the outcome of chronic hepatitis B, J. Infect. 59 (2009) 144–145, https://doi.org/10.1016/j. jinf.2009.06.005 PMID: 19595462.
[43] R. Zhai, G. Liu, C. Yang, C. Huang, C. Wu, D.C. Christiani, The G to C polymorphism at -174 of the interleukin-6 gene is rare in a Southern Chinese population, Pharmacogenetics 11 (2001) 699–701 PMID: 11692078.
[44] B.L. Park, H.S. Lee, Y.J. Kim, J.Y. Kim, J.H. Jung, L.H. Kim, H.D. Shin, Association between interleukin 6 promoter variants and chronic hepatitis B progression, Exp. Mol. Med. 35 (2003) 76–82, https://doi.org/10.1038/emm. 2003.11 PMID: 12754410.
[45] K. Migita, S. Miyazoe, Y. Maeda, M. Daikoku, S. Abiru, T. Ueki, K. Yano, S. Nagaoka, T. Matsumoto, K. Nakao, K. Hamasaki, H. Yatsuhashi, H. Ishibashi, K. Eguchi, Cytokine gene polymorphisms in Japanese patients with hepatitis B virus infection–association between TGF-beta1 polymorphisms and hepatocellular carcinoma, J. Hepatol. 42 (2005) 505–510, https://doi.org/10.1016/j.jhep.2004.11.026 PMID: 15763337.
[46] X.Q. Qiu, C.H. Bei, H.P. Yu, X.Y. Zeng, Q.A. Zhong, 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 (2011) 510–513 PMID: 21569738.
[47] W. Song, F. Zhang, Z.A. Li, A quantitative analysis of IL-6 mRNA expression of peripheral blood monocyte cell in patients with chronic hepatitis B, Zhonghua Ganzangbing Zazhi 8 (2008) 346–347 PMID: 11135692.
[48] H. Song le, V.Q. Binh, D.N. Duy, J.F. Kun, T.C. Bock, P.G. Kremsner, A.J. Luty, Serum cytokine profiles associated with clinical presentation in Vietnamese infected with hepatitis B virus, J. Clin. Virol. 28 (2003) 93–103 PMID: 12927756.
[49] A.R. Zekri, A.A. Bahnassy, S.A. Abdel-Wahab, M.M. Khafagy, S.A. Loutfy, H. Radwan, S.M. Shaarawy, Expression of pro- and anti-inflammatory cytokines in relation to apoptotic genes in Egyptian liver disease patients associated with HCV-genotype-4, J. Gastroenterol. Hepatol. 24 (2009) 416–428, https://doi.org/10.1111/j.1440-1746.2008.05699.x PMID:19054267.
[50] C. Xia, Y. Liu, Z. Chen, M. Zheng, Involvement of interleukin 6 in hepatitis B viral infection, Cell Physiol. Biochem. 37 (2015) 677–686, https://doi.org/10.1159/000430386 PMID: 26343270.
[51] M. Hosel, M. Quasdorff, K. Wiegmann, D. Webb, U. Zedler, M. Broxtermann, R. Tedjokusumo, K. Esser, S. Arzberger, C.J. Kirschning, et al., Not interferon, but interleukin-6 controls early gene expression in hepatitis B virus infection, Hepatology 50 (2009) 1773–1782, https://doi.org/10.1002/hep.23226.
[52] G.A. Palumbo, C. Scisciani, N. Pediconi, L. Lupacchini, D. Alfalate, F. Guerrieri, L. Calvo, D. Salerno, S. Di Cocco, M. Levrero, et al., IL-6 inhibits HBV transcription by targeting the epigenetic control of the nuclear cccDNA minichromosome, PLoS One 10 (2015) e0142599.
[53] R. Saxena, J. Kaur, Th1/Th2 cytokines and their genotypes as predictors of hepatitis B virus related hepatocellular carcinoma, World J. Hepatol. 7 (2015) 1572–1580, https://doi.org/10.4254/wjh.v7.i11.1572 PMID: 26085916.