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The Effect of IP-10 Level and HLA-DP/DQ Polymorphisms on Response to Nucleoside/ Nucleotide Analogues Treatment among Hepatitis B Egyptian Patients

Amany Abdel-Kader Ghazy[1], Amel Gaber El-Sheredy[2*], Khaled Mohei Al-Din[3], Mohammed Khatab[1] and Zakia Ahmed Abdel-Rahman[1]

[1]Department of Immunology and Allergy, Medical Research Institute, Alexandria University, Egypt.
[2]Department of Microbiology, Medical Research Institute, Alexandria University, Egypt.
[3]Department of Tropical Medicine, Faculty of Medicine, Alexandria University, Egypt.

Authors' contributions

This work was carried out in collaboration between all authors. Authors AAKG, AGES and MK designed the study, wrote the protocol, performed the laboratory work, performed the statistical analysis, managed literature searches and wrote the first draft of the manuscript. Author KMAD recruited the cases and performed critical review of the manuscript. Author ZAAR participated in the planning of the study, performed the statistical analysis, shared in management of the literature searches and critical review of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Background: The efficacy of anti-viral drugs used in treatment of HBV is high but these drugs are expensive, associated with many side effects and drug resistance has evolved. Thus selection of patients with the highest probability of response is essential for clinical practice. Recent genome-wide association studies have revealed some polymorphisms in the HLA region to be associated with liver diseases, susceptibility for HBV infection, chronic HBV infection or even spontaneous HBV clearance. Additionally, IP-10 is one of the main chemokine in the recruitment of immune cells to the liver and induction of cellular immunity against viral infections, especially HBV.
Aims: This study aimed to investigate HLA-DQ.rs3920, HLA-DP.rs3077 SNP and IP-10 serum

**Corresponding author: E-mail: amelelsheredy@yahoo.com;*

levels in relations to nucleoside/ nucleotide analogues (NAs) treatment among CHB infected Egyptian patients.
Subjects and Methods: The current study was conducted on 30 HBV infected patients (naïve, responder and non-responder) and 10 healthy volunteers, as control group. Measuring HBV DNA levels using real time PCR, IP-10 levels using ELISA and genotyping of HLA-DQ.rs3920, HLA-DP.rs3077 SNP using 5' nuclease assay were done.
Results: The expression of HLA-DP rs3077 allele A was higher among naïve and non-responder HBV patients while expression of rs3077 allele G was higher among responder HBV patients and healthy controls. While for HLA-DQ rs3920, the GG genotype was highly expressed in the different groups of HBV patients. Correlation analysis revealed association between HLA-DQ.rs3920 AA genotype and HBV-DNA in non-responder. IP-10 level was significantly increased in non-responder group compared with naïve, responder control groups, especially in patients carrying HLA-DP-rs3077 AG allele and those carrying HLA-DQ-rs3920 GG allele.
Conclusion: Genetic variations of HLA-DP.rs3077 and HLA-DQ.rs3920 could be associated with HBV risk and resistance to treatment with nucleoside/ nucleotide analogues. Moreover, IP-10 could be a promising predictor of response to treatment among HBV infected patients.

Keywords: Hepatitis viruses; IP-10; HLA polymorphisms; nucleoside/nucleotide analogues.

ABBREVIATIONS

HBV: Hepatitis B virus, CHB: chronic Hepatitis B, IP-10: Interferon gamma-induced protein 10, SNP: single nucleotide polymorphism, NAs: nucleotide analogues, ETR: end of treatment response.

1. INTRODUCTION

Hepatitis viruses infect the liver, causing both acute and chronic diseases. HBV infection is a major global health problem [1], according to WHO, approximately 2 billion people worldwide have been infected with HBV, 350 million people are chronic carriers of the virus and 600 000 die each year as a result of either acute or chronic infections with the virus [2]. In addition, approximately 60% of liver cancers are related to CHB and the subsequent liver cirrhosis. These HBV-related diseases impose a considerable economic burden as well as morbidity on patients, families, and society [3].

CHB sufferers are treated with interferon and anti-viral drugs. Alfa-interferon suppresses the viral replication and enhances T lymphocyte response, but it has a lot of side effects and only 40 % efficacy rate of eliminating CHB infection. Current antiviral strategies for CHB aim at effective viral suppression as well as restoration of HBV-specific immune responses [4]. Unfortunately there have been reports of resistance among CHB patients [5].

IP-10 is CXC chemokine that bind to the cell surface chemokine receptor CXCR3, which is highly expressed on effector T cells and plays an important role in T cell trafficking and function [6]. It can be secreted by hepatocytes and sinusoidal endothelium in the liver of hepatitis patients [7]. This chemokine plays important roles in appropriate immune response and is one of the main chemokines in the recruitment of immune cells to the liver and induction of cellular immunity against viral infections, especially HBV [6].

Recent genome-wide association studies have revealed some polymorphisms in the HLA region on chromosome 6p21 to be associated with liver diseases [8], susceptibility for HBV infection [9], chronic HBV infection [10,11] or even spontaneous HBV clearance [12]. Furthermore, given the complexity of HBV infection and its progression, gene-gene and gene-environment interactions should also be taken into consideration.

But because of population heterogeneity, different races would have different causative polymorphisms. Therefore, this study aimed to investigate HLA-DQ.rs3920, HLA-DP.rs3077 SNP and IP-10 serum levels relations to nucleoside/ nucleotide analogues (NAs) treatment among CHB infected Egyptian patients.

2. SUBJECTS AND METHODS

This study was conducted on 40 subjects; 30 HBV patients selected from Tropical Medicine

Department, Faculty of Medicine, Alexandria University and 10 healthy volunteers. Subjects were divided into 4 groups; group 1 (naïve HBV patients) included 10 HBV infected patients (6 males vs. 4 females) who did not received any treatment, group 2 (responder HBV patients) included 10 CHB patients (6 males vs. 4 females) who responded to nucleoside /nucleotide analogues (NAs) after 6 months of treatment, group 3 (non-responder HBV patients) included 10 CHB patients (6 males & 4 females) who did not responded to NAs treatment or had a relapse after 6 months of the treatment and group 4 (control group) included 10 healthy persons (5 Males & 5 Females) matched in age and sex with patients in the other groups. Relapse was defined as the reappearance of HBV DNA during follow-up in subjects with previous end of treatment response (ETR), which is undetectable serum HBV DNA at the end of therapy.

Inclusion criteria included HBV patients aged between 20 and 55 years old, untreated or receiving NAs for 6 months. Chronic HBV infection was confirmed by positive serology for HBs and HBe Ags while active viral replication by the detection of HBV-DNA in the serum. Exclusion criteria included HCV infection, blood transfusion, renal, cardiac, neoplasic or immunological disorders. All participants were asked to freely volunteer to the study and informed written consents were gathered prior to their inclusion in the study protocol, according to ethical guidelines of the Medical Research Institute, Alexandria University (Informed Written Consent for Patient Participation in a Clinical Research, 2011).

Liver functions tests including serum albumin, ALT (SGPT) (RANDOX; alanine aminotransferase EC 2.6.2 IFCC kit), AST (SGOT) (RANDOX; aspartate aminotransferase EC 2.6.2 IFCC kit) and alkaline phosphatase were performed for all subjects under the study.

2.1 Hepatitis B Markers Tests

HBs-Ag and HBe-Ag were measured in the serum samples, using AcuuDiag™ enzyme linked immunosorbant assay (ELISA) kit (USA, Diagnostic Automation/ Cortez Diagnostics, Inc.) [13]. Anti-HBe was measured in the serum samples, using EXPRESSBio® ELISA kit (USA, Express Biotech International) [14]. Total anti-HBc was measured in the serum samples, using Accu-Tell ®ELISA kit (AccuBioTech Co., China) [14].

2.2 Quantitative Detection of HBV-DNA by Real Time PCR

Assessment of active viral replication was done by measuring serum HBV DNA levels in serum, using Real Artus ® HBV RG/TM PCR kit (Artus-Biotech, Qiagen, Hamburg, Germany) [15].

DNA extraction was performed using QIAamp® viral DNA mini kits. In brief, 20 µl of proteinase K were pipetted into a 1.5 ml micro centrifuge tube, then 200 µl serum were added, followed by 200 µl of AL buffer. All components were mixed by pulse-vortex for 15 sec and incubated at 56℃ for 10 min. 200 µl of ethanol (96%) were added to the sample, and mixed by pulse-vortex for 15 sec. The mixtures were carefully applied to the QIAamp® spin column (in 2-ml collection tube). After centrifugation at 8000 rpm for 1 min., the QIAamp® spin column was placed into a clean 2-ml collection tube. 500 µl of buffer AW1 were added then centrifugation at 10000 rpm for 2 min. was done twice. Finally, QIAamp® spin column was placed in a clean 2-ml collection tube, 50 µl of buffer AE were added, incubated at room temperature for 1 min, and centrifuged at 8000 rpm for 1 min. The eluted DNA was stored at -20℃ till used.

For PCR amplification, 15 µl TaqMan universal PCR master mix (Artus ® HBV RG PCR) were added in PCR tubes with 10 µl of extracted DNA to bring the reaction to a final volume of 25 µl. 10 µl of five quantitation external standard (RG/TM QS, Qiagen) ranging from 105 I.U/ml to 101 I.U/ml were added in separate tubes containing 15 µl of the HBV TM RG Master Mix. Real time PCR was performed with the Mx3000P TM (Stratagene) real time PCR system. Thermal profile was adjusted as follow: 95℃ for 10 min, followed by 40 cycles of two PCR-step amplification, denaturation for 95℃ for 15 sec, followed by annealing and extension at 60℃ for 1 min.

Results were interpreted by drawing a standard curve for the different DNA standard concentrations and their corresponding cycle threshold (CT). The viral load was measured in international unit per ml by blotting the different CT against the standard curve.

2.3 Measurement of Serum Levels of IP-10 Using Human ELISA Kit

IP-10 levels were measured in all serum samples, using commercial human IP-10 Instant

ELISA kit (eBioscience, USA) [16]. Colour intensity was measured by spectrophotometer at 450 nm. A standard curve was created by plotting the mean absorbance for each standard concentration on the ordinate against the human IP-10 concentration on the abscissa. The best fit curve was drawn through the points of the graph. The concentration of circulating human IP-10 was determined by finding the mean absorbance value on the ordinate and a horizontal line was extended to the standard curve. At the point of intersection, a vertical line was extended to abscissa to read the corresponding human IP-10 concentration.

2.4 Assessment of HLA-DP-rs3077 and HLA-DQ-rs3920 SNP

HLA-DP-rs3077 and HLA-DQ-rs3920 SNPs were genotyped using 5' nuclease assay with a TaqMan MGB probe in an StepOne™ Real-Time PCR System (Applied Biosystems, Life Technologies, USA) [17,18].

SNPs were performed following the work of Al-Qahtani et al. [19]. Genomic DNA was extracted from EDTA whole blood samples using the PureLink® Genomic DNA Kits # K1820-01 (Invitrogen, Life Technologies) followed by assessment of DNA concentration and purity using Nano drop spectrophotometer. The extracted DNA was stored at -80ºC till used.

HLA-DP-rs3077 and HLA-DQ-rs3920 SNPs were analyzed using 5' nuclease assay with a TaqMan MGB (minor groove binder) probe in a StepOne™ Real-Time PCR System (Applied Biosystems, Life Technologies). SNP Genotyping Assays contain VIC® dye-labeled probe, FAM™ dye-labeled probe, and two target-specific primers. TaqMan® probes incorporate MGB technology at the 3' end to deliver superior allelic discrimination. The MGB molecule binds to the DNA helix minor groove, improving hybridization based assays by stabilizing the MGB probe-template complex. This increased binding stability permits the use of probes as short as 13 bases for improved mismatch discrimination and greater flexibility when designing assays for difficult or variable sequences. All MGB probes also include a non-fluorescent quencher (NFQ) that virtually eliminates the background fluorescence and provides excellent signal-to-noise ratio for superior assay sensitivity.

HLA-DP-rs3077 and HLA-DQ-rs3920 SNP primers and TaqMan MGB probes were provided by the assay on-demand ™ service by Applied Biosystems, Life Technologies. The 5′ nuclease assay was performed using 2 µl genomic DNA, 7 µl DNase-free water, 10 µl TaqMan Universal PCR Master Mix (2X) and 1 µl working stock of SNP genotyping assay (20X). The assay contain sequence-specific forward and reverse primers to amplify the polymorphic sequence of interest, two TaqMan® MGB probes with NFQ (one VIC®-labeled probe to detect Allele 1 sequence, one FAM™-labelled probe to detect Allele 2 sequence). Negative controls (without DNA samples) were included in each run. Thermal cycling conditions were specified to be 10 min. at 95℃ followed by 40 PCR cycles each consists of 15 sec at 92℃ and 1 min. at 60℃. Each reaction plate was loaded into StepOne™ Real-Time PCR System, and then the run started.

When probes that have hybridized to the complementary sequence are cleaved, an increase in fluorescence signal occurs. Thus, the fluorescence signal generated by PCR amplification indicates which alleles are present in the sample.

2.5 Statistical Analysis of the Data

Data were analysed using IBM SPSS software package version 20.0. Qualitative data were described using number and percent. Quantitative data were described using mean, standard deviation and median. Comparison between different groups regarding categorical variables was tested using Chi-square test. When more than 20% of the cells have expected count less than 5, correction for chi-square was conducted using Monte Carlo correction. For normally distributed data, comparison between > two population were analyzed F-test (ANOVA) to be used. Correlations between two quantitative variables were assessed using Pearson coefficient. For abnormally distributed data, comparison between different groups were done using Kruskal Wallis test. Significance of the obtained results was judged at the 5% level [20].

3. RESULTS

3.1 Subject's Demographic Data

Age and sex distributions among naïve, responder and non-responder HBV patients and healthy control group didn't show any statistically significant differences (Table 1).

3.2 Liver Functions & Viral Markers

Results of liver functions (Albumin, ALT, AST and Alkaline phosphatise) were markedly deteriorated in HBV patients compared to control group where there was statistical significant differences between responder group compared to naïve and control groups in albumin levels (P=0.006). In addition, there were statistical significant increase in AST values among naive and non-responder groups compared to responder and control groups (P=0.003) and increase in ALT values among naïve and non-responder groups compared to control group (P=0.002) (Fig. 1).

Regarding viral markers, there were statistical significant differences between HBV responder patients and other groups with respect to HBe-Ag (where all responders showed negative HBe-Ag, P=0.005), HBe-Ab (where most of them were HBe-Ab positive, P=0.027) and HBc-Ab (where nearly all were HBc-Ab positive, P=0.004) (Fig. 2).

3.3 Viral Load "HBV- DNA"

HBV-DNA quantification was done using real time PCR. Results were expressed as IU/ml and summarized in (Figs. 3, 4). Among naive HBV infected patients, HBV-DNA levels ranged between $370*10^3$-$64000*10^3$ IU/ml with a median of $4250 *10^3$ IU/ml, in responder group it ranged between 110 - 197 $*10^3$ IU/ml with a median of 1600 IU/ml, in the non-responder group it ranged between 1600 - 830 * 10^3 IU/ml with a median of 22500 IU/ml. There was a statistical significant increase in HBV-DNA level among naïve HBV patients compared to other groups (P=0.001).

Table 1. Comparison between the studied groups as regard age and sex

Characteristics	Groups								*MCP*
	Naive		Responder		Non responder		Control		
	No	%	No	%	No	%	No	%	
Age (years)									
▪ <35	6	60.0	4	40.0	4	40.0	5	50.0	
▪ >35	4	40.0	6	60.0	6	60.0	5	50.0	0.064
Range	19-46		25-56		22-53		25-52		
Mean ± SD	29.8±3.8		36.1±9.9		37.9±9.5		32.1±5.8		
Gender									
▪ female	4	40.0	4	40.0	4	40.0	5	50.0	
▪ male	6	60.0	6	60.0	6	60.0	5	50.0	0.781

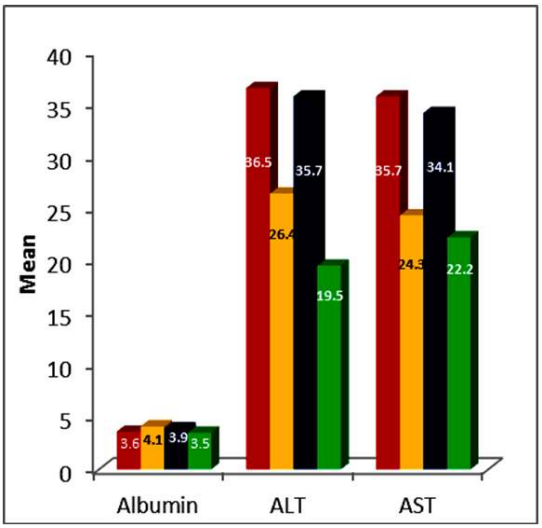

Fig. 1. Liver functions among studied groups

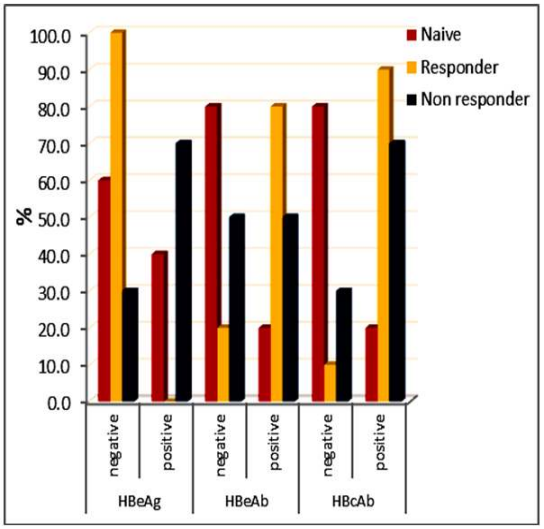

Fig. 2. Viral markers among HBV studied groups

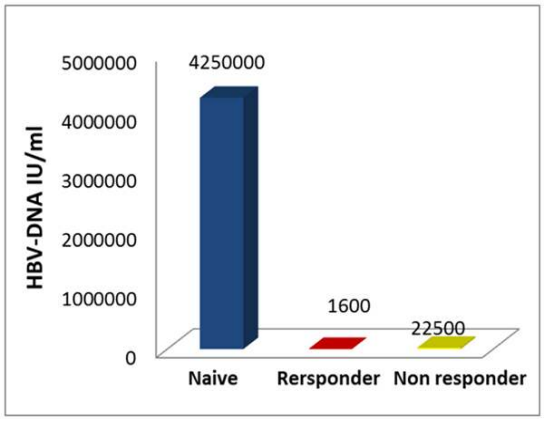

Fig. 3. HBV-DNA among naïve, responder and Non-responder groups

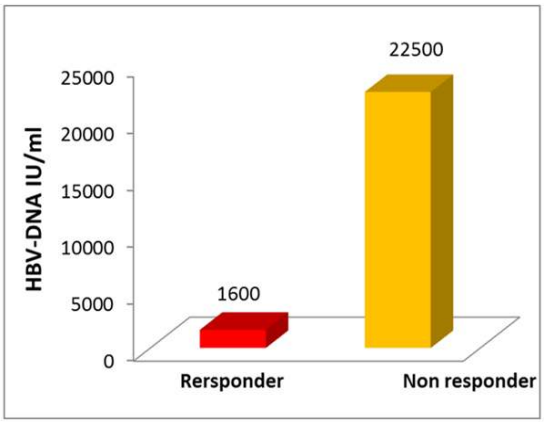

Fig. 4. HBV-DNA among responder and non-responder groups

3.4 IP-10 Serum Levels among Different Groups

The mean levels of serum IP-10 among naive, responder, non-responder HBV patients and healthy controls were 97.2±26.7, 135.0±163.5, 227.6±123.9 and 92.4±43.8 pg/ml, respectively. There was statistically significant increase in IP-10 levels among the non-responder group when compared with naïve, responder and control groups (P = 0.011) (Fig. 5). In addition, correlation analysis of the present data, using spearman correlation coefficient, demonstrates that IP-10 is an intermediate indicator for response to treatment of HBV (r = 0.54) (Fig. 6).

3.5 HLA-DP.rs3077 and HLA-DQ.rs3920 Variants

HLA-DP and HLA-DQ SNPs at rs3077 and rs3920 loci, respectively, were identified using step one real-time PCR (Fig. 7). Concerning HLA-DP rs3077, AA genotype was expressed in 100%, 40%, 50% and 40% of naïve, responder, non- responder HBV infected patient groups and healthy control group, respectively. AG genotype was expressed in 60%, 50% and 60% of responder, non- responder HBV patients and healthy control group, respectively. Correlation analysis showed statistically significance association between HLA-DP-rs3077 and both ALT level in responder HBV group (P = 0.022) and Albumin level in non-responder HBV group (P = 0.036).

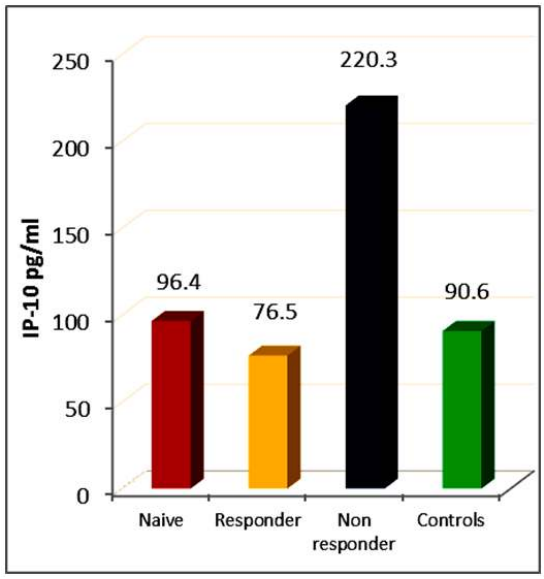

Fig. 5. Median IP-10 levels among the different studied groups

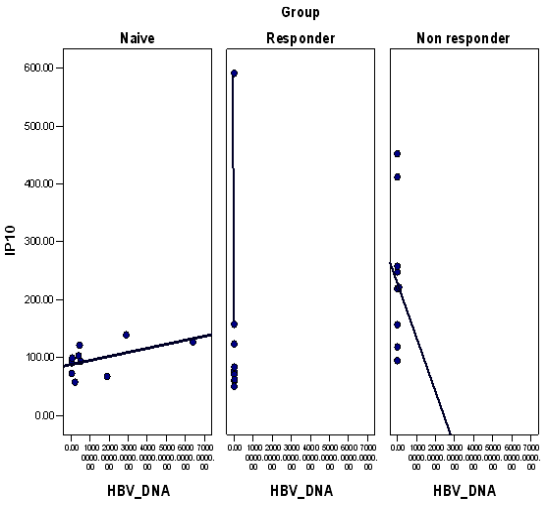

Fig. 6. Relation between HBV-DNA and IP-10 levels among the studied groups

While for HLA-DQ rs3920, the present study showed that AA genotype was expressed in 10% for each of responder, non-responder and control groups but not expressed in naïve group. AG genotype was expressed in 20%, 20%, 10% and 60% of naïve, responder, non- responder HBV patients and healthy control group, respectively. GG genotype was highly expressed in the different groups of HBV patients; where it was expressed in 80%, 70% and 80% of naïve, responder, non- responder HBV patients, respectively, compared to 30% only in the control group (Table 2).

There was statistical significant correlation between HLA-DQ-rs3920 genotype and HBV-DNA in non-responder group (P = 0.047); where the median of HBV-DNA was significantly increased in HLA-DQ-rs3920 (AA) genotype (830000 IU/ml) than both (GG) genotype (22500 IU/ml) and (A/G) genotype (7100 IU/ml) (Fig. 8). In addition, There was statistically significant increase in IP-10 levels among the non-responder HBV patients carrying HLA-DP-rs3077 AG (P = 0.047) and/or HLA-DQ-rs7453920 GG alleles (P = 0.001).

4. DISCUSSION

HBV is non-cytopathic itself and the liver damage caused by HBV infection is mainly immune-mediated [21]. The HBV-specific immune response is directed and primed by APCs in association with viral antigen and MHC molecules. Impairment in the immune response can causes asymptomatic or chronic inflammation in the liver [22]. Successful clearance of HBV is a promising event in which host's immune system will attempt to get rid of the virus. The immunological events of HBs-Ag seroclearance have attracted great attention in both natural history investigations and therapeutic trials [12].

The function of HLA-DP is to present bound peptide antigens, e.g. from HBV, at the surface of antigen presenting cells. CD4+ T cells recognize these antigens and initiate the adaptive immune response. They assist the MHC class I restricted CD8+ T cells which are the primary cellular effectors mediating HBV clearance from the liver during acute viral infection [23]. HBV infection will either be cleared by these means, or establish itself as a chronic infection. The reason for the latter is unclear but may be related to variation of HLA-DP alleles. Thus, the position of HLA-DP SNPs might be associated with possibility of clearance or chronicity. HLA-DP.rs3077 SNPs are located within the 39 untranslated regions (UTR) of HLADPA1.

In the present study, the associations of variations at the HLA-DP rs3077 gene in HBV infected Egyptian patients were studied and found that the homozygous genotype "AA" was significantly associated with HBV susceptibility and carrier status. This risk allele of HLA-DP rs3077 might thus influence the antigen presentation, incurring immune evasion of the virus and resistance to nucleoside/ nucleotide analogues therapy. So allele A of HLA-DP.rs3077 could be identified as HBV risk allele and may have a role in resistance to treatment. Moreover, the expression of HLA-DP.rs3077 allele G was higher among responder HBV patients and healthy controls than non-responders and naïve groups, but no statistically significant association was found between HLA-DP.rs3077 SNP and response to nucleoside/ nucleotide analogues treatment. This may be due to the small sample size included in this study. So allele G of HLA-DP.rs3077 may have a role in resistance to treatment. While for HLA-DQ rs3920, there was no statistical significant differences between the studied groups where P value = 0.175. This could be attributed to the small sample size. Correlation analysis revealed that there was statistical significant relation between HLA-DQ.rs3920 AA genotype and HBV-DNA in non-responder group, while there wasn't any statistical relation between liver functions & HLA-DQ-rs3920 variants among HBV patients. So the current study could identify allele G of rs3920 as HBV risk allele as it was strongly associated with HBV infection while allele A of rs3920 was strongly associated with resistance to treatment when compared to GG or GA genotypes.

In accordance with our results, Posuwan et al. [23] have concluded that genetic variations of HLA-DP.rs3077 were significantly associated with HBV carriers in Thai population. Also, Al-Qahtani et al. [19] have reported that the haplotype that included the risk allele (A) of SNPs rs3077 was found to be significantly associated with HBV susceptibility. But, they identified HLA-DQ.rs3920 G as non-risk allele among Saudi Arabian patients as it was dominantly associated with HBV clearance. This was in agreement with the observations of Hu et al. [24].

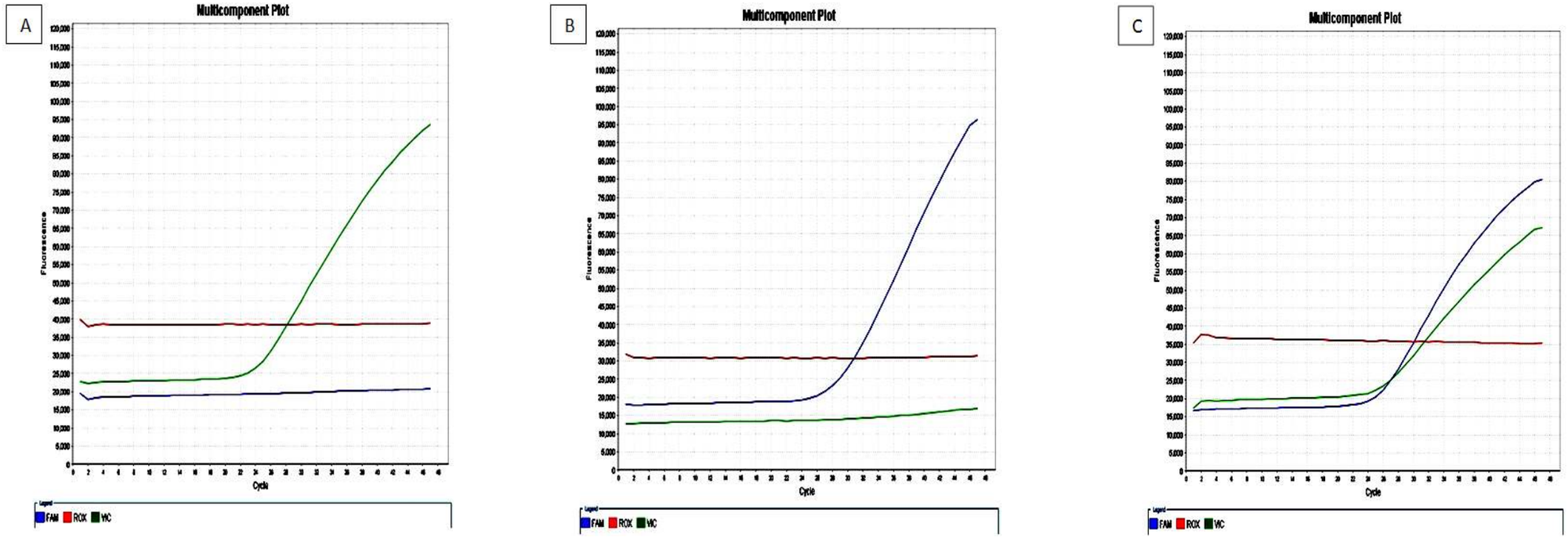

Fig. 7. Real-time PCR picture displaying A) homogenous expression of HLA-DP-rs3077 AA or HLA-DQ-rs3920 AA (VIC® dye is associated with the A allele), B) homogenous expression of HLA-DP-rs3077 GG or HLA-DQ-rs3920 GG (FAM™ dye is associated with the G allele), C) heterozygous expression of both alleles (HLA-DP-rs3077 AG or HLA-DQ-rs3920 AG)

Table 2. Distribution of HLA-DP-rs3077 and HLA-DQ-rs3920 alleles; among the studied groups

HLA	Group								*MCP*
	Naive		Responder		Non responder		Controls		
	No	%	No	%	No	%	No	%	
HLA-DP-rs3077									
H (A/G)	0	0.0	6	60.0	5	50.0	6	60.0	
VIC (AA)	10	100.0	4	40.0	5	50.0	4	40.0	0.018*
HLA-DQ-rs3920									
FAM (GG)	8	80.0	7	70.0	8	80.0	3	30.0	
H (A/G)	2	20.0	2	20.0	1	10.0	6	60.0	0.175
VIC (AA)	0	0.0	1	10.0	1	10.0	1	10.0	

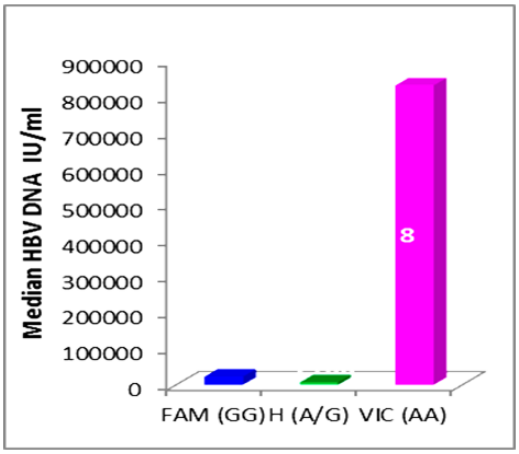

Fig. 8. HBV-DNA in relation to HLA variants

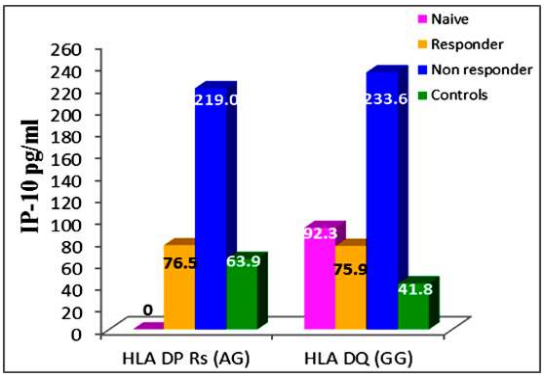

Fig. 9. Relation between HLA-DP and HLA-DQ variants and IP-10 among different groups

On contrary to the present results, Mbarek et al. [25] have reported a lack of association between SNP rs3920 that belongs to intron 1 of HLA-DQB2 region and susceptibility to HBV infection. Su et al., [26] have investigated the association of SNP in the HLA-DP and DQ genes with the outcome of chronic HBV infection. They concluded that the presence of A allele at rs3077 of the HLA-DP gene may decreased the risk for chronic HBV infection. Furthermore Liao et al. [8] have correlated HLA-DP rs3077 polymorphisms with HBV natural clearance among Tibetans and Uygurs. They reported that HLA-DP/DQ polymorphisms associated with HBV natural clearance in both ethnicities. Moreover, in Tibetan patients, HLA-DQ rs3920 GG had a higher frequency in HBe-Ag positive patients. All these results could verify that host genetics may be useful for the prediction of HBV susceptibility and genetic variations of HLA-DP.rs3077 and HLA-DQ.rs3920 could be associated with HBV risk and resistance to treatment with nucleoside/ nucleotide analogues, but according to less number of samples that might influence statistical power in this study; a large-scale study should be required.

Several studies have shown the potential roles of cytokines and chemokines in chronic viral hepatitis. IP-10 has been reported to play important roles during hepatitis flares in CHB. An animal study showed that HBV-specific CTLs induced IP-10 production and subsequently recruited host inflammatory cells responsible for liver damage. In human study, elevated IP-10 involves in liver inflammation during hepatitis flares in CHB. It was reported to correlate with hepatic injury during hepatic flares in CHB and a strong correlation between serum IP-10 concentrations and ALT levels was also noted. Xu et al. [10] have reported that elevation of IP-10 affects host immune responses to infection by altering activity of cells that expresses CXCR3 receptors. For example, crosstalk between Tregs and NKT cells was mediated by IP-10: when activated, the NKT cells secrete large amounts of IFN-γ, which induces the production of IP-10 and IP-10 chemoattract Tregs (which express CXCR3 receptors). In healthy organism, Tregs suppress the activation of effector T cell to maintain immune homostatus. However, in CHB, Tregs suppressed the immune response locally and contributed to the persistence of HBV infection. Therefore, higher level of IP-10 means more recruited Tregs, especially within the liver, and suggests a stronger inhibition on immune responses. Particularly, the liver-derived Tregs

express higher level of CXCR3 comparing with blood-derived Tregs, which make the inhibition even stronger.

In the current study, there was statistically significant increase in IP-10 levels among the non-responder HBV patients carrying HLA-DP-rs3077 AG (P = 0.047) and/or HLA-DQ-rs7453920 GG alleles (P= 0.001). These results clarify that IP-10 levels in serum of non-responder HBV patients are markedly higher than these of responder HBV patients. So IP-10 could be used as a predictor for response to nucleoside/ nucleotide analogues treatment among HBV infected patients.

In accordance with the present results, Wang et al. [27] have verified that levels of IP-10 and IP-10 mRNA in the peripheral blood of patients with cirrhosis increase and are closely correlated with the load of HBV DNA in serum, and play a key role in the progression of post-hepatitis cirrhosis. Papatheodoridis et al. [28] have proved that serum IP-10 levels represent a promising predictor of HBs-Ag decline in CHB patients. Furthermore, Keating et al. [29] have reported that earlier initiation of immune responses may mediate vaccine-induced protection preventing symptomatic acute and chronic HBV infections in individuals with HBV vaccine breakthrough infections. They identified common early induction of several cytokines and chemokines as IP-10 that may play a role in triggering earlier adaptive cellular responses, thus regulating inflammation and inducing anamnestic anti-HBs antibodies to help control viremia.

5. CONCLUSION

According to our study findings, we can conclude genetic variations of HLA-DP.rs3077 and HLA-DQ.rs3920 could be associated with HBV risk and resistance to treatment with nucleoside/ nucleotide analogues, but according to less number of samples that might influence statistical power in this study. Additionally, IP-10 level may be a promising predictor of response to treatment among HBV infected patients. So, we recommend a wide scale study of HLA genetic variations and its association with HBV infection and/or responses to treatment. Further studies, based on a larger number of patients, are necessary to explore the roles of HLA-II SNPs in other infectious diseases.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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