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STUDY OF PROTHROMBIN TIME (PT) AND CYTOGENETIC CHANGES IN PATIENTS WITH HBV IN WASIT PROVINCE

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ABSTRACT

Viral hepatitis type B is a disease caused by entry of hepatitis B virus (HBV) to the body, reaching the liver, multiplication, and stimulates the patient's immune system to attack the infected cells therefore caused inflammation of hepatocytes. The study included 40 blood samples from patients with HBV compared with 25 blood samples from control. Patients were divided according to their age into two groups. Levels of prothrombin time (PT) was measured and cytogenetic tests: mitotic index (MI), micronucleus assay (MN) and nuclear division index (NDI) were measured for patients lymphocyte. The study showed a significant increase ($P \leq 0.05$) in prothrombin time of the patients when compared with control to all age groups, as well as the results of mitotic index, micronucleus assay and nuclear division index were significantly increasing ($P \leq 0.05$) indicates a defect in cells division.

KEYWORDS: Hepatitis B Virus, Prothrombin Time, Mitotic Index, Micronucleus Assay, Nuclear Division Index.

INTRODUCTION

Hepatitis is a general term refers to inflammation of liver tissue, from Latin "heap" the liver and "titis" meaning inflammation, microscopically; a spotty formation as a result of liver parenchymal cell degeneration and necrosis.[1] There are many agents that can cause liver inflammation such as viruses, bacteria, fungi, parasites, mycotoxins, tumors, and chemical agents including drugs, toxins and alcohol.[2] Viral hepatitis type B (serum hepatitis) is one of the most common viral diseases which are a global health problem and common cause of liver disease such as cirrhosis, hepatocellular carcinoma (HCC), it's a slow or silent disease characterized by rare symptoms in the early stages of infection.[3] Hepatitis B virus causes both acute and chronic infection in human, in acute stage (AHB) the inflammation develops rapidly but ends quickly and takes less than six months, if the infection continues more than six months, the disease turns from acute to chronic stage (CHB), this condition may be continue forever and sometimes lead to death.[4] According to the world health organization (WHO), two billion people worldwide have evidence of past or present infection with HBV, 248 million are chronic carriers of HBV, worldwide, it is estimated that around 686,000 people die each year from the complications of CHB. Overall, HBV infection accounts for around 45% of cases of hepatocellular carcinoma and 30% of cirrhosis.[5] HBV documented to be second only to tobacco as a potent environmental carcinogen.[6] AHB is without symptoms in 70% of cases, and the complications associated approximately 1% of them, about 10% of adults with severe infection develop CHB if they continue for more than six months.[1] Symptoms of the AHB include nausea, fever, anorexia, yellowing of the skin and abdominal pain, CHB are more serious complications involving cirrhosis and HCC, The routes of HBV transmission are blood transfusion, sexual, perinatal, percutaneous, nosocomial, and organ transplantation.[2] Prothrombin time (PT) is valuable prognostic factor of liver failure that measures the extrinsic pathway of hemostasis. All coagulation factors, except factor VIII, are synthesized in the liver, factors II, V, VII and X are clotting factors involved in prothrombin production. When the liver cannot perform its function in some way, the manufacture and secretion of these factors decrease in blood.[7] Cytogenetic tests have ability to detect factors that cause genetic damage by interacting with enzymes and microtubules, the damage in nucleic acid are detected by the lymphocytes of patient depend on fact that white blood cells (WBCs) in the circulatory system constantly monitor the body and look for signs of exposure to genotoxic factor. The cytogenetic tests used in this study include: mitotic index (MI), micronucleus assay (MN) and nuclear division index (NDI). MI used to assess the chromosomal change in cells exposure to physical factor or diseases that can effect on cell division time.[8] Micronucleus assay (MN) widely used to detect micronucleus in cytoplasmic cells during interstitial phase and estimate the genetic toxicity of chemicals in

living organisms as well as to detect the factors that mutate and change in the composition of chromosomes. The nuclear division index (NDI) is ratio of the nuclear division cells to the total number of cells with condition that all of them not contain micronucleus.[9] Aims of this study include measure the duration of blood clotting by use prothrombin time (PT) test, as well as study the patient's cytogenetic changes and compare them with healthy (control group), which include: mitotic index (MI), micronucleus assay (MN) and nuclear division index (NDI).

MATERIALS AND METHODS

Blood samples

Blood samples were collected from hospitals and the central health laboratory in Wasit province during the period from November 2016 to April 2017. This study included 40 patients of both sexes, (20) males and (20) females, compared with a control group which included 25 people (13) male and (12) female, 2.8 ml of venous blood were withdrawn from both patient and control, divided into 2 groups: the first group (1.8) ml of blood placed in sodium citrate tube to prothrombin time (PT) test, the second group (1) ml of blood placed in sodium heparin tube for cytogenetic tests. The data was taken in both patients and control as well as their divided into two groups depending on sex, each group was divided to two age groups (less than 35 years and older or equal to 35 years) for all studied criteria.

Prothrombin time (PT) test

The first stage of prothrombin time PT is measure the clotting time of plasma after adding the source of tissue factor (thromboplastin) and calcium. The recalcification of plasma in the presence of tissue factor generates active factor Xa, which the consequent formation of thrombin and ultimately an insoluble fibrin clot.[10]

Cytogenetic tests

Cytogenetic test includes mitotic index (MI), micronucleus assay (MN) and nuclear division index (NDI). The lymphocyte cells in human used to determine shape and number of chromosomes because it's easily stimulate to division and growth in blood culture, karyotypes method developed to provide information about chromosomal normalities. Lymphocyte cells don't normally undergo subsequent cell divisions so it's stimulated for division by use phytohemagglutinin-M (PHA-M) to enter cells into mitosis by DNA replication.[11]

The in vitro micronucleus assay is a mutagenic test system for detection chemicals that stimulate formation of small membrane bound DNA fragments and the cytokinesis block micronucleus assay (CBMA) was used as a useful technique to evaluate cytogenetic damage in peripheral blood lymphocytes. The purpose of the micronucleus assay is to detect the agents that modify chromosome structure and segregation in a way leads to the formation of micronuclei in cells at interphase phase.[12]

Statistical analysis

The results were statistically analyzed by using the statistical program for social science 13 (SPSS 13) by finding (mean $\pm$ SD) and using the LSD (least significant difference) test. Two way ANOVA method was used to compare between results to identifying significant differences between patient and healthy people, and the results are significant if the value of P-value is less than 0.05 ($P \leq 0.05$).[13]

RESULTS

The results of prothrombin time (PT) in patients with hepatitis B virus are significantly different when compared with control group, the table (1) for males show a significant increase ($P \leq 0.05$) in mean value of prothrombin time (PT) in males with hepatitis B compared with control in both age group, The results of male patients (mean + SD) were (16,792 $\pm$ 2.082) (16.022 $\pm$ 2.258).

compared with control (13.362 $\pm$ 2.240) (13.356 $\pm$ 0.388) for the first and second age groups respectively (less than 35 years and older or equal 35 years), and table (2) for females show same result in same age groups, (16.590 $\pm$ 1.119) (15.762 $\pm$ 1.879) compared to control (13.110 $\pm$ 0.202) (12.925 $\pm$ 0.345) for the first and second age groups respectively. According to tables (1) and (2) age have no effect on PT in both males and females.

Table (1): Prothrombin time (PT) in male's patient with hepatitis B virus (HBV) compared with control.

Parameter / Age \ years	Prothrombin time (PT) (mean±SD) sec.	
	< 35	≥ 35
Patients	A‹a 16.792±2.082	A‹a 16.022±2.258
Control	B‹a 13.362±2.240	B‹a 13.356±0.388
LSD	2.12	2.50
P-value	0.037	0.003

***Different capital letters show significant difference (p≤0.05) between patients and control.**
***Different small letters show significant difference (p≤0.05) between age groups.**
***LSD: Least significant difference. *PT: Prothrombin time.**

Table 2: Prothrombin time (PT) in female's patient with hepatitis B virus (HBV) compared with control.

parameter / Age \ years	Prothrombin time (PT) (mean±SD) sec.	
	< 35	≥ 35
Patients	A‹a 16.590±1.119	A‹a 15.762±1.879
Control	B‹a 13.110±0.202	B‹a 12.925±0.345
LSD	0.62	1.83
P-value	0.0002	0.001

***Different capital letters show significant difference (p≤0.05) between patients and control.**
***Different small letters show significant difference (p≤0.05) between age groups.**
***LSD: Least significant difference. *PT: Prothrombin time.**

The results of cytogenetic studies in patients with hepatitis B virus show significant differences when compared with control group, table (3) showed a significant increase (P≤0.05) in rate of mitotic index (MI) in male with hepatitis B virus compared with control in both age group, result of patient (9.638 ± 1.339) (8.522 ± 1.491).

compared to control (1.777 ± 0.478) (2.222 ± 0.172) for the two age groups less than 35 years and older or equal 35 years respectively, the number of dividing cells in the metaphase phase is increase, fig (1) show the shape and number of chromosomes in lymphocyte cell of patients with hepatitis B (HBV) under light microscope stain with Giemsa stain. In same table results also showed a significant increase (P≤0.05) in micronucleus assay (MN), the results of patients (0.0381 ± 0.0062) (0.0477 ± 0.0104) compared to control group (0.0111 ± 0.0006) (0.0166 ± 0.0022) for the first and second age groups respectively, the micronucleus (MN) in lymphocytes of HBV patient can see in fig (2) under a light microscope stains with Giemsa stain, also there are a significant increase (P≤0.05) in a nuclear division index (NDI) in patients compared with control, the patients results were (2.057 ± 0.566) (1.6722 ± 0.4877) compared to the control (0.962 ± 0.145) (0.7856 ± 0.0557), fig (3) show the nuclear division index (NDI) in lymphocytes of patients with hepatitis B virus (HBV) under a light microscope stains with Giemsa stain. Table (4) for females show same result in same age groups, MI result of patients female (8.400 ± 1.534) (7.437 ± 1.335) compared to control (1.570 ± 0.295) (2.212 ± 0.294) for the two age groups respectively, and MN (0.0440 ± 0.0083) (0.0576 ± 0.0143) compared to control (0.0147 ± 0.0067) (0.0186 ± 0.0029). The comparison between the two age groups according to tables (3, 4) for male and female show a significantly different between the two age groups in all criterion in both sex except female NDI has no significant difference (P>0.05) between age group, the result were (1.6530 ± 0.4047) (1.6912 ± 0.2175) compared to control (0.8350 ± 0.1484) (0.8225 ± 0.0198).

Table (3): Cytogenetic change in male's patient with hepatitis B virus (HBV) compared with control.

Parameter / Age \ years	MI (mean±SD) %		MN (mean±SD)		NDI (mean±SD)	
	<35	≥35	<35	≥35	>35	≥35
Patients	A‹a 9.638 ± 1.339	A‹b 8.522 ± 1.491	A‹a 0.0381 ± 0.0062	A‹b 0.0477± 0.0104	A‹a 2.057 ± 0.566	A‹b 1.6722 ± 0.4877
Control	B‹a1.777±0.478	B‹b 2.222±0.172	B‹a 0.0111±0.0006	B‹b 0.0166 ± 0.0022	B‹a 0.962 ± 0.145	B‹b 0.7856 ± 0.0557
LSD	2.37	3.24	0.00012	0.0575	0.741	0.57
P-value	0.0002	0.001	0.0003	0.0003	0.0001	0.0001

Table (4): Cytogenetic change in female's patient with hepatitis B virus (HBV) compared with control

Parameter / Age \ years	MI (mean±SD) %		MN (mean±SD)		NDI (mean±SD)	
	<35	≥35	<35	≥35	>35	≥35
Patients	A‹a 8.400 ± 1.534	A‹b 7.437 ± 1.335	A‹a 0.0440 ± 0.0083	A‹b 0.0576 ± 0.0143	A‹a 1.6530 ± 0.4047	A‹a 1.6912 ± 0.2175
Control	B‹a 1.570 ±0.295	B‹b 2.212 ± 0.294	B‹a 0.0147 ± 0.0067	B‹b 0.0186 ±0.0029	B‹a 0.8350 ± 0.1484	B‹a 0.8225 ± 0.0198
LSD	2.31	2.85	0.00349	0.0108	0.529	0.258
P-value	0.0003	0.0004	0.0005	0.0003	0.0003	0.004

***Different capital letters show significant difference (p≤0.05) between patients and control.**
***Different small letters show significant difference (p≤0.05) between age groups.**
***LSD: Least significant difference. *PT: Prothrombin time.**
***MI : Mitotic Index. *NDI : Nuclear division index. *MN : Micronucleus.**

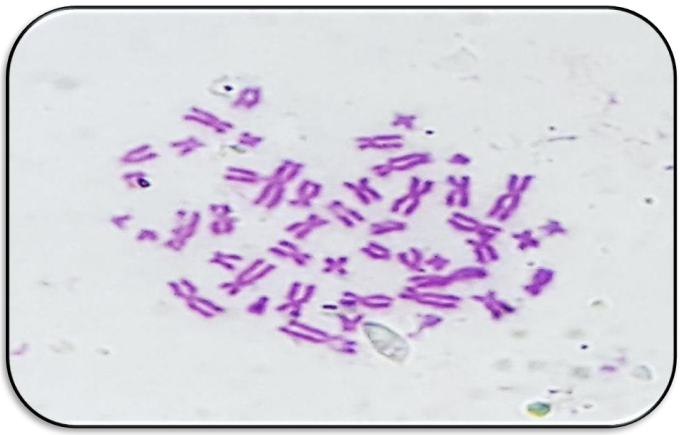

Fig (1): Shape and number of chromosomes in lymphocyte cell of patients with hepatitis B (HBV) under light microscope (X100) stain with Giemsa stain.

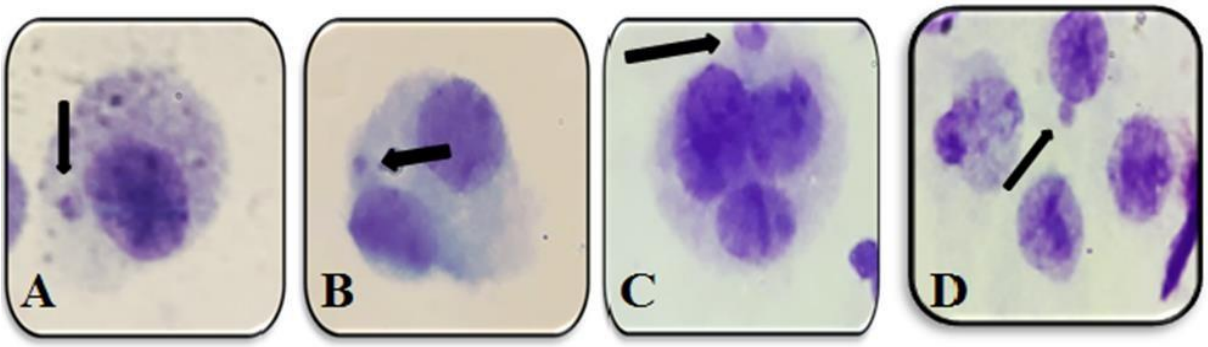

Fig (2): Micronucleus (MN) in lymphocytes of patient with hepatitis B virus (HBV) under a light microscope (100X) stains with Giemsa stain. Arrow signal to micronucleus, A: mononucleated, B: binucleated, C: Trinucleated, D: tetranucleated.

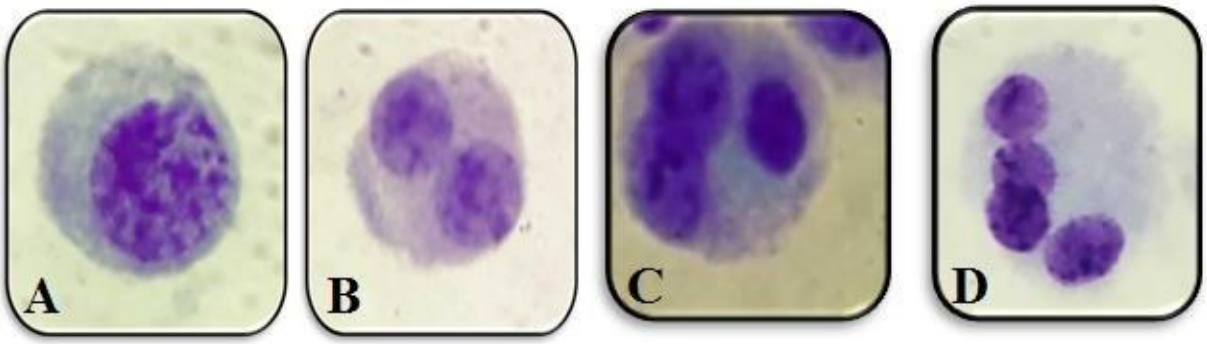

Fig (3): Nuclear division index (NDI) in lymphocytes of patients with hepatitis B virus (HBV) under a light microscope (100X) stains with Giemsa stain: A: mononucleated, B: binucleated, C: Trinucleated, D: tetranucleated.

DISCUSSION

The results in table (1) and (2) show a significant increase in PT mean value in male and female patients compared with control for all age groups, without significant difference between age groups, these results are consistent to findings by Min and others.[14] Parenchymal hepatic cells are responsible for synthesis of most coagulation factors such as anticoagulant proteins and components of the fibrinolytic system, so the liver plays an important role in hemostasis. When the liver is infected, these functions are impaired and the time of coagulation becomes longer.[15] PT is elevated in other conditions such as vitamin K deficiency, warfarin intoxication, and primary fibrinolysis. All liver patients have coagulopathies, and a statistical change observed in all coagulation factors in HBV patients according to[16], when the liver is infected, the virus stimulates production of tumor necrosis factor (TNF). Therefore, the liver loses part of its ability to synthesis coagulation factors. Loss of liver function after viral infection can also result from the inflammation caused by HBX (hepatitis B virus x protein). In addition, liver disease caused by vitamin K deficiency also reduces liver productivity of prothrombin and fibrinogen, therefore prolongs PT.

Cytogenetic criteria have ability to detect factors that cause genotoxicity effect in cells by interacting with enzymes and microtubules that play an important role in DNA replication as well as in the dissociation of chromosomes during cell division, so the increase in the mean value of cytogenetic criteria indicates a genotoxic factor affecting cell division.[17] The damage in nucleic acid are detected by the lymphocytes of patient depend on fact that white blood cells (WBCs) in the circulatory system constantly monitor the body and look for signs of exposure to genotoxic factor.[18] The results in table (3) and (4) show there was a significant increase in the mean value of MI in male and female patients with HBV compared with control for all age groups, that's caused by direct genotoxic effects of HBV virus on genetic material of host cells, which can effectively contribute caused of hepatocellular carcinoma (HCC) in addition to apoptotic[19], some study found that the virus causes the DNA double - strand breaks (DSBs) in the genetic material of host cells[20], these results are consistent to[21] that evidence increase of MI in HBV patient as well as HCC compared to control people. The duration of infection, age, sex, smoking, alcohol and exposure to toxins are partly due to differences in the value of MI between patients[22], the results did not agree with.[23]

The results also show a significant increase in the mean value of MN in male and female patients with HBV compared with control for all age groups; most cells found are mononucleated, in second place binucleated and less trinucleated and tetranucleated. The increase in the number of cells containing micronuclei MN in lymphocytes is a sign of structural or numerical abnormalities in the chromosomes; this indicates the effect of the disease on the host's nuclear material. MN is also affected when cells are treated with chemicals or when the disease is developed.[24] The results of the study are consistent with[25], which found an increase in MN in lymphocytes of patients with HBV compared to lymphocytes of control, the infected with HBV represents the instability of chromosomes in lymphocytes, and this's characterized by formation of decentralized pieces or fragments, in addition to a defect in the number of chromosomes (Aneuploidy).

MN formed from chromosome fragments or loss of chromosomal centrosome, so its fails to bind to the spindle, that's making them not merged with the nuclei of the cells resulting after cell division[26], The results showed that the average value of MN in females in both age groups was increased compared with males for both patients and control, this in line with[27], that indicating there are several factors causes the difference in average value of MN including: age, sex, smoking and alcohol, as well as the results showed a significant difference between the age groups, MN increased in second age group of both sexes for both patient and control.[25] In two tables above (3) and (4), the nuclear division index (NDI) showed a significant increase in the mean values for male and female patients compared to the control to both age groups, most cells found are mononucleated, in second place binucleated and less trinucleated and tetranucleated. NDI is a measure of general cytotoxicity and a marker of cell proliferation; cells with high chromosomal damage either die before cell division or less likely to enter this phase.[28] As explained above, these changes as a result of viral integration is considered to have direct mutagenic potential in hepatocytes. The integration of HBV into the human genome affects the expression of genes located near the site of insertion and also causes more widespread alterations of chromosomal stability, because the genome of viral integration into the host DNA also frequently occurs in the peripheral blood mononuclear cells PBMCs of chronically HBV-infected patients, this process may contribute to genomic instability in these cells.[25] The observed difference between male and female patients caused by female sex hormones that play an important role in increasing protein and RNA in a some tissues such as muscles of glands mammary and uterine, any defect occur in synthesis of RNA will cause an imbalance in composition and duplication of DNA that will send incorrect instructions lead to the formation of cells randomly.[29] The results of the study did not agree with.[23]

CONCLUSIONS

From this study we find that prothrombin time (PT) increased in patients with hepatitis B virus (HBV) disease compare with control, the liver loses part of its ability to synthesis coagulation factors during HBV infection, as well as the result finding that mitotic index, micronucleus assay and nuclear division index of patient were increasing indicates a defect in cells division.

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