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IFN-γ AND IP-10 LEVELS IN HBV INFECTED PATIENTS

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Background: Hepatitis B virus (HBV) infection causes a global health problem. WHO reported that Two billion individuals worldwide have been infected with HBV. Chronic HBV carriers are about Three hundred and fifty million individuals. About 600,000 die due to either acute or chronic infections of HBV every year. Almost 90% of children infected and 10% of young adults with HBV develop chronic infection. In Egypt, the most prevalent genotype is four[1].

Aim of work: We aimed to investigate serum levels of IP-10 and IFN-γ in cirrhotic and non-cirrhotic in chronic HBV infected patients. A total of 53 chronic HBV infected patients and 25 healthy controls were enrolled in this study. Mean serum IP-10 levels were higher in patients than healthy controls and cirrhotic patients had higher IP-10 than non-cirrhotic patients (457 vs 236 pg/ml; $p< 0.005$), Mean IFN-γ levels of cirrhotic patients had higher IFN-γ than non-cirrhotic patients (35 vs 8 pg/ml; $p< 0.005$). Interestingly, IFN-γ levels correlated with HBV DNA level ($r= -0.692$, $p= 0.0001$*).

In conclusion: IP-10 and IFN-γ may be used to predict cirrhosis in chronic HBV infected patients.

Keywords: IP-10, IFN-γ, cirrhosis, chronic HBV.

INTRODUCTION

Hepatitis B virus (HBV) is an enveloped virus which causes acute and chronic hepatitis. While there is a vaccine against HBV, it still a serious health problem, particularly in Africa, Asia, and South America and may cause death[2]. Chronic HBV (CHB) leads to activation of the compensatory mechanism for death of liver cell by a sustained inflammatory response triggering, which gradually cause liver cirrhosis (LC) and also hepatocellular carcinoma (HCC)[3]. Infection of HBV may cause many of clinical forms, like acute hepatitis, chronic hepatitis, fulminant hepatitis, (HCC), (LC), carrier state and asymptomatic[4]. Each year there are over 1.5 million new cases of HBV and 296 million people, or about 4% of the world's population, are living with CHB as of 2019. Also, HBV is the cause of about 820 000 deaths, direct or indirect, due to LC and HCC[5].

If virus was not clear after acute infection, it can cause CHB, which may development to liver cirrhosis, fibrosis, and HCC. Some viral elements such as viral load, HBV genotype,

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HBV genetically mutations and serum hepatitis B surface antigen (HBsAg) and serum hepatitis B envelope antigen (HBeAg) levels which used to expect clinical results after persistent infection[6].

Interferon-γ inducible protein-10 (IP-10) was known by Luster *et al.*[7] as a 10-kD protein and it is a type of the CXC chemokine. Although an N-terminal ELR (Glu-Leu-Arg) motif CXC chemokines, are specific neutrophils chemo-attractants, which have not the N-terminal ELR motif, as monokine and IP-10 which represented by interferon-gamma (IFN-γ)[8].

The aim of the present study was to investigate serum levels of IP-10 and IFN-γ in chronic HBV-infected patients with and without liver cirrhosis and possible association with different clinical and laboratory parameters.

PATIENT'S, MATERIAL AND METHODS

Ethics statement

The study protocol followed the 1975 Helsinki Declaration ethical guidelines and was approved by the ethics committee of the Faculty of Medicine, Assiut University, (no: IRB00008718) Informed written consent was obtained from enrolled patients.

Study subjects and clinical parameters

In our study we were 78 enrolled. Chronic HBV infected patients were presented to the outpatient clinic of the Liver Hepatitis Centre, Assiut governorate, Ministry of Health, Egypt, in the period from May 2017 to May 2018. enrolled Twenty-five healthy blood Were selected as control in the study.

Chronic HBV infection was considered if the patient had negative IgM anti-HBc test, and positive HBsAg, HBeAg, or HBV DNA ≥ 6 months[9]. If patients had coinfection with HIV viruses, or other types of hepatitis, or had other causes of chronic liver injuries such as autoimmune hepatitis, or excessive alcohol intake, were excluded from the study.

HBV infected patients Were divided two main categories; non-cirrhotic HBV patients (*n*= 33) and patients complicated with cirrhosis (*n*= 20). Besides, a control group (*n*= 25) that included healthy blood donors was included. They were negative for HBV antibodies and HCV.

All patients had clinical examination, abdominal ultrasonography, fibro scan testing for assessment of liver pattern, laboratory tests like liver function tests including, aspartate transaminase (AST), alanine transaminase (ALT), Platelets count, total bilirubin, direct bilirubin. HBV-related Liver cirrhosis was identified based on the clinical examination and laboratory tests. The Fibrosis-4 (FIB-4) score was used to evaluated liver cirrhosis[10&11].

Sample collection and processing

A blood sample (5 ml) was collected to separate the serum which was used for ELISA measurements[12]. Using the COBAS Amplicor HBV Monitor test (Roche Diagnostics, Mannheim, Germany) Hepatitis markers (HBeAg and HBeAb) were evaluated by ELISA supplied by Alpha Diagnostic International (USA). Also screened for HAV, HCV, HEV, HDV, or HIV antibodies in serum using ELISA technique (Ortho Diagnostics Systems, USA).

Determination of serum IP-10 and serum IFN-γ levels

In non-cirrhotic and cirrhotic HBV patients, serum levels of IP- 10 and IFN-γ were measured by direct sandwich ELISA according to the manufacturer instructions (KOMAbiotech, Korea). Frist, in wells of 96-well ELISA microplate pre-coated with rabbit antihuman IP10 and IFN-γ in duplicates, 100 ul of serum or standard samples were added. After incubated for 2 hours, washed 3 times, Detection antibodies (biotinylated anti-Human IP-10 and IFN-γ) were added to wells and incubated for two hours then washed. Then, streptavidin-coupled HRP conjugate was added. Color development reagents were added and at 450 nm wavelength, the plate was read using a microplate reader (Biotek, USA). The unknown serum samples were calculated by a standard curve and expressed as pg/ml[13].

Statistical analysis

Test was used Mann-Whitney U test to compare the changes in serum IP-10 and serum IFN-γ expression levels among the study groups. We were categorized data by Fisher's exact test. Spearman's correlation coefficient

(r) was used to correlations between serum (IP-10 and IFN-γ) and laboratory parameters were estimated. Statistical significance was considered if $p< 0.05$.

RESULTS

In table 1, the demographics and baseline laboratory parameters of the study groups are shown. There were 78 subjects enrolled in this study. There were 53 chronic HBV infected patients that classified into two groups; non-cirrhotic HBV infected patients ($n= 33$), and cirrhotic HBV infected patients ($n= 20$), and there were 25 healthy controls (Fig. 1).

Mean serum IP-10 levels were higher in patients than healthy controls and cirrhotic patients had higher IP-10 than non-cirrhotic patients (457 vs 236 pg/ml; $p< 0.005$) (Fig. 2), Mean IFN-γ levels of cirrhotic patients had higher IFN-γ than non-cirrhotic patients (35 vs 8 pg/ml; $p< 0.005$) (Fig. 3). Interestingly, IFN-γ levels correlated with HBV DNA level ($r= -0.692$, $p= 0.0001$*) (Fig. 4).

Table 1: Summary of the clinical characteristics of enrolled individuals.

	Cirrhotic HBV patients ($n= 20$)	Non-cirrhotic HBV patients ($n= 33$)	Healthy controls ($n= 25$)
Age (years)	45.68 ± 12.29	37.6 ± 12.2	36.96 ± 11.31
Male/female (no)	25/8	15/3	19/6
Mean HBV DNA level (IU/ml)	$8.7x10^7 \pm 6.1x10^6$	$4.9x10^6 \pm 1.2x10^5$	NA
HBeAg-positive (%)	8 (40%)	14 (42.4%)	NA
ALT (IU/L)	87 ± 32	118 ± 42	18.1 ± 8.2
AST (IU/L)	33.1 ± 12.6	75 ± 42	23.9 ± 10.6
Platelets $x10^3/mm^3$	108.96 ± 23.93	200.4 ± 50.87	208.86 ± 128.5
Serum albumin g/dL	3.46 ± 0.09	3.74 ± 0.8	3.95 ± 0.08
Serum total bilirubin (mg/dl)	0.99 ± 0.42	0.82 ± 0.4	0.7 ± 0.5
FIB-4 classification			
<1.45	0	30 (90.9%)	NA
≥1.45- ≤3.25	5 (25%)	3 (9.1%)	NA
>3.25	15 (75%)	0	NA

Abbreviations: NA: not applicable; FIB-4: Fibrosis-4; ALT: alanine transaminase; AST: aspartate transaminase. Data are presented as mean ±SD.

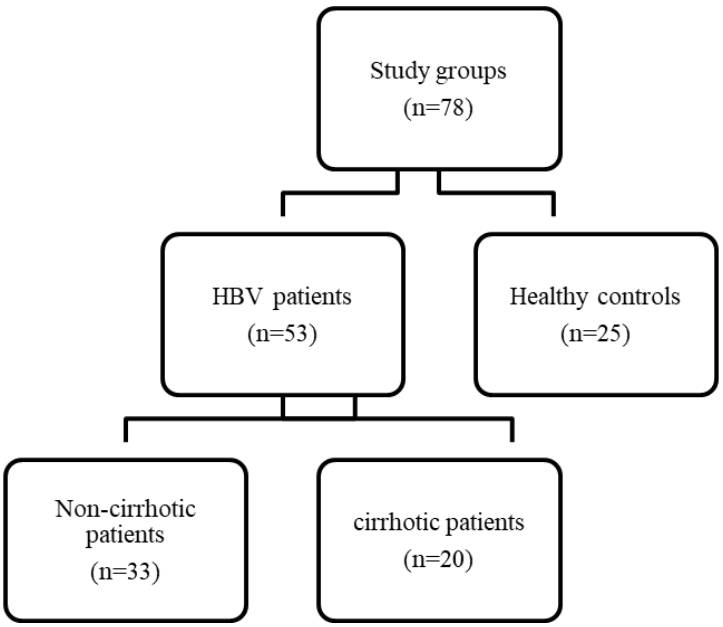

Fig. 1: Summary of study groups.

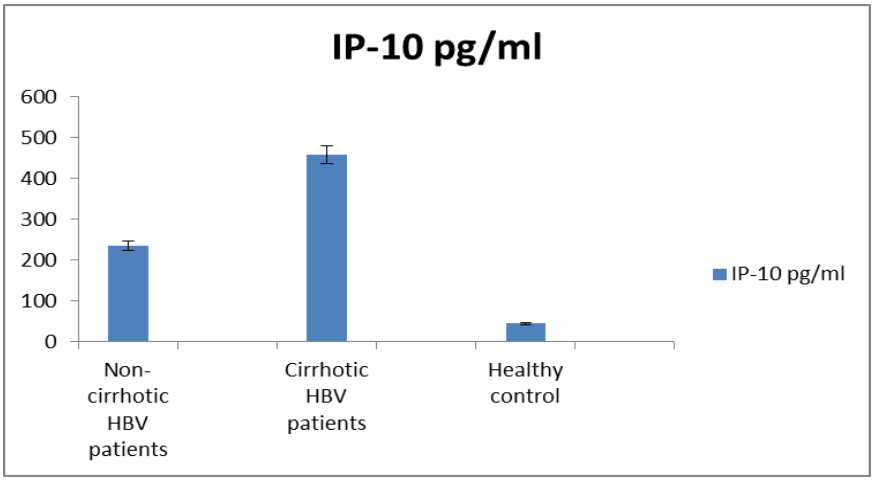

Fig. 2: Serum IP-10 profile. Error bars represent standard deviation. *; indicates $p< 0.05$ using Mann-Whitney test.

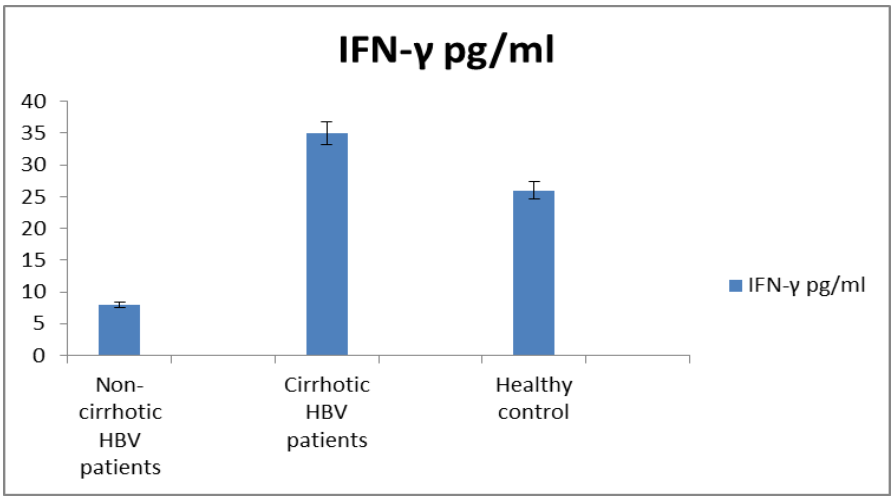

Fig. 3: Serum IFN-γ profile. Error bars represent standard deviation. *; indicates $p< 0.05$ using Mann-Whitney test.

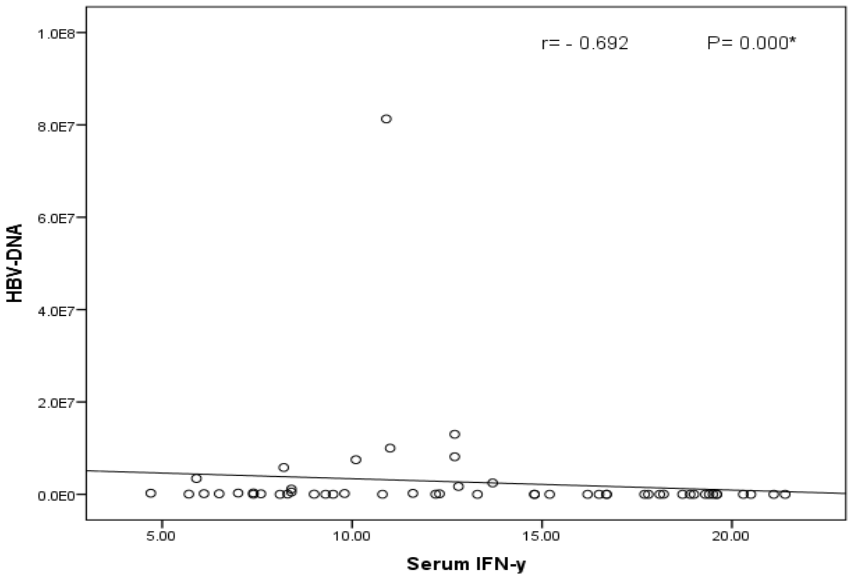

Fig. 4: Correlation between serum levels of IFN-γ (pg/ml) and HBV DNA (IU/ml) load in patients with CHB.

DISCUSSION

Our study aimed to measure the level of IFN-γ and IP-10 in serum in non-cirrhotic and cirrhotic HBV patients. HBV DNA level were found in patients with non-cirrhosis was significantly lower than cirrhotic, the same result found in Chu *et al.*[14] also showed that the HBV DNA levels in serum significantly decreased in the CHB patients without fibrosis significantly in contrast with those with hepatic fibrosis.

In this study, significantly lower levels of peripheral platelet count (PPC) were found in patients with hepatic cirrhosis than non-cirrhotic and healthy control, the same result found in Lemoine *et al.*[15] that show the levels of PPC in serum significantly increased in the CHB patients without fibrosis compared to those with hepatic fibrosis, Karasu *et al.*[16] shown that an increase in the degree of fibrosis during chronic viral hepatitis B and C may be a sing to decrease in PPC; and the etiology of chronic hepatitis does not play a role in the decrease in PPC, although the mechanism(s) needs to be elucidated.

In our study, we found in hepatic cirrhosis a significantly lower of levels of both AST and ALT levels than non-cirrhotic, the same result found in Zhao *et al.*[2] also showed that both levels of AST and ALT in serum significantly increased in the CHB patients without hepatic fibrosis compared to those with fibrosis.

Th1 cells secret IFN-γ as a cytokine, it inhibits liver fibrosis by inhibition of the proliferation and activation of Hepatic stellate cells (HSCs) and synthesis of collagen also activation of natural killer cells that can destroy activated HSCs[17].

In this study, significantly lower levels of IFN-γ were found in patients with hepatic non-cirrhosis than cirrhotic and healthy control, the same result found in Wang *et al.*[18] also showed that the IFN-γ levels in serum significantly reduced in the CHB patients without hepatic fibrosis compared to those with fibrosis. Other studies like Rakela and Ishizawa[19] show serum IFN-γ levels in HBV infection have given conflicting results. Early study Yumoto *et al.*[20] did not detect elevated in IFN-γ levels in HBV patients, while more other recent studies Song *et al.*[21] show either very low levels in different clinical groups or increase in levels in those with chronic HBV.

Vietnamese HBV infected patients study reported lower IFN-γ levels than that of the controls, which represents the susceptibility of patients to develop chronic HBV infection[17], Akpolat *et al.*[22] also found that the different in serum level of Th1 response (IFN-γ) in both study and control groups show significantly lower in the levels of IFN-γ of the study group more than controls groups ($p< 0.001$). However, they were not correlated with inflammation in the liver ($p> 0.05$). Lower levels of IFN-γ in patients with CHB more than the level of the controls may be due to the decrease in IFN-γ efficiency during the development of chronicity in the infection[22].

In this study, we observed that IP-10 levels were increased in cirrhotic and non-cirrhotic HBV patients compared to healthy controls, Karin *et al.*[5] showed the same result. this is due to the fact that these chemokines have immune attractive properties, these chemokines may cause inflammation in the liver by attracting macrophages and neutrophils which participates in the hepatic damage in chronic HBV infection[23].

The IP-10 level in the CHB patients was associated with the liver cirrhosis as the serum IP-10 levels were significantly increased in the CHB patients with cirrhosis compared to the CHB patients without cirrhosis, Wang *et al.*[18] showed the same result that IP-10 shows an significant role in the progression and development of liver sickness.

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مستويات IP-10 والانترفيرون جاما في مرضى التهاب الكبد الوبائي المزمن

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[٢]قسم الميكروبيولوجيا الطبية والمناعة ، كلية الطب ، جامعة أسيوط ، أسيوط ٧١٥٢٦ ، مصر

[٣]قسم طب المناطق الحارة والجهاز الهضمي ، كلية الطب ، جامعة أسيوط ، أسيوط ٧١٥٢٦ ، مصر

تمثل عدوى فيروس التهاب الكبد (ب) مشكلة صحية عالمية. وفقًا لمنظمة الصحة العالمية تم إصابة ملياري شخص في جميع أنحاء العالم بفيروس التهاب الكبد (ب). حاملي فيروس التهاب الكبد بي المزمن حوالي ثلاثمائة وخمسون مليون فرد. يموت ٦٠٠٠٠٠ بسبب الالتهابات الحادة أو المزمنة من الالتهاب الكبدي بي كل عام. ما يقرب من ٩٠٪ من الأطفال و١٠٪ من الشباب المصابين بفيروس التهاب الكبد (ب) يصابون بعدوى مزمنة. النمط الجيني الأكثر انتشارًا في مصر هو النمط الجيني الرابع[١].

نحن نهدف إلى التحقيق في مستويات المصل من بروتين ١٠ المحفز بانترفيرون جاما وانترفيرون جاما في التليف الكبدي وغير التليف الكبدي في المرضى المصابين بفيروس التهاب الكبد (ب) المزمن. تم تسجيل مجموعه من ٥٣ مريضًا مصابًا بفيروس التهاب الكبد (ب) و ٢٥ من الأصحاء في هذه الدراسة. كان متوسط مستويات من بروتين ١٠ المحفز بانترفيرون جاما في المصل أعلى في المرضى من الضوابط الصحية ، وكان لدى مرضى التليف الكبدي مستوي بروتين ١٠ المحفز بانترفيرون جاما أعلى من المرضى غير المصابين بالتليف الكبدي (٤٥٧ مقابل ٢٣٦بيكوغرام/مل ؛ $p<$ 0.005، كان متوسط مستويات انترفيرون جاما أقل في المرضى من كان لدى الضوابط الصحية ، ومرضى التليف الكبدي أعلى انترفيرون جاما من المرضى غير المصابين بالتليف الكبدي (٨ مقابل ٣٥ بيكوغرام/مل ؛ $p<$ 0.005) ومن المثير للاهتمام ، ووجد ارتباط بين مستوي الانترفيرون جاما والحمض النووي لفيروس الالتهاب الكبدي (ب) (r = -0.692 ، p= 0.0001*)

فى الختام؛ يمكن استخدام بروتين ١٠ المحفز بانترفيرون جاما والانترفيرون جاما للتنبؤ بتشمع الكبد لدى المرضى المصابين بفيروس التهاب الكبد (ب) المزمن.