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Determination of levels of Cytokines (HGF ,TNF- ,CXCL-13) in serum of hepatitis B patients in Wasit Province

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ABSTRACT

HBV infection is one of the major concerns and a constant threat to public health. Cytokines are used in biological studies as vital signs in the diagnosis, treatment, and follow-up of progression a number of diseases such as CHB and HCC .

This study aimed to evaluation the levels of HGF , TNF- α , CXCL-13 in HBV patients .Studies have shown that the hepatocyte growth factor is the engine for the development of HCC caused by infection with HBV, and may serve as an effective biomarker for targeted therapy. The study included 100 patients with positive HBs Ag (100% positive). Three cytokines (HGF , TNF- α , CXCL-13) were analyzed in the

serum of patients ,and the results showed highly significant increase inthe levels of those cytokines in patients comparing to healthy individuals($p \leq 0.01$). Also ,the results showed there is a highly significant difference($P<0.001$) of HGF levels when comparing between acute andchronicHBV patients .Our results concluded HGF , TNF- α , CXCL-13 play a key role in development of HBV.

KEYWORDS: HBV, CXCL-13, HGF, TNF-α, ELISA, Cytokines

INTRODUCTION

Hepatitis B virus (HBV) belongs to Orthohepadnavirus genus a member of the Hepadnaviridae family that leads to hepatitis B, liver cancer, and liver cirrhosis (Sadik *et al*, 2014).

Cytokines are small proteins that are excreted from different cells and although they regulate a number of vital functions in the human body, including innate and acquired immunity, hematopoiesis, inflammation and repair, and proliferation ,they play an important role in protection against viral infections and enhancing viral clearance. Cytokines are also used in biological studies as vital signs in the diagnosis, treatment, and follow-up of progression a number of diseases such as CHB and HCC .Studies have showed that the hepatocyte growth factor is the engine for the development of HCC caused by infection with HBV, and may serve as an effective biomarker for targeted therapy and plays a major role in liver repair damage (Xie *et al* ., 2013). The tumor necrosis factor alpha decrease the entry of HBV in the cells, and reduces the replication of virus ,also may help in immune response to HBV clearance (Valaydon*et al* ., 2016) and responsible for multiple cell signaling events leading to necrosis or apoptosis (Parameswaran and Patial. 2010). . Also, the studies demonstrated that HBV increased the production of CXCL13serum level ,which might be associated with immune control of chronic HBV infection, and HBV might increase the

percentage of T cells through promoting the expression of CXCL13, which lead to the induction of immune tolerance (Liu *et al*., 2017) and it can recruit B cells and directly regulate the maturation and development of B cells in vivo, and can participate in the transmission of immune system signals (Schiffer *et al* ., 2015).

Cytokines has antiviral and immune-modulatory activity play an significant role in direct elimination of the HBV replication of hepatocytes (Phillips *et al*., 2010), and modulation of antiviral T-cell function in controlling adaptive immune response to HBV, such as TNF-α (Freeman *et al*., 2012), and IL21 (Phillips *et al*., 2010)(Lin and Young, 2014; Gehring and Protzer, 2019). In order to avoid primary HBV infection, IFN- γ and TNF-α are key antiviral mediators of specific CD8+T cells (Phillips *et al*., 2010; Bertoletti and Ferrari, 2016). Up to date, Single nucleotide polymorphisms (SNPs) in the host genes IFN-γ, TNF-α, CXCL13, PDCD1, TLR3, and TLR9 were should to be relevant for intrauterine transmission (mother-to-child transmission) (Yu *et al*., 2006; Gao*et al.*, 2015; Wan *et al*., 2016; Liu *et al*., 2018).

Liver regeneration is a phase requiring at least two crucial steps: the entry of the quiescent hepatocyte into the cell cycle (priming) and the development beyond the limitation point in the cycle's G1 level. These steps have been proposed to be regulated separately, priming by the tumor necrosis factor alpha and interleukin-6 family cytokines, and progression of the cell cycle by growth factors, mainly by hepatocyte growth factor (HGF) (Fausto , 2000;Zimmermann , 2004).

MATERIALS AND METHODS

Samples collection

This study included 100 of hepatitis B patients , 72 males and 28 females, ranging from (10-70) year , with median age 37 year. Patients were outpatients attending Al-Karama Teaching Hospital and Central Health Laboratory in AL-Kut City/ Wasit Province / Iraq. This work was carried out in the College of Science, Department of Biology, College of Medicine, PCR unit at AL-Karama Teaching Hospital and Central Health Laboratory in Wasit Province/Iraq ,from December 2017 to June 2018,.

Five milliliters of peripheral blood were collected from patients. Three milliliters of blood were used to isolate the serum that was frozen at-40Ċ and which was used in the study of proteins while the remaining blood of patients was used to isolate the viral DNA and was frozen at a temperature of -70 Ċ for use in the molecular study .Patients co-infected with hepatitis C and HIV and cancer ,also patients undergo dialysis and other autoimmune diseases, as well as the pregnant women were excluded from our study.

Screen of HBV infection by using ELISA kit for HBsAg

Serum samples were carried out according to the designation (Foresight, USA) on the ELISA worksheet.

Evaluation of Human (CXCL- 13 , TNF- , HGF) Concentrations byELISA kits

These ELISA kit from (SinoGene Clone Biotech / China) used Sandwich detection method to assay the quantitative level of Human HGF (TNF- α , CXCL-13) in the sample, adopt purified HGF antibody to coat microtiter plate, made solid-phase antibody, then added HGF (TNF- α , CXCL-13) to wells, Combine HGF (TNF- α , CXCL-13) antibody with labeled HRP to form

antibody-antigen-enzyme-antibody complex, after washing completely, added TMB substrate solution, TMB substrate became blue color at HRP enzyme-catalyzed, reaction is terminated by the addition of a stop solution and the color change is measured at a wavelength of 450 nm. The concentration of HGF TNF- α , CXCL-13 then determined by comparing the O.D. of the samples to the standard curve.

Statistical Analysis

The data was analyzed statistically using SPSS 26 program. The comparisons between groups were performed with analysis of variance (ANOVA) test. Chi-square test was used to significant compare between percentage and T-Test was used to significant compare between means. P-Value less than 0.05 was considered significant. Correlation between protein and other variables such as stages and viral load was done using sperman Correlation.

RESULTS

Detection of HGF , TNF- α , CXCL-13 in HBV patients

Levels of HGF , TNF- α , CXCL-13 anti-bodies in patients and control group

Table (1) was showed the levels of cytokines in HBV-infected patients compared with healthy individuals as control group .The results showed a high significant increase of the three proteins (HGF , TNF- α , CXCL-13) in the patients compared to the control group ($P< 0.01$).

Table (1):Levels of HGF , TNF-α , CXCL-13 anti-bodies in patients and control group

Protein	Gender	Mean	Standard Deviation	Standard Error	t	Sig
HGF pg/ml	Control	146.65	81	18.11	-8.27	0.000
	Patients	581.26	409.77	49.33		
TNF- αng/L	Control	30.16	20.2	4.52	-3.2	0.002
	Patients	49.39	33.01	3.97		
CXCL -13 pg/ml	Control	62.2	25.01	5.59	-5.08	0.000
	Patients	169.45	169.1	20.36		

Detection the levels of HGF , TNF- α , CXCL-13 in HBV patients according gender

Our results revealed there were no significant differences in the levels of HGF,TNF- α , CXCL-13 between male and female patients as showed in table (2).

Table (2): Distribution of HGF , TNF- α , CXCL-13 anti-bodies levels in patients according to gender

Protein	Gender	Mean	Standard Deviation	Standard Error	t	Sig
HGF pg/ml	Male	681.03	279.33	39.12	-1.15	0.255
	Female	676.33	655.15	154.42		
TNF- αng/L	Male	46.62	29.78	4.17	-1.18	0.238
	Female	57.25	40.79	9.61		
CXCL -13 pg/ml	Male	187.13	186.15	26.07	1.48	0.148
	Female	119.34	94.1	22.18		

However , the results in table (3) showed that there were highly significant differences between male patients compared to male healthy individuals for HGF and CXCL13 ($P \leq 0.05$),while the TNF-α did not show a clear significant difference between the patients and healthy males.

It was also noted that the patient males showed higher levels of all proteins compared to the healthy males

Table (3): Differences of HGF, TNF- α , CXCL-13 levels in male patients and control group

Protein	Cases (Males)	Mean	Standard Deviation	Standard Error	t	Sig
HGF pg/ml	Control	163.62	89.07	24.7	-8.3	0.000
	Patients	547.71	279.33	39.11		
TNF- αng/L	Control	33.4	22.06	6.12	-1.5	0.140
	Patients	46.62	29.78	4.17		
CXCL -13 pg/ml	Control	56.71	22.17	6.15	-4.87	0.000
	Patients	187.13	186.15	26.07		

In the same context, the level of HGF and TNF- α were tested, as in table (4), to find out the effect of female sex hormones on those cytokines in females infected with the virus, and it was found that there were significant differences for the HGF and TNF- α ($P \leq 0.05$), unlike CXCL13, which did not show a significant difference. And all the proteins showed an increase in their levels in the infected females compared to the healthy females (table 4)

Table (4): Differences of HGF , TNF- α , CXCL-13 levels in female patients and control group

Protein	Cases (females)	Mean	Standard Deviation	Standard Error	t	Sig
HGF	Control	115.14	55.94	21.14	-2.23	0.036
	Patients	676.33	655.15	154.42		
TNF	Control	24.16	15.97	6.04	-2.92	0.008
	Patients	57.25	40.79	9.61		
CXCL	Control	72.38	28.5	10.77	-1.91	0.070
	Patients	119.34	94.1	22.18		

Detection the levels of HGF , TNF- α , CXCL-13 in HBV patients according activity

The treatment strategy depends on determining the effectiveness of infection with the virus, whether it is chronic or acute. Many biological changes occur to the virus and the hepatocells associated with the degree of activity of the virus. And since the liver is an important organ for the production of cytokines on the one hand and the metabolism of cytokines on the other hand, therefore, studying the level of some cytokines and their relationship with the degree of virus activity is very important to determine the effect of the interaction between the level of cytokines and the activity of the virus. Our results in table(5) showed the levels of HGF,TNF and CXCL13 in acute and chronic infection with HBV. Through the table below, it is evident that there is a highly significant difference in ($P<0.001$) when comparing HGF levels in HBV acute and chronic patients, this is due to the presence of an increase in the level of HGF concentration (629.33) in the serum of chronic HBV patients than those of acute HBV patients (471.38),while the other factors, TNF-α and CXCL-13, did not show any significant differences when compared between patients based on virus activity($P\geq 0.05$).

Table (5): Distribution of HGF , TNF- α , CXCL-13 anti-bodies in patients according to activity

Protein	Activity	Mean	Standard Deviation	Standard Error	t	Sig
HGF pg/ml	Acute	471.38	283.40	61.84	-1.76	<0.001
	Chronic	629.33	1097.6	158.4		
TNF- α ng/L	Acute	42.43	27.66	6.04	-1.16	0.25
	Chronic	52.44	34.93	5.04		
CXCL -13 pg/ml	Acute	189.42	162.01	35.35	0.646	0.521
	Chronic	160.72	173.04	24.98		

Detection the levels of HGF , TNF-α , CXCL-13 in HBV patients according to genotyping

The disease is classified into several genotypes based on the genome sequence and ten genotypes have been identified so far depending on the percentage of changes in the nucleotides of the virus sequences when it is more than 8%. Also, several subtypes have been identified for this virus when the variation of the nucleotides is less than 4-8%.

Over time, there became a clear understanding of the relationship between the genotypes of the virus and the development of viral hepatitis (Sunbul , 2014) .When we compare the mean concentration of HGF, TNF-α and CXCL-13 distribution according to HBV genotyping , As showed in tables (6), (7), (8) regression analysis, we note that there are significant differences between the genotypes of the virus with regard to the levels of protein concentrations mentioned above. Regarding to HGF , It turns out that B, C, and D genotype have a significant effect ($p \leq 0.05$), while the rest have an insignificant effect. These significant variables explain about 52% of the effects on the results of the HGF protein. It is evident from the standard treatments that B is the most influential, followed by D and then C (table 7).

Table (6): The effect of genotype on the level of HGF anti-body in patients

Genotype	Coefficient	Standard Error	t	P-Value
A (Pure + Mixed)	511	308	1.66	0.102
B (Pure + Mixed)	459	91.2	5.03	0.000
C (Pure + Mixed)	260	104	2.5	0.015
D (Pure + Mixed)	455	142	3.2	0.002
E (Pure + Mixed)	-212	154	-1.37	0.175
F (Pure + Mixed)	-445	268	-1.66	0.102
G (Pure + Mixed)	-921	597	-1.54	0.128

As for TNF-α , the results in table (7) showed that both B and D have a significant effect, while the rest are not significant. These significant variables explained about 50% of the effects on the results of TNF-α protein and it is clear from the standard treatments that B is the most influential.

Table (7): The effect of genotype on the level of TNF-α anti-body in patients

Genotype	Coefficient	Standard Error	t	P-Value

A (Pure + Mixed)	4.8	26.4	0.18	0.857
B (Pure + Mixed)	35.26	7.81	4.52	0.000
C (Pure + Mixed)	12.96	8.89	1.46	0.150
D (Pure + Mixed)	42.9	12.1	3.53	0.001
E (Pure + Mixed)	14.9	13.2	1.12	0.265
F (Pure + Mixed)	-31.1	22.9	-1.36	0.179
G (Pure + Mixed)	-53.5	51.1	-1.05	0.299

Table (9) shows CXCL-13 levels according to HBV genotypes, and all genotypes have a significant effect, except for D and E. These significant variables accounted for about 71% of the effects on the results of CXCL-13 protein, and it is clear from the standard treatments that C is the most influential. It is followed by F, then B, A, and G.

Table (8): The effect of genotype on the level of CXCL-13 anti-body in patients

Genotype	Coefficient	Standard Error	t	P-Value
A (Pure + Mixed)	221.5	85.9	2.58	0.012
B (Pure + Mixed)	91.7	25.4	3.6	0.001
C (Pure + Mixed)	144.4	29	4.98	0.000
D (Pure + Mixed)	-25.2	39.6	-0.64	0.527
E (Pure + Mixed)	-12.6	43.1	-0.29	0.770
F (Pure + Mixed)	302	74.6	4.05	0.000
G (Pure + Mixed)	-408	167	-2.45	0.017

Distribution the levels of HGF , TNF-α , CXCL-13 cytokines in HBV patients according to viral load

When infected with the virus, immune cells secrete large quantities of cytokines, which either eliminate the virus or continue infection, which may lead to liver damage (Ming *et al* ., 2016) or cause hepatocellular carcinoma .

Our results in figure (1) showed the distribution of three cytokines (HGF,TNF-α and CXCL-13) according to viral load in patients with HBV. The results reported no significant differences in the three cytokines when comparing between the levels of HBV viral load P > 0.05. However, the lower levels of viral load (< 2000 IU/ml) showed high levels of the HGF comparing with the high levels of viral load(> 2000 IU/ml) as determined by PCR (Figure 1) .

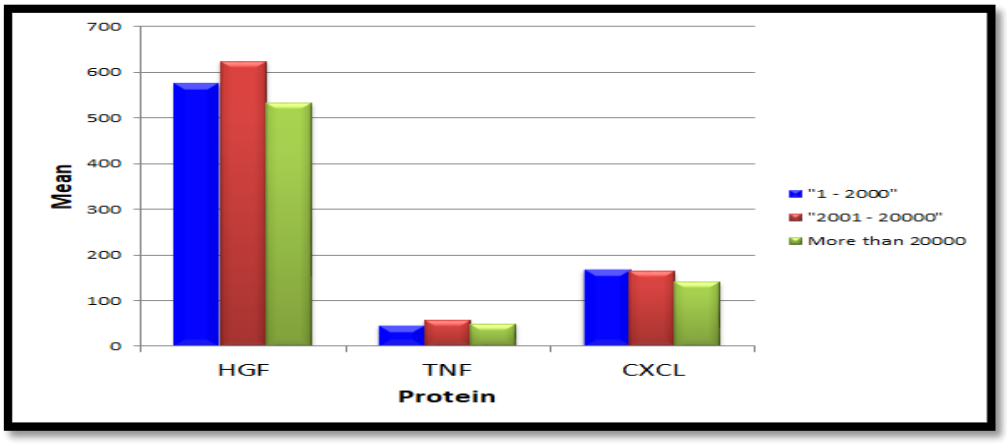

Figure (1): Distribution the levels of HGF , TNF-α , CXCL-13 cytokines in HBV patients according to viral load

Correlation between HGF,TNF-α and CXCL13 in patients with HBV

The cytokines did not show significant correlation between **HGF,TNF-α and CXCL13** in HBV patients as showed in table (9).Several components of the immune system are associated with inflammatory factors that affect the hepatitis process in CHB (Lian*et al* ., 2014) Figure (2,3).

Table (9): Correlation between HGF,TNF and CXCL13 in patients with HBV

Protein		TNF	CXCL
HGF	R	0.102	0.115
	Sig	0.405	0.347
TNF	R		-0.196
	Sig		0.107

R: Correlation Sig : Significance

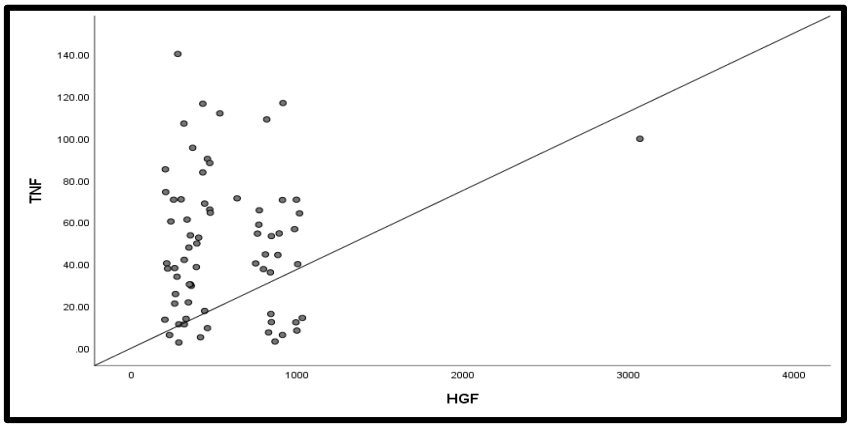

Figure (2): The correlation between TNF-α and HGF antibodies

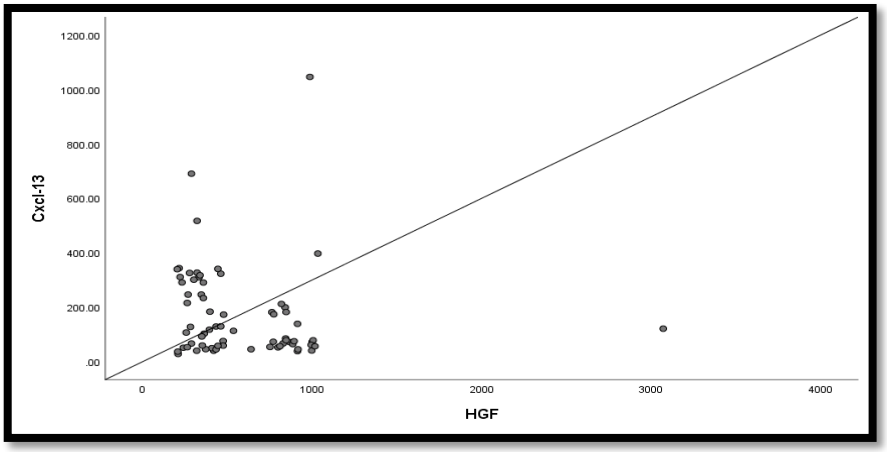

Figure (3): The correlation between CXCL-13 and HGF antibodies

DISCUSSION

Virus infection stimulates the proinflammatory immune response, including cytokines , ((Mogensen and Paludan ., 2001) so the three cytokines HGF , TNF- α , CXCL-13 were proposed for this study to detection their effect upon infection with HBV virus. The results showed a high significant increase of the three proteins (HGF , TNF- α , CXCL-13) in the patients compared to the control group ($P< 0.01$).The immune system is a network of cells, tissues, and organs that work together to protect the body from infection by all pathogens, including viruses. However, the immune system can not only control most of the viruses and pathogens that infect humans, but it can also provide lifelong immunity after successful removal of the infection (Nicholson, 2016). This allows the innate immune system to act as the first line of defense. It has a large number of fast-reacting, non-specific components that can recognize and eliminate invading pathogens and viruses.

In essence, the two host responses both rely on the elimination of infected cells by apoptosis and control HBV infection. One is the self-destruction of infected cells by apoptosis, which acts to limit infection and protect the host (Barreiros *et al*., 2009). The other is the recognition and elimination of infected cells that present viral peptides to cytotoxic T lymphocytes (CTLs), which can transmit apoptosis-inducing signals . CXCL13 is defined as a chemokine 1 or B-lymphocyte chemoattractant-attracting B cell (Worthmann *et al*., 2014).It is chemotactic for mature B cells and T follicular helper (Tfh) cells by working via its cognate receptor C – X – C chemokine receptor type 5 (CXCR5) (Liu *et al*., 2017). Importantly, CXCL13 facilitates the co-migration of B cells and Tfh cells to B cell follicles and germinal centers (GCs), where high-affinity antibody-secreting memory B and plasma cells are generated (Ansel*et al* ., 2000).The stromal cells found in B cell follicles of lymphoid organs normally produce CXCL13 that signals through its cognate receptor, CXCR5, to recruit B cells and Tfh cells to these specialized structures (Allen *et al*., 2004).

Changes that contribute to the survival of virus-infected cells ultimately determine the course of HBV infection. In addition to an inadequate immune response, the current paradigm of viral persistence includes the role played by viral proteins and the induction of cell survival pathways as a way to escape apoptosis (Rapicetta *et al*., 2002; Mateescu *et al.,* 2004). In addition to direct interference with the apoptotic signaling pathway by the viral gene product, host factors activated in response to chronic relentless intrahepatic inflammation and concomitant tissue destruction also protect virus-infected cells from apoptosis. there is a possibility.

TNF-α is a pleiotropic cytokine important for inflammation, maintenance of lymphatic organ structure, and host defense against various pathogens (Pfeffer, 2003). Dysregulated TNF production can be detrimental and is associated with a variety of inflammatory diseases such as rheumatoid arthritis, septic shock, and inflammatory bowel disease (Andreakos*et al*., 2002). For many years, TNF was only considered as a soluble pro-inflammatory cytokine produced by innate immune cells such as macrophages and neutrophils (Kassiotis*et al*., 2001).

A multifunctional growth factor that controls both cell proliferation and cell motility is the hepatocyte growth factor (HGF). On hepatocytes and primary epithelial cells, it exerts a strong

mitogenic impact. Nonparenchymal cells like Kupffer cells, sinusoidal endothelial cells, and hepatic stellate structures are generated by HGF (Nakamura and Mizuno, 2010). In addition, in organs other than the liver, HGF is formed, so the source of serum HGF may be extra-hepatic. In response to tissue injury, HGF is massively deposited on the hepatic biomatrix and activated (Vejchapipat *et al*., 2004). Barreiros *et al*., (2009) study showed elevated HGF levels in patients with HBV infection compared to healthy controls.

Some studies have showed that sex hormones play an important role in the development of viral hepatitis, as well as in the emergence and development of liver cancer (Liu and Liu , 2014).Our results revealed there were no significant differences in the levels of HGF, TNF- α , CXCL-13 between male and female patients . Although there is a slight increase in HGF and CXCL13 in males compared to females, and slight increase in TNF-α in females compared to males. Despite the influence of sex on the cytokines significantly, our results did not indicate any significant differences between the studied samples, and this may be due to the wide variation between the levels of cytokines in the patients serum .
On the other side ,the results of Karabulu *et al.* , (2014) indicated that there was a significant increase in the levels of HGF in the serum of patients males compared to females .While Borawaski and Mysliwiec (2002) showed no association between HGF and gender .In a study conducted by Fujiwara *et al*., (2018) about the survivors because they suffer from liver failure due to their infection with the HB virus, it was found that there is no significant differences between the sexes regarding to HGF in survivor and no survivor patients .Wungu *et al*., (2019) were reported in their study that women patients with liver cirrhosis due to chronic HBV infection were showed higher level of TNF than men .

The results showed that there were highly significant differences between male patients compared to male healthy individuals for HGF and CXCL13 ($P \leq 0.05$),while the TNF-α did not show a clear significant difference between the patients and healthy males. But when compare the female patients with healthy to find out the effect of female sex hormones on those cytokines in females infected with the virus, and it was found that there were significant differences for the HGF and TNF- α ($P \leq 0.05$), unlike CXCL13, which did not show a significant difference. And all the proteins showed an increase in their levels in the infected females compared to the healthy females . Many studies have shown that male subjects are more likely than female subjects to exacerbate chronic hepatitis B due to sex hormones (MacekJilkova *et al*., 2017;Wang *et al*., 2015). This may indicate a strong immune response in men. As well as, the expression of genes controlled by sex hormones differs in different lights and has a special importance in liver metabolism, where the liver is targeted by sex hormones, and its cells are receptors for the hormones estrogens and androgens in males and females. Therefore, males are more susceptible to liver cancer compared to females, while females are more susceptible to autoimmune diseases in the liver as well as acute liver failure (Kur*et al* ., 2020). Another study reported that HBV-related liver cancer occurs more in males than in females (Wang*et al* ., 2015). Also, monitoring the progression of viral B hepatitis and the life cycle of the virus shows the difference between both sexes .The difference between the males and females in the predisposition to HBV infection is due to the effect of sex hormones in addition to the doses of genes carried on the sex chromosomes (Ghosh and Klein, 2017).

Our results showed the levels of HGF,TNF and CXCL13 in acute and chronic infection with HBV. It is evident that there is a highly significant difference ($P<0.001$) when comparing HGF levels in HBV acute and chronic patients, while the other factors, TNF-α and CXCL-13, did not show any significant differences when compared between patients based on virus activity($P\geq 0.05$).

Previous studies have reported that HGF is elevated in active HBV because it protects the liver from loss of mitochondrial membrane potentials and interfere with CD95-mediated apoptosis (Barreiros *et al* .,2009).This increase leads to negative consequences for the liver, as this increase leads to the progression of hepatocellular carcinoma (HCC)(Xie *et al* .,2013). Our results come in contrast to the results recorded by Ozden *et al*., (2004) which indicated a significant increase in the level of HGF in acute patients compared to the healthy control and chronic group. In chronic liver injury, increased oxidative stress, hepatocyte mitochondria-generated reactive oxygen species, and the level of per-oxidized lipids from necrotic hepatic tissue can maintain constant activation of hepatic stellate cells, favoring hepatic tissue fibrogenesis (Jiang *et al*., 2010). In patients with liver disease, plasma HGF levels were elevated that correspond with the degree of liver damage and the histological activity index score during liver regeneration can be a good measure of the extent of liver damage (Tsubouchi *et al*., 1991; Shiota *et al*., 1995).

In connection with TNF-α , it was elevated in chronic than acute patients without significant difference. TNF-α is the first inflammatory mediator that is produced in the body and plays an important role in the occurrence and development of CHB (Li *et al* ., 2018).Our result was coming in agreement with results of Bahramabadi *et al*., (2017) who showed no significant difference for TNF-α between CHB patients and control group. TNF has long been considered an important cytokine in the eradication of HBV. High intrahepatic levels of TNF-α increase the expression of HLA class I molecules, enhance the response of CD8 + T cells to HBV, and more effectively destroy HBV-infected hepatocytes (Hussain *et al*., 1994). CD8 + T cells lack the capacity to secrete sufficient TNF to kill HBV-infected hepatocytes, the so-called "depleted phenotype" in chronic HBV infection (Peng*et al* ., 2008; Fisicaro*et al* ., 2010). With regard to CXCL13,our study reported no significant difference between acute and chronic hepatitis, although chronic HBV slightly increased than acute hepatitis. The elevation of some cytokines such as CXCL13 might be a hallmark of functional cure of chronic hepatitis patients (Yoshio *et al* ., 2018)

Liu *et al* ., (2017) reported the CXCL13 was higher in the serum of CHB than control group. Changes in Tfh cells are associated with autoimmune and immunodeficiencydiseases and solid tumors (Bindea*et al*., 2014; Gu-Trantien*et al*., 2013). The frequency of circulating CXCR5 + CD4 + Tfh cells is increased in patients with chronic hepatitis B (Hu *et al*., 2014).

CXCL-13, the only ligand for CXCR5, promotes the migration of CXCR5-expressing B and Tfh cells to lymphoid tissues and is required for the development of secondary lymphoid tissues (Kim *et al*., 2001; Takemura*et al* ., 2001). A previous study by (Duan *et al*., 2014) suggested that polymorphisms in CXCL-13 were associated with a lack of response to the hepatitis B vaccine.

When we compare the mean concentration of HGF, TNF-α and CXCL-13 distribution according to HBV genotyping , by regression analysis, we note that there are significant differences between the genotypes of the virus with regard to the levels of protein concentrations. The possible influence of HBV genotype on cytokines unknown ,and to our knowledge, there are no studies concerned with the level of cytokines in the different genotypes of the HBV virus, so we cannot compare with other studies . Rizi *et al*., (2013) reported the level of TNF-α was higher in genotype D than healthy control . Some have reported that HBV genotype associated with clinical features, including fibrosis, cirrhosis and hepatocellular carcinoma . For example , genotype C is associated with HCC in younger patients ,fibrosis is more significantly in C patients aged ≤45 years ,while genotype C is more associated with rapid progression to advanced fibrosis and higher HCC development (Guirgis*et al* ., 2010).

The results reported no significant differences in the three cytokines when comparing between the levels of HBV viral load $P > 0.05$.It is well known the infection with HBV virus often leads to damage to liver tissue, which leads to the liver releasing the HGF for repair mechanism (You *et al* ., 2011).These results may provide a new view on HBV viral load as an independent factor when infected with the virus. Previous studies revealed that TNF- α is the main cytokine in defense against local infections caused by parasites, bacteria and viruses, and it has a major role in inflammation and immune regulation and in cellular differentiation and proliferation and the absence or inhibition of this factor means failure of the immune system to control the infection (Valaydon*et al* ., 2016) . This cytokine also showed that it was not influenced by the levels of viral load , although TNF- α concentrations began to rise gradually with the increase in the levels of viral load without reaching the significant differences .Two studies done by Tzeng*et al*.(2014) and Chyuan*et al*.(2015), were explained that blockage of TNF- α leads to an increase in viral load, enhances gene expression of the virus, and increases the presence of the virus in mice.

The current results revealed that the mean of CXCL13 concentration was not significant differ according to the levels of viral DNA , despite it was gradually decline as the level of viral load increases .Liu *et al*.(2017) were demonstrated that CXCL13 was constantly increased in patients' serum with CHB and negatively correlated with the level of virus DNA , also an increase in CXCL-13 level contributed to controlling viral load during the treatment with nucleos(t)ide analogues

In collective , no significant differences of three cytokines were found depending on the levels of viral load in HBV patients . The reason may be due to that the measurement of these cytokines was in the peripheral blood and not in the area affected by the virus, which reflects the general level of cytokines rather than the in situ inflammatory cellular expression (Ming*et al*., 2016).

The cytokines did not show significant correlation between them in HBV patients. Several components of the immune system are associated with inflammatory factors that affect the hepatitis process in CHB (Lian *et al* ., 2014) . Previous studies have examined the association of cytokines levels with various factors such as T-cells and CD4 in HBV patients. In a 2016 study conducted by Lian and his colleagues , they observed no correlation between some cytokines and chemokines (CXCL9, CXCL10, CXCL11, and IL-10) and Tregs .

CONCLUSION

This study concluded that the cytokines (HGF ,CXCL-13,TNF-α)play a key role in development of HBV .

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