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DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Assessment of coagulation profiles among chronic liver disease patients attending Worabe Comprehensive Specialized Hospital, Central Ethiopia Region, Ethiopia

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This is to certify that the thesis prepared by Temesgen Sheferaw, entitled:

Assessment of coagulation profiles among chronic liver disease patients attending Worabe Comprehensive Specialized Hospital, Central Ethiopia Region, Ethiopia, and submitted in partial fulfillment of the requirements for a Master of Science degree in Clinical Laboratory Sciences (Hematology and Immunohematology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Abbreviations

ACLF	Acute-on-chronic liver failure
ALD	Alcohol-related liver diseases
APTT	Activated Partial Thromboplastin Time
BT	Bleeding time
CBC	Complete blood count
CLD	Chronic Liver Diseases
HBV	Hepatitis B Virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C Virus
HMWK	high-molecular-weight kininogen
INR	International normalized ratio
MELD	Mayo End-Stage Liver Disease
MRN	Medical Record Number
NAFLD	Non-alcoholic fatty liver disease
PT	Prothrombin time
WCSH	Worabe Comprehensive Specialized Hospital
vWF	Von Willebrand Factor

Abstract

Background: Liver damage from chronic liver disease with substantial changes in the hemostatic system is frequently observed and can develop multiple coagulation abnormalities that disturb the balance between clotting and fibrinolysis.

Objective: To assess the coagulation profiles among Chronic liver disease patients attending at Worabe Comprehensive Specialized Hospital from February to May 2024, Central Ethiopia Region, Ethiopia.

Methods: A comparative cross-sectional study was conducted among chronic liver disease patients from February to May 2024 at Worabe Comprehensive Specialized Hospital, Central Ethiopia Region. A total of 170 study participants (85 chronic liver disease patients (CLD) and 85 apparently healthy controls) were enrolled using a convenient sampling technique. The socio-demographic and clinical data were collected by using a pretested structured questionnaire and from patient's medical records, respectively. From venous blood samples, the HumaClot Plus coagulation analyzer and the CELL-DYN Ruby automated hematology analyzer were used to determine coagulation parameters and platelet count, respectively. The data was analyzed using SPSS version 27 software. Independent T-test was used to compare coagulation parameters between cases and controls. A P-value less than 0.05 was considered statistically significant.

Results: The present study found a Prothrombin time (PT), 15.83 ± 1.49 versus 14.22 ± 0.90 seconds, activated partial thromboplastin time (APTT), 35.99 ± 4.01 versus 33.86 ± 1.33 seconds and international normalization ratio (INR), 1.40 ± 0.18 versus 1.35 ± 0.09 were significantly higher in CLD patients than healthy controls, respectively ($P<0.001$). While the mean [SD] value of platelet count was significantly lower in CLD patients than in healthy controls ($P<0.001$). The present study also showed non-significant difference in the mean(SD) value of PT, INR, APTT, and platelet count between etiologies of CLD ($P>0.05$).

Conclusion: In this study, CLD patients had significantly increased PT/INR and APTT values, and significantly lower platelet count compared to healthy controls. Therefore, monitoring coagulation profiles and platelet count are suggested for detecting hemorrhagic complications and better management of CLD patients.

Key words*:* Coagulation profiles, Hemostasis, Platelet, Chronic liver disease

1. Introduction

1.1 Background

The liver is an important organ of the body that performs a wide range of crucial bodily functions; these include the synthesis of coagulation factor proteins to control bleeding within a damaged blood vessel and the production of blood coagulation inhibitors to prevent blood clots in normal circulation. Furthermore, the liver is involved in the reticuloendothelial system, which plays an important role in the clearance of active coagulation products (1, 2). The physiology of blood coagulation is closely linked to liver function as the liver synthesizes most of the factors of the coagulation cascade and fibrinolytic proteins. In addition, the liver is also involved in facilitating the clearance of activated clotting and fibrinolytic factors (3, 4).

Chronic liver disease (CLD) is a progressive decline in liver function, including the synthesis of important proteins, detoxification of metabolites, and bile excretion, continuing for more than half a year (5). A variety of diseases are involved, including cirrhosis, hepatocellular cancer, and chronic hepatitis (6, 7). Various clinical manifestations, including pruritus, joint pain, abdominal pain, muscular cramps, depression, fatigue, and anxiety, are brought on by this disease, which lowers the affected people's health-related quality of life (8, 9).

There are many different causes of CLD; however, the most often found ones worldwide are drug use, autoimmune hepatitis, nonalcoholic fatty liver disease (NAFLD), viral hepatitis B, C, and D infections, and chronic alcohol consumption (10). Liver plays a central role in the maintenance of hemostasis as it is the main site for the synthesis of the vast majority of proteins required for regulation of coagulation and fibrinolysis. Thus, impairment of liver parenchymal cell function disturbs hemostasis resulting in the development of multiple coagulation abnormalities that, depending on the degree of hemostatic impairment, can predispose the patient to bleeding or thrombus formation (11, 12).

Hemostasis is a physiological process that involves a range of cellular and molecular mechanisms to preserve blood's fluid state and stop it from leaking out through the formation of clots (13). The coagulation system, which is made up of several liver-produced protein components, is involved in the conversion of soluble fibrinogen into fibrin clot (14, 15). Platelets are involved in preventing bleeding at the site of endothelium disruption by means of platelet adhesion, aggregation, and coagulation system activation (13). Primary hemostasis consists of the activation of blood platelets that can be triggered by endothelial damage and the formation of a platelet plug. Activated platelets release adenosine diphosphate (ADP), which induces vasoconstriction, stimulates secondary coagulation, and promotes further platelet activation and aggregation (14).

Coagulation factors are crucial in secondary hemostasis in conjugation with platelet and vascular endothelium. After an injury to the blood vessels, two pathways are activated to initiate the blood clotting process. The intrinsic pathway is triggered when coagulation factor XII binds to collagen or other negatively charged substances. On the other hand, the extrinsic pathway is initiated by tissue factor, which is produced in endothelial tissue (14). After endothelial injury, platelets stick to the exposed subendothelial matrix using two specific platelet-collagen receptors known as glycoproteins Ia, IIa, and VI (16).

Most of these coagulation factors are serine proteases, which are enzymes that work by breaking down other proteins. However, there are a few exceptions to this, including tissue factor, FV, FVIII, and FXIII. Tissue factor, FV, and FVIII are glycoproteins, while Factor XIII is a transglutaminase. These factors are typically found in an inactive form known as zymogens as they circulate in the bloodstream (17).

The contact activation pathway is initiated by the formation of the primary complex on collagen, which is facilitated by high-molecular-weight kininogen (HMWK). Subsequently, Prekallikrein is transformed into kallikrein, and FXII is converted into FXIIa. FXIIa then catalyzes the conversion of FXI into FXIa. FXIa, in turn, activates FIX, and together with its co-factor FVIIIa, forms the tenase complex. The tenase complex then catalyzes the activation of FX to FXa (18).

After a blood vessel is damaged, a series of events known as the tissue factor pathway are set into motion. The first step involves Factor VII (FVII) leaving the bloodstream and binding to tissue factor (TF) on certain cells, forming a complex known as TF-FVIIa. This complex then goes on to activate Factor IX and Factor X. FVII itself can be activated by several factors, including thrombin,

Factor XIa, Factor XII, and Factor Xa. Factor Xa, along with its co-factor Factor Va, forms the prothrombinase complex, which ultimately converts prothrombin into thrombin (17).

A layer of platelets covers the entire injured area, and the activated platelets express the receptor GPIb-IX-V complex, which enhances adhesion by binding to von Willebrand factor (vWF) on the subendothelial matrix (19). A platelet monolayer covers all the injured area, and more activated platelets are recruited and aggregated to form a platelet plug by binding to fibrinogen molecules through another receptor, GPIIb/IIIa (20). Formation and deposition of fibrin occurs by activation of the clotting cascade concomitantly with platelet plug formation. A sequential activation of a series of inactive precursors leads ultimately to the formation of thrombin that cleaves fibrinogen to fibrin (21).

In general, liver disease results in variable impairment of hemostasis by multiple causes: quantitative and qualitative platelet defects; decreased production of coagulation and inhibitor factors; vitamin K deficiency; synthesis of abnormal clotting factors, decreased clearance of activated factors by the reticuloendothelial system, hyperfibrinolysis, and disseminated intravascular coagulation.

1.2 Statement of the Problem

Chronic liver disease (CLD) is a global public health issue affecting about 1.5 billion people globally in 2020 (22) and liver disease accounts for two million deaths annually and is responsible for 4% of all deaths in 2023 (23). In 2017, 2.4% of all deaths worldwide were attributed to cirrhosis, which is liver fibrosis brought on by chronic liver injury (22). The exact prevalence of chronic liver disease (CLD) in Ethiopia is not well-documented, but it is widely believed to be quite high (24). In Kersa, located in eastern Ethiopia, CLD emerged as the primary cause of death within the age group of 15-49 years, accounting for 13.7% of all fatalities (25) and in Butajira in central Ethiopia (11.3%) (26).

Liver disease affects both primary and secondary hemostasis by impairing the synthesis of all blood coagulation factors, activators, and inhibitors; which are essential to the blood coagulation pathway and fibrinolytic systems synthesizing plasma proteins and producing a range of blood clotting factors (2). Hemorrhagic complications due to liver disease patients are a major consequence and significant reason for intensive care unit admission (27). Liver damage from chronic liver disease showing substantial changes in the hemostatic system is frequently found (28) and can develop multiple coagulation abnormalities that disturb the balance between clotting and fibrinolysis (11).

The alterations encompass thrombocytopenia and platelet dysfunction, reduced levels of coagulation factors and inhibitors in circulation, and diminished levels of proteins engaged in fibrinolysis. These coagulation abnormalities can predispose patients from minor localized bleeding to massive life-threatening hemorrhage or thrombosis formation (29), and it is a major cause of public health problems, which results in morbidity or mortality worldwide (30). The abnormalities in CLD are usually measured through the prolongation of first-line global screening tests such as the prothrombin time (PT) and the activated partial thromboplastin time (aPTT) (31).

PT is related both to bleeding risk and mortality, and Patients with moderately or severely prolonged PT have 5 to 10-fold higher mortality rates than patients with normal PT (32). Thrombocytopenia is a common feature of chronic liver disease and is seen in 30 to 64% of cirrhotic patients (33, 34). Despite these derangements, the coagulation profiling service is very limited in Ethiopia and there is paucity of published data to inform health policy makers to strengthen the coagulation laboratory service.

1.3. Significance of the study

The findings of this study can be used as baseline information for researchers and policymakers interested in doing a study on the topic around Worabe Comprehensive Specialized Hospital. It could be an input for health care providers to intervene in the identified factors to monitor and manage CLD complications. The hospital can also plan for the sustainable availability of coagulation profile tests once the magnitude of the problem is identified and communicated to them. Patients can benefit from availability of the basic coagulation profile workup tests in the management of CLD.

2. Literature review

2.1 Introduction

To identify all relevant publications presented in different sections related to CLD and coagulation tests specifically PT, aPTT and platelets count, databases including PubMed, Google Scholar, Web of Science, and the Director of Open Access Journals were used, and papers published until 2024 were searched carefully using the title and abstract.

2.2 The Liver and its role in coagulation

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 the abdomen just under or lower right ribs. It has a larger right side 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 (35).

The liver is a very important organ concerning blood clotting, with the help of vitamin K, it produces proteins that are essential to allow blood to properly clot when needed to prevent excess bleeding. The liver plays a crucial role in breaking down and eliminating old or damaged blood cells. Liver failure happens when extensive damage renders large portions of the liver irreparable, leading to a loss of functionality (36).

2.3 The previous studies on the coagulation profiles of CLD

A comparative study done in 2020 by Prajapati DS Dr et al, revealed a Prothrombin time showing marked significant prolongation in all etiologies of chronic liver diseases. Activated Partial Thromboplastin Time(APTT) is quite significant in cirrhosis. In viral hepatitis, 25.3% rise in APTT while in Alcoholic liver diseases, 25.9% cases show rise in APTT (37). Another study by Bhatia G et al found about 62% of liver disease patients had prolonged PT and 39.3% had prolonged APTT. Thrombocytopenia was seen in 46% of liver disease patients (38).

A study conducted by Rai V et al., on hemostatic profiles of patients with Chronic Liver Disease on 60 cases found a mean platelet count in chronic liver disease group significantly lower than that of control group (p-value<0.001). In this study 33 (55%) out 60 cases of Chronic Liver Disease

(CLD) had thrombocytopenia (platelet count <150x103/µl). Thrombocytopenia were found in five cases (16.7%) of chronic hepatitis, 25 cases (93.3%) of cirrhosis patients and in none of the controls. PT, aPTT and thrombin time (TT) in cirrhosis group were significantly higher when compared to control group (p-value<0.001) (39).

A 2020 study in India by Rupela C et al., on liver disease diagnosed patients revealed that 86.6% (39/45) patients had prolonged Prothrombin Time, INR was raised in 86.6% (39/45) patients. There were 82.2% (37/45) patients of liver diseases having prolonged Activated Partial Prothrombin Time (40).

A study done by Kishore S et al., on 300 patients in Tertiary Care Hospital in Uttarakhand, India in 2017, determined the coagulation abnormalities using prothrombin time (PT), and activated partial thromboplastin time (APTT). 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 (41).

According to a study done in Pakistan in 2011 by Siddiqui SA et al., on 171 CLD cases, prothrombin time was prolonged in 150 (88%) cases. The mean PT was 23 ± 14.8 seconds Whereas, activated partial thromboplastin time (aPTT) was raised in 122 (71%) cases with mean of 44 ± 17.5 seconds. Platelet count was decreased in 63 (37%) CLD cases (42).

A 2017 study done in Nigeria by Oledinma S et al., revealed the mean PLT count of CLD patients (108 ± 32x10^9/L) was lower than that of the controls (236 ± 63 x10^9/L). The mean PT in CLD patients was 24 ± 14.1 seconds while it was 12.3 ± 1.40 seconds in the control patients. The aPTT values of CLD patients was 44 ± 17.2 seconds and that of the control was 32 ± 2.70 seconds. The fibrinogen level of CLD patients was 1.4 ± 0.8g/L and 3 ± 1.5g/L in the control groups (43).

According to a 2023 study done by Melkamu et al, in Ethiopia, the magnitude of prolonged Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT) were 68.08% and 63.51%, respectively among their 307 patients recruited from a teaching hospital in northwest Ethiopia (7). Apart from this study as far as the literature research goes, there is paucity of published studies in Ethiopia. This study is an effort to provide additional data to bridge the literature gap which can inform decision makers.

2.4 conceptual framework

The analysis of the study was based on the following modified conceptual framework.

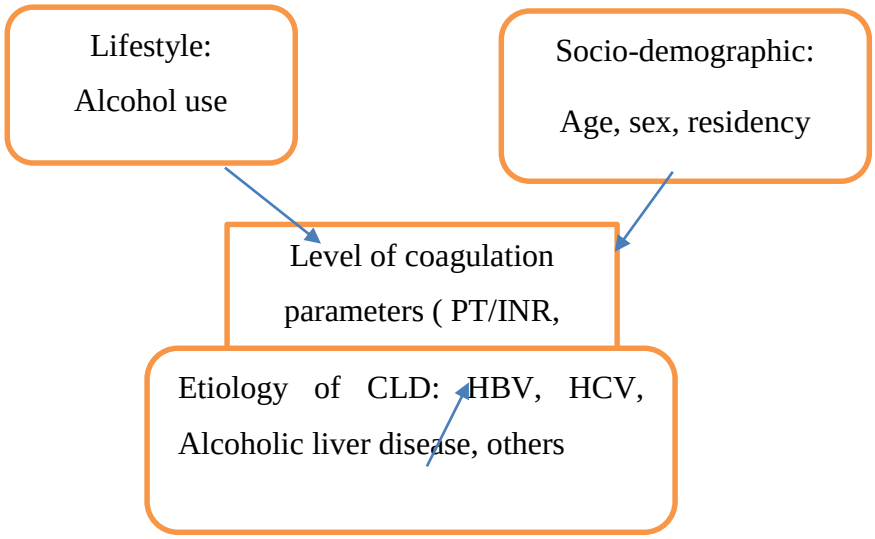

Figure 1The conceptual framework of the study

3. Objectives

3.1 General Objective

To assess the coagulation profiles among chronic liver disease patients in Worabe Comprehensive Specialized Hospital, Central Ethiopia Region, Ethiopia from February to May 2024.

3.2 Specific objectives

- To compare the coagulation parameters_(PT, INR, and APTT) between CLD patients and apparently healthy controls
- To compare the platelet count between CLD patients and apparently healthy controls
- To compare the coagulation parameters and platelet count between the Etiologies of CLD patients

4. Hypothesis

Null hypothesis: The values of coagulation parameters between chronic liver disease patients and apparently healthy controls is the same.

Alternative hypothesis: There is a significant difference between chronic liver disease patients and apparently healthy controls for the values of coagulation parameters (PT, INR, APTT), and Platelet.

5. Materials and Methods

5.1 Study area

The study was conducted in Worabe Comprehensive Specialized Hospital (WCSH), a tertiary hospital in the Central Ethiopia Region. It was established and started service provision officially in 2014. The hospital is located 176 kilometers away from Addis Ababa, the capital city of Ethiopia. Currently, WCSH has over 658 clinical, 516 administrative, and support staff providing medical specialty services to patients referred from across the country, serving a catchment population of 5 million. While the inpatient capacity is more than 427 beds, the hospital sees an average of 957 emergency and outpatient clients daily. Under the Department of Internal Medicine, the Gastroenterology unit offers both inpatient and outpatient clinical services. WCSH was chosen due to its status as the largest referral hospital in the region for liver cases seen at its Gastroenterology clinic. The hematology laboratory of the hospital is equipped a coagulometer.

5.2 Study design and period

A comparative cross-sectional study was conducted from February to May 2024, in Central Ethiopia Region, Ethiopia

5.3 Population

5.3.1 Source population

All patients with Liver disease who were attending Worabe Comprehensive Specialized Hospital.

5.3.2 Study population

Patients with chronic liver disease attending Worabe Comprehensive Specialized Hospital during the study period and who fulfilled the inclusion criteria were the study population.

5.4 Eligibility criteria

5.4.1 Inclusion criteria

Volunteer patients with chronic liver disease including cirrhosis, hepatitis, alcoholic liver disease; and adult patients of all sexes and ages ranging from 18-70 years at WCSH were included.

Apparently healthy peoples who were visiting WCSH for checkups, as care givers and staff who were volunteer to participate were included as controls.

5.4.2 Exclusion criteria

Patients with a history of hereditary coagulation disease, critically ill patients, pregnant women, patients who took oral contraceptives, and patients who took drugs such as aspirin, heparin, and warfarin were excluded from the study; and patients of Diabetes Mellitus, Tuberculosis, Chronic Kidney Disease, and malignancy were excluded.

5.5 Study variables

5.1 Dependent variables

The level of coagulation parameters (PT/INR, APTT, and PLT)

5.2 Independent variables

- Age
- Sex
- Alcohol use
- HBV
- HCV
- Alcoholic liver disease

5.6 Measurement and Data Collection

5.6.1 Sample size determination

The sample size was determined using a double population proportion formula based on the largest sample size by calculating PT, aPTT, and PLT. So, from the previous study from Nigeria, the mean (SD) of APTT level in the case group and control group was 54.29(4.3) and 39.47(5.3), respectively (44). A total sample of 170 was needed with a 95% confidence level and 10% non-response rate.

Sample size n= $\frac{\left(\alpha 1^2+\alpha 2^2\right)^2\left(Z1-\frac{\alpha}{2}+Z\,1-\beta\right)^2}{d^2}$ n = $\frac{\left(4.3^2+5.3^2\right)^2(0.84+1.96)^2}{14.82^2}$

N=77+8=85(including 10% contingency)

N=170 (total number for case and control)

- d = difference in means of two groups (effect size)
- σ1 = SD of Group 1
- σ2 = SD of Group 2
- Zα/2 = Z0.05/2 = Z0.025 = 1.96 (From Z table) at type 1 error of 5%
- Zβ = Z0.20 = 0.842 (From Z table) at 80% power

5.6.2 Sampling method

To choose study participants, a convenient sampling method was used for those who were eligible for the study.

5.6.3 Data collection procedure

After obtaining informed consent, a data collector with the nurse profession was used to collect the data. Interviewer-administered techniques were used to collect data from volunteer participants using a pretested structured questionnaire developed based on other relevant literature that included the variables of interest. Clinical information was reviewed and collected from the patient's medical records. The calculated sample size was collected both for the case and control group.

5.6.4 Specimen collection

After cleaning the venipuncture area with (70%) ethanol, 5ml of venous blood was collected using an EDTA tube for platelet and blue top tubes containing 3.8% sodium citrate as an anticoagulant for coagulation parameters. Plasma was obtained following the centrifugation of the anticoagulated blood at 3000rpm for 5 min following the standard protocol in the hospital**.**

5.6.5 Principles of Laboratory Tests

Principle of PT/INR: It was done by using a HumaClot Plus coagulation analyzer: Its principle is that citrated plasma is incubated at 37^{o}C for 3 minutes mixed with PT reagent which contains excess of calcium used to activate the coagulation cascade (extrinsic pathway). The result is detected through optical sensors which can sense fibrin or clot formation by the changes in light absorbance. The result is displayed in seconds and the INR is a ratio calculated from the patient result divided by control values.

APTT Principle: The principle of the test is that citrated plasma and APTT (with contact activator and pro-coagulant phospholipid) are mixed and incubated at 37ºC. The contact activator agent activates the factors XI & XII and the phospholipid provides a surface for the interaction of coagulation factors. Finally, Calcium is added and the time for clot formation is measured, and reported in seconds.

Platelet principle: It was done by using the CELL-DYN Ruby automated hematology analyzer (Abbott, USA) which uses flow cytometric techniques to analyze the RBC/PLT, WBC, and NOC populations. In flow cytometry individual cells as a suspension injected into a fast-moving cell-free fluid (sheath fluid) without mixing in a single file (hydrodynamic focusing) as they pass through the laser beam light, hit at different angles which is called multi-angle polarized scatter separation (MAPSS). It measures cells or other biological particles' size (0^o to 3^o), complexity (10^o), lobularity (90^o-polarized) and granularity (90^o-depolarized). Light scattering or fluorescence emission (if the particle is labeled with a fluorochrome) provides information about the particle's properties. The optical signals the cells generate are detected and converted to electrical impulses which are then stored and analyzed by the computer.

5.7 Data quality assurance and management

After being created in English, the data collection tool was translated into the Amharic local working language of the region. The two versions' content was kept coherent and consistent by translating back to the original version. The study participants' socio-demographic and clinical information was extracted with utmost care from the registration logbook using a standardized data collection form, ensuring the quality of the extracted data. Throughout the data collection process, including data entry and analysis, the collected data was continuously monitored and checked for completeness by the Principal Investigator (PI). One data collector with a nurse profession and one with medical laboratory technology were assigned to the study area to collect the data and blood samples, respectively. Before attempting to enter and analyze the data, the consistency and completeness of the collected data were checked for completeness immediately after data collection.

5.8 Laboratory Quality Assurance

Pre-Analytical: All blood samples were collected using the appropriate tube and volume, labeled correctly, and processed according to the Standard Operating Procedures to ensure their quality.

Analytical: All testing reagents and controls were stored and handled as per the manufacturer's instructions. Before using them, the machines utilized for coagulation analysis and platelet count underwent a thorough check to ensure their proper functioning. Following the daily control running, three level hematology control for platelets and internal control for PT and APTT, all samples were analyzed.

Post-analytical: The Principal Investigator checked the collected blood sample results daily to ensure its completeness and recorded them for entry.

5.9 Data analysis and interpretation

After being coded, the collected data was entered into Epi-Info version 7 then stored and exported to SPSS version 27 for analysis. Descriptive data was presented using tables and graphs. To determine the mean difference between the CLD and control group, an independent t-test was employed. One-way ANOVA (Analysis of variance) was used to determine the effect of etiologies of CLD on the value of coagulation parameters and platelet count. A P value less than 0.05 was considered to be statistically significant with a confidence level of 95%.

5.10 Ethical considerations

The study was carried out following the approval of the Department of Medical Laboratory Science research and ethical review committee (DRERC) with a protocol number of DRERC/733/23/MLS, College of Health Sciences, Addis Ababa University. Permission to conduct the study was obtained from the administrative office of WCSH. Moreover, an informed oral consent was sought from study participants after assessing their level of consciousness and explaining them the aim, benefits and risks as well as right to withdraw from the study if they do not wish to participate. They were also informed that the principal investigator has the sole access to the collected data and confidentiality would be maintained throughout the project report. They were notified that their laboratory results will be given to the attending physician.

5.11 Dissemination of Result

The final results of this study will be submitted to Addis Ababa University, College of Health Science, Department of Medical Laboratory Sciences as partial fulfillment of a master's degree in Hematology and Immunohematology. Furthermore, it will be shared with WCSH to develop interventional strategies. Finally, the manuscript of the research will be published on appropriate peer-reviewed scientific journals for wider communication.

5.12 Operational Definitions:

Chronic liver disease: Defined as patients with liver disease of HBV, HCV, Alcoholic liver disease, and cirrhosis for more than 6 months.

Coagulation profiles: The evaluation of the coagulation profiles of cases and controls are based on the value of PT/INR, aPTT, and platelet count.

Alcohol consumption: Study participants were classified if they had/hadn't a history of alcohol drinking within the last 6 months or a year.

6. Results

6.1 Socio-demographic and clinical profiles of chronic liver disease patients and apparently healthy controls

A total of 170 study participants comprising 85(57.64% male and 42.35% female) CLD patients and 85(62.35% male and 37.64% female) apparently healthy controls were recruited for the study (Table 1). Out of 85 for each group 57.64% of cases and 52.94% of controls were living in rural areas, and 20% of cases and 12.94% of controls had a habit of drinking alcohol. The mean age (±SD) of CLD patients and healthy controls were 39.64 ±11.63 years and 40.95 ± 9.01 years both ranging from 18-70 years, respectively (Table 1). The Mean ± SD of SBP and DBP for cases and controls were 118.62 ± 5.95, 79.06 ± 4.74, and 119.49 ± 2.32, 79.06 ± 1.82 mm Hg, respectively.

Table 1. Socio-demographic and clinical profiles of chronic liver disease patients and apparently healthy controls at WCSH, Southern Ethiopia, 2024

Variables		**Study participants (n= 170)**		**P-value**
		CLD case group	**Apparently healthy controls**	
		n= 85	**n= 85**	
Age group (years)	18-34	28(32.94%)	37(43.52%)	0.410
	35-54	45(52.94%)	40(47.05%)	
	≥55	12(14.11%)	8(9.41%)	
	Mean ± SD	39.64 ±11.63	40.95 ± 9.01	
Sex	Male	49(57.64%)	53(62.35%)	0.531
	Female	36(42.35%)	32(37.64%)	
Residence	Rural	49(57.64%)	45(52.94%)	0.537
	Urban	36(42.35%)	40(47.05%)	
	Yes	17(20%)	11(12.94%)	0.215

Alcohol consumption	No	68(80%)	74(87.05%)	
SBP	Mean ± SD	118.62 ± 5.95	119.49 ± 2.32	0.211
DBP	Mean ± SD	79.06 ± 4.74	79.06 ± 1.82	1.000

Note: SBP, Systolic blood pressure; DBP, Diastolic blood pressure

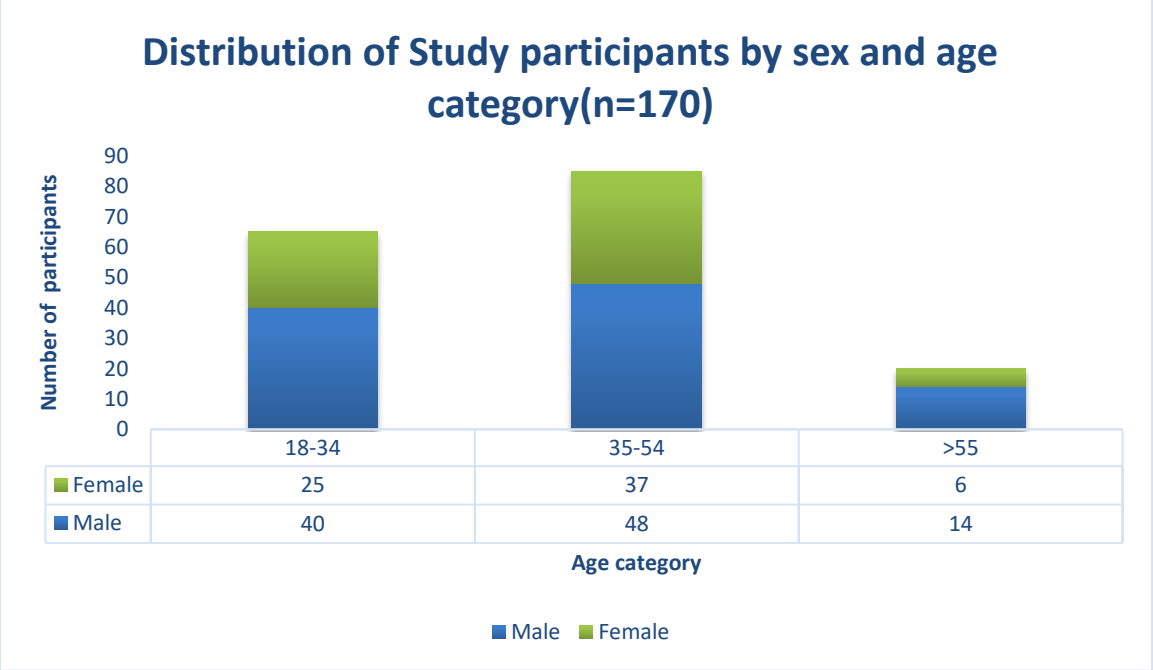

Figure 2 Distribution of Study participants at WCSH by sex and age category

6.2 Clinical profile among patients with Chronic liver disease at WCSH

The most frequently identified cause of chronic liver disease in this study was Hepatitis B virus infection affecting 52(61.2%) of the study participants followed by CLD of other causes in 17(20.0%) and 13(15.3%) HCV; and ALD was documented in 3(3.5%). (Table 2).

Table 2. Clinical profile among patients with CLD at WCSH, Southern Ethiopia, 2024

Etiology	Number(n=85)	Frequency(percentage)
HBV	52	61.2%
HCV	13	15.3%
ALD	3	3.5%
Other	17	20.0%

6.3 Comparison of Coagulation Parameters and platelet count among CLD patients and apparently healthy controls

Study participants who were in CLD groups experienced significantly increased mean value of Prothrombin time (15.83 ± 1.49 versus 14.22 ± 0.90 seconds, p <0.001), INR 1.40 ± 0.18 versus 1.35 ± 0.09, p <0.001, and activated partial thromboplastin time 35.99 ± 4.01 versus 33.86 ± 1.33 seconds, p <0.001) compared with the healthy control groups. The mean platelet count was significantly lower among CLD case groups than healthy controls (232.89 ± 30.63 versus 251.24 ± 38.69 x 10^9/L, p <0.001) (Table 3).

Table 3 Comparison of Coagulation Parameters and platelet count among CLD patients and apparently healthy controls at WCSH, Southern Ethiopia, 2024

Test Parameters	**Study participants (n= 170)**		
	CLD case **n= 85** **Mean ± SD**	**apparentlyHealthy controls** **n= 85** **Mean ± SD**	**p-value**
Platelet(10^9 /L)	232.89 ± 30.63	251.24 ± 38.69	<0.001
PT(sec)	15.83 ± 1.49	14.22 ± 0.90	<0.001
APTT(sec)	35.99 ± 4.01	33.86 ± 1.33	<0.001
INR	1.40 ± 0.18	1.35 ± 0.09	<0.001

Note: Independent-t test was utilized for comparisons, and P- value < 0.05 was considered statistically significant, SD- Standard deviation. PT, Prothrombin time; APTT, Activated partial thromboplastin time; INR, International normalized ratio.

6.4 Association between etiology of CLD and coagulation parameters among chronic liver disease patients

Analysis of coagulation parameters by etiology of chronic liver disease (HBV, HCV, ALD, and others) revealed no statistically significant difference as displayed below (Table 4).

Table 4 Association between etiology of CLD and coagulation parameters of the CLD case group at WCSH, Southern Ethiopia, 2024

Test parameter	Etiology of CLD				P value
	HBV (mean± SD)	HCV (mean± SD)	ALD (mean± SD)	Others (mean± SD)	
Platelet count	236.55±33.0	233.61±18.62	205±3.6	226.05±30.86	0.255
PT	15.81±1.58	15.65±1.27	15.26±0.97	16.11±1.49	0.756
APTT	35.97±3.64	36.25±3.61	31.76±0.41	36.61±5.35	0.289
INR	1.41±0.19	1.35±0.17	1.32±0.08	1.42±0.19	0.616

- An F-test (One way-ANOVA) with post hoc multiple comparisons was utilized to assess the differences in means, while SD represents the standard deviation.

7. Discussion

A total of 170 study participants, 85 with chronic liver disease at the GI clinic, and 85 healthy controls were included at WCSH from February to May 2024. The majority of the participants in both groups; case (57.64%) and control (62.35%) were male and the average age of the participants was 39.64 $\pm$ 11.63 and 40.95 $\pm$ 9.01 years respectively. This is similar to a study conducted by Oledinma S et al. from Nigeria including 270 study participants, where 56% of cases were male and the mean age of their CLD patients was higher compared to the current study 46 ± 14 years (43).

The most frequently identified cause of chronic liver disease in this study was Hepatitis B virus infection affecting 52(61.2%) of the study participants followed by CLD of unknown causes in 17(20.0%) and 13(15.3%) HCV. Another study from Ethiopia carried out at St Paul's Hospital Millennium Medical College revealed that 44.4% of the 117 admitted chronic liver disease patients were diagnosed with the hepatitis B virus, while 18% of the cases were attributed to the Hepatitis C Virus (45), reflecting the remarkable contribution of hepatis B virus in CLD, although the proportion of HBV in the current study is higher compared to theirs.

The findings of this study showed that PT, INR, and APTT were significantly higher in CLD patients than in healthy controls. The significant increase in the PT, INR, and APTT compared with the control in the present study agreed with the findings in previous studies conducted by Melkamu et al in Ethiopia, Oledinma S et al in Nigeria, Siddiqui SA et al in Pakistan, and Prajapati DS Dr et al in India (7, 37, 42, 43)

This study finding is also supported by a study conducted by Rai V et al which reported a PT, and aPTT in the chronic liver disease group were significantly higher when compared to the control group (39). The scientific justification could be due to, the circulating levels of most of the coagulation factors can be significantly reduced in patients with chronic liver disease who have extensive hepatocellular damage, and hence, substantial loss of hepatic parenchymal cell functions(46) which are responsible for the formation of fibrinogen, factors II, V, VII, IX, XI, XII, and XIII. In liver disease, these proteins normally have a very short half-life; and the decreased production of these blood proteins significantly prolongs the value of PT/INR and aPTT (46).

Furthermore, in the present study, the mean (SD) platelet count for chronic liver disease patients was significantly lower compared to apparently healthy controls. This agrees with research conducted by Oledinma S et al in Nigeria and Rai V et al in India which found significantly lower platelet count in CLD patients compared to the healthy controls (39, 43). This study is also in line with a study done in Pakistan by Siddiqui SA et al, that reported 37% of CLD cases had a decreased platelet count (42). Splenomegaly secondary to portal hypertension is considered the main cause of low platelet count in chronic liver disease (47). This might also be due to decreased production of thrombopoietin (TPO) by the liver; since TPO is produced by the liver at a constant rate and is cleared from circulation upon binding to its receptor (c-Mpl) on both megakaryocytes and platelets. Thus, circulating TPO levels depend upon hepatic synthesis and peripheral uptake it is evident that impairment of the liver functioning mass may cause a decrease in TPO production and then a decrease in platelet count (48). This later explanation related to reduced TPO production could be a plausible explanation in our case since the CLD patients in this study have SBP and DBP, measures of hypertension, which are not significantly different from the values in the comparative control groups. Thus, the explanation of splenomegaly related to portal hypertension as a main cause could be unlikely.

Additionally, our findings showed no statistically significant difference in the mean(SD) value of PT, INR, APTT, and platelet count between etiologies of CLD(HBV, HCV, ALD, and others) among chronic liver disease groups. This is in line with a study by Dangana A et al in 2023, which showed that there was a non-statistically significant difference in the level of PT/INR and aPTT among patients with chronic HBV, HCV, HIV, and patients with liver cirrhosis (49). In contrast, according to the study by Prajapati DS Dr et al the value of PT/INR and APTT were significantly different among patients with Alcoholic liver diseases, viral hepatitis, and cirrhosis of CLD cases (37). Another study by Rai V et al showed that, there was a significant difference in the platelet count between etiologies of CLD, which contradicts with the present study (39). The possible reasons for the difference might be associated with differences in the study population, or the way the tests were determined.

8. Strengths and Limitations

8.1 Strengths

The study addresses an area which is less investigated in our setup due to the limited availability of coagulation assays in the country.

8.2 Limitations

In this study, only basic coagulation parameters such as PT, APTT, INR, and platelet count were measured to assess the coagulation profiles, additional specific coagulation factor assays like D-dimer, fibrinogen and others were not performed as they were not available. Due to the nature of the study design being cross-sectional, factors associated with coagulopathy were not assessed in detail.

9. Conclusion and Recommendations

9.1 Conclusion

The present study showed that CLD patients at the WCSH were significantly higher in PT/INR and APTT; and lower platelet count compared to control groups. CLD patients may experience numerous types of coagulopathies including hypercoagulation, a tendency to bleed, and abnormal platelet count.

9.2 Recommendations

It is recommended that healthcare providers should regularly monitor and assess coagulation function in these patients. Further large-scale study is recommended to predict how abnormality levels of these and other additional coagulation tests predict patient outcomes and also identify any modifiable factors associated with the abnormalities. Therefore, coagulation test and platelet count should be used to monitor and manage CLD complications.

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Annexes

Annex I; Information sheet and informed consent

Information sheet

Name of institution; Worabe Comprehensive Specialized Hospital

Name of working section; Gastroentrology(GI) unit

Greeting;

Good morning/ afternoon; I'm Temesgen Sheferaw graduate student in Clinical laboratory science at Addis Ababa University, College of Health Sciences, Hematology & Immunohematology unit and studying on coagulation profile of chronic liver disease patients at Worabe Comprehensive Specialized Hospital in Central Ethiopia Region, Ethiopia 2024 " as a part of master's degree in CLS(Hematology & Immunohematology track). Now I will give you more details about the research.

Advisors name:

1. Prof Aster Tsegaye(Professor of Immuno-hematology)
2. Zemenu Tamir (MSc,PhD fellow ,Ass prof)

Sponsor: Wolaita Sodo University

Consent form

Purpose of the research project: This research will help us to assess the coagulation profile of chronic liver diseased patients.

Procedures: You are randomly selected to participate in this study because you are currently diagnosed in liver disease here. If you agree to participate in this study, you will be asked to answer some questions about yourself and the knowledge you have about CLD and its association with coagulation profile.

Benefits: There might not be direct benefit to you or you will not be given any incentives or payments to take part in this study, but the result of this study will help us as an input to identify the coagulation profile of chronic liver patients.

Risk: By participating in this study, there are no anticipated social and physical risks, but it might take 10-15 minutes for the interview.

Confidentiality: The information collected for this research project will be kept confidential stored in a file, without your name. But a code number assigned to it. In addition, it will not be revealed to anyone except the investigator.

The right to refusal: Your participation is voluntary and if you fill discomfort in the interview please fills free to drop it any time you want. If you choose to take part, you have the right to stop at any time. If you are willing to refuse or decide to withdraw later, you will not be subjected to any ill treatment.

Contacts and questions: If you have any question about the study, please ask me now**.** If you have questions and complains later or want additional information pleases contact the investigator based on the address provided below.

Name of principal investigator; Temesgen Sheferaw (BSc,CLS)

Email; temesgensheferaw80@gmail.com

Cell phone; +251913723438

Department of Medical Laboratory Sciences

Annex II; English version of questionnaire

As I have told you before, your answers to the questions will not be given to anyone else and no reports of this study ever identify you in any way. Therefore, I am asking your willingness to participate in this study.

Are you willing to participate in this study?

1. Yes 2. No

Thank you!!

If the study subject agrees to participate in the study, start the interview.

The respondent has given interviewer signature certifying that informed consent verbally.

Respondent's signature _____Date _____

If not agree, thank him/her and go to the next respondent by writing the reason why not volunteers.

Checked by:

Supervisor name_____ _____ Signature _____

Date___, ___, ____E.C

Interviewer; Code _____ , Name_____ , Signature_____

Date of interview _____ time started _____Time completed _____

Name of interviewer _____

Date _____, _____ _____Signature; _____

Structured questionnaire:

Participant name & Identification Number: _____

1. Age: _____

2. Sex: ☐ Male ☐ Female

3. Residency ☐ Rural ☐ Urban

4. Blood pressure _____ (mmHg)

5. Have you ever experienced prolonged bleeding that lasted for an unusually long period?

☐ Yes ☐ No

6. Duration of chronic liver disease: _____

7. Etiology of chronic liver disease

☐ HBV ☐ HCV ☐ Alcoholic liver disease ☐ cirrhosis

☐ Others: Specify _____

8. Presence of comorbidities

☐ Diabetes mellitus ☐ Hypertension ☐ HIV ☐ Heart failure ☐ Others: Specify _____

9. Assessment of alcohol intake:

a. Does the patient consume alcohol currently?

☐ Yes ☐ No

10. Laboratory parameters: Please tick on all that apply to the participant

	At presentation
CBC	
Platlet counts/ul	
coagulation	
PT/INR/sec	
aPTT/sec	

Annex III Amharic version of information sheet and questionnaire

የመረጃ ወረቀት እና የስምምነት ቅጽ

የተሳትፎ ስምምነት ፎርም

የመስሪያበቱስም ——————————————

የስራክፍል ——————————————

እኔ ተመስገን ሽፈራው በአዲስ አበባ ዩኒቨርሲቲ፣ ሜዲካል ላብራቶሪ ሳይንስ ትምህርት ቤት፣ በክሊኒካል ላብራቶሪ ሳይንስ የሁለተኛ አመት **የሂማቶሎጂ እና ኢሚውኖሄማቶሎጂ ትምህርት ክፍል** ድህረምረቃ ተማሪ ነኝ። በአሁኑ ሰዐት **ወራቤ ኮምፕሬንሲቭ ስፔሻላይዝድ ሆስፒታል ሥር በሰደደ የጉበት በሽተኞች የደም መርጋት ፕሮፋይል** በሚል ጥናታዊ የመመረቂያ ጽሁፍ ለመስራት መረጃ እየሰበሰብኩ ነው። በ CLS (ሄማቶሎጂ እና ኢሚውኖሄማቶሎጂ ትራክ) የማስተርስ ዲግሪ አካል በመሆን፣ **ስለ ጥናቱ ተጨማሪ ዝርዝሮችን እሰጥዎታለሁ**።

የአማካሪዎቼ ስም፤

1. ፕሮፌሰር አስቴር ፀጋዬ(የኢሚኖ-ሄማቶሎጂ ፕሮፌሰር)

2. ዘመኑ ታምር (ኤምኤስሲ፣ ፒኤችዲ ተማሪ፣ ረዳት ፕሮፌሰር)

ስፖንሰር፤ ወላይታ ሶዶ ዩኒቨርሲቲ

የፍቃድ ቅፅ

የምርምር ፕሮጀክቱ ዓላማ፡- ይህ ጥናት ሥር የሰደደ የጉበት በሽተኞች የደም መርጋት ሁኔታን ለመገምገም ይረዳናል።

ሂደቶች፡ በዚህ ጥናት ላይ ለመሳተፍ በዘፈቀደ ተመርጠዋል ምክንያቱም በአሁኑ ጊዜ እዚህ በጉበት በሽታ ስለተመረመሩ ነው። በዚህ ጥናት ላይ ለመሳተፍ ከተስማሙ ስለራስዎ አንዳንድ ጥያቄዎችን እንዲመልሱ እና ስለ CLD እና ከደም መርጋት መገለጫ ጋር ስላለው ግንኙነት ያለዎትን እውቀት እንዲመልሱ ይጠየቃሉ።

ጥቅማ ጥቅሞች፡ ለእርስዎ ቀጥተኛ ጥቅም ላይኖረው ይችላል ወይም በዚህ ጥናት ላይ ለመሳተፍ ምንም አይነት ማበረታቻ ወይም ክፍያ አይሰጥዎትም ነገር ግን የዚህ ጥናት ውጤት ሥር የሰደደ የጉበት በሽተኞች የደም መርጋት መገለጫን ለመለየት እንደ ግብአት ይረዳናል.

ስጋት፡ በዚህ ጥናት ውስጥ በመሳተፍ፣ የሚጠበቁ ማህበራዊ እና አካላዊ አደጋዎች የሉም፣ ግን ለቃለ መጠይቁ ከ10-15 ደቂቃ ሊወስድ ይችላል።

ምስጢራዊነት፡- ለዚህ የምርምር ፕሮጀክት የሚሰበሰበው መረጃ ያለእርስዎ ስም በፋይል ውስጥ በሚስጥር ይቀመጣል። ግን ኮድ ቁጥር ተመድቦለታል። በተጨማሪም, ከመርማሪው በስተቀር ለማንም አይገለጽም.

እምቢ የማለት መብት፡ ተሳትፎዎ በፈቃደኝነት ነው እና በቃለ መጠይቁ ላይ ምቾት ማጣት ከሞሉ እባክዎን በፈለጉት ጊዜ ለመጣል በነጻ ይሞሉ። ለመሳተፍ ከመረጡ በማንኛውም ጊዜ ለማቆም መብት አልዎት። እምቢ ለማለት ፍቃደኛ ከሆኑ ወይም በኋላ ለመልቀቅ ከወሰኑ ምንም አይነት ህመም አይደረግብዎትም።

አድራሻዎች እና ጥያቄዎች፡- ስለ ጥናቱ ምንም አይነት ጥያቄ ካሎት፣ እባክዎን አሁን ይጠይቁኝ። በኋላ ላይ ጥያቄዎች እና ቅሬታዎች ካሉዎት ወይም ተጨማሪ መረጃ ከፈለጉ እባክዎን ከዚህ በታች በተገለጸው አድራሻ መሰረት መርማሪውን ያነጋግሩ።

የዋና ተመራማሪ ስም; ተመስገን ሸፈራው (ቢኤስሲ፣ሲ.ኤል.ኤስ.)

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ተንቀሳቃሽ ስልክ; +251913723438

አስቀድሜ እንደነገርኮ ለጥያቄዎቹ የሚሰጡት መልስ ለማንም አይሰጥም እና የዚህ ጥናት ዘገባ በምንም መልኩ እርሶን ለይቶ አያውቅም። ስለዚህ በዚህ ጥናት ለመሳተፍ ፈቃደኛ መሆንዎን እጠይቃለሁ።

በዚህ ጥናት ለመሳተፍ ፈቃደኛ ኖት?

1. **አዎ** 2. **አይደለም**

አመሰግናለሁ!!

የጥናቱ ርዕሰ ጉዳይ በጥናቱ ውስጥ ለመሳተፍ ከተስማማ, ቃለ-መጠይቁን ይጀምሩ.

ምላሽ ሰጪው ያንን በመረጃ የተደገፈ ፈቃድ በቃላት የሚያረጋግጥ ፊርማ ለጠያቂው ሰጥቷል።

የተጠሪ ፊርማ ቀን

ካልተስማሙ አመስግኑት እና በጎ ፈቃደኞች ያልሆኑበትን ምክንያት በመጻፍ ወደ ቀጣዩ ምላሽ ሰጪ ይሂዱ።

የተረጋገጠው በ፡

የተቆጣጣሪ ስም ፊርማ

ቀን , , ኢ.ሲ.

ጠያቂ; ኮድ ፣ ስም ፣ ፊርማ

የቃለ መጠይቁ ጊዜ የጀመረበት ቀን የተጠናቀቀው ጊዜ

የጠያቂው ስም

ቀን ፣ ፊርማ;

መግለጫ

እኔ፣ ከዚህ በታች የተፈረመው ለምርምር ፕሮጀክቱ ሳይንሳዊ ስነ-ምግባር እና ቴክኒካል ምግባር ሃላፊነቱን ለመቀበል ተስማምቻለሁ እናም ወቅታዊውን የእድገት ሪፖርት ለአማካሪዎቼ እሰጣለሁ እና በምርምር ህትመቶች ጽ / ቤት ውሎች እና ሁኔታዎች መሠረት አስፈላጊውን ምክር እና ፈቃድ እጠይቃለሁ ። ለአማካሪዎቼ እና በጥናቱ ውስጥ ለሚሳተፉ ሁሉም ባለድርሻ አካላት ለዚህ ምርምር ማንኛውንም የገንዘብ ምንጭ ጨምሮ ።

ኤም.ኤስ.ሲ. እጩ፣ ተመስገን ሽፈራው (ቢ.ኤስ.ሲ.)

ፊርማ፡ _____

የማስረከቢያ ቀን፡- _____

ይህ ፕሮፖዛል እንደ አማካሪዎቻችን ተቀባይነት አግኝተናል።

አማካሪ፡ ፕሮፌሰር አስቴር ፀጋዬ(የኢሚኖ-ሄማቶሎጂ ፕሮፌሰር)

ፊርማ፡ _____

ቀን፡ _____

ቦታ፡ አዲስ አበባ፡ ኢትዮጵያ

አማካሪ፡ ዘመኑ ታምር (ኤምኤስሲ፣ ፒኤችዲ ተማሪ፣ ረዳት ፕሮፌሰር)

ፊርማ፡ _____

ቀን፡_____

ቦታ፡ አዲስ አበባ፡ ኢትዮጵያ

የተዋቀረ መጠይቅ፡-

የተሳታፊ ስም እና መለያ ቁጥር፡ _____

1. ዕድሜ፡ _____

2. ጾታ፡ ☐ ወንድ ☐ ሴት

3. የመኖሪያ አካባቢ ☐ የገጠር ☐ ከተማ

4. ለረጅም ጊዜ የዘለቀ የደም መፍሰስ አጋጥሞህ ያውቃል?

5. የደም ግፊት (mmHG) ፡ _____

6. ሥር የሰደደ የጉበት በሽታ የሚቆይበት ጊዜ፡- _____

7. ሥር የሰደደ የጉበት በሽታ ኤቲዮሎጂ

☐ HBV ☐ HCV ☐ የአልኮል ጉበት በሽታ ☐ cirrhosis

☐ **ሌሎች፡** _____ ***ይግለጹ***

8. ***ተጓዳኝ በሽታዎች መኖር***

☐ የስኳር በሽታ mellitus ☐ የደም ግፊት ☐ ኤች አይ ቪ ☐ የልብ ድካም

☐ ሌሎች፡ _____ ይግለጹ

9. የአልኮሆል መጠንን መገምገም;

ሀ. በሽተኛው በአሁኑ ጊዜ አልኮል ይጠቀማል? ☐ አዎ ☐ አይደለም

10. የላቦራቶሪ መለኪያዎች፡ እባክዎን ለተሳታፊው የሚመለከተውን ሁሉ ላይ ምልክት ያድርጉ

	ክሊኒካዊ አቀራረብ
ሲቢሲ	
ፕላትሌት ቆጠራዎች/ul	
የደም መርጋት	
PT/INR/ሰከንድ	
aPTT/ሰከንድ	

Annex IV: Principles and procedure of each laboratory analysis

Principle of PT/INR: It was done by using a HumaClot Plus coagulation analyzer: Its principle is that citrated plasma is incubated at 37^{o}C for 3 minutes mixed with PT reagent which contains excess of calcium used to activate the coagulation cascade (extrinsic pathway). The result is detected by means of optical sensors which are able to sense fibrin or clot formation by the changes occur in light absorbance. The result displayed in seconds and the INR as a ratio calculated from the patient result divided by control values.

Test procedures:

1. A gently inverted well-mixed 3.8% citrated proportionate whole blood is centrifuged at 3000rpm for 5min
2. Turn on the analyzer
3. Pre-warm the PT reagents to 37^{o}C
4. Pipette 50uL plasma to the cuvette
5. Incubate for 3minutes, while incubating press ”optic” twice till display the message “active” and channel 1 is ready to read.
6. Add 100uL PT reagent and simultaneously press “optic” and display the result within 300 seconds. If not clot formed it will display ****
7. Record the result in second and INR unit
8. After finishing the test return the reagent to 2-8^{o}C

Reference Range: 10-14 sec

APTT Principle : The principle of the test is that citrated plasma and APTT (with contact activator and pro-coagulant phospholipid) mixed and incubated at 37^{o}C. The contact activator agent activates the factors XI&XII and the phospholipid provide surface for the interaction of coagulation factors. Finally Calcium is added and the time for clot formation is measured.

Test Procedures

1. A gently invert well-mixed 3.8% citrated proportionate whole blood is centrifuged at 3000rpm for 5min
2. Turn on the analyzer
3. Pre warm the $CaCl_2$ (reagent 2) to 37^{o}C
4. Pipette 50uL plasma to the cuvette pre-warm for 2 minutes.
5. Add 50uL APTT (reagent 1) and incubate for 4 minutes press 'Timer 2' to set time. while incubating double press "optic" to display the message "active" and channel 1 is ready to read.
6. Add 50uL $CaCl_2$ (reagent) and testing automatically start. The instrument will read the result within 300 seconds. If no clot formed it will display ****
7. Record the results in unit of seconds.
8. After finishing the test return the reagent to 2-8^{o}C

Reference Range: 26.1-36.3 sec

Platelet principle:

It was done by using the CELL-DYN Ruby automated hematology analyzer which uses flow cytometric techniques to analyze the RBC/PLT, WBC, and NOC populations. In flow cytometry individual cells as a suspension injected into a fast moving cell free fluid (sheath fluid) without mixing in a single file (hydrodynamic focusing) as they pass through the laser beam light, hit at different angle which is called multi angle polarized scatter separation (MAPSS). It measures cells or other biological particles' size (0^o to 3^o), complexity (10^o), lobularity (90^o-polarized) and granularity (90^o-depolarized). Light scattering or fluorescence emission (if the particle is labeled with a fluorochrome) provides information about the particle's properties. The optical signals the cells generate are detected and converted to electrical impulses which are then stored and analyzed by the computer.

Test Procedure

First they mixed the whole blood with EDTA anticoagulant by placing in mixer machine. Then the Hand-Held Bar Code Reader was used to identify the specimen barcode and select the test in the Next Open Tube Entry region. In the CBC routine mode, then the sample aspirated in to the analyzer CELL-DYN Ruby and after specified period of time the result can be displayed on the screen with numerical and scatter or histogram data.

Reference Range: 135-435 x 10^9/L

Declaration

I, the undersigned, declare that this M.Sc. thesis is my original work, has not been presented for a degree in this or any other university and that all sources of materials used for the thesis have been duly acknowledged.

M.Sc. candidate: Temesgen Sheferaw(B.Sc.)

Signature: _____

Date of submission: _____

This Thesis has been submitted with our approval as advisors.

Advisor: Prof. Aster Tsegaye (Professor of Immuno-hematology)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: Zemenu Tamir(MSc,PhD fellow, Ass prof)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia