بسم الله الرحمن الرحيم

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Title

Evaluation of Prothrombin Time and Partial Thromboplastin Time in Patients with Liver Diseases in Atbara City

A Thesis Submitted for the Partial Fulfilling of the MSc Degree in Haematology

By

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بسم الله الرحمن الرحيم

قال تعالي :

(يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ)

صدق الله العظيم

سورة المجادلة الآية (11)

Dedication

To dear my mother, who taught me the meaning of life.

To my dear father, who gave me love and respect.

To my dear husband, who support me in all the time.

To my sons, whom bring happiness to my life.

To my brother and sister, whom led me to the way of success.

To my friends and colleagues,

I dedicate this study.

Acknowledgements

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I would like to express endless thanks to my supervisor, Dr. Hamza Ahmed Hassan Mohammed Eltoum for his great efforts and guidance.

Also I would like to thank the staff of El Rebat Hospital specially the staff of laboratory of El Rebat Hospital.

I would like to thank Osama Mahmoud who helped me in the statistical analysis and typing for this research. I would also like to thank all the participants (patients) who gave the allowance to give samples of this research and many others whom I could not mention here, but their direct and indirect supports had already contributed in this study.

Abstract

Background: Liver disease, is a group of diseases that includes: cirrhosis, hepatitis, alcohol intake, fatty liver, liver cancer, jaundice, haemochromatosis.

The aim of the study is to summarize the available data on the impact of coagulation disorder in patient with chronic liver disease.

Methods: This is cross sectional descriptive study to evaluate the PT and APTT in liver disease in Atbara town in the period between (April 2018—September 2018). The study included (40) patients who were diagnosed as liver disease patients and the study groups were compared with (20) healthy volunteers as a control group.

Blood samples were collected from the two groups. PT prolonged, APTT prolonged , were measured. Data were collected using a structured face to face questionnaire and the (SPSS) version (21) program was used for data analysis.

Results: The study revealed that the liver disease patients were; (67.5%) male and (32.5%) female, the mean of age was (30.50 ± 5.762) distributed as (32.5%) have (25-35) years old.

The mean values of PT and APTT were (2.65) and (3.33) respectively.

Conclusions: Liver disease is responsible for significant changes in coagulation test like prothrombin time (PT), activated partial thromoplastin time (APTT).

المستخلص

مدخل: أمراض الكبد هو مجموعة من الامراض تضم التليف، التهاب الكبد الفيروسي، تعاطي الكحول، تشمع الكبد، سرطان الكبد، اليرقان بأنواعه، ترسب الحديد في الكبد،

وتهدف الدراسة الي تقييم وظائف عملية الإرقاء الدموي عند مرضى الكبد.

منهجية الدراسة: أجريت هذه الدراسة المقطعية الحالة-الضابطة التحليلية المتقدمة في مستشفى الرباط بمدينة عطبرة لتحديد مدى تأثير أمراض الكبد علي وظائف الإرقاء الدموي (الفحوصات الابتدائية لعملية الإرقاء الدموي) في الفترة ما بين (إبريل 2018- سبتمبر 2018م). وكانت عينة الدراسة عبارة عن (40) مريض تم اختيارهم بصورة عشوائية. وقورنت نتائج الدراسة مع (20) متطوع سليم كمجموعة ضابطة.

تم جمع عينات الدم من جميع المرضي وتم تحليلها معمليا لإجراء زمن البروثرومبين وزمن الثرومبوبلاستين النشط. تم جمع المعلومات بواسطه الاستبيان ومن ثم استخدام برنامج الحزمة الإحصائية للعلوم الاجتماعية الذي يعرف ببرنامج (SPSS) لتحليل بيانات الدراسة.

النتائج: أظهرت الدراسة أن المرضي (67.5٪) منهم ذكور و (32.5٪) منهم اناث وكان متوسط أعمارهم (30.50 ± 5.762)، (32.5٪) منهم أعمارهم من (25-35) سنة.

متوسط قيم زمن البروثرومبين وزمن الثرومبوبلاستين النشط هو (2.65) و (3.33) على التوالي.

الخلاصة: أمراض الكبد مسئولة عن إحداث تغييرات مهمة في وظائف جهاز الإرقاء الدموي مثل زمن البروثرومبين وزمن الثرومبوبلاستين النشط.

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List of Abbreviations

APTT	Activated Partial Thromboplastin Time
BT	bleeding time
Ca	Calcium
CH	Chronic Hepatitis
CT	clotting time
ELISA	Enzyme Likened Immune Sorbent Assay
HMWK	high-molecular-weight kininogen
INR	international normalized ratio
KCCT	kaolin-cephalin clotting time
MELD	Mayo End-Stage Liver Disease
PIVIKAs	proteins formed in vitamin K absence
PL	Platelet
PPP	Poor Platelet Plasma
PR	prothrombin ratio
PT	Prothrombin Time
TF	Tissue Factor
TFPI	Tissue Factor Pathway Inhibitor
TT	Thrombin Time
UK	United Kingdom
VKORC	Vitamin K epoxide reductase
vWF	Von Willebrand Factor

1. Introduction, Rationale and Objectives

1.1 Introduction

Liver disease is on the increase and is the fifth leading cause of death in the UK. The patients often show symptoms only at advanced stages when it is too late to cure.[1]

Liver diseases can be caused by a multitude of factors, including genetic predisposition, infections and the environment, therefore requiring diverse and targeted treatment options. The increasing incidence of some hepatic conditions worldwide is due to lifestyle actions, including alcohol intake and drug treatments. Concurrently, numerous infections and disorders are challenging to treat, as the causes and progression of a number of illnesses are not yet well understood.[1]

The liver plays a central role in the maintenance of haemostasis. It serves as the site of synthesis of all clotting factors and their inhibitors. Thus liver damage from chronic liver disease can develop multiple coagulation abnormalities that disturb the balance between clotting and fibrinolysis. The causes are multiple: quantitative and qualitative platelet defects; decrease production of coagulation and inhibitor factors; vitamin K deficiency; synthesis of abnormal clotting factors; decreased clearance of activated factors; hyperfibrinolysis and disseminated intravascular coagulation[2]. These coagulation abnormalities can predispose patients from minor localized bleeding to massive life-threatening haemorrhage or thrombosis formation[3].

Coagulation abnormalities in chronic liver disease usually measured through the prolongation of first-line global screening tests such as the prothrombin time (PT) and the activated partial thromboplastin time (aPTT)[4]. The PT consists of the time needed for the platelet-poor plasma to clot after the addition of tissue extracts (thromboplastin) and calcium chloride. Whereas aPTT is the time needed for the platelet-poor plasma to clot

when mixed with a particulate or soluble activator of the contact coagulation factors (factor XII, pre-kallikrein and high-molecular-weight kininogen) and negatively charged phospholipids such as platelet substitutes[5]. PT determines vitamin K dependent extrinsic factors VII, X, II, V and fibrinogen. The aPTT measures the activities of intrinsic and common pathways of coagulation cascade most sensitive to factor VIII, IX, XI, XII and those of the contact system[6]. The clotting factors measured by the common screening test are in the normal range until plasma levels of procoagulants would be reduced below 30 to 40%.

The prolonged PT is related to the severity of liver failure and is one of the parameter used in commonly used prognostic indices of chronic liver disease such as Child-Pugh or Mayo End-Stage Liver Disease (MELD) scores. The PT is considered as a simple, inexpensive, qualitative and accurate prognostic marker of liver impairment and also a predictor of bleeding. The degree of PT impairment an expression of decreased liver synthesis predicts the severity of portal hypertension and the presence of esophageal varices[7]. PT is related both to bleeding risk and mortality. Patients with moderately or severely prolonged PT have 5 to 10 fold higher mortality rates than patients with normal PT[8]. The aPTT is also prolonged in advance chronic liver disease[9].

1.2 Rationale

There are previous studies conclude that there are a strong association between liver disease and coagulation disorder exactly in PT and PTT tests.

There are no study in river Nile state done to confirm that. So that this study attempt to determine the association between PT and PTT among liver disease patients.

1.3 Objectives

1.3.1 General Objective:

To evaluate prothrombin time and partial thromboplastin time in patients with liver diseases in the study population.

1.3.2 Specific Objectives:

1. To evaluate coagulation abnormalities associated with chronic liver diseases.

2. To determine the coagulation abnormalities using various coagulation studies (PT/ APPT).

3. To determine the association between PT & PTT and liver diseases according to gender, age and diagnosis.

2. Literature Review

2.1 Physiology of Liver:

Our liver is a vital organ that performs many functions in our body. The liver is one of the largest organs in our body, and the average healthy human liver weighs about 3 pounds. It is located in the upper right side of our abdomen just under or lower right ribs. If one could look at the human liver from the outside, we would see a larger right side of the liver and a smaller left side. These two sides are anatomically called the right lobe and the left lobe of the liver. These two lobes are separated by a band of connective tissue that anchors the liver to the abdominal cavity. The gallbladder, where the bile manufactured in the liver is stored, is found on the underside of the liver[10].

2.2 The Role of Liver in Coagulation System:

Our liver is also very important with regard to blood clotting. With the help of vitamin K, our liver produces proteins that are essential to allow our blood to properly clot when needed to prevent excess bleeding. Our liver is also one of the organs that will break down and remove old or damaged blood cells.

Liver failure occurs when large parts of the liver become damaged beyond repair and the liver is no longer able to function

Liver failure is a life-threatening condition that demands urgent medical care. Most often, liver failure occurs gradually and over many years. However, a more rare condition known as acute liver failure occurs rapidly (in as little as 48 hours) and can be difficult to detect initially[11].

2.3 Causes of Liver Disease:

The most common causes of chronic liver failure (where the liver fails over months to years) include:

- Hepatitis B.
- Hepatitis C.

- Long-term alcohol consumption.
- Cirrhosis.
- Hemochromatosis (an inherited disorder that causes the body to absorb and store too much iron).
- Malnutrition.
- Alcohol abuse[12].

2.4 Symptoms of liver diseases: include:

- Weakness and fatigue,
- Weight loss,
- Nausea,
- Vomiting, and
- Yellow discoloration of the skin (jaundice).

❖ The treatment of a particular liver disease depends on its specific cause[13].

2.5 Coagulation (**Clotting**):

Is the process by which blood changes from a liquid to a gel, forming a blood clot. It potentially results in hemostasis, the cessation of blood loss from a damaged vessel, followed by repair. The mechanism of coagulation involves activation, adhesion, and aggregation of platelets along with deposition and maturation of fibrin. Disorders of coagulation are disease states which can result in bleeding (hemorrhage or bruising) or obstructive clotting (thrombosis).

Coagulation begins almost instantly after an injury to the blood vessel has damaged the endothelium lining the vessel. Exposure of blood to the subendothelial space initiates two processes: changes in platelets, and the exposure of subendothelial tissue factor to plasma Factor VII, which

ultimately leads to fibrin formation. Platelets immediately form a plug at the site of injury; this is called primary hemostasis. Secondary hemostasis occurs simultaneously: Additional coagulation factors or clotting factors beyond Factor VII (listed below) respond in a complex cascade to form fibrin strands, which strengthen the platelet plug.

Coagulation is highly conserved throughout biology; in all mammals, coagulation involves both a cellular (platelet) and a protein (coagulation factor) component. The system in humans has been the most extensively researched and is the best understood[14].

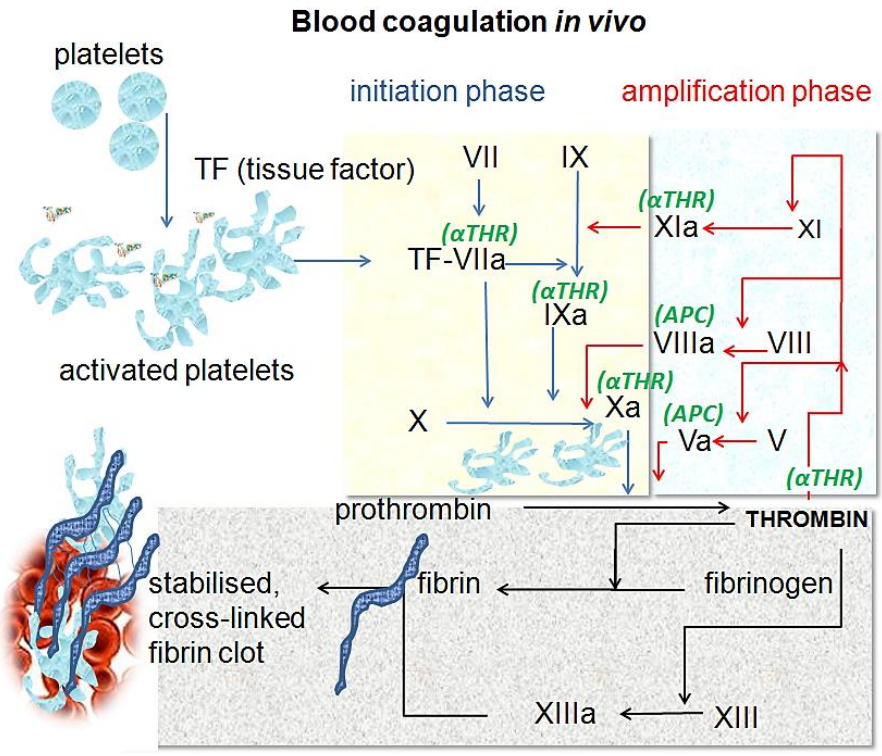


Figure (2.1) Blood coagulation pathways in vivo showing the central role played by thrombin[15]

2.6 Coagulation cascade:

The coagulation cascade of secondary hemostasis has two initial pathways which lead to fibrin formation. These are the contact activation pathway (also known as the intrinsic pathway), and the tissue factor pathway (also known as the extrinsic pathway), which both lead to the same fundamental reactions

that produce fibrin. It was previously thought that the two pathways of coagulation cascade were of equal importance, but it is now known that the primary pathway for the initiation of blood coagulation is the tissue factor (extrinsic) pathway. The pathways are a series of reactions, in which a zymogen (inactive enzyme precursor) of a serine protease and its glycoprotein co-factor are activated to become active components that then catalyze the next reaction in the cascade, ultimately resulting in cross-linked fibrin. Coagulation factors are generally indicated by Roman numerals, with a lowercase a appended to indicate an active form[16].

2.7 The classical blood coagulation pathway:

The coagulation factors are generally serine proteases (enzymes), which act by cleaving downstream proteins. The exceptions are tissue factor, FV, FVIII, FXIII. Tissue factor, FV and FVIII are glycoproteins, and Factor XIII is a transglutaminase. The coagulation factors circulate as inactive zymogens. The coagulation cascade is therefore classically divided into three pathways. The tissue factor and contact activation pathways both activate the "final common pathway" of factor X, thrombin and fibrin[17].

2.7.1 Tissue factor pathway (extrinsic):

The main role of the tissue factor pathway is to generate a "thrombin burst", a process by which thrombin, the most important constituent of the coagulation cascade in terms of its feedback activation roles, is released very rapidly. FVIIa circulates in a higher amount than any other activated coagulation factor. The process includes the following steps:

1. Following damage to the blood vessel, FVII leaves the circulation and comes into contact with tissue factor (TF) expressed on tissue-factor-bearing cells (stromal fibroblasts and leukocytes), forming an activated complex (TF-FVIIa).

2. TF-FVIIa activates FIX and FX.

3. FVII is itself activated by thrombin, FXIa, FXII and FXa.

4. The activation of FX (to form FXa) by TF-FVIIa is almost immediately inhibited by tissue factor pathway inhibitor (TFPI).

5. FXa and its co-factor FVa form the prothrombinase complex, which activates prothrombin to thrombin.

6. Thrombin then activates other components of the coagulation cascade, including FV and FVIII (which forms a complex with FIX), and activates and releases FVIII from being bound to vWF.

7. FVIIIa is the co-factor of FIXa, and together they form the "tenase" complex, which activates FX; and so the cycle continues. ("Tenase" is a contraction of "ten" and the suffix "-ase" used for enzymes)[17].

2.7.2 Contact activation pathway (intrinsic):

The contact activation pathway begins with formation of the primary complex on collagen by high-molecular-weight kininogen (HMWK),

prekallikrein, and FXII (Hageman factor). Prekallikrein is converted to kallikrein and FXII becomes FXIIa. FXIIa converts FXI into FXIa. Factor XIa activates FIX, which with its co-factor FVIIIa form the tenase complex, which activates FX to FXa. The minor role that the contact activation pathway has in initiating clot formation can be illustrated by the fact that patients with severe deficiencies of FXII, HMWK, and prekallikrein do not have a bleeding disorder. Instead, contact activation system seems to be more involved in inflammation, and innate immunity. Despite this, interference with the pathway may confer protection against thrombosis without a significant bleeding risk[18].

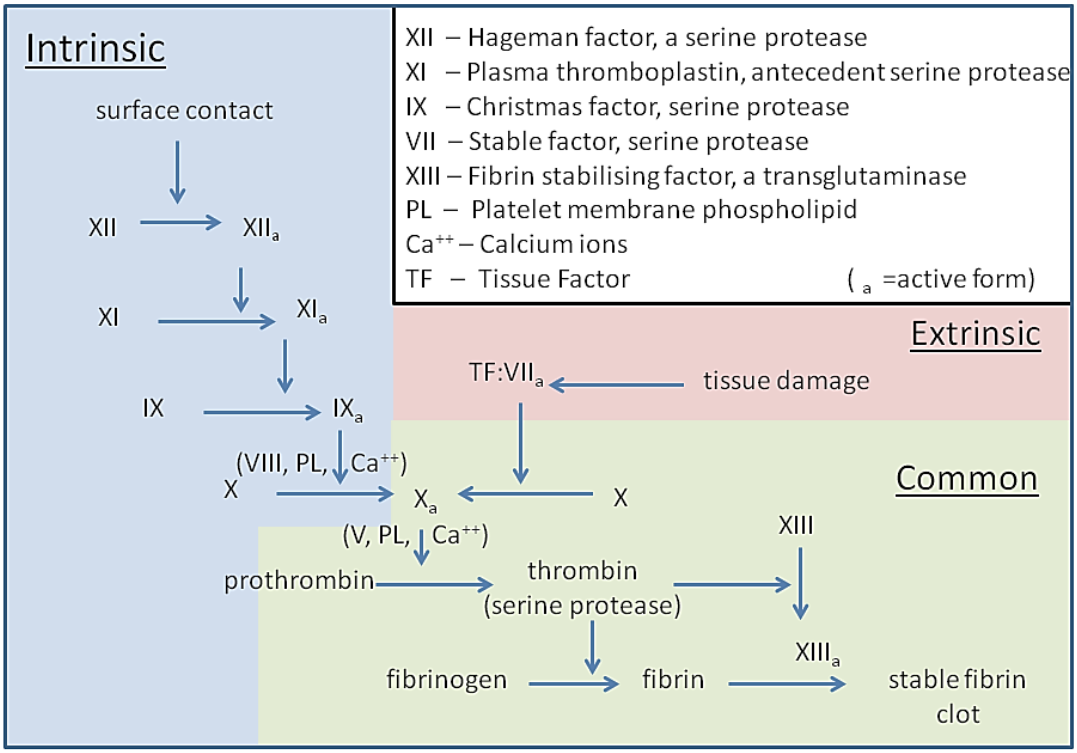
2.7.3 Final common pathway:

The division of coagulation in two pathways is mainly artificial, it originates from laboratory tests in which clotting times were measured after the clotting

was initiated by glass (intrinsic pathway) or by thromboplastin (a mix of tissue factor and phospholipids). In fact thrombin is present from the very beginning, already when platelets are making the plug. Thrombin has a large array of functions, not only the conversion of fibrinogen to fibrin, the building block of a hemostatic plug. In addition, it is the most important platelet activator and on top of that it activates Factors VIII and V and their inhibitor protein C (in the presence of thrombomodulin), and it activates Factor XIII, which forms covalent bonds that crosslink the fibrin polymers that form from activated monomers.

Following activation by the contact factor or tissue factor pathways, the coagulation cascade is maintained in a prothrombotic state by the continued activation of FVIII and FIX to form the tenase complex, until it is down-regulated by the anticoagulant pathways[18].

The three pathways that makeup the classical blood coagulation pathway



(15)

2.7.4 Cofactors:

Various substances are required for the proper functioning of the coagulation cascade:

- **Calcium and phospholipid:**

Calcium and phospholipid (a platelet membrane constituent) are required for the tenase and prothrombinase complexes to function. Calcium mediates the binding of the complexes via the terminal gamma-carboxy residues on FXa and FIXa to the phospholipid surfaces expressed by platelets, as well as procoagulant microparticles or microvesicles shed from them. Calcium is also required at other points in the coagulation cascade[18].

- **Vitamin K:**

Vitamin K is an essential factor to a hepatic gamma-glutamyl carboxylase that adds a carboxyl group to glutamic acid residues on factors II, VII, IX and X, as well as Protein S, Protein C and Protein Z. In adding the gamma-carboxyl group to glutamate residues on the immature clotting factors Vitamin K is itself oxidized. Another enzyme, Vitamin K epoxide reductase, (VKORC) reduces vitamin K back to its active form. Vitamin K epoxide reductase is pharmacologically important as a target of anticoagulant drugs warfarin and related coumarins such as acenocoumarol, phenprocoumon, and dicumarol. These drugs create a deficiency of reduced vitamin K by blocking VKORC, thereby inhibiting maturation of clotting factors. Vitamin K deficiency from other causes (e.g., in malabsorption) or impaired vitamin K metabolism in disease (e.g., in liver failure) lead to the formation of PIVKAs (proteins formed in vitamin K absence) which are partially or totally non-gamma carboxylated, affecting the coagulation factors' ability to bind to phospholipid[18].

2.8 Prothrombin Time:

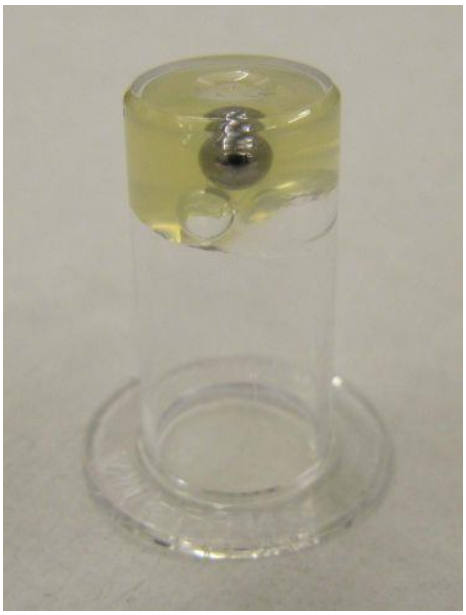


Figure (2.2) Blood plasma after the addition of tissue factor[15]

The prothrombin time (PT)—along with its derived measures of prothrombin ratio (PR) and international normalized ratio(INR)—are assays evaluating the extrinsic pathway of coagulation. This test is also called "ProTime INR" and "PT/INR". They are used to determine the clotting tendency of blood, in the measure of warfarin dosage, liver damage, and vitamin K status. PT measures factors I (Fibrinogen), II (Prothrombin), V (Proaccelerin), VII (Proconvertin), and X (Stuart–Prower Factor). It is used in conjunction with the activated partial thromboplastin time (aPTT) which measures the intrinsic pathway and common pathway[19].

2.8.1 Laboratory measurement:

The reference range for prothrombin time depends on the analytical method used, but is usually around 12–13 seconds (results should always be interpreted using the reference range from the laboratory that performed the test), and the INR in absence of anticoagulation therapy is 0.8–1.2. The target range for INR in anticoagulant use (e.g. warfarin) is 2 to 3. In some cases, if

more intense anticoagulation is thought to be required, the target range may be as high as 2.5–3.5 depending on the indication for anticoagulation[19].

2.8.2 Methodology:

Figure (2.3) Blue Top Vacutainer tube used for PT and APTT blood tests[15]

Prothrombin time is typically analyzed by a laboratory technologist on an automated instrument at 37 °C (as a nominal approximation of normal human body temperature).

- Blood is drawn into a test tube containing liquid sodium citrate, which acts as an anticoagulant by binding the calcium in a sample. The blood is mixed, then centrifuged to separate blood cells from plasma (as prothrombin time is most commonly measured using blood plasma). In newborns, a capillary whole blood specimen is used.
- A sample of the plasma is extracted from the test tube and placed into a measuring test tube (Note: for an accurate measurement, the ratio of blood to citrate needs to be fixed and should be labeled on the side of the

measuring test tube by the manufacturing company; many laboratories will not perform the assay if the tube is underfilled and contains a relatively high concentration of citrate—the standardized dilution of 1 part anticoagulant to 9 parts whole blood is no longer valid).

- Next an excess of calcium (in a phospholipid suspension) is added to the test tube, thereby reversing the effects of citrate and enabling the blood to clot again.
- Finally, in order to activate the extrinsic / tissue factor clotting cascade pathway, tissue factor (also known as factor III) is added and the time the sample takes to clot is measured optically. Some laboratories use a mechanical measurement, which eliminates interferences from lipemic and icteric samples. The prothrombin ratio (aka international normalized ratio) is the prothrombin time for a patient sample divided by the result for control plasma[20].

2.9 Partial Thromboplastin Time:

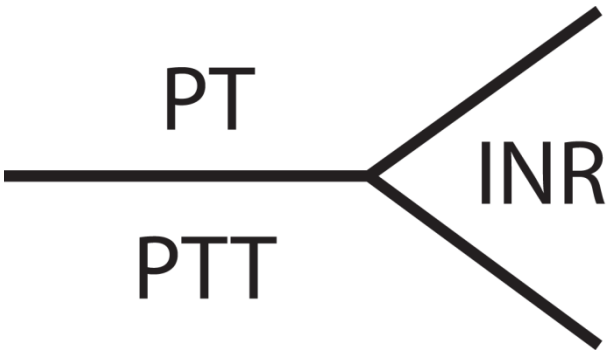


Figure (2.4) Common notation of coagulation times in medical records[15]

The partial thromboplastin time (PTT) or activated partial thromboplastin time (aPTT or APTT) is a medical test that characterizes blood coagulation. A historical name for this measure is the kaolin-cephalin clotting time (KCCT), reflecting kaolinand cephalin as materials historically used in the test. Apart from detecting abnormalities in blood clotting, partial

thromboplastin time is also used to monitor the treatment effects with heparin, a widely prescribed drug that reduces blood's tendency to clot.

Partial thromboplastin time (APTT) measures the overall speed at which blood clots by means of two consecutive series of biochemical reactions known as the "intrinsic" (now referred to as the contact activation pathway) and common coagulation pathways.

The partial thromboplastin time (APTT) is used in conjunction with another measure of how quickly blood clotting takes place called the prothrombin time (PT). The prothrombin time measures the speed of clotting by means of the extrinsic pathway (also known as the tissue factor pathway)[21].

2.9.1 Methodology:

Figure (2.5) Blue Top Vacutainer tube used for PT and APTT blood tests[15]

Partial thromboplastin time is typically analyzed by a medical technologist or a laboratory technician on an automated instrument at 37 °C (as a nominal approximation of normal human body temperature). The test is termed "partial" due to the absence of tissue factor from the reaction mixture.

- Blood is drawn into a test tube containing oxalate or citrate, molecules which act as an anticoagulant by binding the calcium in a sample. The blood is mixed, then centrifuged to separate blood cells from plasma (as partial thromboplastin time is most commonly measured using blood plasma).
- A sample of the plasma is extracted from the test tube and placed into a measuring test tube.
- Next, an excess of calcium (in a phospholipid suspension) is mixed into the plasma sample (to reverse the anticoagulant effect of the oxalate enabling the blood to clot again).
- Finally, in order to activate the intrinsic pathway of coagulation, an activator (such as silica, celite, kaolin, ellagic acid) is added, and the time the sample takes to clot is measured optically. Some laboratories use a mechanical measurement, which eliminates interferences from lipemic and icteric samples[22].

2.9.2 Interpretation:

The typical reference range is between 30 seconds and 50 s (depending on laboratory). Shortening of the PTT is considered to have little clinical relevance, but some research indicates that it might increase risk of thromboembolism. Normal APTT times require the presence of the following coagulation factors: I, II, V, VIII, IX, X, XI and XII. Notably, deficiencies in factors VII or XIII will not be detected with the PTT test[23].

❖ **Prolonged APTT may indicate:**

- use of heparin (or contamination of the sample).
- antiphospholipid antibody (especially lupus anticoagulant, which paradoxically increases propensity to thrombosis).
- coagulation factor deficiency (e.g., hemophilia).
- sepsis — coagulation factor consumption.
- presence of antibodies against coagulation factors (factor inhibitors).

To distinguish the above causes, mixing tests are performed, in which the patient's plasma is mixed (initially at a 50:50 dilution) with normal plasma. If the abnormality does not disappear, the sample is said to contain an "inhibitor" (either heparin, antiphospholipid antibodies or coagulation factor specific inhibitors), while if it does disappear a factor deficiency is more likely. Deficiencies of factors VIII, IX, XI and XII and rarely von Willebrand factor (if causing a low factor VIII level) may lead to a prolonged aPTT correcting on mixing studies[23].

2.9.3 History:

The aPTT was first described in 1953 by researchers at the University of North Carolina at Chapel Hill explaining the Carolina blue Vacutainer tube top color[24].

2.10 Previous Studies:

2.10.1 Study One:

Relevance of clotting test in liver disease, Thachil J.[25], 2008, UK.

Liver disease is associated with impairment of the haemostatic function due to the abnormal and decreased synthesis of the clotting factors. It is thus only logical to have considered assessment of the clotting profile (to include prothrombin time (PT) and activated partial thromboplastin time (aPTT)) to

be an integral part of the comprehensive assessment of a patient who presents with liver impairment. Laboratory abnormalities of coagulation are considered to be a predictive risk factor for bleeding.

2.10.2 Study Two:

Coagulation profile in liver disease, Shah Shaila N., Trrupti Jansari[26], India.

Objective : To study the alteration in coagulation profile in various liver diseases which helps to evaluate the risk of bleeding in patients with liver disease and to study the association of coagulation abnormality with the extent of liver disease.

Conclusion : There are more chances of bleeding when coagulation parameters are altered in cases of cirrhosis but more studies are required in this field to evaluate the thrombotic events seen in patients with liver diseases.

2.10.3 Study Three:

Coagulation Profile in Liver Disease, Gautam Bhatia et al.[27], Tertiary Care Hospital in Uttarakhand, India.

Objective: The objective of this study was to evaluate coagulation abnormalities associated with chronic liver diseases and determine the coagulation abnormalities using various coagulation studies prothrombin time (PT), activated partial thromboplastin time (APTT).

Results: Out of the 300 patients, 156 were diagnosed with cirrhosis, 75 were of viral hepatitis, and 69 were of other liver diseases. About 62% (186/300) had prolonged PT. About 39.3% (118/300) had prolonged APTT. The BT was prolonged in 34% (102/300).

Conclusion: We concluded that various abnormalities of coagulation tests vary greatly with different liver disorders, duration of the disorders, and their severity. Prolongation of PT and APTT in advancing liver cirrhosis indicates damage to the liver parenchyma resulting in decreased production of

coagulation proteins with increased risk of bleeding tendencies, which can be detected before these ensue.

2.10.4 Study Four:

Haemostatic Profile of Patients with Chronic Liver Disease-its Correlation with Severity and Outcome, Varnika Rai, et al. , August 2017, India[28].

Results:

In cirrhosis group PT, aPTT, were significantly increased compared to Chronic Hepatitis (CH) and control group ($p<0.001$ for all comparisons).

2.10.5 Study Five:

Assessment of coagulation parameters in liver cirrhosis, Shaikh Saeed, et al., Sudan, 2014[29].

This study was carried out to assess the haemostatic defects in patients of liver cirrhosis by estimating prothrombin time (PT), activated partial thromboplastin time (APTT). It was carried out at the Department of Pathology, King Edward Medical College, Lahore. A total of 50 patients from all age groups of both gender with cirrhosis of liver were selected from Mayo Hospital, Lahore. All the investigations were carried out by standard procedures. Results were analyzed statistically with appropriate tests of significance. The mean values of PT and APTT were 14 second and 19 seconds longer than the control values respectively. These prolongations were highly significant statistically ($p<0.0001$). Prolongation of PT and APTT indicates plasma clotting factors deficiency due to impaired hepatic synthesis. Liver cirrhosis causes significant morbidity and mortality in our country, however early diagnosis prevents complications and carries good prognosis 2 .

3. Material and Methods

3.1 Study Design

This is a case control descriptive study to evaluate the PT and APTT (Coagulation test) in Atbara town during a period of (April 2018—September 2018).

3.2 Study area:

The study was conducted at Elribat Hospital which located in Atbara town in Sudan. Atbara (sometimes Atbarah) is a city of 111,399 (2007) located in River Nile State in northeastern Sudan. It is located at the junction of the Nile and Atbara rivers. It is an important railway junction and railroad manufacturing centre, and most employment in Atbara is related to the rail lines. It is known as the "Railway City,' and The Sudanese National Railway Company's headquarters are located in Atbara.

3.3 Study population and Sample size:

A total of (40) samples collected of Study group of liver disease patients and (20) samples collected of healthy individuals as control group.

3.4 Inclusion criteria:

Primary criterion of inclusion was presence of liver disease including cirrhosis, hepatitis and all other liver disease. All patients of all sex age ranging from (18-70) years and irrespective of socioeconomic status, were included.

3.5 Exclusion Criteria:

Patients with previous history of coagulation disorder or who took any of the following drugs in the previous week were excluded: (aspirin, non-steroidal anti-inflammatory drugs, antihistaminics, penicillin, thiazides, sulfonamides and anticoagulants).

3.6 Data Collection Tool:

Data were collected using face to face questionnaire which specifically designed to obtain information that helped in study.

3.7 Blood Sampling:

Venous blood collected using sterile disposable plastic syringe after cleaning the venipuncture area with (70%) ethanol, and added vacationer containing 3.2% sodium citrate as anticoagulant. While taking the sample, tourniquet was not tied, as it can change the hemoconcentration and result may vary. The raio of volume of blood to anticoagulant 9:1. Plasma was obtained following the centrifugation of the anticoagulant blood at 300 rpm for (10-15) minutes.

3.8 Methods:

3.8.1 PT was done by using coagulation analyzer (URIT-600):

3.8.1.1 Principle of PT:

Tissue Thromboplastin in the presence of calcium activates the extrinsic pathway of human blood coagulation mechanism. When BioMed-LIQUIPLASTIN regent is added to normal citrated plasma, the clotting mechanism is initiated, forming a solid gel clot within a specific period of time. The time required for clot formation would be prolonged if there is acquired or congenital deficiency of factors/ factor activity in the extrinsic pathway of the coagulation mechanism or reduction in the activity of Vitamin K dependent clotting factors during oral anticoagulant therapy.

The arrest of bleeding depends upon primary platelet plug formed along with the formation of stable fibrin clot. Formation of this involves the sequential interaction of series of plasma proteins in a highly ordered and complex manner and also the interaction of these complex with blood platelets and materials from the tissues.

Tissue Thromboplastin, in the presence of calcium, as an activator , which initiates the extrinsic pathway of coagulation factors VII, X, V, Prothrombin and Fibrinogen.

During oral anticoagulant therapy most of the Vitamin K dependent factors such as II, VII, IX, X, Protein S are depressed, also during the deficiencies of clotting factor activity which may be hereditary or acquired.

Prothrombin Time determination is the preferred method for presurgical screening, as a liver function test, determination of congenital deficiency of factors II, V, VII and X and for monitoring of patients on oral anticoagulant therapy.

3.8.1.2 PT Manual method (by using BIOMED regent):

Patients sample should be tested in parallel with pooled fresh normal plasma (FNP) and suitable controls.

1. Brings the reagent vial to room temperature (20-30^{o}C). Mix the contents of the vial to homogenise the suspension completely.
2. Aspirate from the reagent vial enough reagents for immediate requirements in a thoroughly clean and dry test tube.. (Plastic test tubes are preferred).
3. Pre warm the reagent and bring to 37^{o}C before use in test procedure (5-10 minutes may be required depending on the regent volume to attain 37^{o}C before testing).
4. Recap the regent vial and replace immediately to 2-8^{o}C.
5. To a 12 × 75mm tube add 0.1ml of plasma (PPP) and place the tube in a water bath for 3 to 5 minutes at 37^{o}C.
6. To the tube forcibly add 0.2ml of BioMed-LIQUIPLASTIN regent (pre-warmed at 37^{o}C for at least 10 Minutes) and simulatenously start a stopwatch. Shake the tube gently to mix contents.
7. Gently tilte the tube back and forth and stop the stopwatch as soon as the first fibrin strand is visible and the Gel/ clot formation begins record the time in seconds.
8. Repeat steps from 4 to 6 for a duplicate test on the same sample.
9. Find the average of the duplicate test values. This is the Prothrombin time (PT).

3.8.2 APTT was done by using coagulation analyzer (URIT-600):

3.8.2.1 APTT Principle:

Cephaloplastin activates the coagulation factor of the intrinsic pathway of the coagulation mechanism in the presence of calcium ions.

APTT is prorolonged by a deficiency of one or more of these clotting factors of the intrinsic pathway and in the presence of coagulation inhibitors like heparin.

3.8.2.2 APTT Manual method (by using BIOMED regent):

1. Pre-incubate the Calcium Cloride Regent to 37^{o}C for at least 10 minutes. Pipette 100µl of test or control plasma into a test cuvette.
2. Incubate the plasma at 37^{o}C for 1 to 2 minutes.
3. Pipette 100µl of the APTT reagent, into reagent cuvette containing the plasma. Maintain the suspension of the APTT reagent by magnetic stirring or mixing by inversion immediately prior to use.
4. Incubate at 37^{o}C for 3 minutes.
5. Add 100µl preincubated Calcium Chloride solution and simultaneously start the timer.
6. Record the clotting time in seconds.

3.9 Ethical Consideration:

Permission was obtained from the Ministry of Health – River Nile State. Confidentiality was maintained during the process of the study by ensuring face to face and direct interviews by each interviewer without a third party and information obtained during the study was kept under confidential.

3.10. Data analysis:

The collected data proceed for analysis using SPSS version 21 (mean, standard deviation, standard error mean, P.value by using independent T.test).

4. Results

4.1 Demographic and clinical data:

A total of (40) blood sample collected from liver disease patients and (20) samples collected as control from healthy individuals include frequency of sex was 27 males (67.5%) and 13 females (32.5%), frequency of age groups 25-35 years 13(32.5%). Frequency of residence Atbara locality 37 (92.5%) in the study group.

The average age of patients with liver disease in the study was (30.50 ± 5.762), with a range of (25-35) years.

Furthermore, the majority of patients, 27(67.5%) from male, while the remaining 13(32.5%) from female.

According to residence, most of them from Atbara locality 37(92.5%), while the remaining 3(7.5%) from Eldamer locality.

Table (4.1).

Table (4.1): Distribution of study population according to age, sex, and residence:

Characteristic		Frequency	Percent %
Study groups	Case	40	66.7%
	Control	20	33.3%
Age/yrs	< 25 years	7	17.5%
	25 - 35 years	13	32.5%
	36 - 45 years	10	25.0%
	46 - 55 years	3	7.5%
	> 55 years	7	17.5%
Gender	Male	27	67.5%
	Female	13	32.5%
Residence	Atbara Locality	37	92.5%
	Eldamer Locality	3	7.5%

Table (4.2): Distribution of study population according to age group:

Age group	Frequency	Percent
< 25 years	7	17.5%
25 - 35 years	13	32.5%
36 - 45 years	10	25.0%
46 - 55 years	3	7.5%
> 55 years	7	17.5%
Total	40	100.0%

Table (4.3): Distribution of study population according to gender:

Gender	Frequency	Percent
Male	27	67.5%
Female	13	32.5%
Total	40	100.0%

Table (4.4): Distribution of study population according to Residence:

Residence	Frequency	Percent
Atbara Locality	37	92.5%
Eldamer Locality	3	7.5%
Total	40	100.0%

Participation to diagnosis to liver disease reflected that; 15 (37.5%) were Hepatitis patients, while 25 (62.5%) were not. On the other hand, 15 (37.5%) were Cirrhosis patients, while the remaining 25 (62.5%) were not.

Furthermore, 5 (12.5%) of the patients were Alcoholism, while 35 (87.5%) of them were not. Concerning Jaundice, 5 (12.5%) of them were Jaundice patients, while 35(87.5) were not. table (4.5).

Table (4.5): Distribution of study population according to diagnosis:

Characteristic		Frequency	Percent %
Hepatitis	Yes	15	37.5%
	No	25	62.5%
Cirrhosis	Yes	15	37.5%
	No	25	62.5%
Alcoholism	Yes	5	12.5%
	No	35	87.5%
Jaundice	Yes	5	12.5%
	No	35	87.5%

According to patients who takes permanent treatment, we found that all of them 40(100%) not take any permanent treatment. table (4.6).

Table (4.6): Distribution of study population according to taking permanent treatments:

Characteristic		Frequency	Percent %
Taking permanent treatments	Yes	0	0%
	No	40	100%

Concerning coagulation disease, we found that all patients 40(100%) not suffer from coagulation disease. table (4.7).

Table (4.7): Distribution of study population according to suffering from coagulation disease:

Characteristic		Frequency	Percent %
Coagulation disease	Yes	0	0%
	No	40	100%

Laboratory Data:

The mean values of PT and APTT in case group were (2.65) and (3.33) respectively and in control group the mean values of PT and APTT were (1.00) and (2.00) respectively. Table (4.8).

Table (4.8): Comparison between case and control in PT and APTT:

Group		Number	Mean	SD	P.value
PT	case	40	2.65	1.610	0.000
	control	20	1.00	0.000	
APTT	case	40	3.33	1.328	0.000
	control	20	1.00	0.000	

Table (4.9): Comparison between PT and age group:

		Age					Total	P. Value
		< 25 years	25 - 35 years	36 - 45 years	46 - 55 years	> 55 years		
PT	10 - 15	4	6	3	1	4	18	0.004
	21 - 25	0	1	2	1	2	6	
	26 - 30	1	5	3	1	0	10	
	> 30	2	1	2	0	1	6	
Total		7	13	10	3	7	40	

Table (4.10): Comparison between PT and gender:

		Gender		Total	P. Value
		Male	Female		
PT	10 - 15	13	5	18	0.01
	21 - 25	4	2	6	
	26 - 30	6	4	10	
	> 30	4	2	6	
Total		27	13	40	

Table (4.11): Comparison between PT and Diagnosis:

		Diagnosis				Total	P. Value
		Hepatitis	Cirrhosis	Alcoholism	Jaundice		
PT	10 - 15	8	3	2	5	18	0.079
	21 - 25	0	3	3	0	6	
	26 - 30	6	4	0	0	10	
	> 30	1	5	0	0	6	
Total		15	15	5	5	40	

Table (4.12): Comparison between APTT and age group:

		Age					Total
		< 25 years	25 - 35 years	36 - 45 years	46 - 55 years	> 55 years	
PTT	28 - 40	4	6	3	1	4	18
	41 - 50	0	2	1	0	0	3
	51 - 60	1	2	1	1	2	7
	> 60	2	3	5	1	1	12
Total		7	13	10	3	7	40

P.value = 0.0041

Table (4.13): Comparison between APTT and gender:

		Gender		Total
		Male	Female	
PTT	28 - 40	13	5	18
	41 - 50	2	1	3
	51 - 60	7	0	7
	> 60	5	7	12
Total		27	13	40

P.value = 0.0066

Table (4.14): Comparison between APTT and Residence:

		Residence		Total
		Atbara Locality	Eldamer Locality	
PTT	28 - 40	16	2	18
	41 - 50	3	0	3
	51 - 60	6	1	7
	> 60	12	0	12
Total		37	3	40

P.value = 0.00568

Table (4.15): Comparison between APTT and Diagnosis:

		Diagnosis				Total
		Hepatitis	Cirrhosis	Alcoholism	Jaundice	
PTT	28 - 40	8	3	2	5	18
	41 - 50	0	3	0	0	3
	51 - 60	3	1	3	0	7
	> 60	4	8	0	0	12
Total		15	15	5	5	40

P.value = 0.005

5. Discussion, conclusion and Recommendations

5.1. Discussion:

Liver disease, is a group of diseases that includes liver cirrhosis, hepatitis, alcoholism, jaundice and liver cancer.

The patients' age range from (18-70) years old. The maximum patients were in the age group ranging from (25-35) years. Thus, all the patients above 18 years. The present study age group is similar to that of Shaikh Saeed, et al., Sudan, 2014 include all age groups of both gender with cirrhosis of liver[28].

All liver disease patients diagnostic by liver test such as liver enzymes, bilirubin, for hepatitis patients conformity by (ELISA).

The results of this study denoted that the liver disease patient were in high risk to coagulopathy disorder.

The results of this study obtained demonstrated that there was significant in PT and APTT compared to control. (P value < 0.05).

Results of this current study are similar to study done by Shah Shaila N., Trrupti Jansari in India whom revealed that: high PT and APTT were risk factors for liver disease[25].

Finding of the parameters examined, reflected an increase in the mean of PT and APTT compared to control group and there was strong significant statistical value depicted among study population; (P.value 0.000). This result agreed with the study conducted by Shah Shaila N., Trrupti Jansari, that showed a significant association between PT and APTT liver disease[25].

5.2. Conclusion:

- Prothrombin time test (PT) was prolonged in liver disease patient when compared to healthly indiviuals in the control group.
- Activated partial thromboplastin test (APTT) was prolonged in liver disease patient when compared to healthly indiviuals in the control group.

5.3. Recommendations:

1-Coagualtion tests should be checked regularly in liver disease patients.

2-More investigations should be done for liver disease patients, to determine which risk factors, bleeding and thrombotic are important predictors among liver disease patients.

3- More studies should be done for liver disease patients to help them preventing complications of this disease.

References:

1. https://www.biomedcentral.com/collections/liverdisease.

2. Amitrano L, Guardascione MA, Brancaccio V, Balzano A. Coagulation disorders in liver disease. Semin Liver Dis 2002; 22: 83-96.

3. Peck Radosavljevic M. Review article: coagulation disorders in chronic liver disease. Aliment Pharmacol Ther 2007; 26 Suppl 1: 21-8.

4. Rverter JC. Abnormal hemostasis tests and bleeding in chronic liver disease: are they related? Yes. J Thromb heamost 2006; 4: 717-20.

5. Tripodi A. Tests of coagulation in liver disease. Clin Liver Dis 2009; 13: 55-61.

6. Thachil J. Relevance of clotting tests in liver disease. Postgrad Med J 2008; 84: 177-81.

7. Pugh RN, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. Br J Surg 1973; 60: 649-9.

8. Garrison RN, Cryer HM, Howard DA, Polk HC. Clarification of risk factor for abdominal operations in patients with hepatic cirrhosis. Ann Surg 1984; 199: 648-55.

9. Hedner U, Erhardtsen E. Hemostatic disorders in liver disease. In: Schiff ER, Sorrell MF, Maddrey WC, editors. Diseases of the liver. Philadelphia: Lippincott Williams and Wilkins; 2003; pp 625-35.

10. *David Lillicrap; Nigel Key; Michael Makris; Denise O'Shaughnessy . Practical Hemostasis and Thrombosis. Wiley-Blackwell (2009). pp. 1–5.* ISBN 1-4051-8460-4.

11. *Furie B, Furie BC.* "Thrombus formation in vivo". *J. Clin. Invest (2005). 115 (12): 3355–62.* doi*:* 10.1172/ JCI26987. PMC 1297262 .

PMID 16322780.

12. *Alan D. Michelson.* Platelets. *Academic Press (26 October 2006). pp. 3–5.* ISBN 978-0-12-369367-9. Retrieved 18 October 2012.

13. *Schmaier, Alvin H.; Lazarus, Hillard M. Concise guide to hematology. Chichester, West Sussex, UK: Wiley-Blackwell (2011). p. 91.* ISBN 978-1-4051-9666-6.

14. *Pallister CJ, Watson MS. Haematology. Scion Publishing (2010). pp. 336–347.* ISBN 1-904842-39-9.

15. Shaikh Saeed, et al., Assessment of coagulation parameters in liver cirrhosis Sudan, 2014.

16. "COAGULATION FACTOR". *Clotbase.bicnirrh.res.in*. Retrieved 20 May 2018.

17. *Hoffbrand, A. V.. Essential haematology. Oxford: Blackwell Science (2002). pp. 241–243.* ISBN 0-632-05153-1.

18. *Long, Andrew T.; Kenne, Ellinor; Jung, Roman; Fuchs, Tobias A.; Renné, Thomas. "Contact system revisited: An interface between inflammation, coagulation, and innate immunity". Journal of Thrombosis and Haemostasis (2015). 14: 427–437.* doi*:*10.1111/jth.13235.

19. "Warfarin Therapy Management in Adults" *(PDF).*

20. Fritsma, George A. "Evaluation of Hemostasis." Hematology: Clinical Principles and Applications . Ed. Bernadette Rodak. W.B. Saunders Company: Philadelphia, 2002. 719-53.

21. "KCCT - General Practice Notebook". *GP Notebook. Oxbridge Solutions Ltd*. Retrieved 2010-06-08.

22. "Medline Plus Medical Encyclopedia: Partial thromboplastin time (PTT)". Retrieved 2009-01-01.

23. *Korte, Wolfgang; Clarke, Susan; Lefkowitz, Jerry B. "Short activated partial thromboplastin times are related to increased thrombin generation and an increased risk for thromboembolism". American journal of clinical pathology (January 2000). 113 (1): 123–7.* doi:10.1309/G98J-ANA9-RMNC-XLYU. PMID 10631865.

24. *Langdell RD, Wagner RH, Brinkhous KM. "Effect of antihemophilic factor on one-stage clotting tests; a presumptive test for hemophilia and a simple one-stage antihemophilic factor assy procedure". J. Lab. Clin. Med. (1953) 41 (4): 637–47.* PMID 13045017.

25. Thachil J., Relevance of clotting test in liver disease, University of Liverpool, UK, 2008.

26. Shah Shaila N, Trupi Jansari, Coagualtion profile in liver disease-a study of 100 cases, Gujarat Medical Journal, March 2014, Vol. 69, No. 1.

27. Gautam Bhatia et al, Coagualtion Profile in Liver Disease, Tertiary Care Hospital, Uttarakhand, India, 2017, 2(2): 61-64.

28. Varnika Rai, et al. , Haemostatic Profile of Patients with Chronic Liver Disease-its Correlation with Severity and Outcome, August 2017, India.

29. , Shaikh Saeed, et al., Assessment of coagulation parameters in liver cirrhosis Sudan, 2014.

Appendix 1

الاستبيان

الاسم : .....

العمر : .....

النوع : ذكر ☐ أنثى ☐

السكن : .....

التشخيص : .....

التهاب كبد فيروسي ☐

تليف ☐

كحوليين ☐

أخرى ☐

هل تتناول أي علاجات مستديمة ؟

نعم ☐ لا ☐

إن وجدت أذكرها : .....

هل تعاني من أمراض سيولة الدم :

نعم ☐ لا ☐

إن وجدت أذكرها : .....

النتائج :

.....

.....

Appendix II

إقـــرار بالموافقـــة

الاسم :----------

العمر :---------- العنوان :----------

أوافق بمحض إرادتي بالمشاركة في البحث العلمي المتعلق بدراسة زمن البروثرومبين والثرومبوبلاستين لدى مرضى الكبد في مستشفى الرباط بمدينة عطبرة.

نهى خوجلي محمد النور

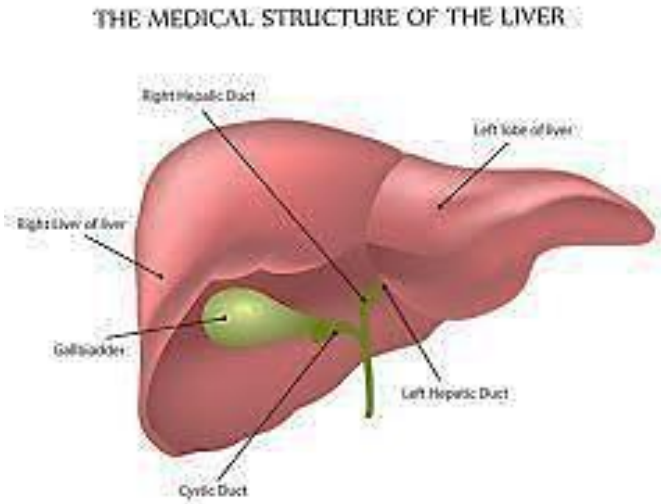
بعد أن شرح لي بأنه لا يترتب عليه أي أذى جسدي او نفسى واعلم أن المشاركة في هذا البحث لن تؤثر بأي حال من الأحوال في الرعاية الطبية التي أتلقاها كما أنه يحق لي بدون ابداء أسباب الإنسحاب من هذا البحث في أي مرحلة من مراحله.

البحث بإشراف :

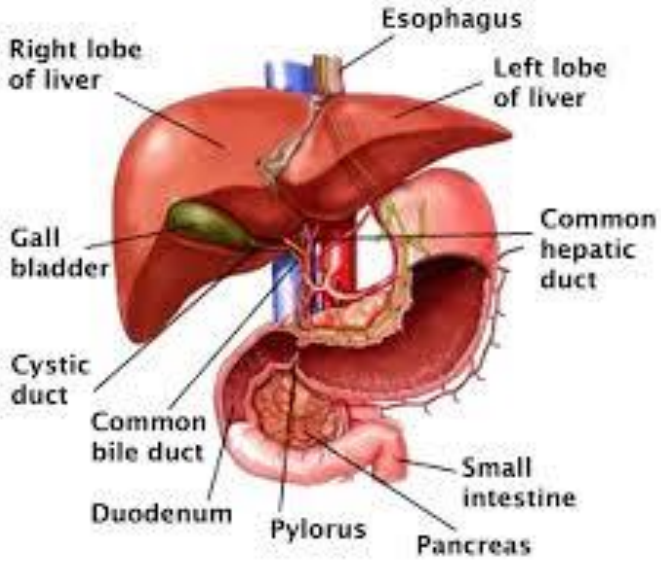
د. حمزة أحمد حسن محمد التوم

التوقيع : ---------- التاريخ :----------

Appendix III



The medical structure of the liver(15)



Appendix IV

Principle: dual-magnetic circuit bead method

Parameters:

- PT (Prothrombin Time)
- APTT (Activated Partial Thromboplastin Time)
- TT (Thrombin Time) FIB (Fibrinogen)
- Protein C, Protein S, HEP (High-molecular-weight Heparin),
- LMWH(Low-molecular-weight Heparin),
- LA(Lupus Anticoagulant),
- Thrombin factor II, V, VII, X, VIII, IV, IX, IX, XI, XII, etc.

Detector: 2 channels

Incubator: 16 sample positions,4 reagent positions (tunable incubation time)

Data storage: 500 test results

Display: 5.1" LCD screen

Print: Built-in printer & external printer

Input/ Output: RS-232 port and parallel interface

Ambient: work temperature: 15~35^{o}C;

relative humidity: $\leq 80\%$RH

Power: AC100V~240V, 50/60Hz,45W

Dimension: 380mm(W)×40mm(D)×130mm(H)

Weight: 5.9 kg

Features:

- 2 detectors up to 2 different parameters analysis.
- Accurate incubator temperature.
- Advanced method to eliminate interference from hemolysis, chyle, icterus, turbidity, plasma viscosity etc.
- Mini-reagent consumption, open reagent system.
- Internal thermal printer with reference value range.

Coggulameter Analyzer[15]