Journal of Wasit for Science and Medicine 2026:19, (4), 34-45

Association of IL-10 (rs1800896) Gene Polymorphism and Serum IL-10 Levels with Chronic Hepatitis B Infection

Hussain Naeim Mohammed[1], **Alaa Ali Matrood**[2]

[1]Department of Biology, College of Science, Wasit University, Wasit, Iraq

alabdyh648@gmail.com

Abstract

Chronic hepatitis B virus (HBV) infection is a main general validity threat. It evolves under the influence of various factors, including immunogenetic ones. This study assessed the correlation between the occurrence of interleukin 10 gene (IL10) polymorphisms and the development of these infections. This retrospective case control study 64 chronic HBV infections, 39 HBV clearance groups and 31 controls. "Tetra-primer ARMS-PCR" is a technique that used to study the single nucleotide polymorphisms (SNPs) at IL10 promoter (-1082A>G; rs1800896). The IL-10 levels were determined in patients with chronic HBV infections, HBV clearance and control group by using an Enzyme-Linked Immunosorbent Assay (ELISA). Based on allele polymorphism analysis, three genotypes were identified: AA (46.8 %), AG (43.7 %) and GG (9.3 %). The level of IL10 in HBV patients was significantly greater than that in other sets. In addition, these findings indicate that polymorphism may not affect initial infection but rather disease progression or disease immune response.

Keywords: Hepatitis B virus, Serum IL-10 Levels, Interleukin 10 Gene Polymorphism.

Introduction

Hepatitis B virus (HBV) infection is a main global general validity problem. Sixty-three percent of the nearly 1.23 million novel infection cases that are recorded each year occur [1]. Hepatitis B surface antigen" (HBsAg) was projected to be present in 291 million people worldwide in 2016; approximately five percent of adult HBV cases develop into chronic status and remain [2].

Numerous factors, including viral and immunogenetic factors, that participate to the onset of a persistent HBV infection were examined. In fact, A diminished immune response significantly influences the prolonged development of illness [2;3]. In individuals with a resolutive infection, memory T and B cells effectively maintain immune surveillance by reacting quickly to the virus and preventing reproduction.

T and B cell dysfunction in a chronic HBV infection is sustained by prolonged exposure to elevated viral antigens and an immunosuppressive liver environment [2].

The normal course of HBV infection is highly diverse: resolution with clearance can occur quickly among some, whereas others chronically continue an asymptomatic course for prolonged periods before progression to debilitating and frequently fatal liver disease [4].

Recently, the focus has progressively moved towards host immune changes and their derived biomarkers. These indicators are particularly interesting as they reflect a more complex host–virus interaction, not merely the detection of a viral component.

Many cytokines and chemokines, soluble mediators of innate and adaptive immunity, exhibit predictable changes during viral infection and liver inflammation, and some have been proposed as diagnostic or prognostic markers in HBV disease [5;6]. Compared to direct viral markers such as HBV DNA, HBsAg and HBeAg, host-derived biomarkers provide earlier indication of immune response, so can signal evidence of emerging immunological control (or impending hepatic injury), delineating between different phases of immunological disease (e.g. immune-tolerant vs. immune-active) that inform treatment decisions [4;7].

These cytokines, Interleukin-10 (IL-10) has emerged as an especially interesting candidate. Many immune cells, including regulatory T cells, monocytes/macrophages, and some B cell subsets, generate the pleiotropic cytokine IL-10, that is primarily anti-inflammatory. IL-10 is among the key cytokines that have an inhibitory and modulatory influence on translational events.

for modulating the activity of proinflammatory cytokines throughout the healing phases of viral infections and inflammation, therefore lessening the damage brought on by detrimental inflammatory factors [8]. Thus, in the setting of HBV infection, IL-10 has been shown to have both protective and harmful roles. Numerous studies show that individuals with chronic HBV have far higher levels of IL-10 than healthy, uninfected controls [9]. These elevations have been associated with ongoing viral replication, immunological tolerance, and the development of cirrhosis and hepatocellular injury [8;10]. Additionally, IL-10 that has already been reported to potentially be a prognostic factor in important clinical situations like acute, chronic failure of the liver caused by HBV,

for its higher expression associated with the risk of adverse outcome and death [11].

However, owing to these findings, the reliability of IL-10 as an independent diagnostic marker for HBV is still unknown. Its elevation is not unique to HBV, as raised levels are also detected in diverse conditions including bacterial sepsis, malignancies, autoimmune disorders, and other viral infections [12].

Furthermore, genetic polymorphisms within the IL10 locus, coinfection with other pathogens, and prior therapeutic interventions additionally confound interpretation of serum concentrations [12;10].

Materials and Methods

2.1 Study Population

The current study comprised 134 human samples, 64 of which were from HBV-infected patients (33 males and 31 females) with ages ranging from 13 to 75 years who were diagnosed by a specialist physician based on clinical symptoms, physical signs, confirmed HBV infection, and Hepatitis B surface antigen (HBsAg) positive test. and 39 samples from people who had recovered from HBV infection, as indicated by positive hepatitis B core antibody (HBcore Ab) and hepatitis B surface antibody (HBsAb).

An additional 31 health samples were collected as the control group. The samples were gathered from Al-Karama Teaching Hospitals, Viral Hepatitis Testing Center at Al-Zahraa Teaching Hospital and the General Public Health Central lab in AL-Kut city in Wasit province.

2.2 Plasma Preparation

A total of 134 people had venal blood drawn from them: 64 patients with HBV infection, 39 people with HBV clearance, and 31 healthy persons who served as the control group. Each participant had around 5 mL of blood extracted into anticoagulant-filled EDTA tubes. For ten minutes, the sample were centrifuged at 3000 rpm. To prevent cellular contamination, the clear plasma was carefully separated and put into sterile, labeled Eppendorf tubes. Prior to analysis, plasma Samples were stored at minus -20 degrees Celsius.

2.3 Laboratory Diagnosis

Hepatitis B core antibody (HBcAb), Hepatitis B surface antigen (HBsAg), and Hepatitis B surface antibody (HBsAb) were identified in HBV patients using the enzyme-linked immunosorbent test (ELISA) following blood collection and centrifugation. DNA viral load was quantified using qRT-PCR in HBsAg positive patient.

An automated extraction equipment (Viral nucleic acid extraction kit, Zybio, China) was used to extract DNA for HBV viral load detection. The HBV viral load Quantification Kit (Sacace, Italy) was then used to quantify the HBV DNA viral load using the qRT-PCR BIORAD CFX-96 system "BIO-RAD Laboratories, Hercules, CA, USA".

2.4 Detection of IL -10 Levels

Interleukin-10 (IL-10) levels in the serum were measured using the ELISA. Instructions from the manufacturer were followed while using commercially accessible ELISA sandwich kits. In short, microtiter plates that had been previously coated with IL-10-specific antibodies were filled with standards and blood samples. After then, The biotinylated human IL-10 antibody binds to the sample's IL-10.

After that, Streptavidin-HRP binds the biotinylated IL-10 antibody. Unbound streptavidin-HRP is removed during a washing phase following incubation. The amount of human IL-10 is shown by the color that emerges when the substrate solution is added. After adding an acidic preventative solution to cease the reaction, wavelength absorption is calculated at 450 nm.

2.5 Genomic DNA Analysis

Genomic DNA was extracted from leukocytes in peripheral blood using the Geneaid - DNA from the genome purification kit (Taiwan) and stored at -20 °C. Two microliters of extracted DNA samples were placed into a micro-volume spectrophotometer (Bio photometer plus) with the ratio of absorption set at 260 and 280 nm in order to determine the DNA's purity and concentration.

All of the genomic DNA samples had an average concentration of 50–70 μg/ml and a purity (OD 260/OD 280) between 1.7 and 1.9. IL-10 (rs1800896) was genotyped utilizing the tetra-primer ARMS PCR technique.

One common technique for SNP genotyping is the "tetra-primer amplification refractory mutation system (ARMS) polymerase chain reaction (PCR)". Regardless of the target gene's genotype, the outside primers amplify a sizable portion of it in a single step reaction. However, each interior primer interacts with a unique opposite outer primer during PCR amplification to produce smaller, allele-specific amplicons that differ in size and are easily recognized as homozygous or heterozygous [13].

2.6 Method validation, genotyping, and primer design

The (rs1800896) SNP were genotyped using the "tetra-primer ARMS–PCR" technique [14]. This technique is rapidly and reliable method for SNPs identification. The T-ARMS-PCR technique uses four primers: "forward outer, reverse outer, forward inner, and reverse inner". For the IL10 rs1800896 polymorphism, we employed two external primers forward outer: "5 CTCCCAGTTACAGTCTAAACTGGAAT G-3" (Tm= 61.4 C) and Reverse outer: (5"-GGATTAAATTGGCCTTAGAGTTTCTT TT-3") (Tm= 5.7 C) and two allele-specific internal primers (forward inner [G allele]: "5-AAGACAACACTACTAAGGCTTCTTTG GTAG-3" (Tm= 61.2 C) and reverse inner [A allele]: "5-TTTCCTCTTACCTATCCCTACTTCCAC T-3" (Tm= 63 C) [15]. Using a commercially available PCR master mix "Go Taq Green Master Mix, Promega, Daejeon, USA", polymerase chain reaction (PCR) was carried out in accordance with the manufacturer's recommended technique. The Go Taq Green Master Mix, 2x, 12.5 µL, 5 µL template DNA, 0.5 µL each primer (10 µM), and 5.5 µL DNase-free water were added to a 0.2 mL PCR tube. The PCR cycling conditions were 5 minutes at 95°C, 35 cycles of 30 seconds at 95°C, 40 seconds at 58°C, 60 seconds at 72°C, and an 8-minute last step at 72°C. Every reaction was verified using an ethidium bromide-containing 1.5% agarose gel. Different amplification band sizes appeared to be located when the "tetra-primer ARMS-PCR" was finished. For the rs1800896 SNP, however, we observed pieces with sizes of 199 bp, 292 bp, as well as 433 bp.

2.7 Statistical Analysis

Chi-squared test was used to compare between HBV patients, HBV clearance, as well as controls. exact test Fisher was used to calculate the Hardy-Weinberg equilibrium (HWE) for SNP in the controls. The odds ratios (ORs) and confidence intervals of 95% (CIs) for genotype analysis were calculated using logistic regression models that were adjusted for confounding factors. Statistical significance was defined as a P-value of less than 0.05. The SPSS (Version 20, IBM) statistics package software was used for all statistical analyses.

Results and Discussion

The distribution of persons with Hepatitis B infection by age and gender HBV clearance and healthy controls demonstrates in Table 1. The highest percentage of (51.7%) at age ≥ 50-years old, followed by (21.8%) at age < 30-years old, (18.7%) at age 40–49-years old, and (7.8%) 30–39-years old in patients with HBV Likewise, the sex distribution

showed significantly difference (P = 0.002), as males constituted 51.6% of the patient group, 64.2% of HBV clearance group and 51.7% of the controls, while females represented 48.4%, 35.8% and 48.3%, respectively.

Table1: Distribution of patients with Hepatitis B infection by age and gender.

	Patients (No. = 64)		Control (No. = 31)		Clearance (No. = 39)	
	Freq	%	Freq.	%	Freq.	%
Age						
< 30	14	21.8	10	32.2	8	20.5
30–39	5	7.8	5	16.1	7	17.9
40–49	12	18.7	9	29	3	7.6
≥ 50	33	51.7	7	22	21	53.8
P value	0.28(N.S)					
Male	33	51.6	16	51.7	25	
Female	31	48.4	15	48.3	14	
p.value	0.002**S					

** Significant differences; NS: not significant; S: significant at P > 0.05; n: number of instances

Overall, age did not significantly differ amongst the three groups (p > 0.05 for all), but sex did significantly differ (P = 0.002), indicating that cases, clearance, and controls were generally well-matched for these demographic factors. Individuals with hepatitis B had considerably higher serum IL-10 levels than both the clearance group and healthy controls as shown in Table 2. The patient group's average IL-10 concentration was 159.6±99.1 ng/L, while the clearance group had a concentration of 111.4±50 ng/L and the control group had a concentration of 96.2±70.9 ng/L.

Table 2: Assessment of the levels of IL-10 between individuals with hepatitis B, HBV clearance and control.

Group IL-10 (ng\L)	Mean ±SD
Patients with HBV n = 64	159.6±99.1
Control n= 31	96.2±70.9
Clearance HBV n = 39	111.4±50
P value	<0.001**

Function of cytokines in a range of infectious illnesses has been extensively proven by several research. They play a complicated part in the pathophysiology of viral infections, including HBV. T cells, B cells, dendritic cells, and monocytes/macrophages all generate the vital anti-inflammatory cytokine IL10.

It primarily targets "antigen-presenting cells" (APCs) to decrease inflammation by suppressing MHC class II molecule expression and cytokine synthesis [16]. Effective viral clearance may be hampered by this reduction of the immune system's adaptive response, which would increase the persistence of HBV.

In this investigation, elevated IL10 levels were significantly associated with CBI, suggesting a potential involvement in the development of chronicity. This result is in line with earlier research showing that CHB had greater IL10 levels than healthy controls, including [9;17]

which reported that individuals with HBV infection had far higher blood IL-10 levels than healthy controls.

A positive relationship among serum IL-10 levels and HBV viral load (P = 0.001) as shown in table 3. The mean levels of IL-10 were 97.6±32.2 for low viremia, 108.6±41.6 for moderate viremia, and 144±50.4 for high viremia.

Table 3: The relationship among HBV viral load and IL10 serum levels.

HBV DNA Viral load (IU/ml) patients n =64	IL-10 (ng/L)
Low viremia n = 27	97.6±32.2
Moderate viremia n= 20	108.6±41.6
High viremia n = 17	144±50.4
P value	<0.001**

Additionally, the current study's findings demonstrated a favorable association between HBV viral load and IL-10 blood levels, these finding are consistent with previous study, conducted by Das A et al [18] revealed a relationship amongst IL-10 levels as well as spontaneous flare-ups of liver disease, besides a relationship among IL-10 levels and the peak of liver damage and a rise in viral load.

[19] This study showed positive a relationship between IL-10 levels and serum HBV-DNA load, that suggesting increased IL-10 serum levels can supress host anti-HBV cell immune activity, which leads increased HBV replications and existence [20]. That indicated a strong, reasonable connection among HBV viral load and IL-10 levels in chronic infection, suggesting that increased IL-10 may coincide with higher viremia across patient.

The current investigation found three genotypes (AA, AG, and GG) that correlate to two alleles (A and G) figure 1 . In both HBV individuals as well as the clearance group, the genotype as well as allele distributions agreed with HWE, demonstrating genetic stability and the accuracy of the genotyping data. The AA genotype showed a slightly higher frequency in HBV patients (46.8%) compared with the clearance group (46.1%), while the AG genotype was slightly lower in HBV patients (43.7%) than in the clearance group (48.7%) table (4) . The GG genotype remained the least frequent in both groups.

However, no significant statistical variation. Odds ratio analysis further confirmed the absence of a strong genetic association,

as the OR values for AA (1.03), AG (0.82), and GG (1.91) were close to unity and the 95% confidence intervals included 1. Similarly, allele frequencies of A and G showed no significant variation between HBV patients and the clearance group. These results suggest that the IL-10 rs1800896 polymorphism does not seem to be a significant independent factor in this cohort's vulnerability to either viral clearance or persistent HBV infection

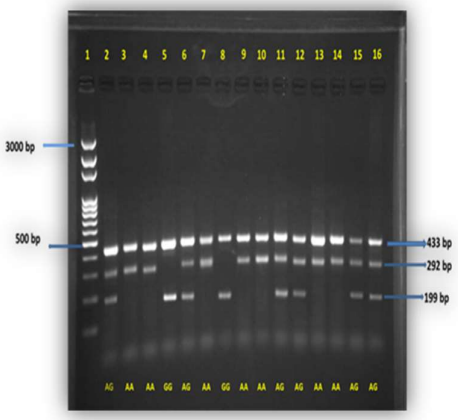


Figure (1). PCR product analysis for IL-10 (rs1800896) gene polymorphism from plasma samples in HBV patients. Lane 1 (Marker ladder 100-3000bp). Lanes (2,6,11,12,15,16) carrying heterozygote genotype (AG) = (292+433+199) bp, Lanes (3,4,7,9,10,13,14) carrying wild type homozygote genotype (AA) = (433+292) bp and Lanes (5,8) carrying homozygous mutant. genotype (GG) = (199+433) bp; separated on Agarose (2%).

Table 4: Observed and expected genotype with allele frequencies of in HBV patients and clearance.

Groups		*IL-10 (rs1800896)*								
		Genotypes			**HWE**	**Alleles**		**chi2**	**A**	G
		AA N/%	**AG**	**GG**		**A**	**G**			
HBV (n=64)	**Observe**	30	28	6	N.S.	88	40			
		46.8	43.7	9.3		68.7	31.2			
	Expect	30.25	27.5	6.25		Not estimated		0.021	0.687	0.312
		47.2	42.9	9.7						
Clearance HBV	**Observed**	18	19	2	N.S.	55	23			
		46.1	48.7	5.1		70.5	29.4			
	Expect	19.3	16.2	3.3		Not estimated		1.47	0.7	0.29
		49.4	41.5	8.4						
(OR)		1.03	0.82	1.91		0.92	1.09			
Fish extra		1	0.6	0.7		0.8	0.8			
95% C.L.		0.43-2.27	0.37-1.8	0.37-9.8		0.5-1.69	0.59-2			

In our investigation, higher viral loads were associated with higher levels of IL-10. Promoter polymorphisms and other host genetic characteristics have been demonstrated to be the most important element in cytokine regulation. Important SNPs include -819C>T, -1082A>C, and -592A>C.

The effects of the three main IL10 regulatory polymorphisms G on cytokine

synthesis and expression of genes have been thoroughly investigated [12]. Due to conflicting findings in previous studies, we concentrated on SNPs -1082A>G. -1082 GG was the only genotype that was substantially linked to chronic infection; its risk was nearly twice that of HBV clearance. The main function of IL-10, which expression may be influenced by gene polymorphisms, is to limit the production of pro-inflammatory cytokines [21;22].

In the present research, the IL-10-1082A>G polymorphism and HBV vulnerability was not significantly correlate, suggesting that this polymorphism may influence disease progression or immune response rather than initial infection. Our findings suggested that IL-10 polymorphism might not be a significant independent factor in predicting this population's vulnerability to chronic infection with HBV or clearance of the virus. The present findings are consistent with Mahavar N., et al[23] But dis agrees with Talaat R. et al [24]. The GG genotype was much higher in HBV individuals than in healthy controls, according to studies done in an Egyptian community. However, neither the HBV patients nor the controls had significantly altered GA/AA genotypes.

5. Conclusion

The result show that patients infected with HBV have significantly elevated levels of IL-10 compared with control and clearance group. IL-10 levels and viral load are positively correlated, according to the current study. HBV susceptibility was not much changed by the IL-10 -1082A>G polymorphism. These indicate that polymorphism may not affect initial infection but rather disease progression or disease immune response

6. References

1. Alberts C. J., Clifford G. M., Georges D., Negro F., Lesi O. A., Hutin Y. J., and de Martel C., (2022). Worldwide prevalence of hepatitis B virus and hepatitis C virus among patients with cirrhosis at country, region, and global levels: a systematic review. The Lancet Gastroenterology and Hepatology. 7,(8), 724-735.
2. Shi Y., and Zheng M., (2020). Hepatitis B virus persistence and reactivation. British Medical Journal. 370, 16.
3. Ribeiro C. R., de A., Beghini D. G., Lemos A. S., Martinelli K. G., de Mello V. da M., de Almeida N. A. A., Lewis-Ximenez L. L., and de Paula V. S., (2022). Cytokines

profile in patients with acute and chronic hepatitis B infection. Microbiology and Immunology. 66,(1), 31–39.

4. Kramvis A., Chang K.-M., Dandri, M., Farci P., Glebe D., Hu, J., Janssen H. L. A., Lau D. T. Y., Penicaud C., and Pollicino T., (2022). A roadmap for serum biomarkers for hepatitis B virus: current status and future outlook. Nature Reviews Gastroenterology and Hepatology. 19,(11), 727–745.
5. Zhong S., Zhang T., Tang L., and Li Y., (2021). Cytokines and chemokines in HBV infection. Frontiers in Molecular Biosciences. 8, 805625.
6. Zhang Q., Huang H., Sun A., Liu C., Wang Z., Shi F., Duan W., Sun X., Wang Q., and Sun P., (2021). Change of cytokines in chronic hepatitis B patients and HBeAg are positively correlated with HBV RNA, based on real-world study. Journal of Clinical and Translational Hepatology. 10,(3), 390.
7. Xiao L., Tang K., Fu T., Yuan X., Seery S., Zhang W., Ji Z., He Z., Yang Y., and Zhang W., (2024). Cytokine profiles and virological markers highlight distinctive immune statuses, and effectivenesses and limitations of NAs across different courses of chronic HBV infection. Cytokine. 173, 156442.
8. Dimitriadis K., Katelani S., Pappa M., Fragkoulis G. E., and Androutsakos T., (2023). The role of interleukins in HBV infection: a narrative review. Journal of Personalized Medicine. 13,(12), 1675.
9. AbdulAl-Abass R. O., (2025). The Significance of Serum IL-10 Levels in Diagnosing Hepatitis B Virus (HBV) Infection. International Journal of Medical Science and Dental Health. 11,(9), 156–163.
10. Jia Y., Jiao X., Shi W., Luo Y., Xiang H., Liang J., and Gao Y., (2024). Expression of 10 circulating cytokines/chemokines in HBV-related liver disease. Infectious Agents and Cancer. 19,(1), 20.
11. Liu L., Xiao N., Chen P., and Zhu X., (2023). IL-10 predicts the prognosis of patients with hepatitis B virus-related acute-on-chronic liver failure combined with spontaneous bacterial peritonitis. Frontiers in Medicine. 10, 1188300.
12. Rybicka M., Woziwodzka A., Sznarkowska A., Romanowski T., Stalke P., Dręczewski M., Verrier E. R., Baumert T. F., and Bielawski K. P., (2020). Genetic variation in IL-10 influences the progression of

hepatitis B infection. International Journal of Infectious Diseases. 96, 260–265.

13. Mesrian Tanha. H., Mojtabavi Naeini. M., Rahgozar S., Rasa S. M. M., and Vallian S., (2015). Modified tetra-primer ARMS PCR as a single-nucleotide polymorphism genotyping tool. Genetic Testing and Molecular Biomarkers. 19,(3), 156–161.
14. Ye S., Dhillon S., Ke X., Collins A. R., and Day I. N. M., (2001). An efficient procedure for genotyping single nucleotide polymorphisms. Nucleic Acids Research. 29,(17), e88–e88.
15. Datta A., Zahora F. T., Aziz M. A., Uddin M. S., Ferdous M., Millat M. S., Sarwar M. S., Barek M. A., Jafrin S., and Nahar S., (2020). Association study of IL10 gene polymorphisms (rs1800872 and rs1800896) with cervical cancer in the Bangladeshi women. International Immunopharmacology. 89, 107091.
16. Saraiva M., Vieira P., and O'garra A., (2019). Biology and therapeutic potential of interleukin-10. Journal of Experimental Medicine. 217,(1), e20190418.
17. Ghanem R. A., and Muzher M. N., (2024). Genetic Polymorphism in the Human IL-10 and Human IL-28 B with Increased Risk of Hepatitis B Virus Infection. Egyptian Journal of Medical Microbiology. 33,(4), 67–73.
18. Das A., Ellis G., Pallant C., Lopes A. R., Khanna P., Peppa D., Chen A., Blair P., Dusheiko G., and Gill U., (2012). IL-10–producing regulatory B cells in the pathogenesis of chronic hepatitis B virus infection. The Journal of Immunology. 189,(8), 3925–3935.
19. Ming D.-S., Lin Z.-Z., and Qiu X.-D., (2016). Analysis of IL-6, IL-10, IL-17 levels in the blood of patients with chronic hepatitis B. Int J Clin Exp Med. 9,(6), 9437–9442.
20. Manea M., Mărunțelu I., and Constantinescu I., (2024). Extended analysis on peripheral blood cytokines correlated with hepatitis B virus viral load in chronically infected patients–a systematic review and meta-analysis. Frontiers in Medicine. 11, 1429926.
21. Shu C., Wang J., He Y., Song T., Chen, Z., Tang S., and Tang X., (2015). Effects of interleukin 10 polymorphisms on the development of hepatitis B virus infection: a systemic review and meta-analysis. International Journal of Clinical and Experimental Medicine. 8,(8), 12028.

22. Gao L., Chen X., Zhang L., Wu D., Zhao H., and Niu, J., (2016). Association of IL-10 polymorphisms with hepatitis B virus infection and outcome in Han population. European Journal of Medical Research. 21,(1), 23.
23. Mahavar N., Naseri M., ANANI S. G., Fereidouni M., Ziaee M., Safari H., Naghizadeh M. S., Tane A., and Mahdavi R., (2021). IL-10-1082 A/G (RS1800896) polymorphism is effective in clearing hepatitis B virus infection.
24. Talaat R. M., Dondeti M. F., El-Shenawy S. Z., and Khamiss O. A. (2014). Association between IL-10 gene promoter polymorphism and hepatitis B viral infection in an Egyptian population. Biochemical Genetics. 52,(9), 387–402.