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COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Hematological abnormalities and their associated factors among Liver disease patients at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia: A cross-sectional study.

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This is to certify that the thesis prepared by Muluken Gashaw entitled: Hematological abnormalities and their associated factors among Liver disease patients at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Hematology and Immunohematology specialty track) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Abbreviations

AAU	Addis Ababa University
ALD	Alcoholic liver disease
CBC	Complete blood count
EDTA	Ethylene diamine tetra acetic acid
HCT	Hematocrit
Hb	Hemoglobin
LMR	Lymphocyte to monocyte ratio
LC	Liver cirrhosis
MCHC	Mean cell hemoglobin concentration
MCV	Mean cell volume
NLR	Neutrophil to lymphocyte ratio
NAFLD	non-alcoholic fatty liver disease
PLR	Platelet to lymphocyte ratio
PCV	Packed cell volume
PI	Principal Investigator
RBC	Red blood cell
RDW	Red cell distribution width
SOP	Standard operating procedure
TPO	Thrombopoietin
WBC	White Blood Cell
WHO	World health organization

Abstract

Background: Liver disease such as alcoholic, hepatitis, and fatty liver are major health problems worldwide that are often accompanied by hematological abnormalities. A variety of disturbances in hemostasis and production of blood cells can occur in the setting of liver dysfunction. A comprehensive understanding of the hematological aspects of liver disease is essential for both diagnosis and management.

Objective: The aim of this study was to assess different hematological abnormalities and their associated factors among liver disease patients at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia.

Methods: A hospital-based cross-sectional study was carried out at St. Paul's Hospital Millennium Medical College, Ethiopia, from January to May 2023. A total of 310 patients with liver disease were included using a convenient sampling technique. Blood samples collected using EDTA tubes were analysed for CBC and ESR by using Beckman Coulter analyzer and Westergren respectively. SPSS software (version 28) was used for analysis. Frequencies and percentages were used to summarize and describe the data. Hematological abnormalities and their associated factors were determined by bivariable and multivariable logistic regression. P-value ≤ 0.05 was considered statistically significant.

Results: The mean age of study participants was 41±12.8 years. From the study participants 79(25.5%) were anemic and 47(15,2%) were leucopenic. Thrombocytopenia and elevated ESR were observed among 105(33.9%) and 77(24.8%) of the study participants, respectively. Anemia was significantly associated chronic liver disease (AOR=11.8;95%CI:4.3,32.1). Alcohol drinking habit(AOR=4.28;95%CI:2.07,8.8)andnothavingphysicalexercisehabit(AOR=2.4,95%CI:1.06,5.5) were significantly associated with thrombocytopenia. Elevated ESR was significantly associated with alcohol drinking habit (AOR=2.24;95%CI:1.04-4.8) and CLD (AOR=4.98;95%CI:2.3-10.7).

Conclusion: Anemia, leukopenia, thrombocytopenia and elevated ESR were common hematological abnormalities among liver disease patients. These hematological abnormalities were more prominent among chronic liver disease patients. Thus it is important to asses and monitor hematological abnormalities during the diagnosis of liver disease patients.

Keywords: *liver disease, hematological abnormalities, erythrocyte sedimentation rate, Ethiopia*

1. Introduction

1.1 Background

Liver is the second largest organ in the human body with impressive regenerative potential and it has more than 500 different bodily functions including, aiding in blood clotting, purifying the blood of toxins, converting food into nutrients, regulating hormone levels, fighting off infections and illness, recovering from injury, and metabolizing cholesterol, glucose, and iron while maintaining their levels(1,2).

Any illness that can harm liver cells and change normal liver function is known as liver disease. Liver disease can be caused by a variety of factors, such as, alcohol abuse which causes alcoholic liver disease (ALD), viral hepatitis, autoimmune conditions, inherited diseases and certain medication or chemicals. The most common causes of the rising burden of abnormal liver function tests in liver disease are nonalcoholic fatty liver disease, alcohol-related liver disease, and viral hepatitis (3,4). Proper diagnosis is essential for identifying the underlying cause to treat liver disease. Some of blood liver function tests include complete blood count (CBC), prothrombin time (PT), activated partial thromboplastin time (APTT), bilirubin (direct and indirect), and the liver enzymes such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (3,5).

The stages of liver disease include; inflammation, fibrosis, cirrhosis and end-stage liver disease (ESLD). In the inflammation stage, there can be mild hematologic abnormalities such as a mild decrease in red blood cells and increase in white blood cells (1). During Fibrosis stage liver begins to develop scars and more severe hematologic abnormalities, such as a significant decrease in RBC and platelet counts as well as dysfunctional WBC can occur. Cirrhosis and ESLD are associated with severe hematologic abnormalities including leukopenia, anemia, thrombocytopenia and sever coagulopathies (1,6).

Hematological profiles are measurable blood indicators that may be used to identify and track clinical and physiological anomalies. Complete blood count (CBC) is one of the most common clinical laboratory tasks which is performed for the analysis of abnormalities within numerous blood cell components including the white blood cells (WBC), red blood cells (RBC), and

platelets (PLT), plus other parameters like MCV, MCH, MCHC and RDW as well as platelet indices, aiding in the clinical diagnosis and identification of blood illnesses and other systemic diseases (7,8).

Due to its distinct portal circulation and synthetic (clotting factors, thrombopoietin) and immune functions, the liver is implicated in or is responsible for various hematological abnormalities (9). Protein production and lipid metabolism are important processes that both depend on the liver. As a result, both the cellular and soluble components of blood may be adversely affected by hepatic synthetic failure (10).

Hematological abnormalities that are related with liver disease may be divided in to 4 major disorders; red blood cell, white blood cell, platelet and coagulation disorders (6,11). Red blood cell production is affected in liver disease because of the damage to the liver cells, resulting a decrease in production of hepcidine, which is the iron regulatory hormone mainly produced by the liver. Absorption of iron from the entrocytes becomes decreased, which results disruption of the heme biosynthesis pathway, and a decrease in erythropoietin production (12,13). These conditions plus hemorrhage, especially into the gastrointestinal tract and hemolysis lead to anemia, a condition where red blood cell count is decreased. Consequently, anemia in liver disease is a critical factor to consider when diagnosing and treating the condition (14,15).

Another hematological complication associated with liver disease are white blood cells. They are a type of immune cell that protects the body from infection, and their numbers can be greatly reduced in cases of liver disease. A decrease in white blood cells can lead to a more severe infection, and thus, it is important to monitor white blood cell count in case of liver disease (16,17).

Another common complication related with liver disease is bleeding disorder. The basic reasons for this disorder are coagulation proteins and platelets. Defects of platelet number and function occur in liver disease due to a number of reasons including hypersplenism, low thrombopoietin, bone marrow suppression and reduced survival. Thrombopoietin which is primarily produced in the liver promotes megakaryopoiesis and thrombopoiesis. In case of liver disease circulating TPO level becomes depleted due to low production related with impaired hepatic synthetic

function as well as increased platelet sequestration in the spleen which accelerates TPO clearance. As a result, thrombocytopenia has become common future among liver disease patients and it is correlated with the severity of the disease (18).

This study was aimed to contribute in proving evidence-based information which could help to suggest that hematological parameters could have diagnostic and prognostic value in certain liver disease complications.

1.2 Statement of the Problem

Liver disease is one of the most critical public health problems in the world. Chronic liver disease (CLD) is one of the most frequent causes of death (19). Approximately 29 million people suffer from a chronic liver condition (20). The liver disease accounts for approximately 3.5% of deaths per year worldwide and cirrhosis is the 11th most common cause of death globally. Viral hepatitis and hepatocellular carcinoma caused 1 million deaths per year (2,21). World health organization reports that the total deaths caused by viral hepatitis, including acute cases, cirrhosis, and liver cancer account for 1.1 million deaths globally in 2019(22).

In central and eastern sub-Saharan Africa in 2017, the most common cause of death due to cirrhosis was mainly hepatitis B and C virus (23). Cirrhosis-related deaths doubled in sub-Saharan Africa between 1980 and 2010, Most cases of cirrhosis were attributed to HBV, alcohol misuse, and HCV, but around 30% of death were unrelated to these causes (24). In Ethiopia, chronic liver disease (CLD) is the 7th leading cause of death, accounting for about 24 deaths per 100000 populations in 2019(25).

Hematologic and coagulation abnormalities are usually linked to chronic liver illnesses. About 40-80% of individuals with chronic liver disease experience anemia of various etiologies (26). Anemia in a patient with cirrhosis is clinically important but often overlooked clinical entity (27).

White blood cell (WBC) counts are often assessed as a simple and convenient indicator of systemic inflammation. Numerous disorders have been linked to increased or decreased WBC

levels including liver disease. The WBC count varies depending on the cause and stage of the liver disease (28,29).

In those with chronic liver illness, coagulation disorders and thrombocytopenia are prevalent hematologic conditions. Thrombocytopenia increases the risk of bleeding and poor prognosis which may affect the outcome of surgeries or liver biopsies (18). Around one-third of patients with acute liver failure died with bleeding consequences (30).

There are still plenty of unresolved issues related to the actual role of hematological indices as potential markers of liver function (12). As of our knowledge, no studies have been published on assessing the hematological abnormalities and their associated factors among liver disease patients in Ethiopia.

1.3 Significance of the study

This study was carried out to see if there is a significant relationship between different hematological abnormalities and associated factors among liver diseases patients. Hematological abnormalities in individuals with liver illness must be investigated in order to correctly identify and manage the condition. Liver disease prognosis depends on how quickly the condition was diagnosed and treated. In beginning stages, liver disease usually responds to treatment, but in advanced liver disease, the damage done by fibrosis, cirrhosis and liver failure cannot be reversed (3,5).

Thus, the findings of this study could enrich the knowledge of clinicians which helps them to indicate whether or not hematological parameters could be perceived as possible indices in the noninvasive evaluation of people with liver disorders as well as it could help to identify some associated factors which relates to different hematological abnormalities among liver disease patients.

This study could provide scientific relevant evidence to update current knowledge on the magnitude of basic hematologic abnormalities and their associated factors among liver disease patients.

This study could be important for non-governmental organizations, governmental bodies, and other stakeholders who seek this information to plan an intervention for liver disease complications in hematological abnormality through routine hematology test screening. Moreover, this study could be used as a reference or base line information for researchers to conduct further studies.

2. Literature Review

Hematological parameters (profiles) are measurable indices of blood that can be used to identify and monitor some pathological and physiological abnormalities. These profiles can be affected by many disease scenarios that affect physiology and immunological responses of the hematopoietic cell and they are often applied in clinical settings (6,8).There are studies performed in different countries which establish a significant relationship between different hematological parameters and liver disease patients(6,10,11).

A cross-sectional study in Singapore conducted by Das S K *et al* (2011) consisted a total of 254 patients who were further classified into three subgroups according to the degree of disease severity, i.e. mild (I), moderate (II) and severe (III). The findings show that hemoglobin concentration, RBC count, hematocrit, lymphocyte count and platelet count were significantly lower, while MCV, MCH were significantly higher in ALD patients compared to the three other groups. When comparing the alcoholics versus the normal participants platelet count was reduced (22.9%) significantly ($p< 0.001$), while ESR (55.4%) were significantly elevated. Also, ESR was significantly elevated ($p < 0.001$) in ALD patients compared to normal participants and NAFLD patients. The HCT level was significantly ($p < 0.01$) higher (8%) and the platelet count was significantly ($p < 0.01$) lower (10.8%) in NAFLD patients compared to the control group. Comparison of liver disease patients between the severe stages and the milder one shows significant reduction in HCT and Hb concentration (31).

A prospective study conducted by Machiwal*et al* (2022) in JLN medical college, Ajmer on 120 chronic liver disease patients found that normocytic normochromic anemia was the common type of anemia. In this study the mean MCV, MCHC and MCH among ALD patients were significantly higher. 30 % of the participants had thrombocytopenia (32).

An observational study among 50 chronic liver disease patients was conducted by Jha *et al* (2019) at Darbhanga Medical College and Hospital, India. The research found that 88% of the participants were anemic. Furthermore, leucopenia was present among 6% of the participants and 48% had thrombocytopenia (11).

Another research conducted prospectivelyin 2016 among 88 chronic liver disease patients with etiology of alcohol in India showed that anemia and thrombocytopenia are common findings among alcoholic liver disease patients. Regarding the WBC count, both leukocytosis and leukopenia had occurred depending on the severity of the disease. The more severe the disease, leukopenia becomes prominent (26).

A crossectional study conducted in India, in 2017 on 50 chronic liver disease patients showed that from the total study participants half (50 %) of the patients had thrombocytopenia(6).

A study done in India by E Halleys et al. (2021) on 100 decompensated chronic liver disease patients found that 86%, 36% and 56% had anemia, leukocytosis and thrombocytopenia respectively. In this study thrombocytosis did not occur in all patients(34).

Wang et al performed a research on 114 HBV related liver disease patients and found that the patients had lower TPO than controls which results the significant thrombocytopenia(35).

A prospective study conducted in china in 1991 among 324 acute viral hepatitis patients indicated that anemia, thrombocytopenia, leukocytosis and leukopenia were 12.6%, 19.3%, 10.8% and 7.4% respectively (36).

A prospective study conducted in Nigeria in 2009 among 120 subjects with 70 CLD cases found that hematocrite and platelet counts were significantly lower among the CLD patients when comparing them to the controls($p<0.001$)(38).

A retrospective cohort conducted by Zhide Hu *et al* on 33 patients with non-cirrhotic HBV chronic hepatitis, 125 patients with liver cirrhosis after HBV infection, 81 newly diagnosed primary hepatocellular carcinoma (pHCC) patients, 17 alcoholic liver cirrhosis patients and 42 patients with primary biliary cirrhosis (PBC) and 66 healthy individuals as a control. Increased RDW was observed in liver disease patients. RDW was positively correlated with serum bilirubin and creatinine levels, prothrombin time, and negatively correlated with platelet counts and serum albumin concentration (39).

Another retrospective research performed by Bashour et al. on 191 cirrhosis patients found that 64% of the study participants had thrombocytopenia. Leukopenia (WBC, <3,500) was relatively rare in the cirrhotic group, having a prevalence of 5% (7.59 +/- 4.3) versus 3.3% (10.62 +/- 14.2) of noncirrhotic patients (46).

A study performed in Nigeria by F.A. Fasola et al. (2009) among 50 patients with acute viral hepatitis found that 12(24%) were anemic. The WBC count was abnormal in 8(16%) of the patients. From the total participants 2(4%) of the patients were leucopenic. On the other hand, 6(12%) of the patients showed neutrophilic and lymphocytic leukocytosis. None of the patients had showen thrombocytopenia (40).

3. Objectives

3.1 General Objective

- To assess hematological abnormalities and its associated factors among liver disease patients at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia, 2023.

3.2 Specific Objectives

- To determine the magnitude of hematological abnormalities among liver disease patients.
- To assess the associated factors of hematological abnormalities among liver disease patients.

4. Materials and Methods

4.1 Study Area

The study was conducted at St. Paul's Hospital Millennium Medical College which is found in Ethiopia's capital, Addis Ababa. The capital, Addis Ababa, is Ethiopia's largest city and contains 608 health institutions (hospitals, health centers, and private clinics) that serve a population of almost three million people based on the report in 2017.St. Paul's Hospital Millennium Medical College is one of the largest hospitals found in the capital which was built by the Emperor Haile Selassie in 1961 with the help of the German Evangelical Church. It currently has 392 beds and 1200 clinical and non-clinical staff members that provide medical specialty services for an annual average of 300,000 patients and a catchment population of more than 5 million(55). The hospital has different departments, one of these being the gastroenterology and hepatology department which serves and treats patients including liver disease.

4.2 Study Design and Period

A hospital-based cross-sectional study was conducted from January to May 2023 at St. Paul's Hospital Millennium Medical College, Ethiopia.

4.3 Population

4.3.1 Source Population

All liver disease patients who were attending at St. Paul's Hospital Millennium Medical College, Ethiopia.

4.3.2 Study Population

Liver disease patients who were attending at St. Paul's Hospital Millennium Medical College, Ethiopia during the data collection period and fulfills the inclusion criteria.

4.4 Inclusion and Exclusion Criteria

4.4.1 Inclusion Criteria

All liver disease patients' including acute, chronic, viral hepatitis, ALD and NAFLD patients whom were attending at St. Paul's Hospital Millennium Medical College during the study period and volunteer patients of both sexes were included.

4.4.2 Exclusion Criteria

Liver disease patients who have comorbidities like HIV, Tuberculosis, diabetes millets, heart disease, a history of hereditary coagulation disease, critically ill, recent transfusion, recent surgery. Additionally, patient who took any oral contraceptives and drugs such as aspirin, heparin, warfarin, pregnant females and patients with known hematological malignancy were excluded from the study.

4.5 Study Variables

4.5.1 Dependent Variables

- Hematological abnormalities

4.5.2 Independent Variables

Socio-demographic characteristics

- Age, sex, religion, residence, marital status, education and occupation.

Clinical characteristics

- Habit of alcohol drinking
- Habit of smoking
- Physical exercise habit
- Nutritional status
- Type of liver disease (stage)
- Etiology of liver disease

4.6 Sample Size Calculation and Sampling Methods

4.6.1 Sample Size Calculation

The required sample size for this study was calculated using single population proportion formula and considering the following assumptions. Since there is no study conducted on this study in Ethiopia, here sample size can be calculated by using 50% proportion with 95% confidence interval and 5% marginal error, sample size (n) was determined using the following statistical formula.

$$n = \frac{Z^2\, p(1-p)}{d^2} = \frac{(1.96)^2 * 0.5(1-0.5)}{(0.05)^2} = 384$$

The total sample size becomes 380.

Where,

d = Margin of error between the sample and the population (d=5%)

n = minimum number sample size

Z α/2 = 95% confident interval (1.96)

Since the total number of liver disease patients in the hospital per year was 1081(data taken from the previous annual report) which is less than 10,000, a reduction formula was used.

$$\text{So, n (final)} = \frac{n}{1+n/N} = \frac{384}{1+(\frac{384}{1081})} = 282$$

By adding 10% non-responsive rate, the final sample size becomes 310.

4.6.2 Sampling Methods

A convenient sampling technique was used for all liver disease patients at St. Paul's Hospital Millennium Medical College during data collection time.

4.7 Measurement and Data Collection

4.7.1 Data Collection Procedure

4.7.1.1 Socio-demographic and clinical data collection

The socio-demographic data and other related medical, lifestyle, and nutritional data were collected by the principal investigator and trained nurse professionals, using a pre-tested structured questionnaire and data collection sheet respectively through face-to-face interview, which was developed following a thorough review of works of literature from different sources.

4.7.1.2 Sample collection procedures

After obtaining consent from the participants, the blood samples were collected from the patients using 5ml EDTA tubes and transported to the hematology laboratory for the investigation of CBC and ESR as soon as possible. Phlebotomists and Laboratory technologists at St. Paul's Hospital Millennium Medical College collected the blood and performed the test according to the standard operating procedure (SOP) of the laboratory under the supervision of the principal investigator (PI).

4.7.1.3 Laboratory Analysis

Hematological parameter analysis

Blood samples were run for complete blood count within two hours of sample collection. Total WBC, absolute and relative neutrophil, lymphocyte, monocyte, eosinophil and basophil counts, RBC, Hb, MCV, MCH, MCHC, RDW, Platelet, MPV and PDW of the study participants was performed by using five differential UnicelDxH 800 (Beckman Coulter, Ireland) automated hematology analyzer. The coulter analyzes the specimen via the principle of impedance, optical method and spectrophotometry as explained in the Annex part. The results were printed and hematological parameters were summarized.

Leucocyte and platelet levels were described based on the laboratory’s reference range which was derived from the output of the Beckman Coulter haematology analyzer. Leucopoenia was defined as having a WBC count of less than 4 x 109/L, whereas leucocytosis was defined as having a number more than 11 x 109/L. When it comes to the platelet count, thrombocytopenia is

defined as having a number less than 150 x 109/L, whereas thrombocytosis is defined as having a value higher than 300 x 109/L. For the determination of anemia, the WHO2011 haemoglobin (Hb) concentration cut-offs for the diagnosis of anemia was utilized. According to this assessment, hemoglobin level less than 13 g/dl for men and 12 g/dl for women was considered anemic.

ESR measurement

Leftover EDTA anti coagulated blood samples were diluted with 3.1 g/L tri–sodium citrate in 1:4 ratio, 1 part of citrate to 4 parts of blood. Tri–sodium citrate diluted blood samples were transferred to a vertical test tube (westergren tube) which is graduated from 0 to 200 in top to bottom form and stand for one hour on the Westergren method of ESR determination set up. The red blood cells settled to the bottom because of gravitational force. The process of falling or settlement of RBCs after forming rouleaux (stack of RBC) is called sedimentation. After one hour the height of plasma which is the clear straw colored fluid at the top of the tube was measured and reported in millimeters per hour (mm/hr).

Elevated ESR was described as, greater than 15mm/hr for men less than 50 years and 20mm/hr for female less than 50 years. For the age group greater than 50 years old elevated ESR was described as, greater than 20mm/hr for men and 30 mm/hr for female.

4.8 Data Quality Assurance

Data quality assurance was implemented on pre-analytical, analytical and post analytical phases of the research.

Pre-analytical

Data quality was ensured by giving short training to data collectors prior to data collection. Data extraction format was used to extract clinical data of patients by nurse health professionals. After data collection process, the data was checked for completeness. The specimens were also checked for serial number, quality and sample acceptance and rejection criteria.

Analytical

Before testing patient samples, reagents were checked for expiration date and instruments quality were checked. During the testing phase data quality was ensured by following the prepared SOP and assay instructions at various activities of the study. Three levels of whole blood controls

(High, Normal, and Low) were tested every day before running patient sample. At the end leftover samples were disposed safely following the laboratory's SOP for waste management.

Post analytical

The data extraction format which should include all the necessary information of the participant was checked for completeness. After the test is done the CBC and ESR results were properly recorded in a well-prepared format.

4.9 Data Analysis and Interpretation

Data was entered, coded, cleaned, sorted, and analyzed using the Statistical Package for Social Sciences (SPSS) software (version 28). Frequencies, proportion, and summary statistics were used to summarize the data for the study population concerning relevant variables. Both bivariate and multivariable logistic regressions were used to assess associations between dependent and independent categorical variables. Important variables with a P value of <0.25 from the bivariate analysis were selected and fitted into multivariable logistic regression analysis. Hosmer and Lemeshow's goodness fit statistic was used to check for the model of fitness. Compiled results were presented in the form of text and tables.

4.10 Operational Definitions

- **Liver cirrhosis:** is scarring (fibrosis) of the liver caused by long-term liver damage.
- **Anemia:** According to WHO guideline for anemia, hemoglobin value less than 13 g/dl for men and 12 g/dl for women.
- **Chronic liver disease:** progressive deterioration of liver function for more than six months.
- **Habit of doing physical exercise:** performing active exercise daily for about 30 min and above.
- **Habit of eating fatty food:** It is the routine consumption of animal-derived proteins as one's main source of nutrition, such as beef, chicken, pork, fish, or other meats.
- **Habit of drinking alcohol:** Daily intake of pure alcohol greater than 30g. It is also defined as drinking more than 2 drinks per day or 14 drinks per week for men and 1 drink per day or 7 drinks per week for female.

- **Habit of smoking:** Smoking at least 1 cigarette per day.
- **Hypersplenism:** is a condition in which the spleen is overactive and removes too many blood cells from the bloodstream.

4.11 Ethical Considerations

This study was conducted after the proposal is reviewed and ethically cleared by the Departmental Ethics and Research Committee of the Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University. Letter of cooperation was written for St. Paul's Hospital Millennium Medical College to get permission to undertake the study. Respondents were informed about the objectives and purpose of the study and verbal consent was obtained from each respondent to use their clinical data and blood samples. Their confidentiality of the information was assured throughout the study. Moreover, all the study participants were informed that they have a full right to participate or decline from participating in the study with no effect on their clinical care in the hospital. All international and institutional research ethics conventions were strictly obeyed during the study process.

4.12 Dissemination of the result

The findings of this research will be submitted to the Department of Medical Laboratory Sciences for public defense in the presence of MLS staffs and students. The final paper will be submitted to Addis Ababa University College of Health Science, Department of Medical Laboratory and to St. Paul's Hospital Millennium Medical College for future reference. In addition, the findings will be presented on scientific conferences to reach the wider medical community and published on reputable and highly accessed journals.

5. Results

5.1 Socio-demographic characteristics of liver disease patients

In this study, a total of 310 study participants were included. Of them, 172 (55.5%) were males. The mean age of the participants was 41±12.8 years, ranging from 18 to 71years. The mean BMI of the study participants was22.74±3 and 76.8 % of the participants were urban dwellers.135 (43.5%), 145(46.5%), 115(37.1%) of the study participants were Orthodox, married and private workers respectively. Participants who were in collage and above were 109(32.4%).

Table 1. Socio demographic characteristics of Liver disease patients at St. Paul's Hospital Millennium Medical College, 2022. (n=310)

Variables		Frequency	Percentage (%)
Age	18-30	72	23.2
	31-45	133	42.9
	46-60	84	27.1
	Above 60	21	6.8
Gender	Male	172	55.5
	Female	138	44.5
Residence	Urban	238	76.8
	Rural	72	23.2
Marital status	Single	118	38.1
	Married	145	46.8
	Divorced	33	10.6
	Widowed	14	4.5
Religion	Orthodox	135	43.5
	Muslim	88	28.4
	Protestant	64	20.6
	Other	23	7.4
Educational background	Cannot read and write	52	16.8
	Primary school	51	16.5
	Secondary school	98	31.6
	Collage and above	109	35.2
Ethnicity	Amhara	87	28.1
	Oromo	127	41
	Tigray	42	13.5
	Others	54	17.4
Occupation	Employee	92	29.7
	Student	43	13.9
	House wife	47	15.2
	Private	115	37.1

	Other	13	4.2
Mean Age of study participants		41±12.8	
Mean weight of study participants		61.5±9.2	
Mean height of study participants		1.64±.08	
Mean BMI of study participants		22.7±.2.9	

NB. Mean weight of study participants is in Kilogram; mean height of study participants is in Meters, BMI: Body Mass Index

5.2 Nutritional and behavioral characteristics of study participants

From the study participants, 95(30.6%) were alcohol drinkers, 56(18.1%) had a habit of smoking cigarette frequently and 74(23.9%) participants had frequent habit of doing physical exercise. Furthermore 63 (20.3%) of the participants frequently consume fatty food like meat.

Table 2.Lifestyle characteristics of liver disease patients at St. Paul's Hospital Millennium Medical College, 2022. (n=310)

Variable	Duration	Frequency
Smoking habit	Yes	56(18.1)
	No	254(81.9)
Alcohol drinking habit	Yes	95(30.6)
	No	215(69.4)
Physical Exercise habit	Yes	74(23.9)
	No	236(76.1)
Fatty food consumption	Yes	63(20.3)
	No	247(79.7)

5.3 Clinical characteristics of study participants

From the total of 310 study participants 61(19.7%) had a family history of liver disease whereas 97(31.3) don’t know whether or not they have a family with liver disease. From the total study participants 28(9%) were acute liver disease patients whereas 165(53.2%) were chronic liver disease patients. Based on the cause (etiology) of the disease 37(11.9%) had ALD, 23(7.4%) had NAFLD, and 206(66.5) had hepatitis B and/or Hepatitis C virus.

Table 3.Clinical characteristics of Liver disease patients at St. Paul's Hospital Millennium Medical College, 2022. (n=310)

Characteristics		Frequency (%)
Family history of liver disease	Yes	61(19.7)
	No	152(49)
	Don't know	97(31.3)
Type of liver disease (stage)	Acute	28(9)
	Chronic	165(53.2)
Type of LD based on cause (etiology)	NAFLD	23(7.4)
	ALD	37(11.9)
	Viral Hepatitis (HBV and HCV)	206(66.5)

5.4 Hematological abnormality of study participants

In this study different hematological profiles were assessed. From the study participants 79(25.5%) were anemic. Elevated ESR were observed among 77(24.8%) of the study participants. Furthermore, leukopenia and thrombocytopenia were observed among 47(15.2%) and 105(33.9%) of the study participants respectively.

5.5 Factors associated with hematological abnormalities

Both bivariable and multivariable logistic regression ware performed to determine the association between different hematological abnormalities and their associated factors. Variables with a p-value of less than 0.25 in the bivariable analysis were selected for multivariable logistic regression. The analysis includes independent variables that satisfy the chi square assumption.

5.5.1 Factors associated with Anemia

In the bivariable logistic regression analysis age, drinking alcohol frequently (p=0.001), habit of consuming fatty food (meat) frequently as well as not doing physical exercise regularly were associated with anemia. Furthermore, both acute and chronic liver disease, alcoholic liver disease and viral hepatitis were significantly associated with anemia.

After performing multivariable logistic regression analysis, chronic liver disease (AOR=11.8; 95%CI:4.3,32.1) and acute liver disease (AOR=17;95%CI:4.8,59.9) were significantly associated

with anemia. The habit of not doing physical exercise (AOR=0.24;95%CI:0.08-0.68) were associated with anemia.

Table 4.Bivariable and multivariable logistic regression analysis of factors associated with anemia among Liver disease patients at St. Paul's Hospital Millennium Medical College, 2022. (n=310)

Variable	Category	Anemia		COR (95% CI)	P-value	AOR (95% CI)
		Yes N (%)	No N (%)			
Age	18-30	16(22.2)	56(77.8)	**1**[a]		
	31-45	31(23.3)	102(76.7)	0.94(0.47-1.86)	0.86	
	46-60	27(32.1)	57(67.9)	0.6(0.29-1.24)	0.17	
	Above 60	5(23.8)	16(76.2)	0.9(0.29-2.88)	0.87	
Gender	Male	42(24.4)	130(75.6)	0.88(0.52-1.47)	0.63	
	Female	37(26.8)	101(73.2)	**1**[a]		
Residence	Urban	64(26.9)	174(73.1)	0.3(0.74-2.64)	0.77	
	Rural	15(20.8)	57(79.2)	**1**[a]		
Educational background	Cannot read and write	12(23.1)	40(76.9)	1.14(0.9-1.44)	0.26	
	Primary school	16(31.4)	35(68.6)			
	Secondary school	32(32.7)	66(67.3)			
	Collage and above	19(17.4)	90(82.6)	**1**[a]		
Smoking habit	Yes	16(28.6)	40(71.4)	1.21(0.63-2.3)	0.56	
	No	63(24.8)	191(75.2)	**1**[a]		
Alcohol drinking habit	Yes	36(37.9)	59(62.1)	2.44(1.43-4.16)	0.001	1.6(0.8-3.3)
	No	43(20)	172(80)	**1**[a]		
Physical Exercise habit	Yes	6(8.1)	68(91.9)	**1**[a]		
	No	73(30.9)	163(69.1)	5.07(2.1-12.2)	0.000	0.24(0.08-0.68) *
Fatty food consumption	Yes	20(31.7)	43(68.3)	0.2(1.48-2.71)	0.2	1.28(0.64-2.6)
	No	59(23.9)	188(76.1)	**1**[a]		
Acute LD	Yes	10(35.7)	18(64.3)	1.71(0.76-3.9)	0.19	17(4.8-59.9) *
	No	69(24.5)	213(75.5)	**1**[a]		
Chronic LD	Yes	65(39.4)	100(60.6)	6.08(3.22-11.46)	0.000	11.8(4.3-32.1) *
	No	14(9.7)	131(90.3)	**1**[a]		
NAFLD	Yes	6(26.1)	17(73.9)	1.04(0.4-2.7)	0.95	
	No	73(25.4)	214(74.6)	**1**[a]		

ALD	Yes	18(48.6)	19(51.4)	3.3(1.6-6.66)	0.001	1.7(0.63-4.6)
	No	61(22.3)	212(77.7)	**1**[a]		
Viral Hepatitis	Yes	44(21.4)	162(78.6)	0.54(0.32-0.9)	0.02	1.77(0.87-3.65)
	No	35(33.7)	69(66.3)	**1**[a]		

N.B: *Shows significant association, **1**[a]= shows reference group**, COR**= crude odds ratio, **AOR**= adjusted odds ratio, ALD=Alcoholic liver disease, NAFLD=Non-alcoholic fatty liver disease, LD=Liver disease

5.5.2 Factors associated with Leucopenia

Leukopenia was associated with residence, habit of frequently drinking alcohol and habit of not doing physical exercise in the bivariable analysis. Moreover, chronic liver disease type was significantly associated with leucopenia in the bivariable analysis (p<0.002).

After performing multivariable logistic regression analysis chronic liver disease (AOR=2.6;95%CI:1.2,5.7) were significantly associated with leucopenia.

Table 5.Bivariable and multivariable logistic regression analysis of factors associated with Leukopenia among Liver disease patients at St. Paul's Hospital Millennium Medical College, 2022. (n=310)

Variable	**Category**	**Leukopenia**		**COR (95% CI)**	**P-value**	**AOR (95% CI)**
		Yes N (%)	No N (%)			
Age	18-30	11(15.3)	61(84.7)	**1**[a]		
	31-45	22(16.5)	111(83.5)	0.9(0.4-2.0)	0.81	
	46-60	11(13)	73(87)	1.19(0.48-2.95)	0.69	
	Above 60	3(14.3)	18(85.7)	1.08(0.27-4.3)	0.91	
Gender	Male	24(14)	148(86)	0.81(0.44-1.51)	0.5	
	Female	23(16.7)	115(83.3)	**1**[a]		
Residence	Urban	40(16.8)	198(83.2)	1.88(0.8-4.4)	0.15	1.88(0.8-4.5)
	Rural	7(9.7)	65(90.3)	**1**[a]		
Educational status	Can't read and write	9(17.3)	43(82.7)	1(0.8-1.3)	0.99	
	Primary school	7(13.7)	44(86.3)			
	Secondary school	13(13.3)	85(86.7)			
	College and above	18(16.5)	91(83.5)	**1**[a]		
Smoking	Yes	8(14.3)	48(85.7)	0.9(0.4-2.09)	0.84	

habit	No	39(15.4)	215(84.6)	**1**[a]		
Alcohol drinking habit	Yes	18(18.9)	77(81.1)	1.5(0.78-2.9)	0.22	1.04(0.52-2.09)
	No	29(13.5)	186(86.5)	**1**[a]		
Physical Exercise habit	Yes	6(8.1)	68(91.9)	**1**[a]		
	No	41(17.4)	195(82.6)	2.38(0.97-5.86)	0.06	1.6(0.6-4.15)
Fatty food consumption	Yes	10(15.9)	53(84.1)	1.07(0.5-2.3)	0.86	
	No	37(15)	210(85)	**1**[a]		
Acute LD	Yes	4(14.3)	24(85.7)	0.93(0.3-2.8)	0.9	
	No	43(15.2)	239(84.8)	**1**[a]		
Chronic LD	Yes	35(21.2)	130(78.8)	2.98(1.48-6)	0.002	2.6(1.2-5.7) *
	No	12(8.3)	133(91.7)	**1**[a]		
NAFLD	Yes	5(21.7)	18(78.3)	1.62(0.57-4.6)	0.37	
	No	42(14.6)	245(85.4)	**1**[a]		
ALD	Yes	7(18.9)	30(81.1)	1.36(0.6-3.3)	0.5	
	No	40(14.7)	233(85.3)	**1**[a]		
Viral Hepatitis	Yes	30(14.6)	176(85.4)	0.87(0.46-1.67)	0.68	
	No	17(16.3)	87(83.7)	**1**[a]		

N.B: *Shows significant association, **1**[a]= shows reference group**, COR**= crude odds ratio, **AOR**= adjusted odds ratio, ALD=Alcoholic liver disease, NAFLD=Non-alcoholic fatty liver disease, LD=Liver disease

5.5.3 Factors associated with Thrombocytopenia

In the bivariate statistics age, alcohol drinking habit, habit of not doing regular exercise ($p<0.000$) and fatty food consumption were significantly associated with thrombocytopenia. Chronic liver disease ($p<0.0001$) and alcoholic liver disease did show significant association with thrombocytopenia in the bivariate analysis.

After performing multivariable analysis participants who had a habit of alcohol drinking were 4 folds (AOR=4.28 %CI:2.07,8.8) more likely associated with thrombocytopenia than nondrinkers. Besides low platelet count than normal were significantly associated with chronic liver disease (AOR=3.04;95%CI:1.59-5.8). From the study participants old adults (age between 46 and 60 years) were 3 fold (AOR=3.1;95%CI:1.77,5.5) more likely associated with thrombocytopenia than young adult age groups(age between 18 and 30 years).

Table 6.Bivariable and multivariable logistic regression analysis of factors associated with Thrombocytopenia among Liver disease patients at St. Paul's Hospital Millennium Medical College, 2022. (n=310)

Variable	Category	Thrombocytopenia		COR (95% CI)	P-value	AOR (95% CI)
		Yes N (%)	No N (%)			
Age	18-30	19(26.4)	53(73.4)	**1**[a]		
	31-45	28(21)	105(79)	1.34(0.68-2.62)	0.386	
	46-60	51(60.7)	33(39.3)	0.23(0.11-0.46)	0.000	3.1(1.77-5.5) *
	Above 60	7(33.3)	14(66.7)	0.71(0.25-2.04)	0.53	
Gender	Male	61(35.5)	111(64.5)	1.17(0.73-1.88)	0.5	
	Female	44(31.9)	94(68.1)	**1**[a]		
Residence	Urban	82(34.5)	156(65.5)	1.12(0.64-1.97)	0.69	
	Rural	23(31.9)	49(68.1)	**1**[a]		
Educational status	Can't read and write	18(34.6)	34(65.4)	1.09(0.88-1.36)	0.41	
	Primary school	16(31.4)	35(68.6)			
	Secondary school	42(42.9)	56(57.1)			
	College and above	29(26.6)	80(73.4)	**1**[a]		
Smoking habit	Yes	21(37.5)	35(62.5)	1.21(0.67-2.21)	0.53	
	No	84(33.1)	170(66.9)	**1**[a]		
Alcohol drinking habit	Yes	52(54.7)	43(45.3)	3.7(2.22-6.15)	0.000	4.28(2.07-8.8) *
	No	53(24.7)	162(75.3)	**1**[a]		
Physical Exercise habit	Yes	12(16.2)	62(83.8)	**1**[a]		
	No	93(39.4)	143(60.6)	3.36(1.72-6.6)	0.000	2.4(1.06-5.5) *
Fatty food consumption	Yes	26(41.3)	37(58.7)	1.5(0.85-2.64)	0.17	1.08(0.55-2.13)
	No	79(32)	168(68)	**1**[a]		
Acute LD	Yes	8(28.6)	20(71.4)	0.76(0.32-1.8)	0.54	
	No	97(34.4)	185(65.6)	**1**[a]		
Chronic LD	Yes	80(48.5)	85(51.5)	4.52(2.66-7.66)	0.000	3.04(1.59-5.8) *
	No	25(17.2)	120(82.8)	**1**[a]		
NAFLD	Yes	7(30.4)	16(69.6)	0.84(0.34-2.12)	0.72	
	No	98(34.1)	189(65.9)	**1**[a]		
ALD	Yes	20(54.1)	17(45.9)	2.6(1.3-5.2)	0.007	0.67(0.24-1.85)
	No	85(31.1)	188(68.9)	**1**[a]		
Viral	Yes	64(31.1)	142(68.9)	0.69(0.42-1.13)	0.14	1.96(0.92-4.18)

Hepatitis	No	41(39.4)	63(60.6)	**1**[a]		

N.B: *Shows significant association, **1**[a]= shows reference group**, COR**= crude odds ratio, **AOR**= adjusted odds ratio, ALD=Alcoholic liver disease, NAFLD=Non-alcoholic fatty liver disease, LD=Liver disease

5.5.4 Factors associated with elevated ESR

In the bivariable logistic regression analysis elevated ESR was associated with age, gender, residence, educational background, fatty food consumption and smoking habit. Furthermore, alcohol drinking (p<0.000), ALD (p=0.001), viral hepatitis (p=0.005) and CLD (p<0.000) showed significant association in the bivariable analysis.

After adjusting for covariates in the multivariable logistic regression analysis alcohol drinking habit (AOR=2.24;95%CI:1.04,4.8) and chronic liver disease (AOR=4.98;95%CI:2.3-10.7) were significantly associated with elevated ESR. Moreover elevated ESR was significantly associated with old age (above 60 years) groups (AOR=2.4;95%CI:1.3,4.4) and urban dwellers in the multivariate analysis.

Table 7.Bivariable and multivariable logistic regression analysis of factors associated with elevated ESR among Liver disease patients at St. Paul's Hospital Millennium Medical College, 2022. (n=310)

Variable	Category	Elevated ESR		COR (95% CI)	P-value	AOR (95% CI)
		Yes N (%)	No N (%)			
Age	18-30	9(12.5)	63(87.5)	**1**[a]		
	31-45	24(18)	109(82)	0.65(0.28-1.48)	0.305	
	46-60	32(38)	52(62)	0.23(0.1-0.53)	0.001	
	Above 60	12(57.1)	9(48.9)	0.1(0.03-0.32)	0.000	2.4(1.3-4.4) *
Gender	Male	55(32)	118(68)	2.48(1.4-4.3)	0.001	1.78(0.93-3.43)
	Female	22(15.9)	116(84.1)	**1**[a]		
Residence	Urban	69(29)	169(71)	0.47(0.23-0.92)	0.03	2.75(1.05-7.17) *
	Rural	8(11.1)	64(88.9)	**1**[a]		
Educational background	Cannot read and write	7(13.5)	45(86.5)	0.44(0.15-1.12)	0.08	1(0.29-3.46)
	Primary school	14(27.5)	37(72.5)	0.39(0.16-0.97)	0.04	0.67(0.23-1.97)

	Secondary school	28(28.6)	70(71.4)	0.45(0.18-1.11)	0.08	0.94(0.3-2.91)
	Collage and above	28(25.7)	81(74.3)	**1**[a]		
Smoking habit	Yes	19(33.9)	37(66.1)	1.74 (0.93-3.25)	0.08	1.21(0.57-2.6)
	No	58(22.8)	196(77.2)	**1**[a]		
Alcohol drinking habit	Yes	41(43.2)	54(56.8)	3.78(2.2-6.5)	0.000	2.24(1.04-4.8) *
	No	36(16.7)	179(83.3)	**1**[a]		
Physical Exercise habit	Yes	11(14.9)	63(85.1)	**1**[a]		
	No	66(28)	170(72)	2.22(1.1-4.5)	0.025	1.05(0.45-2.45)
Fatty food consumption	Yes	15(23.8)	48(76.2)	0.93(0.49-1.78)	0.83	
	No	62(25.1)	185(74.9)	**1**[a]		
Acute LD	Yes	5(17.9)	23(82.1)	0.63(0.23-1.73)	0.37	
	No	72(25.5)	210(74.5)	**1**[a]		
Chronic LD	Yes	64(38.8)	101(61.2)	6.4(3.36-12.3)	0.000	4.98(2.3-10.7) *
	No	13(9)	132(91)	**1**[a]		
NAFLD	Yes	7(30.4)	16(69.6)	1.36(0.54-3.43)	0.52	
	No	70(24.4)	217(75.6)	**1**[a]		
ALD	Yes	18(48.6)	19(51.4)	3.44(1.7-6.7)	0.001	0.84(0.28-2.48)
	No	59(21.6)	214(78.4)	**1**[a]		
Viral Hepatitis	Yes	41(19.9)	165(80.1)	0.47(0.28-0.8)	0.005	1.4(0.64-3.08)
	No	36(34.6)	68(65.4)	**1**[a]		

N.B: *Shows significant association, **1**[a]= shows reference group**, COR**= crude odds ratio, **AOR**= adjusted odds ratio, ALD=Alcoholic liver disease, NAFLD=Non-alcoholic fatty liver disease, LD=Liver disease

6. Discussion

The liver is a vital organ in the human body that serves a wide range of purposes. But a variety of factors can affect these functions, leading to liver disease (1,2). These liver disease patients have a variety of abnormalities. Hematological abnormalities are among the disorders, which are anomalies related to the diverse blood cells (3,6). This hospital based cross sectional study has determined different hematological abnormalities related with their associated factors among liver disease patients. Here we compare our finding with different available studies.

The results of this study indicated that the overall prevalence of anemia, leukopenia, thrombocytopenia and elevated ESR result among liver disease patients were 25.5%, 15,2%, 33.9% and 24.8% respectively. This finding raises a significant issue for liver disease patients in terms of public health. There are various causes for the decrease in blood cells among liver patients.

In the present study, anemia was observed in 39.4% of chronic liver disease patients which was not far from the studies conducted in India(31)(12). In contrast, the finding of this study was lower than a study reported by Scheiner B et al. (66%)(53),Tha et al. (88%), Jha et al (88%) and E Halleys et al. (86%) (34). The difference result regarding anemia might be related with the severity of the disease as such, significantly impaired hematopoiesis usually occurs in people with severe alcoholism who also may suffer from nutritional deficiencies that play a role in blood cell development and socio demography of the study participants.

In our study from the total alcoholic liver disease patients 48.6% had anemia. Previous studies done in India (31,41,42) also showed that alcohol drinking habit and ALD were correlated with anemia. The reason behind this finding might be alcohol which is one of the commonly used drugs in the world has a direct toxic effect on the bone marrow and consequences the suppression of blood cell production or hematopoiesis(43). In many alcoholic patients, blood loss and subsequent iron deficiency are caused by gastrointestinal bleeding (4,41).

In our study chronic liver disease was 11 times (AOR=11.8; 95%CI:4.3,32.1) more likely associated with anemia when compared with non chronic liver disease types. Anemia related with chronic liver disease might be due to variety of reasons, one of these being acute or chronic

blood loss due to hypersplenism secondary to portal hypertension. After spleen becomes overactive following chronic liver disease it starts to breakdown RBCs rapidly (53).

From this study we observed that thrombocytopenia is the most common haematological abnormality among the study participants with 33% prevalence. Thrombocytopenia in the old age group (40-60 years) were 3 fold grater when compared with the young adult age group (18-30 years), which was similar with the studies done in India(11). Furthermore, age group above 60 were 2 fold greater than the young adult age group (18-30 years) regarding elevated ESR value in this study. Different researches deduct the conclusion that liver function and age have inverse relation. With increasing age, liver becomes less functional (54). Consequently thrombopoietin production becomes decreased with less functional liver as liver is the site for TPO production which will in turn decrease the platelet count and leads the patient to thrombocytopenia stage (49).

In this study leukopenia was observed among 15.2% of liver disease patients which is complimentary with a research performed in china (32). This study found that CLD patients were 2 times (AOR=2.6;95%CI:1.2-5.7) more related with leukopenia than other liver disease patients. A similar study performed in India (11) support our finding in which leukopenia is more common in chronic liver disease patients. The reason for low WBC count might be transient bone marrow suppression and increased hemolysis as a result of liver disease which may be caused by viral hepatitis(26). Leucocytosis was less frequent in our patients when compared with other studies performed in Nigeria (12%)(40) and America (45). The difference might be related with the study subject's geographical area as well as stage and severity of the disease.

In this study we found that liver disease patients who didn't perform physical activity regularly were significantly associated with thrombocytopenia. Research performed by Davis et al. also states that participants who perform exercise have high platelet count (56). In case of regular exercise there is a high mobilization of blood cells including platelet from the bone marrow (56). Physical activity has an effect on platelet activation and aggregation even if the mechanism is not well investigated.

In our study thrombocytopenia was significantly associated with alcoholic liver disease. Researches done in Singapore (30) and India (47) also report that thrombocytopenia is significantly associated with ALD. However, alcohol-related thrombocytopenia generally is transient, and platelet counts usually return to normal within 1 week of abstinence (48).In our study from the chronic liver disease patients 48.5% of the study participants had thrombocytopenia which is almost similar to the studies reported in India (50%) (6)and (48%)(11).On the other hand, this result is lower than the study performed by Bashour FN et al(64%) (46). Patients with liver illness who experience thrombocytopenia typically have hypersplenism, in which portal hypertension induces the sequestration of blood cell components, particularly thrombocytes, in the enlarged spleen.TPO, which is primarily produced by the liver, is another possibility that could apply (48,49). Hence, in case of liver disease, decrease in TPO production occurs, this leads to thrombocytopenia (18).

In the present study elevated ESR was observed among 43.2% of alcohol drinkers which is lower than a research done in India (55.4%) (49). In our study elevated ESR and alcoholic liver disease were significantly associated. Although, in this study 48.6% of ALD patients had elevated ESR, it is lower than the studies conducted in United States (55.4%)(50) and India (49). The difference might be related with sociodemography of the study participants as well as severity of the disease. Elevated or abnormal ESR results were due to proinflammatory effect of frequent alcohol drinking (52).

7. Strength and limitation of the study

There are strengths and limitations to this study. This study has the advantage of being the first to identify hematological abnormalities and their associated factors in patients with liver disease in Ethiopian. It tries to identify some hematological abnormalities that will occur among different liver disease cases. On the other hand, this study had some drawbacks, including the fact that it was a cross-sectional study that did not show the causality of hematologic value. Furthermore, the study site being a single hospital and study samples being slightly healthier due to selection bias that only included volunteers as well as lack of validated questionnaire might be limitations.

8. Conclusion and recommendation

8.1 conclusions

Anemia, thrombocytopenia, leukopenia and elevated ESR were common hematological abnormalities among liver disease patients. The abnormalities differ between the different liver disease types as well as they are associated with different factors. In our study generally anemia, thrombocytopenia, leucopenia and elevated ESR were observed with different proportion on most liver disease patients.

In this study, drinking alcohol and a person having alcoholic liver disease were significantly associated with increase in erythrocyte sedimentation rate whereas decrease in platelet and RBC count. Leukopenia was associated with CLD with the etiology of hepatitis B virus.

8.2 Recommendations

Based on the occurrence of different hematological abnormalities in patients with liver disease, it is recommended that healthcare providers should regularly monitor and assess hematological profile in these patients. Assessment of hematological profile and occurrence of hematological abnormalities can aid in easily diagnosis of liver disease patients. Consequently, it can help for timely intervention to prevent complications.

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10. Annexes

Annex I. Participant Information Sheet (English Version)

Addis Ababa University

College of Health Sciences

Department of Medical Laboratory Sciences

Title: Hematological parameters and associated factors among Liver disease patients at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia.

Introduction

This information sheet is prepared by the principal investigator to clarify the study that you are asked to take part in. If there is any unclarity before you decide to participate or not, you can ask freely.

Purpose

This study planned to conduct a study with the objective of assessing Hematological parameters and associated factors among Liver disease patients at St. Paul's Hospital Millennium Medical College, Ethiopia.

Confidentiality

Any information that we are going to collect about you during this research will be kept confidential. Your name and identity on the request paper will be changed to confidentiality code for the purpose of this study. Samples and information given by the participants will serve only for this research not for any other purpose.

Benefit

Findings from this study will be utilized to improve patient care by the medical community including doctors and nurses, managers, administrators, and policy makers. So, you are indirectly benefiting yourself, other patients and the society at large by involving into this research.

Person to contact

Please direct any questions or problems you may encounter during this study to the principal investigator:

Muluken Gashaw, Department of Medical Laboratory, Laboratory Technologist.

Tel: +251948438019 **E-mail:** mlkngashaw@gmail.com

Annex II. Participant Information Sheet (Amharic Version)

አዲስአበባዩኒቨርስቲጤፍሳይንስኮሌጅ

የህክምናላቦራቶሪትምህርትክፍል

ርዕስ፡ Hematological parameters and associated factors among Liver disease patients at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia.

መግቢያ፡

ይህየሚጃወረቀትእንዲሳተፉበትየተጠየቁትንጥናትግልጽለሚድረግሲባልበተሞራሚ ውክኩልተዘጋጅቶየቀረበጽሁፍነው ፡፡

በጥናቱለማሳተፍወይምላለማሳተፍከመወሰንዎበፊትማንኛውምያልገባዎትነገርካለበግልጽማከየቅይችላሉ፡ ፡

የተሳታፊዎች ምክጡር ስለማከበቅ

በዚህጥናትስለእርስዎየ ምንሰበስበውማንኛውምመጂሚክጥራዊነ ቱይጠበቃል፡ ፡

ለጥናቱሲባልበማከየቂያውወረቀትላይያለውንየእርስዎስምምሆነ ማንነ ትወደሚክጥራዊቁጥር ይቀየራል፡ ፡ እንዲሁምየ ሰጡትናሜኛ ምሆነ ሚጃከዚህጥናትውጪለሌላአላማጉቅምአ ይውልም ፡

ጥቅም

ከዚህጥናትየሚኘውውጤትየህመማንንጡፍለማሻሻል፣

በአስተዳዳሪዎችእናበመመሪያአውጬዎችጥቅምላይይውላል፡ ፡

ስለዚህእርስዎምክላቦራቶሪውጤትጥራትጋርበተገናኘየላቦራቶሪጥናታዊምርምርላይበማሳተፍዎ በቀጥታእራስዎትን፣ በተዘዋዋሪደግሞሌሎችህመማን፣

እናበአጠቃላይህብረተሰቡንይጠቅሜልማለትነው ፡

ጥያቄ ካለዎት

ይህንጥናትበተመለከተወይምከዚህጋራበተዛመደመልኩስለሚያጋጥመድንገተኛችግሮችወይምጥያቄ ካለዎትበሚከተለዉአድራሻይጠቀሙ፡

ሞሉቀን ጋሻው፣ ላቦራቶሪ ክፍል ላቦራቶሪ ቴክኖልጂስት፡ ፡

ስልክ፡ +251948438019 **ኢ-ሜይል፡** mlkngashaw@gmail.com

Annex III. Informed Consent Form (English Version)

I, the undersigned, confirm that, as I give consent to participate in the study, it is with a clear understanding of the objectives and conditions of the study and with recognition of my right to withdraw from the study if I change my mind. I give consent to include me in the proposed research. I have been given the necessary information about the research. I have also been assured that I can withdraw my consent at any time without penalty or loss of benefits. The proposal has been explained to me in the language I understand**.**

Name _____

Participant's signature_____

Annex IV. Informed Consent Form (Amharic Version)

ስለጥናቱ ማረጋገጫ

እኔ ስሜ ታችየ ተገለጸውየ ጥናቱ ተሳታፊ ለመሆን ስወስንየ ጥናቱ አላማ
አሰራሮች እና ቅድመሁኔ ታዎች በግልጽ በሚዳ ትእናለጥናቱ ተሳታፊነ ትፍቃደኝነ ቴን በማንኛውም ደረጃየ ማነሳትመብቴን በሚጋገጥነ ው ፡
በመሆኑ ምክ ጥናቱ ተሳታፊ ለመሆን ስወስን በጥናቱ ሲያሊከሰቱየ ሚኖሉአደጋዎችን በማ ባየ ተረዳሁና ከጥናቱ በማንኛውም ደረጃእራሴን ለማ ረዝብወስንተገ ቢየ ሆኑእገዛዎችሁሉእንደሚይነ ፈጉኝ በማመነ ው ፡
እነ ዚህን ሚጃዎችሁሉበ ማ ባበምረዳውቋንቋየ ተገለጸልኝ መሆኑን በፊር ሜ አረጋግጣለሁ፡ ፡

ሞሉስም_____

ፊርማ_____

Annex V. Socio-Demographic, clinical, and nutritional information of Liver patients

ID.No:_____

S. no	Socio-demographic questions please encircle in the correct information you have.		
01.	Age in years	_____	
02.	Sex	Male	1
		Female	2
03.	Where do you live? (Residence)	Urban	1
		Rural	2
04.	What is your occupation?	Employee	1
		Student	2
		Housewife	3
		Private	4
		Others	5
05.	Religion	Orthodox	1
		Muslim	2
		Protestant	3
		Catholic	4
		Others	5
06.	How much is your monthly income?		
07.	What is your highest educational status?	Unable to write and read	1
		Primary School	2
		Secondary school	3
		University/college	4
08.	Marital status	Single	1

		Married	2
		Divorced	3
		Widowed	4

B) Clinical data of a participant

09.	If question 2 is female, have you given birth within 4 months	Yes	1
		No	2
10.	Neoplastic diseases (leukemia)	Yes	1
		No	2
11.	Do you have hypertension	Yes	1
		No	2
12.	Do you have DM	Yes	1
		No	2
Nutritional data of a participant			
13.	Do you eat meat	Yes	1
		No	2
14.	If the answer to question 15 is yes how many times	Daily	1
		Every two day	2
		Every two week	3
		Once a month	4
15.	Do you drink alcohol?	Yes	1
		No	2
16.	If question number 19 yes how many times	Daily	
		Weekly	
		Amount in bottle	
17.	Do you smoke	Yes	1

		No	2
18.	A habit of physical exercise	Yes	1
		No	2
19.	If question number 22 yes how many times	Daily	
		Weekly	
		How long	
20.	Types of liver disease (stage)	Acute	1
		Chronic	2
21.	Types of liver disease (etiology)	Alcoholic	1
		NAFLD	2
		HBV	3
		HCV	4
		HBV and HCV	5
		HBV and NAFLD	6

Annex VI. Socio-Demographic, clinical, and nutritional information of Liver patients (Amharic version)

የ ህ ክ ምና ማ ላ ያ ቁ ጥ ር (MRN)----------

1.ማ ህ በ ራ ዊ ና ህ ዝ ባ ዊ ፤ የ ጤ ፍ (የ ክ ል ኒ ካ ል)ና የ አ ማ ገ ብ ስ ረ ዓ ት ሚ ጃ ዎ ች ማ ኪ ይ ቆ ች

ተ.ቁ	ማ ህ በ ራ ዊ ና ህ ዝ ባ ዊ ማ ኪ ይ ቆ ች ለ እ ር ሰ ዎ ሁ ኔ ታ የ ም ና ኘ ዉ ን ያ ክ ብ ቡ ት		
01.	ዕ ድ ሜ ዎ ት ስ ን ት ነ ው በ ዓ መ ት	_____	
02.	ጾ ታ	ወ ን ድ	1
		ሴ ት	2
03.	መ ኖ ር ያ አ ድ ራ ሻ	ከ ተ ማ	1
		ገ ጠ ር	2
04.	ስ ራ ዎ ት ም ን ድ ነ ዉ	መ ን ግ ስ ት ሰ ራ ተ ኛ	1

		ተማሪ	2
		የ ቤትእ ማኬት	3
		የ ግልስ ራላ ይየ ተሰ ሙ	4
		ሌላ	5
05.	ሐይሞ ት	ኦ ር ቶ ዶክ ስ	1
		ማ ላ ም	2
		ፕ ሮ ቴ ስ ታ ን ት	3
		ካ ቶ ሊ ክ	4
		ሌላ	5
06.	የ ት ም ህ ር ት ደ ረ ጃ ዎ ት ስ ን ት ነ ው?	ማ ፍ አ ና ማ ን በ ብ ሚ ይ ች ል	1
		አ ን ደ ኛ ደ ረ ጃ (1-8ኛ ክ ፍ ል)	2
		ሁ ለ ተ ኛ ደ ረ ጃ	3
		ዩ ኒ ቨ ር ስ ቲ/ኮ ሌ ጅ	4
07.	የ ጋ ብ ቻ ሁ ኔ ታ	ያ ገ ባ/ች	1
		ያ ላ ገ ባ/ች	2
		አ ገ ብ ቶ የ ፈ ቱ	3
		አ ገ ብ ቶ የ ሞ ተ ባ ት/ች በ ት	4

2.የ ክ ል ኒ ካ ል (የ ጡ ፍ ሚ ጃ)

01.	ጥ ያ ቄ ቁ 2 ሴ ትከ ሆ ነ በ ዝ ህ 4ወ ሪ ዉስ ጥ ል ጅ ወ ል ደ ዋ ል	አ ዎ	1
		የ ለ ም	2
02.	የ ደ ም ካ ን ሰ ር በ ሽ ታ አ ለ ብ ወ ት	አ ዎ	1
		የ ለ ም	2
03.	ደ ም ግ ፊ ት አ ለ ብ ወ ት	አ ዎ	1
		የ ለ ም	2
04.	ስ ኳ ር አ ለ ብ ወ ት	አ ዎ	1

		የ ለ ም	2
05.	የ ጉ በ ት በ ሽ ታ አ ይነ ት(በ ደ ረ ጃ ወ ዱ ም በ ጊ ዘ ቆ ይ ታ)	አ ጣ ዳ ፊ	1
		የ ቆ የ	2
06.	የ ጉ በ ት በ ሽ ታ አ ይነ ት(ማ ስ የ)	.በ አ ል ኮ ል	1
		በ ስ ብ	2
		በ ቫ ይ ረ ስ (B)	3
		በ ቫ ይ ረ ስ (C)	4
		በ ቫ ይ ረ ስ (B)እ ና በ ቫ ይ ረ ስ (C)	5
		በ ቫ ይ ረ ስ (B) እ ና በ ስ ብ	6
3.የ አ ሚ ገ ብ ስ ረ ዓ ት ማ ከ ይ ቅ			
07.	ስ ጋ ይ ማ ባ ሉ	አ ዎ	1
		የ ለ ም	2
08.	ተ.ቁ 18 ሜ ል ሰ ዎ አ ጨ ሆ ነ ምን ያ ህ ል ጊ ዜ ይ በ ላ ሉ	በ የ ቀ ኑ	1
		በ የ ሁ ለ ት ቀ ን	2
		በ የ ሁ ለ ት ሳ ምን ት	3
		በ ወ ር አ ን ዴ	4
09.	አ ል ኮ ል ይ ጠ ጣ ሉ	አ ዎ	1
		አ ል ጠ ቀ ም ም	2
10.	ተ.ቁ8አ ዎ ከ ሆ ነ ምን ያ ክ ል	ቀ ን በ ቀ ን	
		በ ሳ ምን ት	
		ምን ያ ክ ል (በ ቢ ራ ጠ ር ማ ት)	
11.	ሲ ጋ ራ ያ ጨ ስ ሉ	አ ዎ	1
		አ ላ ጨ ስ ም	2
12.	የ አ ካ ል ብ ቃ ት እ ን ቅ ስ ቃ ሴ ያ ደ ር ጋ ሉ	አ ዎ	1
		አ ላ ረ ግ ም	2

13.	ተ.ቁ10 ጫእሰዎአ ጪከሆነ ምንያህልጊዜ ይሰራሉ	ቀንበቀን	
		በሳምንት	
		ለምን ያክል ደቂቃ	

Annex VII. Laboratory result record format

ID No**:** _____

Hematological parameters		Result	Remark
RBC parameters	RBC count ($\times 10^{12}$/L)		
	MCV (fl)		
	HCT (%)		
	RDW		
Hemoglobin parameters	Hb (g/dL)		
	MCH (pg)		
	MCHC (g/dL)		
WBC absolute counts ($\times 10^{9}$/L)	Total WBC count		
	Neutrophil		
	Lymphocyte		
	Monocyte		
	Eosinophil		
	Basophil		
WBC differential count (%)	Neutrophil		
	Lymphocyte		
	Monocyte		
	Eosinophil		
	Basophil		
Platelet parameters	PLT count ($\times 10^{3}$/ μL)		
	MPV		
	PDW		

ESR	mm/hr		

Annex VIII: Principles and Laboratory procedure

1. Hematology (CBC) analysis

The reagents utilized are UnicelDxH 800 controls and diluent, lyser, cleaner, and diff pack. The UnicelDxH 800 (BD Coulter, Ireland) coulter system hematology analyzer was used to determine the hematological profiles. The UniCel® DxH 800 Analyzer is a quantitative, automated hematology analyzer used in clinical laboratories for in vitro diagnostic application in screening patient populations. The analyzer offers the following results from whole blood: CBC, WBC 5-part differential, reticulocyte, and nucleated RBC.

Hematological analyser, syringe, EDTA test tube, and personal protection equipment (PEP) are the materials used to determine the hematological profile.

UniCel® DxH 800 (BD coulter, Ireland) analyzerPrinciple

The Coulter Principle is the foundation of the DxH 800 CBC analysis. By detecting and measuring changes in electrical resistance as a particle (such as a cell) in a conductive liquid pass through a tiny aperture, the Coulter Method precisely counts and sizes cells. Each cell serves as an insulator when it is suspended in a conductive liquid (diluent). Each cell temporarily raises the resistance of the electrical route between the submerged electrodes on each side of the aperture as it passes through. A detectable electronic pulse result from this. In that case, the amount of pulses and their height are directly related to the quantity and size of cells, respectively. WBC differential technique uses three measurements: individual cell volume, high-frequency conductivity, and laser-light scatter which is VCS technology (volume, conductive, and light scatter analysis). Numerous cell-by-cell details were obtained using a combination of low-frequency current, high-frequency current, and light-scattering technology; these data were then transformed into data plots using the sample processing module (SPM). Conductivity analysis is the process by which a current is sent through a cell wall and the interior of each cell identifies variations in the insulating qualities of its constituent parts. The current characterizes nuclear and granular constituents and the composition of the cell interior. Light scatter cell analysis measures particle size and refractivity to the angle of side scatter from a laser beam.

Hemoglobin is measured photometrically**. T**he lytic reagent used for the WBC prepares the blood so the system can count leukocytes and measure the amount of hemoglobin. The lytic reagent rapidly and simultaneously destroys the erythrocytes and converts a substantial proportion of the hemoglobinto a stable pigment while it leaves leukocyte nuclei intact. The absorbance of the pigment is directly proportional to the hemoglobin concentration of the sample. Calculated values are derived from measured parameters.

UniCel® DxH 800 (BD coulter, Ireland) complete blood count procedure

1. Collect venous blood specimen with EDTA tube and store properly

2. Ensure the specimen processing module (SPM) is set up for the appropriate test for your workflow

3. Mix the specimen

4. Load the specimens into the cassettes

5. Place the cassettes into the input buffer to the right of the SPM. The SPM automatically begins cycling the cassettes

6. After the SPM cycles the samples, review the sample results at the System Manager

7. Hematological parameters are displayed

8. Inspect for the presence of flags and print out the displayed result and record the result

2. ESR determination

ESR is defined as the rate of RBC sedimentation over an hour. This hematological test is frequently used to detect inflammation that may be brought on by an infection, some malignancies, or certain autoimmune conditions.

The materials used in this study for ESR determination are Westergren tube, Westergren rack, syringe fitted with rubber, syringe and PPE.

The reagent used for ESR determination is tri sodium citrate**.**

Principle for ESR determination

When anticoagulated blood is allowed to stand in a narrow vertical glass tube, undisturbed for some time, the RBCs – under the influence of gravity- settle out from the plasma. The rate at which RBCs settle is measured after one hour (in mm/hr) as the plasma is present at the top of the column.

Procedures of ESR determination

1. Collect venous blood from study participant (asthmatic patient)
2. Mix one part of blood with four parts of 3.1g/L trisodium citrate anticoagulant in a 1:4 ratio (1 part of blood with 4 part of anticoagulant).
3. Fit the syringe fitted with rubber
4. Draw the blood into the Westergren tube up to the '0' mark with the help of a syringe fitted with a rubber
5. Wipe out blood from the bottom of tube with cotton
6. Set the tube upright in the ESR stand. Make sure the pipette fits snugly to eliminate possible leakage and the pipette in a vertical position.
7. Leave the tube undisturbed for one hour
8. At the end of one hour, read the result and report in mm/hr.

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: **MulukenGashaw (B.Sc.)**

Signature: _____

Date of submission: _____

This thesis has been submitted with our approval as advisors.

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Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: **FekaduUrgessa (MSc, PhD fellow)**

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: **JemalAlemu (MSc, PhD fellow)**

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.