

# IMMUNOLOGICAL CHANGES ASSOCIATED WITH CHRONIC HEPATITIS-B-VIRUS INFECTION

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## **Abstract:**

This study was done to illustrate some immunological parameters in chronic hepatitis B viral (HBV) infection in Babylon district. Seventy patients with chronic hepatitis B viral infection were enrolled in this study which lasted from November (2007) to May (2008), and consists of (57 males) and (13 females) with a mean age of ( $31.5 \pm 7.8$  years). Those patients were matched with thirty apparently healthy subjects (as controls), consisted of (21 males) and (9 females) with a mean age of ( $30.5 \pm 6.7$  years). The investigations for those patients and controlled subjects were done in laboratory of Marjan Teaching Hospital, AL-Hilla Teaching Hospital and advanced physiology laboratory in Medical College of Babylon University. The results of this study were as in the following: the total serum immunoglobulin-G (IgG) for patients ( $18373.86 \pm 5629.85$  mg/L) was higher than of controls ( $7893.33 \pm 2589.09$  mg/L), the total serum immunoglobulin-M (IgM) for patients ( $3742.43 \pm 1444.33$  mg/L) was more than in controls ( $1045.00 \pm 611.06$  mg/L). There were no significant differences in the total numbers of leucocytes, eosinophils, basophils, while the mean numbers of neutrophils for patients ( $3324.04 \pm 844.86$  cells/mm<sup>3</sup>) was less than for controls ( $4179.23 \pm 856.47$  cells/mm<sup>3</sup>). The mean numbers of lymphocytes for patients ( $3263.30 \pm 698.00$  cells/mm<sup>3</sup>) was more than controls ( $2104.50 \pm 473.46$  cells/mm<sup>3</sup>) and the mean numbers of monocytes for patients ( $701.70 \pm 192.77$  cells/mm<sup>3</sup>) was higher than controls ( $366.07 \pm 97.76$  cells/mm<sup>3</sup>). This study revealed a significant difference in the mean values of erythrocyte sedimentation rate (ESR) for patients ( $26.29 \pm 5.51$  mm/hr) and controls ( $8.87 \pm 3.95$  mm/hr). The present study illustrated that the incidence of negativity of tuberculin skin test (TST) was higher in patients (84.3%) than in controls (30%).

## التغيرات المناعية المتزامنة مع التهاب الكبد الفيروسي نوع "ب" المزمن

### الخلاصة:

أعدت هذه الدراسة لتوضيح بعض التغيرات المناعية عند المرضى المصابين بالتهاب الكبد الفيروسي نوع "ب" المزمن في محافظة بابل. لقد شملت هذه الدراسة سبعين مريضاً مصاباً بالتهاب الكبد الفيروسي نوع "ب" المزمن والتي استمرت من تشرين الثاني 2007 ولغاية أيار 2008 المكونين من (57 من الذكور) و(13 من الإناث) وكان متوسط أعمارهم (31,5 سنة) وتم مقارنتهم بالأصحاء في مجموعة السيطرة والذي كان عددهم ثلاثون شخصاً مكونين من (21 من الذكور) و(9 من الإناث) وكان متوسط أعمارهم (30,5 سنة).

لقد اجري للمرضى والأصحاء في مجموعة السيطرة تحقيقات مخبرية في مستشفى مرجان التعليمي ومستشفى الحلة التعليمي ومختبر الدراسات العليا لفرع الفلسفة في كلية الطب- جامعة بابل وكانت النتائج كالآتي:

متوسط قيمة الغلوبولين المناعي- G (IgG) للمرضى (18373,86 ملغم\لتر) وهو أعلى من مجموعة السيطرة (7893,33 ملغم\لتر) بالإضافة إلى ذلك إن متوسط قيمة الغلوبولين المناعي- M (IgM) للمرضى (3742,43 ملغم\لتر) وهو أكثر من قيمته عند الأصحاء (1045 ملغم\لتر). أيضاً هذه الدراسة بينت انه لا يوجد اختلاف في العدد الكلي لكريات الدم البيض وعدد الخلايا القعدة والخلايا الحمضة ولكن عدد الخلايا العدلة للمرضى (3324,04 خلية\ملم<sup>3</sup>) وهي اقل من عددها عند مجموعة السيطرة (4179,23 خلية\ملم<sup>3</sup>) أما الخلايا اللمفاوية للمرضى (3263,3 خلية\ملم<sup>3</sup>) هي أكثر من متوسط عددها عند مجموعة السيطرة (2104,5 خلية\ملم<sup>3</sup>) وكذلك متوسط عدد الخلايا الوحيدة للمرضى (701,7 خلية\ملم<sup>3</sup>) أكثر من متوسط عددها عند الأصحاء (366,07 خلية\ملم<sup>3</sup>). كذلك بينت هذه الدراسة إن هنالك اختلاف مهم بين متوسط قيمة سرعة ترسيب الكريات الحمراء (ESR) عند المرضى (26,29 ملم\ساعة) وقيمته عند مجموعة السيطرة (8,87 ملم\ساعة). إن نسبة سلبية فحص الجلد للتوبركولين (TST) أعلى عند المرضى (84,3%) من مجموعة السيطرة (30%).

## **Introduction and Review of Literatures**

### **Background:**

Hepatitis B virus (HBV) infection is a significant public health problem that may lead to acute and chronic liver disease, cirrhosis, and hepatocellular carcinoma (HCC) (Atkinson, *et al.*, 2000). Hepatitis B is a viral disease with a high incidence and prevalence worldwide and it can cause acute and chronic liver disease (Tsuyoshi and Nagayama, 2004). Approximately ( 8%) of the world's population has been infected with HBV, and about (350 million, 5–6%) are persistent carriers of HBV (Inlin, *et al.*, 2005). The role of host genetic factors and their interactions with environmental factors lead to chronic HBV infection and its complications are not well understood. The clinical presentation ranges from subclinical to symptomatic and, in rare instances, fulminant hepatitis (Parveen and Michael, 2006). Perinatal or childhood infection is associated with few or no symptoms, but it has a high risk of becoming chronic (Alexander and Kourtis, 2007). The immunological state of the patients assessed by the following parameters:

#### **1.Total serum immunoglobulin G (IgG):**

IgG is the predominant antibody in secondary responses and constitutes an important defense against bacteria and viruses, it is the only antibody to pass the placenta and is therefore the most abundant relationship between liver damage and immune regulation in HBV infection, spontaneous IgG production was elevated only in those with chronic active hepatitis whilst those with less severe inflammation had values comparable to normal, because of concanavalin A (Con A) induced suppressor cell regulation of IgG producing cells was impaired in those with chronic active hepatitis, and those with chronic persistent hepatitis, and there was a correlation in both groups of patients with the severity of portal tract inflammation (Kayhan, *et al.*, 1986). In a study done to find the relationship between HBsAg, HBeAg and IgG it was found

that the patients who were serologically negative to these markers show frequent elevation of IgG (Segovia, *et al.*, 1996).

## **2. Total serum immunoglobulin M (IgM):**

IgM is the main immunoglobulin produced early in the primary immune response, IgM is present on the surface of virtually all uncommitted B cells it is composed of five H<sub>2</sub>L<sub>2</sub> units (each similar to one IgG unit ) and one molecule of J (joining ) chain, it's the most efficient in defense against bacterial and viruses (Geo, *et al.*, 2007). A study was done on patients with chronic HBV in the United Kingdom showed that there was a increased spontaneous IgM production due to defect in Con A induced suppressor cell function have been identified in association with chronic active hepatitis and a proportion of those with chronic persistence hepatitis, in contrast, patients with minimal liver damage have completely normal profiles (Kayhan, *et al.*, 1986).

## **3. Tuberculin skin test (TST):**

A positive skin test indicates active cell-mediated immunity, whereas a negative skin test indicates a weak cell- mediated immunity (Geo, *et al.*, 2007). (Mcglynn, *et al* 1985) revealed that in hepatitis B carriers there are a statistically significant association between a negative tuberculin skin test and HBV replication, and this finding suggests that BCG vaccination might reduce the prevalence of infectious carriers, thereby ultimately reducing the incidence of HBV.

## **4. Total and differential leucocytes count:**

The absolute amount of blood lymphocytes decreased with the increase of the age of the patients, but in chronic HBV this was accompanied by the decrease of the morphological activity of hepatitis, while in (HBV+HCV) it was accompanied by its increase, and the amount of lymphocytes rise with the increase of liver fibrosis in chronic HBV. In children with chronic hepatitis C and B + C the amount of blood lymphocytes was found to be unrelated to the morphological activity of hepatitis

(Filimonov, *et al.*, 2004). (Vassilopoulos, *et al.*, 2008) revealed that the lymphocytes and monocytes will increase in patients with chronic HBV infection. Another study was found that the peripheral blood monocytes in chronic HBV infection proliferate and increase in number (Vingerhoets, *et al.*, 2000). (Craig, *et al.*, 2002) illustrated that the lymphocytes will increase while the neutrophils will decrease and no changes in total leucocytes count. (Vassilopoulos, *et al.*, 2008) revealed that the lymphocytes and monocytes will increase, while the neutrophils will decrease in patients with chronic HBV infection.

### **5. Erythrocytes sedimentation rate (ESR):**

The ESR is a nonspecific screening test that indirectly measures how much inflammation is in the body. It is a simple and inexpensive laboratory test that is frequently used in clinical medicine (Saadeh, 1998). (Par, *et al.*, 1995) found that the ESR elevated in chronic HBV. Another study was done to find the relationship between ESR and all types of hepatitis and was found that the high ESR were more frequent in HBA than in other types of hepatitis (Kassas, 1997).

### **The aim of this study:**

Chronic HBV is associated with many immunological changes. Owing to the fact that insufficient informations concerning the effect of these factors on Iraqi patients are available, this study was carried out to provide insight to this question and to know some immunological changes in Iraqi patients. Such information is of no doubt necessary as a background for any programs devised in the future for studying chronic HBV, and treating this disease. Also this study will aid in gaining a better understanding of the pathogenesis of the chronic HBV, and this ultimately leads to advances in the design of drugs of choice to prevent and treat this disorder. To fulfill these objectives, the present study has dealt with samples of subjects in Babylon province to determine: (Total serum IgG, Total serum IgM, TST, Total and differential leucocytes count, ESR).

## Materials and Methods

### A. Materials:

#### 1. Subjects of the study:

##### 1.1. Patients:

Seventy patients were included in this study which lasted from November (2007) to May (2008), and consisted of (57 males) and (13 females). The mean age of those patients was (31.5±7.8 years). Those patients were attended to the gastroenterology center in the Marjan teaching hospital and diagnosed by specialist doctors as chronic hepatitis B infection.

##### 1.2. Controls:

Thirty apparently healthy subjects (clinically assessed by specialist doctors) were included as controls in this study, which consist of (21 males) and (9 females). The mean age of those subjects was (30.5±6.7 years). Those subjects were selected randomly from the population.

#### 2. Instruments:

**Table (1): The tools used in this study and their sources**

No.	Tools	Company	Country
1.	EDTA tubes	Afma-dispo	Jordan
2.	Micro-pipette	Oxford,	USA
3.	Westergren tubes	Witeg	Germany
4.	Plain tubes	Afma-dispo	Jordan
5.	Disposable syringes	Witeg	Malaysia
6.	Microscope slides	Sail brand	China
7.	Microscope cover slides	Ataco	China

**Table (2): The main instruments sources**

No.	Instruments	Company	Country
1.	Hemocytometer	Witeg	Germany
2.	Light microscope	Olympus	Japan
3.	Water bath	Memmert	Germany
4.	Centrifuge	Hermle	Japan
5.	Spectrophotometer	Cecil	England
6.	Refrigerator	Concord	Lebanon
7.	Incubator	Memmert	Germany
8.	Water distillator	G.F.L.	Germany
9.	Manual leucocytes counter	Hermle	Japan

**Table (3): The chemical and biological materials and their sources**

No.	Chemical materials	Company	Country
1.	Turk's solution	Crescent	Saudi Arabia
2.	Leishman stain solution	Crescent	Saudi Arabia
3.	Trisodium citrate solution	Crescent	Saudi Arabia
4.	Human IgG plate	Birmingham	U. K.
5.	Human IgM plate	Birmingham	U. K.
6.	PPD	Amertek	USA
7.	HBsAg-kit	Biokit	Spain
8.	HCV-kit	Biokit	Spain

## **B. Methods :**

### **1. Blood collection:**

The collection of blood was done in gastroenterology center in the Marjan teaching hospital at (9-12:am). The brachial vein on the front of the elbow was employed. Two groups of labeled tubes were used; the first tubes contain (EDTA) as anticoagulants to be used for hematological studies. The second group tubes were without anti-coagulant to be used for preparing sera for subsequent immunological tests, the serum samples were frozen at (-20°C) for analysis (Lewis, *et al.*, 2006).

The investigations for those patients and control group were done in the laboratory of Marjan Teaching Hospital and post graduate physiology laboratory of medical college of Babylon university.

### **2. Measurement of total serum IgG and IgM:**

The method involves antigen diffusing radially from a cylindrical well through an agarose gel containing an appropriate monospecific antibody. Antigen-antibody complex are formed which, under the right conditions, will form a precipitin ring. The ring size will increase until equilibrium is reached between the formation and breakdown of these complexes, this point being termed 'completion'. At this stage, a linear relationship exists between the square of the ring diameter and the antigen concentration. By measuring the ring diameter produced by a number of samples of known concentration of the antigen in an unknown sample may then be determined by measuring the ring diameter produced by that sample and reading off the calibration curve according to the procedure recommended by the company (Birmingham ,Germany) (Fahey and Mckelvey, 1965).

### **2. Tuberculin skin test:**

The tuberculin test is performed by intracutaneous injection of 5 tuberculin unit (TU); (0.1ml) of PPD using a gage number 27 needle. The reaction is read at 48-72 hours, and a positive test is indurations of 10mm or more in diameter; erythema is not considered in determining a positive test (Carol, 2007 and Geo, *et al.*, 2007).

### **3. Total leucocytes count:**

Blood was drawn in a clean and dry WBC pipette up to the mark 0.5 and the outside of the pipette was wiped off with a gauze. Then diluting fluid (Turk's solution) was drawn up to mark 11 (dilution 1:20), the contents were mixed for three minutes; 1-2 drops were discarded then counting chamber (Neubaur chamber) was filled. It was left for three minutes to let the cells for setting down and then the chamber was examined under 40X objective lens of the microscope to count WBCs in the four corners secondary squares (Lewis, *et al.*, 2006).

### **4. Differential leucocytes count (DLC):**

Blood film was made immediately by careful mixing of the blood, an appropriate drop was delivered by a glass capillary and placed in the center line of a clean microscope slide about one cm from one end. Then without delay, placed a spreader in front of the drop at an angle of about 45° to the slide and moved it back to make contact with the drop. The drop was spread out quickly along the line of contact. With a steady movement of the hand, the drop of blood was spread along the slide. The spreader should not be lifted off until the last trace of blood has been spread out. After air drying, the blood film was stained by Leishman's stain; the slide was flooded with the Leishman's stain. After two minutes, double the volume of buffered water was added for 5-7 minutes. Then washed in a stream of buffered water until it had acquired a pinkish tinge (up to two minutes), the back of the slide has been wiped and set it upright to dry. Preparation were examined under oil immersion lens (100X). Counted 100 leukocytes were taken and calculated the percentage of each type of cells (Lewis, *et al.*, 2006).

### **5. Measurement of ESR:**

A Westergren tube: length- 300mm (open at both ends), diameter 2.5mm were used. One part of anticoagulant (3.8% trisodium citrate solution) was added to 4 parts of blood (0.5 ml of anticoagulant is added to 2 ml of blood). The mixture was drawn into a

Westergren tube up to the zero mark and the tube was set upright in a stand with a spring clip on the top and rubber at the bottom. The level of the top of the red cell column was read at the end of 1 hour (Lewis, *et al.*, 2006).

## 6. Determination of HBsAg:

Put 100 µl of sample dilutant in a well, then add 50 µl of serum and incubate for 30 minutes at 37°C, then wash 5 times and add 100 µl of conjugate and incubate for 90 minutes at 37°C then wash 5 times and add 100 µl of substrate incubate for 30 minutes at 37°C and add 100 µl of stopping solution and read at 450nm according to (Biokit, Spain). (Lennette, *et al.*, 1974).

## C. Statistical analysis:

All values were expressed as means  $\pm$  SD. The data were analyzed by using of SPSS program and taking  $p < 0.05$  as the lowest limit of significance. Chi square and were applied to determine the differences between one group and another, and between all groups and within group (Wayne, 1999).

## The Results

### 1. Total serum IgG:

The mean values of total serum IgG for patients was (18373.86 $\pm$ 5629.85 mg/L), while in control group it was (7893.33 $\pm$ 2589.09 mg/L). There was a significant difference between patients and control group ( $P < 0.05$ ). (Table 4).

**Table (4):** Mean values of IgG (mg/L) for patients and control group.

IgG		Mean $\pm$ S. D.	No.
Groups	patients	18373.86 $\pm$ 5629.85	70
	Control	7893.33 $\pm$ 2589.09	30
Total			100

Significant differences between patients and control group ( $P < 0.05$ ).

## 2. Total serum IgM:

The mean values of total serum IgM for patients was (3742.43±1444.33 mg/L), while in control group it was (1045.00±611.06 mg/L), and this was statistically significant difference between patients and control subjects enrolled in this study ( $P < 0.05$ ) as demonstrated in (table 5).

**Table (5):** Mean values of IgM (mg/L) for patients and control group.

IgM		Mean ± S. D.	No.
Groups	patients	3742.43 ± 1444.33	70
	Control	1045.00 ± 611.06	30
Total			100

Significant differences between patients and control group ( $P < 0.05$ ).

## 3. Total leucocytes count:

This study revealed that there was no significant difference ( $P > 0.05$ ) in the mean numbers of leucocytes for patients (7462.86±1607.70 cells/mm<sup>3</sup>) and control group (6836.67±1422.84 cells/mm<sup>3</sup>). (Table 6).

**Table (6):** Mean numbers of total leucocytes (cells/mm<sup>3</sup>) for patients and control group.

Leucocytes		Mean ± S. D.	No.
Groups	patients	7462.86 ± 1607.70	70
	Control	6836.67 ± 1422.84	30
Total			100

No significant differences between patients and control group ( $P > 0.05$ ).

#### 4. Leucocytes differential count:

##### 1. Neutrophils count:

The mean numbers of neutrophils for patients ( $3324.04 \pm 844.86$  cells/mm<sup>3</sup>) was lower than in control group ( $4179.23 \pm 856.47$  cells/mm<sup>3</sup>) and the statistical analysis revealed a significant difference between patients and control group ( $P < 0.05$ ). (Table 7).

##### 2. Lymphocytes count:

There was an increase in the mean numbers of lymphocytes for patients ( $3263.30 \pm 698.00$  cells/mm<sup>3</sup>) compared to control group ( $2104.50 \pm 473.46$  cells/mm<sup>3</sup>). There was a significant difference between patients and control group ( $P < 0.05$ ). (Table 7).

##### 3. Monocytes count:

There was a significant difference ( $P < 0.05$ ) between the mean numbers of monocytes for patients ( $701.70 \pm 192.77$  cells/mm<sup>3</sup>) and control group ( $366.07 \pm 97.76$  cells/mm<sup>3</sup>). (Table 7).

##### 4. Eosinophils count:

There were insignificant differences ( $P > 0.05$ ) in the mean numbers of eosinophils for patients ( $110.41 \pm 68.14$  cells/mm<sup>3</sup>) and control group ( $119.07 \pm 64.32$  cells/mm<sup>3</sup>). (Table 7).

##### 5. Basophils count:

There were insignificant differences ( $P > 0.05$ ) in the mean numbers of basophils for patients ( $63.40 \pm 46.84$  cells/mm<sup>3</sup>), and control group ( $67.80 \pm 47.83$  cells/mm<sup>3</sup>). (Table 7).

**Table (7):** Mean numbers of leucocytes differential count (cells/mm<sup>3</sup>) for patients and control group.

Types of leucocytes	Groups	Mean $\pm$ S. D.
Neutrophils	Patients	$3324.04 \pm 844.86^{**}$

	<b>Control</b>	4179.23 ± 856.47**
<b>Lymphocytes</b>	<b>Patients</b>	3263.30 ± 698.00**
	<b>Control</b>	2104.50 ± 473.46**
<b>Monocytes</b>	<b>Patients</b>	701.70 ± 192.77**
	<b>Control</b>	366.07 ± 97.76**
<b>Eosinophils</b>	<b>Patients</b>	110.41 ± 68.14*
	<b>Control</b>	119.07 ± 64.32*
<b>Basophils</b>	<b>Patients</b>	63.40 ± 46.84*
	<b>Control</b>	67.80 ± 47.83*

\*\*Significant differences between patients and control group  
(P < 0.05).

\* No significant difference between patients and control group  
(P > 0.05).

### 5. ESR:

This study revealed that there was a significant difference (P < 0.05) in the mean values of ESR for patients (26.29±5.51 mm/hr) and control group (8.87±3.95 mm/hr). (Table 8).

**Table (8):** Mean values of ESR (mm/hr) for patients and control group

<b>ESR</b>		<b>Mean ± S. D.</b>	<b>No.</b>
<b>Groups</b>	patients	26.29 ± 5.51	70
	Control	8.87 ± 3.95	30
<b>Total</b>			100

Significant differences between patients and control group (P < 0.05).

## 6. Evaluation of tuberculin skin test:

The incidence of negative tuberculin test was higher in patients with chronic HBV (84.3%) than in the subjects control group (30%). There was a significant difference between patients and control group ( $P < 0.05$ ). As illustrated in (table 9).

**Table (9):** Distribution of patients and control group by (response to tuberculin skin test (PPD)).

PPD		+ve	-ve	Total
Groups	patients	11	59	70
	Control	21	9	30
Total		32	68	100

Significant differences between patients and control group ( $P < 0.05$ ).

## Discussion

### 1. Total serum IgG:

This study it was found that the mean values of total serum IgG for patients is ( $18373.86 \pm 5629.85$  mg/L), while in control group it is ( $7893.33 \pm 2589.09$  mg/L). that means, the titer of IgG increased in chronic HBV infection and the results of the present study are in agreement with those obtained by (Alexander, *et al.*, 1983) who found that, elevation in IgG production occurs in patients with chronic HBV infection and explained that due to impaired concanavalin A (Con A) induced suppressor it cell regulation of IgG production. In addition to that, results of this study are consistent with the results of other studies was illustrated that, if IgG concentration was higher than 2000 mg/l it would indicate chronic aggressive hepatitis or cirrhosis has occurred, and exclude all other groups of HBV, thus persistent high levels of IgG following acute hepatitis indicate the development into a chronic hepatitis (Kleinschmidt and Schlicht, 1997). (Segovia, *et al.*, 1996) mentioned in their study that there is a relationship

between HBsAg, HBeAg and IgG; and found that those patients who were serologically negative to these markers showed frequent elevation of IgG. (Par, *et al.*, 1995) illustrated that the IgG elevated in chronic HBV. (Voiculescu, *et al.*, 1993) revealed that in chronic HBV the immunoglobulin (IgG and IgM) would increase. Sequential study of three patients prior to the detection of HBsAg in serum and subsequently during and after acute hepatitis B showed that such abnormalities were transient and closely related to the onset of liver damage (Kayhan, *et al.*, 1986). Explanation of these results as mentioned by the researchers is that spontaneous IgG production was elevated in patients with chronic active hepatitis while those with less severe inflammation had values comparable to normal, because of Con A-induced suppressor cell regulation of IgG producing cells was impaired in those with chronic active hepatitis, and those with chronic persistent hepatitis, and there was a correlation in both groups of patients with the severity of portal tract inflammation, in contrast to those with minimal liver damage who had values in the normal range (Kayhan, *et al.*, 1986).

## **2. Total serum IgM:**

The present study revealed that the mean values of total serum IgM for patients is (3742.43±1444.33 mg/L), while in control group it is (1045.00±611.06 mg/L), i.e. the total serum IgM increased in patients with chronic HBV infection and the current study compatible with another study done for patients with chronic HBV in the United Kingdom, and found that, the increase spontaneous IgM production in association with chronic active hepatitis and a proportion of those with chronic persistence hepatitis. In contrast, patients with minimal liver damage have completely normal profiles (Kayhan, *et al.*, 1986). In addition to that, the present study is in agreement with (Schwarz, *et al.*, 1988) who illustrated in a study done for HBV, that the IgM was found rather frequently in acute hepatitis and chronic aggressive hepatitis and occasionally also in chronic persistent hepatitis, and IgM-test may be used as an easily measurable additional criteria for diagnosis and course of chronic liver diseases. (Zuberi, *et al.*, 1997) found that the elevation of IgG and IgA were frequently observed in healthy subjects and patients with acute viral hepatitis, liver cancer and miscellaneous liver

disorders, whereas IgG and IgM were elevated in cirrhosis of the liver. Elevation of IgM in 35% chronic aggressive hepatitis is believed to be an atavistic primary IgM response, occurring in the case of elevated humoral immune reactions and disorders of lymphatic tissues (Schwarz and Scheurlen, 1995). The finding occurs due to defective in Con A-induced suppressor cell regulation of IgM producing cells was impaired in those with chronic hepatitis (Kayhan, *et al.*, 1986, Schwarz, *et al.*, 1988 and Zuberi, *et al.*, 1997).

### **3. Total leucocytes and differential count:**

This study revealed the following:

- No significant difference in the mean numbers of total leucocytes for patients ( $7462.86 \pm 1607.70$  cells/mm<sup>3</sup>), and controls ( $6836.67 \pm 1422.84$  cells/mm<sup>3</sup>).
- The mean numbers of neutrophils in patients was ( $3324.04 \pm 844.86$  cells/mm<sup>3</sup>) which is less than in controls ( $4179.23 \pm 856.47$  cells/mm<sup>3</sup>).
- There are increase in the mean numbers of lymphocytes for patients ( $3263.30 \pm 698.00$  cells/mm<sup>3</sup>) compared to controls ( $2104.50 \pm 473.46$  cells/mm<sup>3</sup>).
- This study revealed a significant difference in the mean numbers of monocytes for patients ( $701.70 \pm 192.77$  cells/mm<sup>3</sup>) and controls ( $366.07 \pm 97.76$  cells/mm<sup>3</sup>).
- There is no significant differences in the mean numbers of eosinophils for patients ( $110.41 \pm 68.14$  cells/mm<sup>3</sup>) and control group ( $119.07 \pm 64.32$  cells/mm<sup>3</sup>).
- Also there is no significant differences in the mean numbers of basophils for patients ( $63.40 \pm 46.84$  cells/mm<sup>3</sup>), and control group ( $67.80 \pm 47.83$  cells/mm<sup>3</sup>).

The results of this study are in agreement with other studies which found that, the number of lymphocytes rise while the number of neutrophils decrease with the increase of liver fibrosis in chronic HBV (Filimonov, *et al.*, 2004). (Vingerhoets, *et al.*, 2000) found that the peripheral blood monocytes in chronic HBV infection proliferate and increase in number. Another study was done for patients with chronic HBV and showed that the lymphocytes will increase while the neutrophils will decrease and no changes in total leucocytes count (Craig, *et al.*, 2002). (Vassilopoulos, *et al.*, 2008) revealed that the lymphocytes and monocytes will increase, while the neutrophils will decrease in patients with chronic HBV. However mild increment in total leucocytes count showed

no significant difference between patients and control group in statistical analysis, the lymphocytes will increase in viral infection due to activation of lymphocytes proliferation, while monocytosis occur as a result of chronic infection and decrement in neutrophils number due to increment in lymphocytes number upon computation of neutrophils, while the eosinophils and basophils have no value in viral infection.

#### **4. ESR:**

This study revealed that there is a significant difference in the mean values of ESR for patients ( $26.29 \pm 5.51$  mm/hr) and control group ( $8.87 \pm 3.95$  mm/hr). Meaning that the ESR elevated in patients with chronic HBV infection, and this finding is similar to that by (Par, *et al.*, 1995) who found that the ESR elevated in chronic HBV. Also the current study is consistent with another study done to find the relationship between ESR and all types of hepatitis and the researchers found that the high ESR were more frequent in HBV than in other types of hepatitis (Kassas, 1997). Explanation of this finding is that, the ESR is a nonspecific screening test that indirectly measures how much inflammation is in the body (Saadeh, 1998). The amount of fibrinogen and immunoglobulin (specially IgG) in the blood directly correlates with the ESR, so in patients with chronic HBV, the ESR will increase due to increment in total serum IgG (Veldhuijzen, *et al.*, 2008).

#### **4. Tuberculin skin test:**

The present study illustrated that the incidence of negative tuberculin test is higher in patients with chronic HBV (84.3%) than in the subjects of control group (30%). The finding of the present study is similar to a study done by (Larke, *et al.*, 1991) who showed that, a person with tuberculin test negative is about (6.2 times) more likely to be HBsAg-positive than those who were tuberculin test positive, and further analyzed a group whose status was known for HBV serologic markers and PPD negativity, and found that a significantly greater proportion (76.2%) of HBsAg positives were PPD negative in comparison to those with anti-HBs positive (67.2%) or who had no HBV markers (43.2%). Also the results of this study are in agreement with a previous study in 1982-1984 for Southeast Asian refugees in Philadelphia. The

researcher found that HBV carriers who reacted to a PPD skin test were more likely to be negative for the HBeAg than carriers who did not react to PPD. The researchers conducted a further study in 1985 in Alaskan native hepatitis B carriers, a group not vaccinated with BCG, and the inverse association of HBeAg and PPD reactivity was confirmed across all age groups and was similar in magnitude to that observed in the refugee population, so if the host response to BCG vaccine is similar, BCG vaccination may be of therapeutic value in chronic hepatitis B infection (Mcglynn, *et al.*, 1987). (Mcglynn, *et al.*, 1985) revealed that in hepatitis B carriers there are a statistically significant association between a negative tuberculin skin test and HBV replication, and this finding suggests that BCG vaccination might reduce the prevalence of infectious carriers, thereby ultimately reducing the incidence of hepatitis B infection. (Geo, *et al.*, 2007) explained that, the positive skin test indicates active cell-mediated immunity, whereas a negative test indicates a weak cell-mediated immunity. False-negative reaction may be produced by certain viral infection such as chronic hepatitis B infection either due to defects in mononuclear cells or defect in cytokines (IL-12, IFN- $\gamma$ , IL-2 and TNF) that produced in the liver (Vinay *et al.*, 2005). In case of hepatitis the liver function was altered, and this lead to subnormal production of cytokines and in turn it farther leads to abnormal TST.

### **Conclusions and Recommendations:**

Chronic HBV infection altered the immune system of the patients, the ESR will increase in chronic HBV infection, in chronic HBV infection, the TST is necessary for checking the immune system of the patients, for early detection of chronic HBV complications; the total serum (IgG and IgM), leucocytes differential count, ESR and TST should be added to routine investigations of those patients. A farther study on the "role of TST in the diagnosis of chronic HBV infection" is recommend.

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