Determination of some Hematological Parameters in Hepatitis B Patients in Najaf City

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Abstract

This study was carried out on 50 patients, who were admitted to Al-Hakeem hospital (Center Laboratory) in AL- Najaf governorate during the period from January to March (2015), their age ranged between (12-65) years. Fifteen healthy individuals without any evidence of chronic inflammatory disease depended as control. Subjects of this study were investigated importance of measuring the blood parameters in hepatitis B patients, and detection of the patient's immune response against viral infection.The obtained results showed that 50 were seropositive hepatitis B in ELISA technique. , the age groups (32-41) and (42-51) years showed a highly significant (P<0.05) elevation in patients than other patients. In contrast white blood cell count indicate that the age groups (12 – 21) and (42 – 51) years recorded high significant difference (p<0.05) compared with healthy groups and other patients while Lymphocyte and monocyte percentage increase in in more groups of patients suffering from hepatitis B compared with healthy control group specific in age groups (42 – 51) and (52 – 61) years in comparison to other patients. In the other hand the result of granulocytes, the basophilis showing high – significant (p<0.05) increase in patients group as compared to healthy control group and other granulocytes.
Keyword: Hepatitis B, Hematological Parameters, Differential leukocytes Count

الخلاصة

أجريت هذه الدراسة على 50 مريضا من الوافدين الى مستشفى الحكيم (المختبر المركزي) في محافظة النجف خلال الفترة الممتدة من كانون الثاني(2015) الى اذار (2015). وقد تراوحت اعمارهم بين (12–65) سنة ,وقد اختير خمسة عشر شخصا أصحاء غير مصابين بأي مرض التهابي مزمن كمجموعة سيطرة. وكان الغرض من هذه الدراسة بيان اهمية قياس المعايير الدموية عند مرضى التهاب الكبد الفايروسي نمط ب والكشف عن الاستجابة المناعية في المرضى ضد الاصابة الفايروسية. أظهرت النتائج ان 50 شخصا مصابا بالتهاب الكبد الفايروسي (ب) عن طريق إجراء إختبار الاليزا . وسجلت الفئات العمرية (32–41) و(42–51) ارتفاعا معنويا عن بقية الفئات العمرية. وبالمقابل في تعداد كريات الدم البيضاء سجلت الفئات العمرية (12–21)و(42–51) ارتفاعا معنويا مقارنة مع مجموعة السيطرة وبقية مجاميع المرضى. بينما الخلايا اللمفية والوحيدة النواة ارتفاعا معنويا في معظم مجاميع المرضى وخصوصا الفئات العمرية (42–51) و(52–61) ارتفاعا معنويا عن بقية الفئات. ومن جهة اخرى اضهرت نتائج كريات الدم البيضاء الحبيبية ان الخلايا القاعدية كانت الاكثر ارتفاعا معنويا مقارنة مع مجموعة السيطرة وبقية الكريات الحبيبية.

الكلمات المفتاحية: التهاب الكبد الفايروسي نمط B ، معايير الدم، عد كريات الم البيضاء المختلفة.

Introduction

The injury with hepatitis B virus of more world problems approximately 350–400 million chronic infected people. Hepatitis B infection could stimulate broad range of clinical manifestations, range from a dormitive conveyor Location invasive the hepatitis, Fibrosis, or liver cancer (Torbenson and Thomas, 2002; Lavanchy, 2004) HBV there is usually at the blood, saliva, vagina secretions and menstrual blood from people infected. Because hepatitis as resistance to drop-out from the body, easily transmit through touch with infected body fluids (Wright, 2006).

Most HBV infections without appear symptoms, this means that there is a risk that people are others without knowing it (Weinbaum *et al*., 2009; WHO, 2012). But some people may be suffering from severe symptoms such as jaundice, fatigue, loss of appetite, nausea and/or abdominal pain, for almost all adults, 90%, the infection heals and they become healthy, but for infants and young children, there is a 90% and 30-

50% risk respectively that the infection leads to chronic hepatitis B .This provides an increased risk, approximately 25% that they later in life will suffer from liver cirrhosis and/or liver cancer, if the infection is not medically managed (Chao *etal*., 2010; WHO 2012; Dahlström and Viberg, 2013).

White blood cells are an importance ingredient of the host defence system, responsible for protection against bacteria, fungi, viruses, and invading parasites. The complex cytokine network and hierarchy of progenitor cells maintain baseline myelopoiesis and also allow rapid
amendment in the rates of production of these cell types that occur in response to acute and chronic stress. (Levinson *etal*., 2004).

Materials and Methods
This study was include (50) samples (35 male and 15 female) age ranging from 12 - 65 years were collected from AL- Hakeem Hospital (Center Laboratory) in AL- Najaf city during the period from January to March (2015) .Those patients were matched with 12 healthy as controls were enrolled into the study. Approximately (5 ml) of fresh blood was drawn from each patient, placed in two types of tubes; the first tubes contain disodium ethylene diamine tetra acetic acid (EDTA) as anticoagulants to prevent clotting of blood to be used for hematological studies. The second types tubes were without anti-coagulant as plain tubes, for blood to be used for preparing sera for subsequent serological tests. Each sample was labeled and given a serial number together with the patient name, the serum samples were frozen at (-20°C) (Lewis *et al*., 2006). Until used for Virological Investigation**.**

ELISA technique for detection of HBsAg (biokit- SPAIN)
A- Assay procedure
1. Reserved 8 wells for blank and controls. 100 μl of negative control were transferred to 2 wells and 100 μl of high positive calibrator to 2 wells and 100 μl of low positive calibrator to 3 wells and leaved a well empty for the substrate blank.
2. For each sample 100 μl were transferred to be tested into the assigned well. For each sample 3 wells were used: transferred 100 μl of each sample undiluted, diluted 1/10 and diluted 1/100 to the appropriate wells.
3. The plate was covered with the adhesive seal, mixed gently and incubated for 1 hour at 37°C.
4. The adhesive seal was removed and discarded. The contents of the wells were aspirated and filled them completely (approximately 350 μl) with the diluted washing solution. The process of aspiration and washing repeated 3 more times. Ensured that each column of wells soaks for at least 15 seconds before the next aspiration cycle. After the last washing blot the inverted microplate on absorbent tissue to remove any excess liquid from the wells.
5. Added 100 μl of conjugate to all wells accepted the blank with avoided bubbles upon addition, and covered with the adhesive seal, mixed gently and incubated for 30 minutes at 37°C.
6. During the last 5-10 minutes of this incubation prepared the substrate-chromogen solution. 280 μl of chromogen (TMB) added to the bottle containing the substrate buffer (14 ml) and mixed well.
7. The adhesive seal was removed and discarded. The plate was washed as in step 4.
8 Added 100 μl of substrate-TMB solution to each well, including the blank, and Incubated for 30 minutes at room temperature (20-25°C).

9. The reaction stopped by adding 100 µl of stopping solution in the same sequence and time intervals as for the substrate-TMB.

10. The absorbance of each well reader at 450 nm with the blank well and read within 30 minutes.

Qualitative assay

1. Calculate the mean absorbance of the low positive calibrator. This is the cut-off.

Cut-off: LPCx

2. The sample absorbance were divided into the cut-off values.

Positive: ratio absorbance/cut-off ≥ 1.0

Negative: ratio absorbance/cut-off < 0.9

Equivocal: ratio absorbance/cut-off ≥0.9 < 1.0

Plot in linear-linear graphic coordinates the anti-HBs concentrations of the negative control (0 IU/ml), low positive calibrator (10 IU/ml) and high positive calibrator (100 IU/ml) on the abscissa (x axis) against their corresponding mean absorbance values on the ordinate (y axis). Draw a line through these three points. The concentration in IU/ml of each sample can be derived from its absorbance using the calibration curve. If the sample was diluted the result should be multiplied by the dilution factor in order to obtain the actual anti-HBs concentration present in the serum.

Hematological Investigation

The hematological parameters were performed on EDTA blood using Mythic^{TM}18(RINGELSAN CO. Turkey) in Hematology of Laboratory of AL- Hakeem Teaching Hospital in AL-Najaf city.Mythic^{TM}18 is a fully automatic hematology analyzer performing complete blood count (CBC) on EDTA anticoagulated blood(Wasmuth ,2010), for counting the cellular blood components, theMythic^{TM}18 uses the impedance technique only.

Cyanide free spectrophotometry method was used to measure hemoglobin by formation of oxyhemoglobin at 555 nm. Hematocrit was measured by volume integration. The sample volume was 10µl. The instrument can determine 18 parameters in the research mode: white blood cells (WBC) with absolute number and percentage of lymphocytes (LYM), monocytes (MONO), neutrophilis (NEU), eosinophilis (EOS) and basophilis (BASO).

Procedure:

EDTA blood sample (10µl) was placed in the aspirator of the instrument. And the blood sample was aspirated. The results were provided within 1 minute on the LCD display, printed out on the printer and stored in the resident memory.

Statistical Analysis

All values were expressed as means ± SE. The data were analyzed by using of SPSS (T test) version 17 and Microsoft Excel computerized programs and taking P value less than 0.05 (p <0.05) as the lowest limit of significance (Paulson ,2008) .

Results and Discussion
The Acute Hepatitis B Virus (HBsAg)

The Results indicated that a significantly difference (p<0.05) in the diffusion of hepatitis B among groups (32-41) and (42-51) years compared to older age groups (P<0.05). This can be explication that these age groups are more exposure to the risk factor of infection may be during the work, sexual activity or travel. This result was also mentioned by Al-Awady *et al*., (2008) and agrees with some local researches

such as Al-Jaaf,(2006) who was found that most of patients were located within third and fourth decade (30-49 y) with a percentage of (50%). The statistical analysis relieved significant difference between the mean of optical density of patients samples and negative control in all age groups (LSD 0.9) Figure (1).
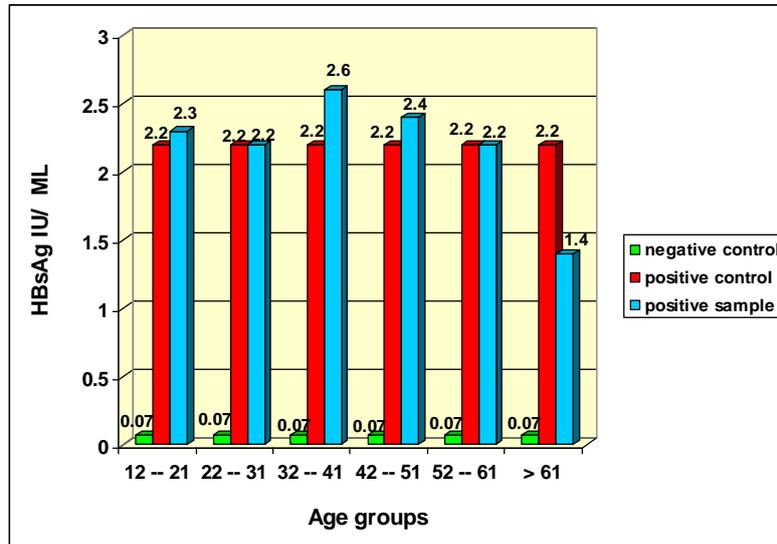

Figure (1): The status of anti-HBs Ag in different age groups in patients by ELISA test.

White Blood Cell (WBC)

This study indicates that the age groups (12 – 21) and (42 – 51) years recorded high significant difference (p<0.05) and LSD = 1.2 compared with healthy groups and other patients Figure (2). .WBC is usually raised due to infectious disease (viral and bacterial infection) and cause inflammation in infected via infiltrate of inflammatory cells to the site of inflammation (Goldsby *et al*., 2007). Abnormalities in hematological allusion into so much come across in cirrhosis.Several reasons contribute to the incidence of hematological abnormalities.According to new studies that the existence of hematological cytopenias is linked to poor prognosis in cirrhosis (Qamar and Grace, 2009).
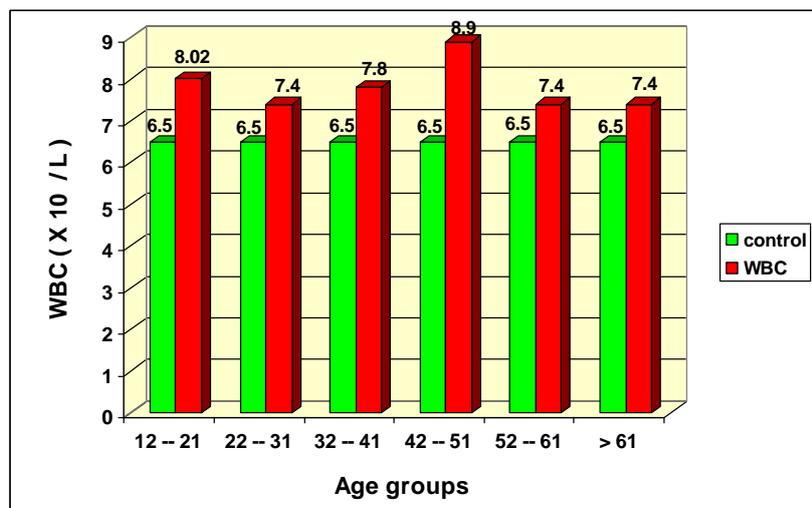

Figure (2): White blood cell count in the hepatitis B patients compare with healthy control.

Differential leukocytes Count (DLC):
### Lymphocyte (LYM %) and Monocytes (MON %)

There was increase in lymphocyte percentage in the most groups of patients suffering from hepatitis B compared with healthy groups. This increases statistically significant (p<0.05) and LSD= 3.2 Figure (3). With regard to monocyte percentage, the study shows a significant increase (p<0.05) and LSD= 2.2 in age groups (42 – 51) and (52 – 61) years in comparison to healthy control group Figure (4).

Lymphocytes form about a third of the white blood cells in the peripheral blood (1·0–4·0_109/L). Mostly circular nucleus or made lightly integrated with little a granular cytoplasm. Two thirds of a Tcells, which are involved in cellular immune responses (Stock and Hoffman, 2000). Also the Lymphocytes are the central cells of the immune system which responsible for adaptive immunity. The haemopoietic stem cells produce Lymphocyte during viral infection to neutralize the virus by antibody production, so the Lymphocyte will increase significantly (Al-Jaifry, 2012)

These results are in agreement with results obtained by Yang *et al*., (2014) who found that monocytes expression of the different receptors, and sensors which monitoring of environmental changes. Monocytes are extremely plastic and diversified, and changed their physical appearance and functional responding to the environmental stimulus, because of the evidence from mice and human studies pointed out that the large subunit may be an indication of different inflammatory diseases. Monocytes it can differentiate into sub-inflammation or anti-inflammatory collections. Also agreed with the results Zhu *et al.*, (2011) who showed that the Cell Population Data (CPD), it carries significant mutation in responding to hepatitis B virus (HBV).
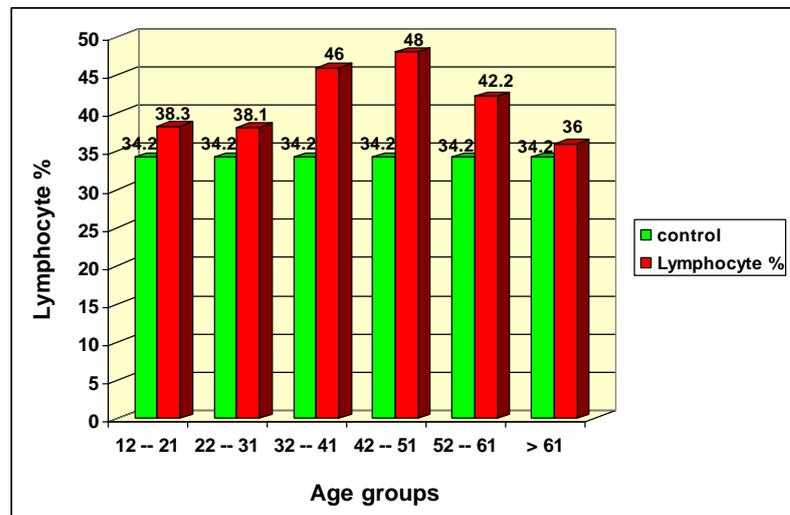
Figure (3): Lymphocyte in the patients groups compare with healthy control
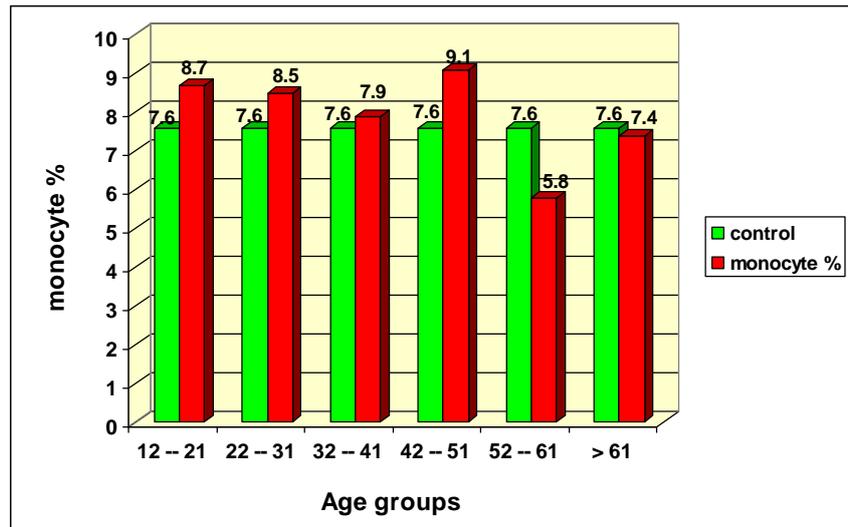
Figure (4): Monocytes in the hepatitis B patients compare with healthy control

Granulocytes (GRA %)

The result of differential type of Granulocytes, Neutrophilis, Eosinophilis and Basophilis
is shown that the significant increase (p<0.05) in patients suffering from hepatitis B compared to healthy control group. Figures (5, 6 and 7) consecutive .But the Basophilis showing high – significant (p<0.05) change in patients group as compared to healthy control group.

these results in agreement with Park-Min *et al*., (2005) & Heydtmann, (2009) who found that most of Macrophages are single-core phagocytic cells of the innate immune responsiveness whichever downright the adaptive responses.Also the absolute basophil cell counting higher than 0·2_109/L , this can be seen with hypersensitivity acute sensitivity, chronic inflammation, and inflammatory disorders (TB disease, ulcerative colitis and rheumatoid arthritis) or with virus infections(Stock and Hoffman, 2000).

Measuring hematological parameter in patients with hepatitis B is an important step in the knowledge of the affected state of the immune. This study similar to recent study by Xu, (2015)   how founds that the specific changes in Leukocyte morphological parameters, also defined the cell population data (CPD) in viral infection, providing prospect new blood parameters for Virus infections. The clinical application of these leukocyte morphologic parameters offers many advantages. These parameters are generated during automated differential analysis without additional specimen requirements.
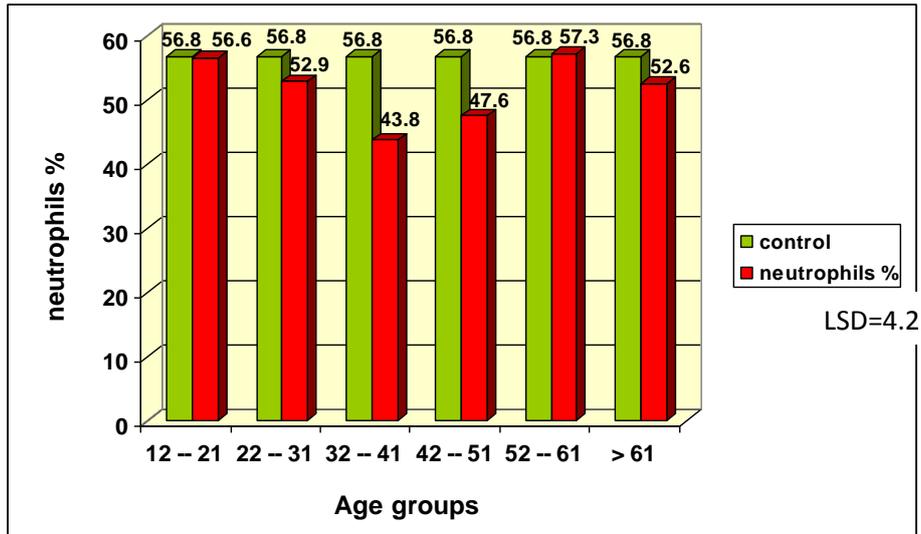
Figure (5): Neutrophilis in the hepatitis B patients compare with healthy control.
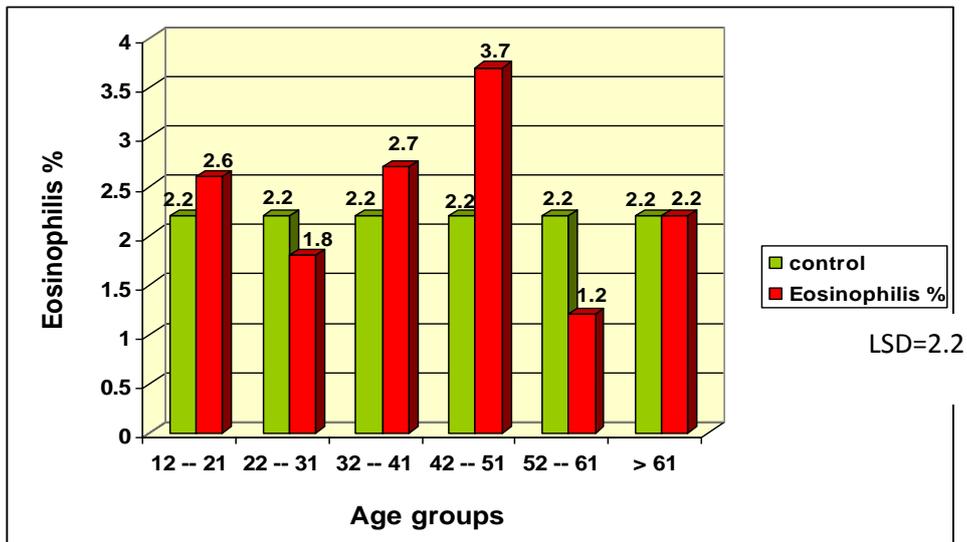

Figure (6): Eosinophilis in the hepatitis B patients compare with healthy control.
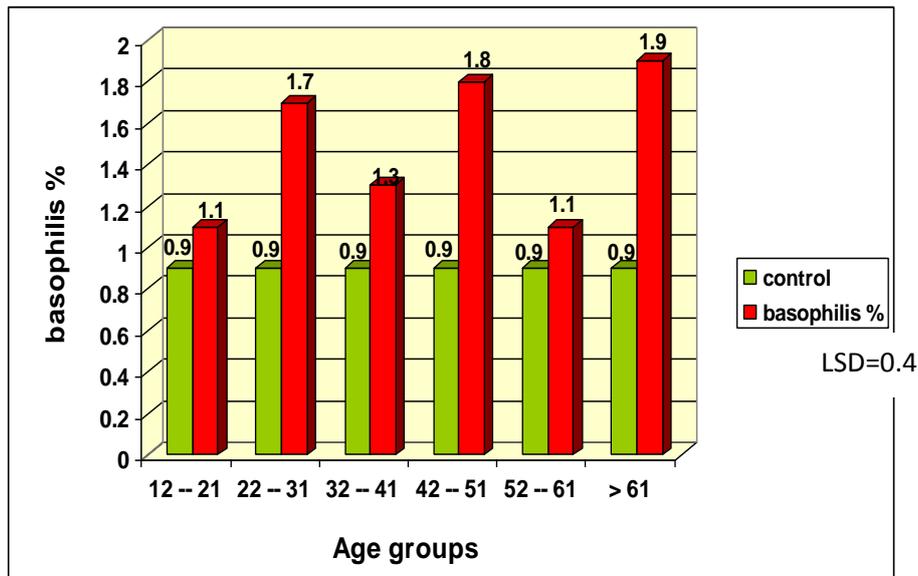
Figure (7): Basophilis in the hepatitis B patients compare with healthy control.

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