EVALUATION OF PLATELETS COUNT AND COAGULATION PARAMETERS AMONG PATIENTS WITH LIVER DISEASE

Mohammed Elamin Mustafa¹, Mansoor Mohammed Mansoor², Asaad Mohammed Ahmed Abd Allah Babker³

¹,²Department of Hematology and Immunohaematology College of Medical Laboratory Science Sudan University of Science and Technology.
³Al-Ghad International Medical Sciences Colleges - Al-Madinah Al-Munawarah (Sudia Arabia) - Department of Medical Laboratory Science.

ABSTRACT

We hypothesized a strong association between hemostatic changes and liver disease because hemostasis is intimately related to liver function, because most coagulation factors are protein synthesized by liver the extent of coagulation abnormalities depends upon the degree of disturbed liver function. The aim of this study was to evaluate hemostatic mechanism change especially PT, PTT and PLTs count among patients with liver disease. Method: We enrolled Forty patients with liver disease (cases) include: tow gall choleystitis (5%), five obstructive jaundice (13%), ten liver cirrhosis (25%), fifteen HBV (38%), six HCV (15%) and liver metastasis (5%) those were compared them to fifteen healthy individuals as (control) group neither affected by liver diseases nor receiving any kinds of therapy that can affect in liver. Cases and controls were tested for the above investigation. Informed consent was obtained from all participants before enrolment. The automated method used to measure PT, APTT, by Coagulometer and PLTs count used (Sysmex KX 21N). Results: The PT was showed significant differences in these values between patient and control in hypertension (p= 0.01) and diabetes (p = 0.03) the no significant PT result was showed in renal failure group (p = 0.2).The APTT was tend to be prolonged in diabetes group (43.2) only the normal APTT result was showed in hypertension (40.0) and renal failure group (40.0) the statistical analysis showed significant differences in these values between patient and control in hypertension group (p = 0.01) and diabetes (p =
0.006) and renal failure (p= 0.008). The platelet count was found to be lower in hypertension group (141.0), diabetes (147.7) and in renal failure (135.8). The statistical analysis showed significant values in all groups, in hypertension (p = 0.000) diabetes (p= 0.002) and in renal failure (p= 0.002). **Conclusion:** PT showed significantly prolonged in all cases except renal failure, APTT showed significant among all cases and PLTs showed lower with significant value among cases. We conclude that from our study that there was a considerable association between the liver diseases and related disorder with abnormality of different haemostatic mechanism.

**KEYWORDS:** Liver disease, Prothrombin, Thromboplastin, Platelets.

**INTRODUCTION**

The liver is the body’s largest gland and it is a vital organ that supports nearly every other organ in the body in some facet. The liver is a gland and plays a major role in metabolism with numerous functions in the human body, including regulation of glycogen storage, decomposition of red blood cells, plasma protein synthesis, hormone production, and detoxification.\(^1\) The liver plays a central role in the hemostatic system as it synthesizes the majority of coagulation factors and proteins involved in fibrinolysis. Furthermore, the liver produces Thrombopoietin, which is responsible for platelet production from megakaryocytes. Consequently, chronic or acute liver diseases frequently have a profound impact on the hemostatic system.\(^2\) The liver supports almost every organ in the body and is vital for survival. Because of its strategic location and multidimensional functions, the liver is also prone to many diseases.\(^3\) Liver disease leads to a form of "rebalanced" hemostasis, in which diminished hepatic function leads to both procoagulants and anticoagulant effects. All stages of the hemostatic process may be abnormal, including primary hemostasis (platelet adhesion and activation), coagulation (generation and cross linking of fibrin), and fibrinolysis (clot dissolution).\(^4\) Patients with chronic liver disease frequently have major and multiple alterations in their hemostatic system, including a decreased platelet count and decreased plasma levels of pro- and anti-hemostatic proteins produced by the diseased liver.\(^2\) The rebalanced hemostatic system is more fragile as compared to healthy individuals and may decompensate towards hypo- or hypercoagulability by factors such as renal failure, trauma, infection, and surgery\(^1\). Besides bleeding complications, patients with cirrhosis are also at risk for thrombotic complications and this particular clinical scenario has only recently been fully appreciated.\(^5\) Individuals
with liver disease frequently have abnormalities in routine laboratory tests of coagulation, including prolongations of the prothrombin time (PT), international normalized ratio (INR), and activated partial thromboplastin time (aPTT), along with mild thrombocytopenia, elevated D-dimer, especially when liver synthetic function is more significantly impaired and portal pressures are increased. However, these tests are very poor at predicting the risk of bleeding in individuals with liver disease because they only reflect changes in procoagulants factors.\[^{6}\]

**MATERIAL AND METHOD**

**Study design**
Analytical case control study.

**Study area**
The study was conducted in Khartoum teaching hospital.

**Study population**
Patient with liver diseases.

**Inclusion criteria**
All patients who had confirmed diagnosis as liver disease patients. Both sexes, age between 10-70 years.

**Exclusion criteria**
All patients who were not diagnosed as liver disease patient.

**Sample size**
The samples (cases) were selected by a simple random sampling method (probability sampling). The study sample size was set as fifty (40) patients with liver disease, against ten (15) healthy individual as control.

**Ethics**
The study was approved by the Ethics Committee at the Khartoum teaching Hospital (Sudan).

**Sample and data collection**
Interview was used to obtain all information about age, sex, family history clinical features and treatment using questionnaire as instrument then 5 ml of blood was taken from the
patient, 2.5 ml was collected in tri- sodium citrate anticoagulant container to obtain plasma for PT and APTT testing and other 2.5 ml was collected in EDTA for platelet count.

Data analysis
The collected data was analyzed to obtain the mean, standard deviation and the probability (p-value) between patients and control using SPSS computer program.

METHODOLOGY
Plaletes (PLT) Counts
Sysmex (KX 21N) Haematology analyzer was used for platelets count, this technique was based on the modification of the impedance of calibrated aperture soaking in an electrolyte and going through a constant course delivered by two electrodes located on both sides of the aperture then count sample were counted automatically by PLT counted normal value: (150,000, 450,000 /cumm).[7]

Determination of Prothrombin time (PT) and Activated Partial Thromboplastin Time (APTT)
Coagulometer in various ranges and specifications was used for measuring of Prothrombin and partial thromboplastin measures the clotting time of plasma in the presence of an optimal concentration of tissue extract (thromboplastin) and kaolin (PTTK) and the kaolin cephalin clotting time (KCCT), in presence of calcium chloride (CaCl$_2$) Norma value of PT: (12-16 second) and Norma value of APTT: (20-40 second).

STATISTICAL METHOD
Data were entered and analyzed by SPSS programme. All demographic data of the study population were presented as mean ± SD in the text and Odds Ratio was used for detecting the power of relationship between the determinant and the outcome and 95% confidence interval was calculated.

RESULT
The cases in this study divided into six groups according to the types of liver disease, tow gall choleystitis (5%), five obstructive jaundice (13%), ten liver cirrhosis (25%), fifteen HBV (38%), six HCV (15%) and liver metastasis (5%) (Table 1). The prothrombin time (PT) was tending to be prolonged in liver cirrhosis (21.1), HBV (17.2) and gall cholecystitis (18.3), the normal result was showed in obstructive jaundice (15.5%), HCV(14,7) and liver metastasis
(13.7) but statistical analysis indicate significant differences in these values between patient and control (P.value < 0.05) in liver cirrhosis (P = 0.008), HBV (P = 0.08), gall cholecystitis (P = 0.000) and obstructive jaundice (P = 0.03) but showed no significant (P.value > 0.05) in HCV (P = 0.31) and liver metastasis (p = 0.07). The APTT was tending to be prolonged in HBV (41.8), obstructive jaundice (43.9) and liver metastasis (43.5). The normal result was in liver cirrhosis (36.3), gall cholecystitis (32.0) and HCV (38.4) but statistical analysis showed significant differences in these values between patient and control in HBV (p = 0.002), obstructive jaundice (p =0.000), HCV (p = 0.04) and liver metastasis (p = 0.003) but showed no significant differences in liver cirrhosis (p = 0.3) and gall cholecystitis (p = 0.7). The platelet count found to be lower in liver cirrhosis (112.2), HBV (147.0) and HCV (146.6). The normal result showed in gall cholecystitis (211.0), obstructive jaundice (202.6) and liver metastasis (215.0). But the statistical analysis showed differences values was significant in liver cirrhosis (p =0.000), HBV (p = 0.000) and HCV (p = 0.007). But showed no significant differences in gall cholecystitis (p = 0.5), obstructive jaundice (p = 0.1) and liver metastasis (p = 0.6). PT- INR was indicating abnormal values in liver cirrhosis (2.0), HBV (1.4) and gall cholecystitis (1.4) also. The normal values showed in obstructive jaundice (1.2), HCV (1.3) and liver metastasis (1.0). But the statistical analysis showed significant differences in these values between patient and control in liver cirrhosis (p = 0.01), gall cholecystitis (p = 0.003), obstructive jaundice (p = 0.03) and HCV (p = 0.008) but showed no significant values in HBV (0.1) and liver metastasis (0.7) (Table 2 and 3).

Table 1: shows the frequency of liver diseases among the study population.

<table>
<thead>
<tr>
<th>Liver disease</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>cholecystitis Gall</td>
<td>2</td>
<td>5.0</td>
</tr>
<tr>
<td>Obstructive jaundice</td>
<td>5</td>
<td>12.2</td>
</tr>
<tr>
<td>Liver cirrhosis</td>
<td>10</td>
<td>25.0</td>
</tr>
<tr>
<td>HBV</td>
<td>15</td>
<td>37.5</td>
</tr>
<tr>
<td>HCV</td>
<td>6</td>
<td>15.0</td>
</tr>
<tr>
<td>liver metastasis</td>
<td>2</td>
<td>5.0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>40</strong></td>
<td><strong>100.0</strong></td>
</tr>
</tbody>
</table>
Table 2: shows descriptive statistic of age, first time of diagnosis, and coagulation tests among the study population.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT</td>
<td>40</td>
<td>12</td>
<td>45</td>
<td>17.5</td>
<td>6.6</td>
</tr>
<tr>
<td>PTT</td>
<td>40</td>
<td>26</td>
<td>65</td>
<td>39.8</td>
<td>9.3</td>
</tr>
<tr>
<td>Platelet counts</td>
<td>40</td>
<td>15</td>
<td>280</td>
<td>151.8</td>
<td>68</td>
</tr>
<tr>
<td>Age</td>
<td>40</td>
<td>25</td>
<td>77</td>
<td>49</td>
<td>14.2</td>
</tr>
<tr>
<td>INR</td>
<td>40</td>
<td>0.9</td>
<td>5.5</td>
<td>1.5</td>
<td>0.9</td>
</tr>
<tr>
<td>First diagnosis</td>
<td>40</td>
<td>1</td>
<td>84</td>
<td>14</td>
<td>17.7</td>
</tr>
</tbody>
</table>

Table 3: shows the statistical test of hypothesis (95% of confidence and 0.05 of confidence interval) for patient and control among study population (Student T. tests for independent sample test).

<table>
<thead>
<tr>
<th>Variable</th>
<th>t</th>
<th>Sig. (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT</td>
<td>2.029</td>
<td>0.048</td>
</tr>
<tr>
<td>PTT</td>
<td>2.736</td>
<td>0.008</td>
</tr>
<tr>
<td>Platelet count</td>
<td>-4.037</td>
<td>0.000</td>
</tr>
<tr>
<td>INR</td>
<td>1.853</td>
<td>0.069</td>
</tr>
</tbody>
</table>

**DISCUSSION**

It is well known that chronic liver diseases are characterized by variable haemostatic defects that affect primary haemostasis, fibrinolysis and coagulation. The extent of coagulation abnormalities depends upon the degree of disturbed liver.\(^9\) In our study we found that a significant prolongation in prothrombin time among the patients with liver cirrhosis (p value 0.008), gall cholecystitis (p value 0.00), obstructive jaundice (p value 0.03). The activated partial thromboplastin time among the patients with hepatitis B virus (p value 0.002), obstructive jaundice (p value 0.000), hepatitis C (p value 0.04) and liver metastasis (p value 0.003). Platelet counts among the patients with liver cirrhosis (p value 0.000), hepatitis B (p value 0.000), hepatitis C (p value 0.007). and lastly for INR also there were a higher significant of variation among the patients with liver cirrhosis (p value 0.01), gall cholecystitis (p value 0.003), obstructive jaundice (p value 0.003), and hepatitis C (p value 0.008). Our results were agreed with many studies conducted among patients with liver disease. One of these done by Shah and Trupti among patient with liver disease and concluded that there was a significant alteration (p<0.05) of PT, APTT and Platelet count in patients of liver cirrhosis.\(^10\) Also our result was supported by another study done by Sheikh and Raza among patient with liver disease to evaluate the activity of coagulation.\(^11\) Another study done by Mahmoud among Sudanese patients with liver disease he concluded that there were significant increased in PT and APTT associated with liver diseases and significant
decreased in fibrinogen level among patients of liver disease when compared to normal control.[12] PT is commonly increased in liver diseases because liver is unable to manufacture adequate amount of clotting factors. Factor VII is the rate limiting factor in this pathway and thus has the greatest influence on the PT. As the liver function worsens, the APTT may become abnormal, the reason being that factors X, XI, XII and fibrin stabilizing factors are also produced by the liver.[10] Our result also showed there was sever thrombocytopenia among cases with a higher significant of variation among the controls, supported that thrombocytopenia is a common finding in advanced liver disease. It is predominantly a result of portal hypertension and platelet sequestration in the enlarged spleen, but other mechanisms may contribute. The liver is the site of Thrombopoietin (TPO) synthesis and reduced TPO production further reduces measurable serum platelet counts.[13] INR also was shoed a higher significant of variation among the patients with liver cirrhosis (p value 0.01), gall cholecystitis (p value 0.003), obstructive jaundice (p value 0.003), and hepatitis C (p value 0.008 ), this support that The liver specific causes of an incorrect INR are related to the fact that the ISI has been standardized for patients on warfarin therapy Since patients with liver disease have more complex coagulopathy, the ISI as calibrated for warfarin therapy may not be applicable to liver disease.[14] In Sudan there were many studies conducted about evaluation of coagulation mechanism about liver disease and pregnancy, the affect among patient with liver disease were clearly significant but in pregnancy some studies found significant and other not.[15,16]

CONCLUSION
The study is concluded with the fact that the coagulation profile was significantly altered in liver diseases. There were a considerable variation in haemostatic parameter among the patients with liver diseases even within different clinical groups, in which there were a significant prolongation in prothrombin time among the patients with liver cirrhosis (p value 0.008), gall cholecystitis (p value 0.00), obstructive jaundice (p value 0.03), and the activated partial thromboplastin time among the patients with hepatitis B virus (p value 0.002), obstructive jaundice (p value 0.000), hepatitis C (p value 0.04) and liver metastasis (p value 0.003). and platelet counts among the patients with liver cirrhosis (p value 0.000), hepatitis B (p value 0.000), hepatitis C (p value 0.007). and lastly for INR also there were a higher significant of variation among the patients with liver cirrhosis (p value 0.01), gall cholecystitis (p value 0.003), obstructive jaundice (p value 0.003), and hepatitis C (p value 0.008) (Table 3). Lastly the study concludes that there was a considerable association
between the liver diseases and related disorder with abnormality of different haemostatic mechanism.

ACKNOWLEDGMENTS
I would like to thank all of the patients and normal individuals involved in the study and special thank to the staff of Khartoum teaching hospital.

COMPETING INTERESTS
None of the authors has conflicts of interest.

REFERENCES


