International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2016): 79.57 | Impact Factor (2017): 7.296

Interleukin 10: A Serum Level and Profile of Gene Polymorphism in HBV and HCV Iraqi Patients

Osama B. Al-Saffar[1], Ali H. Ad'hiah[2], Jwan Sabah Bajlan[3]

[1]Department of Biology, Madenat Al-elem University College

[2]Tropical-Biological Research Unit, College of Science, University of Baghdad, Iraq

[3]Ministry of Science and Technology, Baghdad-Iraq

Abstract: ***Serum level and gene polymorphism of Interleukin 10 were investigated in 76 Iraqi Arab hepatitis patients; 38 for each of hepatitis B virus (HBV) and hepatitis C virus (HCV) infection, as well as 24 ethnicity, age and gender-matched controls. IL-10 was showed significant variations, Serum level of IL-10 was significantly increased in HBV patients compared to controls (22.32 ± 4.09 vs. 18.90 ± 5.49 pg/mL). Distributing patients and controls according to age and gender revealed that there was no significant variation between the age groups < 40 and ≥ 40 years or males and females. Cytokine gene polymorphism analysis revealed that some genotypes showed a significant departure from Hardy-Weinberg equilibrium in HBV and HCV patients but not in controls. The comparisons between patients (HBV and HCV) and controls revealed some significant variations in the distribution of cytokine genotypes or alleles. $IL10_{-819}$ CT genotype showed a significant decreased frequency in HBV patients compared to controls, and the PF of such negative association was 0.33. At the three investigated positions of IL10 gene, no significant impact of genotypes was observed, in positions ($IL10_{-1082}$, $IL10_{-819}$ and $IL10_{-592}$), with some exceptions. At $IL10_{-1082}$, GA and AA genotypes shared a similar mean of IL-10 (22.2 ± 6.5 and 22.6 ± 5.8 pg/mL, respectively) in HBV patients, but both means were significantly higher than the mean of GG genotype (20.6 ± 0.1 pg/mL). For $IL10_{-819}$, the TT genotype of HBV patients demonstrated a significant increased IL-10 level (24.1 ± 3.9 pg/mL) compared to CC and CT genotypes (21.9 ± 3.1 and 18.5 ± 7.6 pg/mL).***

Keywords: Hepatitis B virus, Hepatitis C virus, IL-10, $IL10_{-1082}$, $IL10_{-819}$, $IL10_{-592}$, Gene Polymorphism

1. Introduction

Hepatitis is an inflammation of the liver that is most commonly caused by one of the five types of hepatitis viruses; A, B, C, D and E. These types are of a greatest concern because of the burden of illness and death they cause and the potential for outbreaks and epidemic spread worldwide; in particular, types B (HBV) and C (HCV) **[1].** The balance between virus and host defense defines the course of viral infection and pathogenesis, and persistent viruses such as HBV and HCV are generally not directly cytopathic and have developed immune evasion mechanisms to survive without destroying the host **[2]**. For the host, the goal is to prevent, eliminate, or at least control viral infection while limiting undue collateral damage. These interactions are influenced by various host genetic, immunological and viral factors **[3]**.

IL-10 was discovered in 1980 as a cytokine with inhibitory factors, and exhibits various immunomodulatory functions. It is coded by a gene located on chromosome 1, and it is mainly secreted by Th2 type T cells, but also macrophages/monocytes, dendritic cells, B cells, and even Th1 cells, Th2 cells, lung mast cells, B cell derived tumor cell lines, and keratinocytes are also able to secrete IL-10, but recently, it is considered as T-regulatory (Treg) cell cytokine **[4]**. IL-10 is 18.5 KDa protein of 160 amino acids, and exists in the form of a non-covalent homodimer **[5]**. It is also an anti-inflammatory cytokine, and during infection, it inhibits the activity of Th1 cells, NK cells, and macrophages, all of which are required for optimal pathogen clearance but also contribute to tissue damage **[6]**. IL-10 is encoded by a gene located on chromosome 1 at position 1q31.32. Three SNP has been found on the promoter region of *IL10* gene; $IL10_{-1082}$ G/A, $IL10_{-819}$ C/T and $IL10_{-592}$ C/A. These genes exhibited a strong effect on the transcription of *IL10* gene **[7,8]**.

From the genetic point of view, the existence together of many forms of DNA sequences (polymorphism) at a locus within a population, or a discontinuous genetic variation may results in different forms or types of individuals among the members of a single species that differ in their immune resonse **[9]**. In this regard, many studies have examined the relationship between certain cytokine gene polymorphisms (single nucleotide polymorphisms; SNPs), cytokine gene expression, and susceptibility to and clinical severity of diseases **[10]**. One of these SNPs of *IL10* gene; $IL10_{-1082}$ G/A, $IL10_{-819}$ C/T and $IL10_{-592}$ C/A, its alleles or genotypes have been suggested to effect susceptibility to several human diseases **[11]**. Therefore, the present study was planned to determine the role of IL-10 in etiopathogenesis of HBV and HCV in terms of serum level and gene polymorphism.

2. Materials and Methods

Subjects

After ethical clearance, the study was carried out at the Gastroenterology and Hepatology Teaching Hospital/ Baghdad. The study was carried out on 100 subjects; 76 of them were suffering from viral hepatitis, and were divided into two clinical groups. The first group consisted of 38 HBV patients (20 males and 18 females), and their age ranged between 13–57 years (Mean ± SD: 43.36 ± 10.68) years. The second groups involved 38 HCV patients (13 males and 23 females), and their age ranged between 17-73 years (Mean ± SD: 33.63 ± 15.35 years). A control sample of 24 individuals (6 males and 18 females) was also included in the study, and their age ranged between 17-60 years (Mean ± SD: (39.20 ± 11.32) years. The controls were blood donors and their laboratory profile in the Central Blood

Bank (Baghdad) revealed that they were negative for HBV and HCV infections.

From each participating subject, 5 ml were drawn and distributed into plain tube (3 ml) and EDTA tube (2 ml). After isolation of serum, it was tested by ELISA method to detect anti-viral (HBV and HCV) antibodies (Biomerieux HBs Ag HBV kit; France), and if it was positive, the diagnosis was confirmed further by real-time PCR analysis to detect the viral genetic material (COBAS® AmpliPrep/COBAS® TaqMan® HBV and COBAS® AmpliPrep/COBAS® TaqMan® HCV kits; USA). All patients were firstly diagnosed and none of them was under therapy.

Assessment of IL-10 serum level

Sera of hepatitis patients and controls were assessed for the level of IL-10 using a commercially available kit (PeproTech; UK), and the instructions of muanfacturer were followed.

Detection of *IL10* gene polymorphism

Genomic DNA was extracted from EDTA blood using AccuPrep® Genomic DNA Extraction Kit (Bioneer Corporation, Korea). The polymorphism was detected at one position of the promoter region (*IL10*$_{-1082}$, *IL10*$_{-819}$ and *IL10*$_{-592}$) by polymerase chain reaction-specific sequence primer (PCR-SSP) assay, followed by electrophoresis on 2% agarose-gel, by using CTS-PCR-SSP Tray Kit (**Heidelberg, Germany).** The thermocycling conditions were: initial denaturation at 94˚C for 2 minutes, followed by denaturation at 94˚C for 15 seconds, and then 10 cycles of annealing and extension at 65˚C for 60 seconds. This was followed by denaturation at 94˚C for 15 seconds, and then 20 cycles of annealing 61˚C at 50 seconds and extension at 72˚C for 30 seconds.

Statistical Analysis

Serum level of IL-10 was given as mean ± SD, and significant differences between means were assessed by ANOVA (Analysis of Variance) followed by either LSD (Least Significant Test) or Duncan using the computer software SPSS (Statistical Package for Social Sciences) version 13.

Genotypes of IL-10 were presented as percentage frequencies, and significant differences between their distributions in hepatitis patients and controls were assessed by two-tailed Fisher's exact probability (P). In addition, the relative risk (RR), etiological fraction (EF) and preventive fraction (PF) were also estimated to define the association between a genotype with the disease. These estimations were calculated by using the WINPEPI computer programs for epidemiologists. The latest version of the WINPEPI package is available free online at http://www.brixtonhealth.com.

3. Results and Discussions

Serum Level of IL-10

Serum level of IL-10 was significantly increased in HBV patients compared to controls (22.32 ± 4.09 vs. 18.90 ± 5.49 pg/mL). The investigated cytokine IL-10 showed no significant differences in their serum levels between the two age groups (< 40 and ≥ 40 years) in HBV and HCV patients or controls. In additin, IL-10 showed an opposite picture in HBV patients, in which females demonstrated a significant increased level of IL-10 compared to female controls (22.82 ± 4.13 *vs*. 18.18 ± 5.85 pg/mL) (Table 1).

Table 1: Serum level of IL-10 and in hepatitis B and C patients and controls distributed by age group snd gender

Groups	IL-10 Serum Mean Level ± SD (pg/ml)		
	Patients		Controls (No.= 24)
	Hepatitis B (No.= 38)	Hepatitis C (No.= 38)	
Total	22.32 ± 4.09^{A}	20.31 ± 5.89^{AB}	18.90 ± 5.49^{B}
< 40 years	22.72 ± 3.26^{A}	22.53 ± 5.38^{A}	18.75 ± 5.75^{A}
≥ 40 years	21.63 ± 6.98^{A}	19.15 ± 5.91^{A}	19.05 ± 5.46^{A}
p	N.S.	N.S.	N.S.
Males	21.78 ± 5.55^{A}	19.27 ± 6.62^{A}	21.06 ± 3.82^{A}
Females	22.82 ± 4.13^{A}	20.98 ± 5.40^{AB}	18.18 ± 5.85^{B}
p	N.S.	N.S.	N.S.

Different superscript letters: Significant difference ($P \leq 0.05$) between means of rows.
p: Probability of difference between males and females of each group. N.S.Not significant ($p > 0.05$)

There is suggestive evidence that T-cell immune-regulatory cytokines may play a key role in influencing the persistence of hepatitis viral infection and the extent of liver damage **[12,13]**, and the view point is in favor of that the immune response, which is associated with a Th1 cytokine profile, is augmented with cell-mediated immunity to enhance recovery, while Th2 cytokine response is regarded to be associated with the development of persistent infection **[14]**. Furthermore, in HBV related hepatitis, some Th1 phenotype cytokines are positively correlated with hepatic inflammatory activities, especially those underlined by CD4+ T cells **[15]**. Activated CD4+ T cells can be distributed into two subsets based on their cytokine secretion profiles, which are Th1 subset that produces IFN-γ, TNF-α and IL-2, and participates in cell-mediated immune responses. In contrast, Th2 subset produces IL-4 and IL-13, and mediates humoral immune responses, in which IL-10 is an up-regulator produced by Treg cells **[16]**. Therefore, an increased level of IL-10 may encounter cell-mediated immunity, which is effective in virus clearing.

Previous studies have demonstrated a correlation between cytokine serum levels and gender, and this may have been influenced by various factors, and among them is the hormonal status **[17]**. In this regard, immune defense capacity has shown differences between human males and females. In addition, males are found to be more prone to infections, while females are at greater risk to develop autoimmune diseases **[18]**. These findings were correlated with humoral responses to the foreign antigenic challenge, and the suggestion was that sex hormones may influence immune functions **[19]**. Such results may suggest that the age is not critical factor that affect the serum level of the five investigated cytokines; however, caution must be considered in interpreting these results because the sample size in patients and controls may not permit a firm conclusion. In addition, age can be considered an effected factor when we have subjects at age more than 60 years, because it is often that a dysregulation in the immune functions, and a decline

in health and increased sensitivity to various diseases are associated with advanced ages **[20]**, and since cytokines are central to immune cell communications, age-associated changes in cytokine production may contribute to these alterations **[20]**.

Genetic Polymorphism of *IL-10* Gene

The genetic polymorphism of IL10 gene was explored at three positions; $IL10_{-1082}$, $IL10_{-819}$ and $IL10^{-592}$, which were presented with three genotypes (GG, GA and AA for IL10-1082; CC, CT and CC for $IL10_{-819}$; and CC, CA and AA for $IL10_{-592}$) in HBV and HCV patients and controls. Genotypes for HBV and HCV patients showed a significant difference between the observed and expected frequencies at $IL10_{-819}$ and $IL10_{-592}$; therefore a departure from H-W equilibrium was recorded, while at $IL10_{-1082}$, no significant deviation from the equilibrium was observed in both groups of patients. Among controls, the genotypes at the three positions were in a good agreement with H-W equilibrium (Table 2,3,4).

In addition, the frequency distribution of the recorded genotypes and alleles at the three positions showed no significant differences between patients (HBV or HCV) and controls, with one exception. The CT genotype frequency at $IL10_{-819}$ was significantly decreased in HBV patients (18.4 vs. 45.9%; P = 0.05). Such difference was associated with RR value of 0.27 and EF value of 0.33(Table 5,6,7).

Table 2: Observed numbers and percentage frequencies and Hardy-Weinberg (H-W) equilibrium of $IL10_{-1082}$ genotypes and alleles in hepatitis B, hepatitis C patients and controls.

Groups			$IL10_{-1082}$ Genotype or Allele					HWE
			GG	***GA***	***AA***	***G***	***A***	$p \leq$
Hepatitis B (No. = 38)	Observed	No.	2	15	21	19	57	Not significance
		%	5.3	39.5	55.2	25.0	75.0	
	Expected	No.	2.4	14.3	21.3	Not Estimated		
		%	6.3	37.5	56.2			
Hepatitis C (No. = 38)	Observed	No.	5	15	18	25	51	Not significance
		%	13.1	39.4	47.3	32.8	67.2	
	Expected	No.	4.1	16.8	17.1	Not Estimated		
		%	10.8	44.2	45.0			
Controls (No. = 24)	Observed	No.	1	8	15	10	38	Not significance
		%	4.1	33.3	62.5	20.8	79.2	
	Expected	No.	1.0	7.9	15.1	Not Estimated		
		%	4.3	33.0	62.7			

Table 3: Observed numbers and percentage frequencies and Hardy-Weinberg (H-W) equilibrium of $IL10_{-819}$ genotypes and alleles in hepatitis B, hepatitis C patients and controls

Groups			***IL10*$_{-819}$ Genotype or Allele**					**HWE**
			CC	***CT***	***TT***	***C***	***T***	$p \leq$
Hepatitis B (No. = 38)	Observed	No.	13	7	18	33	43	0.001
		%	34.2	18.4	47.4	43.4	56.6	
	Expected	No.	7.1	18.6	12.1	Not Estimated		
		%	18.8	49.1	32.0			
Hepatitis C (No. = 38)	Observed	No.	19	10	9	48	28	0.01
		%	72.2	26.3	23.6	63.2	36.8	
	Expected	No.	15.2	17.6	5.2	Not Estimated		
		%	39.9	46.6	13.5			
Controls (No. = 24)	Observed	No.	7	11	6	25	23	Not significant
		%	29.1	45.9	25.0	52.1	47.9	
	Expected	No.	6.5	12.0	5.5	Not Estimated		
		%	27.1	49.9	23.0			

Table 4: Observed numbers and percentage frequencies and Hardy-Weinberg (H-W) equilibrium of $IL10_{-592}$ genotypes and alleles in hepatitis B, hepatitis C patients and controls.

Groups			***IL10*$_{-592}$ Genotype or Allele**					**HWE**
			CC	***CA***	***AA***	***C***	***A***	$p \leq$
Hepatitis B (No. = 38)	Observed	No.	15	15	11	12	41	0.01
		%	39.4	39.4	28.9	31.5	53.9	
	Expected	No.	11.1	11.1	18.9	Not Estimated		
		%	29.1	29.1	49.7			
Hepatitis C (No. = 38)	Observed	No.	21	11	6	53	23	0.05
		%	55.3	28.9	15.8	69.7	30.3	
	Expected	No.	18.5	16.0	3.5	Not Estimated		
		%	48.6	42.2	9.2			
Controls (No. = 24)	Observed	No.	7	12	5	26	22	Not significant
		%	29.1	50.0	20.8	54.2	45.8	
	Expected	No.	7.0	11.9	5.1	Not Estimated		
		%	29.3	49.7	21.0			

Table 5: Statistical evaluations of associations between *IL10*$_{-1082}$ genotypes or alleles and hepatitis B infection.

IL10$_{-1082}$ Genotype or Allele	Statistical Evaluations			
	Relative Risk	Etiological or Preventive Fraction	Fisher's Exact Probability	95% Confidence Intervals
GG	1.28	0.01	Not significant	0.11-14.25
GA	1.30	0.09	Not significant	0.46-3.73
AA	0.74	0.16	Not significant	0.27-2.07
G	1.27	0.05	Not significant	0.54-3.00
A	0.79	0.16	Not significant	0.33-1.87

Table 6: Statistical evaluations of associations between *IL10*$_{-819}$ genotypes or alleles and hepatitis B infection.

IL10$_{-819}$ Genotype or Allele	Statistical Evaluations			
	Relative Risk	Etiological Or Preventive Fraction	Fisher's Exact Probability	95% Confidence Intervals
CC	1.26	0.07	Not significant	0.43-3.74
CT	0.27	0.33	0.05	0.09-0.82
TT	2.70	0.29	Not significant	0.90-8.12
C	0.71	0.15	Not significant	0.34-1.45
T	1.42	0.16	Not significant	0.69-2.91

Table 7: Statistical evaluations of associations between *IL10*$_{-592}$ genotypes or alleles and hepatitis B infection.

IL10$_{-592}$ Genotype or Allele	Statistical Evaluations			
	Relative Risk	Etiological Or Preventive Fraction	Fisher's Exact Probability	95% Confidence Intervals
CC	1.58	0.14	Not significant	0.56-4.64
CA	0.41	0.29	Not significant	0.14-1.16
AA	1.75	0.13	Not significant	0.54-5.69
C	0.99	0.005	Not significant	0.48-2.03
A	1.01	0.004	Not significant	0.49-2.07

The presented results suggest that polymorphisms of IL10 gene at *IL10*$_{-1082}$, *IL10*$_{-819}$ and *IL10*$_{-592}$ may have no role in susceptibility to HBV and HCV infections in the investigated sample of Iraqi patients, but there was a possibility that the CT genotype of *IL10*$_{-819}$ has some protective effects in HBV infection. Studies on the polymorphism of IL10 gene in HBV or HCV infection have shown conflicting results. In some studies, there was a significant association while in other studies there was no association, and this has been reasoned by the heterogeneity of both viruses, which may cause research results to be inconsistent, and in addition, the statistical power of small sample associated analysis has been suggested to be too low to detect minor gene variations **[21]**. A previous study showed that the frequency of *IL10*$_{-1082}$ GG genotype was significantly higher in HBV patients than in healthy controls, while the distribution of *IL10*$_{-819}$ genotypes was not significantly different between HBV patients and controls **[22]**. Roli et al. (2014) also indicated that IL10 is a potential candidate gene that may be strongly associated with predicting the transition of HBV related disease phases. Afzal et al. (2011) also suggested that the different IL10 gene polymorphisms may lead to an imbalance between the pro-inflammatory and anti-inflammatory cytokine responses, which may in turn influence the susceptibility to HCV infection. Yong-liang et al. (2010) exposed that the A and C alleles of *IL10*$_{-592}$ might be a risk factor for HBV or HCV in Asians but not in Europeans. Gao et al. (2011) also suggested that the polymorphism of *IL10*$_{-1082}$ appears to have some influences on chronic infection of HCV and/or HBV and HCV replication.

Genotype Impact on IL-10 Level

Genotypes at the three *IL10* gene promoter positions (*IL10*$_{-1082}$, *IL10*$_{-819}$ and *IL10*$_{-592}$) recorded no significant differences between the serum level of IL-10 for each position in HBV and HCV patients or controls, with some exceptions. At position -1082, GA and AA genotypes shared a similar mean of IL-10 (22.2 ± 6.5 and 22.6 ± 5.8 pg/mL, respectively) in HBV patients, but both means were significantly higher than the mean of GG genotype (20.6 ± 0.1 pg/mL) (Figure 1). For *IL10*$_{-819}$, the TT genotype of HBV patients demonstrated a significant increased IL-10 level (24.1 ± 3.9 pg/mL) compared to CC and CT genotypes (21.9 ± 3.1 and 18.5 ± 7.6 pg/mL), while CT genotype of controls showed a significant decreased level (16.8 ± 5.8 pg/mL) compared to CC and TT genotypes (20.1 ± 4.6 and 21.4 ± 4.9 pg/mL) (Figure 2).

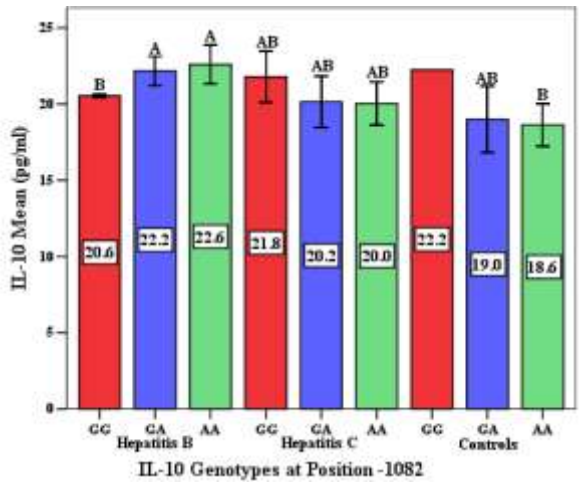

Figure 1: Serum level of IL-10 in hepatitis B and C patients and controls distributed by *IL10*$_{-1082}$ genotypes. (Different

capital letters: Significant difference ($P \leq 0.05$) between means of bars).

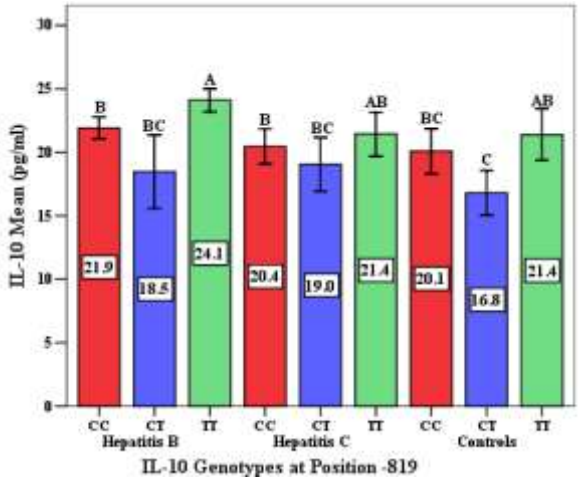

Figure 2: Serum level of IL-10 in hepatitis B and C patients and controls distributed by *IL10*$_{-819}$ genotypes. (Different capital letters: Significant difference ($P \leq 0.05$) between means of bars).

Finally, the CC and CA genotypes of *IL10*$_{-592}$ for controls showed a significant decreased IL-10 level (18.1 ± 6.7 and 18.0 ± 5.4 pg/mL, respectively) compared to AA genotype (22.2 ± 2.4 pg/mL) (Figure 3).

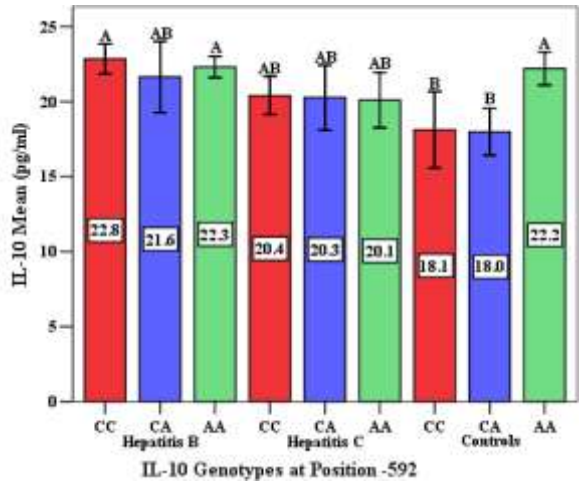

Figure 3: Serum level of IL-10 in hepatitis B and C patients and controls distributed by *IL10*$_{-592}$ genotypes. (Different capital letters: Significant difference ($P \leq 0.05$) between means of bars)

These findings are also shared by the present results, in which some of the investigated polymorphisms demonstrated significant impact on IL-10 production, but such effect was subjected to the sample investigated (patients or controls). However, there have been contradictory reports about the exact effect of IL-10 promoter polymorphisms on the natural outcome of HBV and HCV infection. Some authors have found that the plasma IL-10 level is decreased significantly in chronic HCV patients **[27]**. Gao et al. (2009) demonstrated that the *IL10*$_{-1082}$ AA genotype was associated with an increased risk, but *IL10*$_{-1082}$ GA was associated with a reduced risk of persistent HBV and/or HCV infection. The latter investigators further found that *IL10*$_{-1082}$ AA genotype is not only a potential susceptibility gene for HBV and/or HCV infection, but also potentially determines the disease clinical outcome. *IL10*$_{-1082}$ AA and *IL10*$_{-1082}$ A were associated with an increased risk, but *IL10*$_{-1082}$ GA and *IL10*$_{-1082}$ G were associated with a reduced risk of HCV RNA replication. In a more recent study, Saxena *et al.* (2014) investigated the spontaneous/un-stimulated levels of IL-10 in the peripheral blood mononuclear cells of HBV carriers and correlated that with two polymorphisms of IL10 gene (*IL10*$_{-819}$ and *IL10*$_{-592}$). Their results demonstrated a non-significant elevation in IL-10 levels in individuals with *IL10*$_{-819}$ CC and *IL10*$_{-592}$ TA genotypes when compared to the subjects with the corresponding CC and TA genotypes.

4. Conclusion

The serum profile of IL-10 was upregulated in hepatitis B patients, and the infections were associated with an increased level of this cytokine, Such immunogenetic background not just conferred predisposition but also impacted the serum level of these cytokines in HBV and HCV patients.

5. Acknowledgments

We thank all our friends for the critical support of the manuscript.

References

[1] WHO, (2016). Hepatitis. Retrieved 2016-6-20.
[2] Bertoletti, A. and Gehring, A. (2006). The immune response during hepatitis B virus infection. J Gen Virol 87:1439-1449.
[3] Saxena, R., Chawla, Y., Verma, I., and Kaur, J. (2014). Association of interleukin-10 with hepatitis B virus (HBV) mediated disease progression in Indian population. Indian J Med Res. 139:737-745
[4] Saraiva, M. and O-Garra, A. (2010). The regulation of IL-10 production by immune cells. Nat Rev Immunol., 10:170-181.
[5] Gu, Y., Yang, J., Ouyang, X., Liu, W., Li, H. and Yang, J. (2008) Interleukin 10 suppresses Th17 cytokines secreted by macrophages and T cells. Eur J Immunol., 38:1807-1813
[6] Cope, A., LeFriec, G., Cardone, J. and Kemper, C. (2011). The Th1 life cycle: molecular control of IFN-γ to IL-10 switching. Trends Immunol., 32: 278-286.
[7] Gambhir, D., Lawrence, A., Aggarwal, A., Misra, R., Mandal, S.K. and Naik, S. (2010). Association of tumor necrosis factor alpha and IL-10 promoter polymorphisms with rheumatoid arthritis in North Indian population. Rheumatol Int., 30:1211-1217.
[8] Sofian, M., Ebrahim, K., Arezoo, A., Soudabeh, H., Mohammad, B., Ali, E., Ali, j., Ali, A. and Amitis, R. (2013). No Correlation Between Interleukin-10 Gene Promoter Polymorphisms and Hepatitis B Virus Infection Outcome. Hepatolgy, 13:8800-8803.
[9] Dai, C.Y., Chuang, W.L. and Lee, L.P. (2008). Association between transforming growth factor–beta 1 polymorphism and virologic characteristics of chronic hepatitis C. Translation Res., 152(4):151-156

[10] Ulger, M., Emekdas, G., Aslan, G., Tas, D., Ilvan, A., Tezcan, S., Calıkoglu, M., Erdal, M. and Kartaloglu, Z. (2014). Determination of the cytokine gene polymorphism and genetic susceptibility in tuberculosis patients. Microbiol. bul., 47(2):250-264.
[11] Bouzgarrou, N., Hassen, E. and Farhat, K., (2009). Combined analysis of interferon-γ and interleukin-10 gene polymorphisms and chronic hepatitis C severity. Hum. Immunol., 70:230-236
[12] Gramenzi, A., Andreone, P., Loggi, E., Foschi, F.G., Cursaro, C., Margotti, M., Biselli, M. and Bernardi, M. (2005). Cytokine profile of peripheral blood mononuclear cell from patients with different outcomes of hepatitis C virus infection. J Viral Hepat., 12:525-530.
[13] Horner, S. M. and Gale, M. (2013). Regulation of hepatic innate immunity by hepatitis C virus. Nat Med., 19:879-888.
[14] Schaefer, E. and Chung, R. (2012). Anti-hepatitis C virus drugs in development. Gastroenterology, 142:1340-1350.
[15] Jiang, R., Feng, X., Guo, Y., Lu, Q., Hou, J., Luo, K. and Fu, N. (2002). T-helper cells in patients with chronic hepatitis B virus infection. Chin Med J., 115:422-424.
[16] Bailey-Bucktrout, S. and Bluestone, J. (2011). Regulatory T cells: stability revisited. Mod Trends Immunol., 32:295-300
[17] Franceschi, C., Capri, M., Monti, D., Caruso, C., Candore, G., Vasto, S., Oliveri, F., Marchegiani, F., Sansoni, P., Baggio, G., Mari, D., Passarino, G. and DeBenedictis, G. (2007). Inflammaging and anti-inflammaging: a systemic perspective on aging and longevity emerged from studies in humans. Mech Ageing Dev., 128:92-105.
[18] Zhu, S., Patel, K. V., Bandinelli, S. and Ferrucci, G. J. (2009). Predictors of interleukin-6 elevation in older adults. J Am Geriatr Soc., 57:1672-1677.
[19] Goetzl, E., Huang, M., Kon, J., Patel, K., Schwartz, J., Fast, K., Ferrucci, L., Madara, K., Taub, D. and Longo, D. (2010). Gender specificity of altered human immune cytokine profiles in aging. FASEB J., 24:3580-3589.
[20] Poveshchenko, A., Orlov, N., Kazakov, O., Poveshchenko, O. Kim, I., Bondarenko, N., Miller, T., Strunkin, D., Kabakov, A., Reiter, T. and Konenkov, V. (2014). Age and Gender Differences in Cytokine Profile of Lymph and Blood Serum. Advances in Aging Research, 3:216-221
[21] Fonseca, C., Lindah, G., Ponticos, M., Sestini, P., Renzoni, A. and Holmes, A. (2007). A polymorphism in the CTGF promoter region associated with systemic sclerosis. N Engl J Med., 357:1210-1220.
[22] Talaat, R., Dondeti, M., El-Shenawy, S. and Khamiss, O. (2014). Association between IL-10 gene promoter polymorphism and hepatitis B viral infection in an Egyptian population. Biochem Genet., 52:387-402.
[23] Roli, S., Yogesh, C. and Jyotdeep, K. (2014). Association of interleukin-10 with hepatitis B virus (HBV) mediated disease progression in Indian population. Indian J Med Res., 139:737-745.
[24] Afzal, M., Tahir, S., Salman, A., Baig, T., Shafi, T. Zaidi, N. and Qadri, I. (2011). Analysis of interleukin-10 gene polymorphisms and hepatitis C susceptibility in Pakistan. J Infect Dev., 5:473-479
[25] Yong-liang, L., Xiao, W., Huang, H. and AI, L. (2010). Allele polymorphisms of interleukin-10 and hepatitis B, C virus infection. Chin Med J., 123:1338-1344.
[26] Gao, Q., Zhang, S., Wu, L., Jia, M., Mi, Y. and Liu, D. (2011). Association of IL-10-1082 and IL-10-592 polymorphisms with chronic hepatitis B and/or hepatitis C virus infection among plasma donors in a rural area of Hebei Province, China. Wei Sheng Yan Jiu., 40:709-713.
[27] Zhang, P., Chen, Z., Chen, F., Li, M., Fan, J., Zhou, H., Liu, J. and Huang, Z. (2004). Expression of IFN-gamma and its receptor alpha in the peripheral blood of patients with chronic hepatitis C. Chin Med J., 117:79-82.
[28] Gao, Q., Liu, D., Zhang, S., Jia, M., Wang, L., Wu, L. and Tong, L. (2009). Polymorphisms of some cytokines and chronic hepatitis B and C virus infection. WJG., 15:5610-5619