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RESEARCH ARTICLE

Liver Enzymes and Bilirubin Abnormalities and Associated Factors Among Patients with Liver Disease in a Tertiary Hospital, Northwest Ethiopia: A Cross-Sectional Study

Abateneh Melkamu[1], Yalew Much[1], Abebe Yensew[1], Melaku Laikemariam[1], Befikad Mandefro[2], Adane Adugna[1], Gashaw Azanaw Amare[1], Aytenew Atnaf[1]

1 Debre Markos University
2 Arba Minch University

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Abstract

Background: Liver disease is a disturbance of the normal and healthy functioning of the liver. It is directly linked to liver synthesis, excretion, and metabolism and results in liver dysfunction. Thus, this study aimed to evaluate the extent of liver function abnormalities and their related factors in patients with liver disease.

Methods: This cross-sectional study was conducted on 307 patients with liver disease using consecutive sampling from August 22, 2022, to October 24, 2022, at the University of Gondar Comprehensive Specialized Hospital. Approximately 3 ml of venous blood specimens was collected and analyzed using a *Bechman coulter 700 chemistry analyzer.* Data were analyzed using Statistical Package for Social Sciences version 25 software. Frequencies and proportions were used to characterize the results. Bivariable and multivariable logistic regression analyses were used to investigate factors associated with liver function abnormalities. Statistical significance was set at P <0.05.

Result: Among 307 patients, 117 (38.11%, 95% CI: 32.64%, 43.57%), 212 (69.06%; 95% CI: 63.85%, 74.25%), 168 (54.72%, 95% CI: 49.12%, 60.32%) patients had abnormal ALT, AST, and total bilirubin respectively. Nearly one-fourth (25.40%) of the patients had all liver function test abnormalities. Male sex (AOR = 0.17; 95% CI: 0.08, 0.38) and the presence of viral hepatitis (AOR = 3.85; 95% CI: 1.23, 12.02) were significantly associated with abnormal liver ALT levels. A history of blood transfusion (AOR=0.45; 95%CI: 0.21, 0.95), lack of vegetable feeding habits (AOR=2.73; 95%CI: 1.34, 5.59), lack of physical exercise habits (AOR=2.97; 95%CI: 1.44, 6.11), and alcoholic liver disease (AOR=17.09; 95%CI: 1.96, 148.98) were significantly associated with AST abnormalities. Being anemic (AOR=3.33; 95%CI: 1.58, 7.08), lack of vegetable feeding habit (AOR=1.98; 95%CI: 1.06, 3.69), and lack of physical exercise habits (AOR=4.03; 95%CI: 1.90, 8.57) were significantly associated with total bilirubin abnormality.

Conclusion: In this study, patients with liver disease showed substantial liver function abnormalities. Viral hepatitis was associated with increased ALT levels, whereas alcoholic liver disease was only associated with increased AST levels. A presence of blood transfusion, lack of physical activity, and lack of vegetables are associated with increased bilirubin

levels. Therefore, clinicians should consider these factors in the management and treatment of patients with liver disease.

Abateneh Melkamu[1,*], **Yalew Muche**[1], **Abebe Yensew**[1], **Melaku Laikemariam**[2], **Befikad Mandefro**[3], **Adane Adugna**[1], **Gashaw Azanaw Amare**[1], and **Aytenew Atnaf**[1]

[1]*Department of Medical Laboratory Science, College of Medicine and Health Sciences, Debre Markos University, Debre Markos, P.O. Box 269, Ethiopia.*

[2]*Department of midwifery, College of Medicine and Health Sciences, Debre Markos University, Debre Markos, P.O. Box 269, Ethiopia.*

[3]*Department of medical laboratory science, College of Medicine and Health Sciences, Arba Minch University, Arba Minch, P.O. Box 21, Ethiopia*

[*]**Corresponding Author:** Abateneh Melkamu, Department of Medical Laboratory Science, College of Medicine and Health Sciences, Debre Markos University, Debre Markos, Ethiopia. P.O. Box 269; email: abatenehmelkamu@gmail.com, abateneh_melkamu@dmu.edu.et.

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Background

The liver, which is located in the right upper quadrant below the diaphragm, is the largest organ of the body. It performs approximately 5,000 different physiological activities. The liver detoxifies different metabolites, produces digestive enzymes, and synthesizes proteins to keep other body systems operational [1].

Liver disease is one of the world's most critical public health problems[2] that can affect liver cells and impair normal liver function [3]. It is characterized by increased liver biochemistry, coagulopathy, and hepatic encephalopathy without underlying chronic liver disease [4]. The most common causes of the rising prevalence of liver disease are non-alcoholic fatty liver disease (NAFLD), which is brought on by obesity, diabetes, autoimmunity, hemochromatosis, and chronic viral hepatitis, primarily from the hepatitis B and hepatitis C viruses. Other causes and types of liver disease caused by medication and chemicals [2].

The liver function test (LFT) is among the most commonly used laboratory investigations to measure its normality. Abnormal results indicate liver disease, even when a person does not have symptoms. Despite of this, LFT detects the severity of liver diseases, the effect of therapies, and drug-induced liver injury [5]. 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 [6].

Liver function tests are groups of blood, urine, and stool tests that provide information about a patient's liver condition. Blood LFT include liver enzyme such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) additionally total bilirubin are main valuable biomarkers of liver injury in a patient with some degree of abnormal liver function [7].

Liver illness is becoming increasingly widespread in globally, with an estimated two million fatalities each year and accounting for 4% of all deaths [8]. In 2019, cirrhosis and other chronic liver disorders caused 2·5% of deaths and 1·3% of total disability-adjusted life-years in Sub-Saharan Africa[9]. In Ethiopia it is the 7th leading cause of death, accounting for about 24 deaths per 100000 populations in 2019 [10]. There is research on the prevalence of liver disease in Ethiopia, despite its consequences, there is a scarcity of information on the liver function test abnormalities and associated factors among liver disease patients in the study area. In this study, the authors aim to evaluate the extent of liver function abnormalities, associated factors among liver disease patients at the University of Gondar Comprehensive Specialized Hospital Northwest, Ethiopia, in 2022

Methods and materials

Study design, area, and period

Cross-sectional research was carried out at the University of Gondar Comprehensive Specialized Hospital (UoG-CSH) from August 22,2022, to October 24, 2022. The hospital is located in Gondar. Gondar is located 727 kilometers northwest of Ethiopia's main city, Addis Ababa, and 175 kilometers from Bahir Dar, the seat of the Amhara National Regional State. According to the 2015 report of the central statistical agency of Ethiopia, Gondar has a population of 323,900 [11]. The town has one public comprehensive specialty hospital, which is one of the oldest teaching hospitals in the country and offers health services for more than 7 million people in Gondar town and its catchment regions [12].

Operational definitions

- **Habit of drinking tea or coffee**: Habitual tea/coffee drinkers are defined as tea/coffee 120 mL or more of tea/coffee each day for at least a year [13].
- **Alcohol drinking Habit:** Three drinks or more in a day for men and two drink or more in a day for women[14].
- **AST normal**: If the serum levels were from 8 to 35 IU/L for female and 6 to 31 IU/L for males[15].
- **ALT normal**: If the serum levels were from 4 to 45 IU/L for males and 2 to 34 IU/L for females[15].
- **Normal serum total bilirubin**: If the reference range from 0.1 to 1.2 mg/dl[15].
- **Physical exercise**: Participant who engages in physical exercise for around 30 minutes each day[16].
- **Habit of feeding vegetables**: It is a consistent action to integrate a variety of vegetables into one's diet or meal plan [17].
- **Habit of feeding meat**: It is the use of animal-derived proteins as the main source of food in a person's diet, such as fish, chicken, cattle, or other meats [18].

Sample size determination and sampling techniques

The required sample size for this study was calculated using the single population proportion formula and considering the following assumptions. Since no study has been conducted to show the magnitude of basic coagulation abnormalities in Ethiopia, the sample size was calculated using a 50% proportion with a 95% confidence interval and a 5% margin of error. Sample size (n) was determined by using the following statistical formula.

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

Since the population at UoG-CSH was 1509, which is below 10,000, the sample size was corrected by using the correction formula and given to 307.

$$no = \frac{n}{1 + \frac{n}{N}} = \frac{384}{1 + \frac{384}{1509}} \approx 307$$

Where

- d = Margin of error between the sample and the population (d=5%)
- n_o= minimum number sample size
- N=Population size
- n = uncorrected sample size
- $Z_{\alpha/2}$ = 95% confident interval (1.96)

Study participants were collected using Consecutive sampling method in patients with liver disease

Population and variables

Patients with liver illness who attended the UoG-CSH during the data collecting period and met the inclusion criteria were considered a study population. Although the liver function parameters (ALT, AST, and total bilirubin) were taken as dependent variables, the socio-demographic variables, clinical variables and behavioural related variables were taken as independent variables.

Inclusion and exclusion criteria

All liver disease pathogen attending at UoG-CSH during data collection patient were included in the study. Study participants with critically ill patients and pregnant women, had excluded from the study.

Data collection methods and data quality management

A structured questionnaire was pretested at Maraki Health center, Gondar, after pretest sociodemographic, lifestyle, and nutritional information collected using a face-to-face interview by trained nurses from all ward in UoG-CSH. A data

extraction sheet was used to get the clinical data from the patient's medical files. Three milli liter of venous blood collected using needle and syringe method and dispensed by serum separator tube by medical laboratory technologist professionals. Liver function tests (ALT, AST, and total bilirubin) were analyzed by an automated clinical chemistry analyzer (Beckman Coulter DXC 700, United States of America). The machine determines the bilirubin level by using the diazo method for bilirubin determination. The indirect portion of bilirubin requires a solubilizing agent, such as a surfactant. A stabilized diazonium salt, 3,5-dichlorophenyldiazonium tetrafluoroborate, reacts with bilirubin to form azobilirubin. Caffeine and a surfactant are reaction accelerators, and reactions absorb at 570/660 nm absorbance, which is converted into concentration by the spectrophotometry method [19]. The principle of measuring ALT activity is based on the fact that ALT catalyzes the reversible conversion of L-alanine and -ketoglutarate to pyruvate and L-glutamate. In the presence of lactate dehydrogenase (LDH), pyruvate is reduced to lactate, while NADH (nicotinamide adenine dinucleotide (NAD + hydrogen H)) is simultaneously oxidized to NAD (nicotinamide adenine dinucleotide). The system monitors the absorbance change rate at 340 nm over a fixed time interval. The absorbance change rate is directly proportional to the ALT activity in the sample [20]. In the AST measurement, AST catalyzes the reversible transamination of L-aspartate and α-ketoglutarate to oxaloacetate and L-glutamate. The oxaloacetate is then reduced to malate in the presence of malate dehydrogenase with the concurrent oxidation of NADH to NAD. The system monitors the absorbance change rate at 340 nm over a fixed-time interval. The absorbance change rate is directly proportional to the AST activity in the sample [20].

Data quality management

To maintain the quality of the data, quality control testing was performed for each procedure. Furthermore, standard operating procedures were strictly followed. Training was given to all data collectors prior to the actual data collection. The investigator strictly controls the data collector and general activity during the data collection period.

Statistical analysis

Epidata version 3.1 was utilized for data entry, coding, and cleaning. The statistical package for the social sciences (SPSS) version 25 software was used to import and analyze the data. To summarize the data, summary statistics, proportions, and frequencies were employed. Pearson rank chi-square assumption fulfillment was checked for categorical variables. Bivariable and multivariable logistic regression were used to determine factors associated with liver function abnormalities. To verify the model fitness assumption, the Hosmer-Lemeshow goodness of fit test was performed, requiring a p-value of larger than 0.05. Finally, the odds ratio with a 95% confidence interval was used to express the strength of the association. The multivariable analyses with p-value < 0.05 indicated variables had a significant association with the result.

Results

Socio-demographic and clinical characteristics of study participants

This research had 307 individuals. Among them, 220 (71.66%), 213 (69.38%), 181 (59.28%), and 143 (46.58%) were male, from rural areas, married, and unable to read and write respectively. Furthermore, the average age of the research participants was 38.38 + 15.13 years, with a range of 6 to 82 years.

From a total of study participants, 157 (51.14%), 87 (28.34%), 37 (12.05%), and 26 (8.47%) of study participants had chronic liver disease (CLD), viral hepatitis, acute liver disease, and alcoholic liver disease (ALD), respectively. Among viral hepatitis study participants, about 71.26% and 28.74 had HBV and HCV, respectively. On the other hand, about 51 (16.61%), 39 (12.70%), and 33 (10.75%) were anemic, had a presence of blood transfusions, and had heart disease, respectively additionally about 86 (28.01%) of the study participants had taken medication other than liver disease drugs. (Table 1).

Table 1. Clinical characteristics of liver disease patients at UoG-CSH Northwest, Ethiopia (n =307).

Variable	Category	Frequency	Percent (%)
Use of medication other than liver disease	Yes	86	28.01
	No	221	71.99
Presence of anemia	Yes	51	16.66
	No	256	83.34
History of tuberculosis	Yes	12	3.90
	No	295	96.10
Presence of DM	Yes	13	4.20
	No	294	95.80
Presence of cardiac disease	Yes	31	10.10
	No	276	89.90
History of blood transfusion	Yes	39	12.70
	No	268	87.30
History of surgery	Yes	10	3.30
	No	297	96.70
Presence of cancer	Yes	8	2.60
	No	299	97.40
Presence of hypertension	Yes	21	6.80
	No	286	93.2
Presence of HIV/AIDS	Yes	6	2.0
	No	301	98.0
Types of liver disease	Acute liver disease	37	12.05
	CLD	157	51.14
	ALD	26	8.47
	Viral hepatitis	87	28.34
Presence of viral hepatitis	Yes	87	28.34
	No	220	71.66

Abbreviations: ***ALD****: Alcoholic Liver Disease,* ***CLD****: Chronic Liver Disease,* ***HIV****; Human Immunodeficiency Virus,* ***DM****; Diabetes Miletus*

Nutritional and life style characteristics of study participants

Of the study participants, about 269 (87.91%), 287 (93.49%), and 237 (77.1%) had a habit of feeding meat, drinking tea or coffee, and consuming vegetables, respectively. despite this, many of the study participants about 262 (85.34%) had not regularly physical exercise habit, and only 11 (3.58%) of study participant had smoking habit (Table 2).

Table 2. Lifestyle characteristics of liver disease patients at UoG-CSH Northwest, Ethiopia (n=307).

Variable	Category	Frequency	Percent (%)
Tea or coffee drinking habit	Yes	270	88.24
	No	37	11.76
Meat feeding habit	Yes	284	92.51
	No	23	7.49
Vegetable feeding habit	Yes	237	7.2
	No	70	22.8
Alcohol drinking habit	Yes	205	66.8
	No	102	33.2
Cigarette smoking habit	Yes	11	3.58
	No	296	96.42
Physical exercise habit	Yes	45	14.66
	No	262	85.34

Magnitude of liver function abnormalities

From the total study participants, 117 (38.11%, 95% CI: 32.64% - 43.57%), 212 (69.06%; 95% CI: 63.85% - 74.25%), 168 (54.72%, 95% CI: 49.12% - 60.32%), of the study participants had abnormal ALT, AST, and total bilirubin respectively. Furthermore, 78 (25.40%) study participants had all liver function abnormalities (Figure 1).

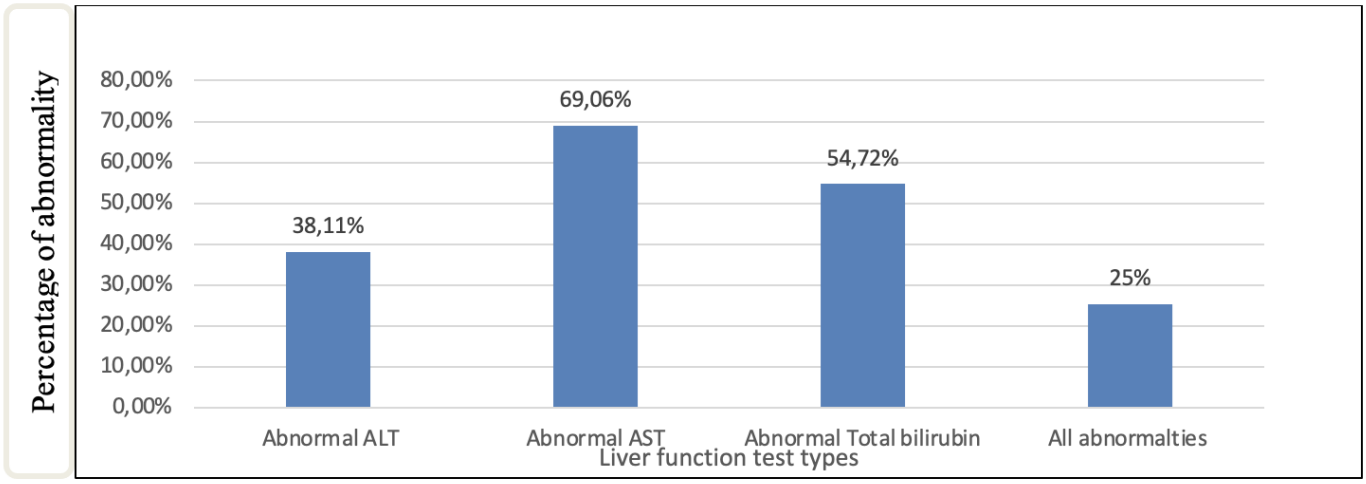

Figure 1. Magnitude of liver function test abnormalities among liver disease patients at the UoG-CSH, Northwest Ethiopia (n=307)

Table 3. Bivariable and multivariable logistic regression of ALT among liver disease patients attending at the UoG-CSH, Northwest, Ethiopia (n=307)

Variables	Category	ALT		Bivariable analysis		Multivariable
		Abnormal (%)	Normal (%)	COR (95%CI)	P-value	AOR (95%CI)
Sex	Male	70 (31.82)	150(61.18)	0.39 (0.23, 0.66)	<0.001	0.17(0.08,0.38) *
	Female	47(54.02)	40(45.98)	1		1
	<18 (children)	4(16.66)	24(83.34)	1		1

Age	18-45 (young adult)	70(37.24)	118(62.76)	3.55(1.18, 10.68)	0.024	2.92(0.49, 17.24)
	>45 (old adult)	43(47.23)	48(52.77)	5.35(1.72, 16.73)	0.004	4.66(0.73, 29.68)
Residence	Rural	36(38.29)	58(61.71)	0.98(0.59, 1.62)	0.964	NA
	Urban	81(38.02)	132(61.98)	1		
Occupation of study participants	Farmer	74(41.81)	103(58.19)	2.29(0.80, 6.55)	0.119	3.21(0.53,19.2)
	Housewife	16(48.49)	17(51.51)	3.01(0.89, 10.14)	0.075	0.84(0.11,6.24)
	Merchant	5(21.74)	18(78.26)	0.88(0.21, 3.64)	0.870	1.11(0.14,8.45)
	Private employee	10(52.63)	9(47.37)	3.55(0.92, 13.69)	0.065	2.95(0.40,21.52)
	Other	7(20.59)	27(79.41)	0.82(0.22, 3.05)	0.779	1.46(0.20,10.34)
	Government employee	5(23.81)	16(76.19)	1		1
Educational level	No education	60(41.95)	83(58.05)	2.02(0.69, 5.92)	0.198	1.48(0.21,10.13)
	Primary school	41(34.45)	78(65.55)	1.47(0.49, 4.37)	0.752	1.84(0.27,12.43)
	High school	11(42.31)	15(57.69)	2.05(0.56, 7.41)	0.272	2.73(0.37,20.08)
	University & college	5(26.32)	14(73.68)	1		1
Marital status	Unmarried	66(36.46)	115(63.54)	0.84(0.52, 1.34)	0.477	NA
	Married	51(40.47)	75(59.53)	1		
Use of medication other than liver disease	Yes	33(38.37)	53(61.63)	1.01(0.60, 1.69)	0.953	NA
	No	84(38.01)	137(61.99)	1		
Presence of anemia	Yes	26(50.98)	25(49.02)	1.88(1.02, 3.45)	0.040	1.53(0.76, 3.10)
	No	91(35.55)	165(64.45)	1		1
Presence of DM	Yes	5(41.66)	7(58.34)	0.71(0.21, 2.36)	0.579	NA
	No	112(37.96)	183(62.04)	1		
Presence of cardiac disease	Yes	9(29.04)	22(70.96)	0.63(0.28, 1.43)	0.275	NA
	No	108(39.14)	168(60.86)	1		
History of blood transfusion	Yes	11(28.21)	28(71.79)	0.60(0.28, 1.25)	0.176	0.70(0.30, 1.61)
	No	106(39.55)	162(60.45)	1		1
Presence of hypertension	Yes	7(33.34)	14(66.66)	0.8(0.31, 2.04)	0.641	NA
	No	110(38.46)	176(61.54)	1		
Tea or coffee drinking habit	No	104(38.52)	166(61.48)	0.86 (0.42, 1.77)	0.691	NA
	Yes	13(35.14)	24(64.86)	1		
Meat feeding habit	Yes	107(37.67)	177(63.33)	0.78(0.33, 1.85)	0.582	NA
	No	10(43.47)	13(56.53)	1		
Vegetable feeding habit	No	35(50.00)	35(50.00)	1.89(1.10, 3.24)	0.021	1.54(0.83, 2.86)
	Yes	82(34.60)	155(65.40)	1		1
Alcohol drinking habit	Yes	73(35.61)	132(64.39)	0.72(0.44, 1.18)	0.202	0.65(0.37,1.16)
	No	44(43.14)	58(56.86)	1		1
Cigarette smoking habit	Yes	4(36.36)	7(63.64)	0.92(0.26, 3.23)	0.903	NA
	No	113(38.18)	183(61.82)	1		
Physical exercise habit	No	15(33.33)	30(66.67)	0.78(0.40, 1.52)	0.476	NA
	Yes	102(38.93)	160(61.07)	1		
	CLD	48(30.57)	109(69.43)	1.59(0.68, 3.74)	0.283	0.99(0.33, 2.98)

Types of liver disease	ALD	7(26.93)	19(73.07)	1.33(0.41, 4.29)	0.627	1.38(0.32, 5.83)
	Viral hepatitis	54(62.06)	33(37.94)	5.93(2.42, 14.51)	<0.001	3.85(1.23, 12.02) *
	Acute liver disease	8(21.62)	29(78.38)	1		1
Presence of virus hepatitis (HBV and HCV)	Yes	30(34.48)	57(65.52)	0.80 (0.47, 1.35)	0.411	NA
	No	87(39.54)	133(60.46)	1		

Abbreviation: **= Significant Variable,* ***ALD****: Alcoholic Liver Disease,* ***CLD****: Chronic Liver Disease* ***DM****=Diabetes millets* ***AOR****= Adjusted odds ratio,* ***COR:*** *Crude Odd Ratio,* ***CI****: Confidence Interval,* ***1****= reference group other= includes student and have no jobs,* ***NA****=not applicable.*

Factors associated with liver function abnormalities

The bivariable logistic regression analysis, patients' age group, gender, habit of eating vegetables, viral hepatitis, and anemia were linked to abnormal liver ALT levels. After multivariable logistic regression analysis, gender being male (AOR = 0.17; 95% CI: 0.08-0.38), presence of viral hepatitis (AOR = 3.85; 95% CI: 1.23-12.02) were significantly associated [3].

Using bivariable regression analysis, AST abnormalities was linked to independent factors such as the presence of blood transfusion, vegetable eating habits, physical activity, and ALD. After multivariable logistic regression analysis, presence of blood transfusion history (AOR=0.45; 95%CI: 0.21-0.95), lack of vegetable feeding habit (AOR=2.73; 95%CI: 1.34 - 5.59), and not doing physical exercise (AOR=2.97; 95%CI: 1.44 - 6.11) and ALD (AOR=17.09; 95%CI: 1.96 -148.98) remained significantly associated (Table 4).

Table 4. Bivariable and multivariable logistic regression of abnormal AST among liver disease patients attending at the UoG-CSH, Northwest, Ethiopia, 2022 (n=307)

Variable	Category	AST		Bivariable analysis		Multivariable analysis
		Abnormal (%)	Normal (%)	P-value	COR (95%CI)	AOR (95%CI)
Sex	Male	148(67.27)	72(32.73)	0.284	0.73 (0.42, 1.28)	NA
	Female	64(73.56)	23(26.44)		1	
Age	<18 (children)	18(64.29)	10(35.71)		1	
	18-45 (young adult)	127(67.55)	61(32.45)	0.731	1.15(0.50, 2.65)	NA
	>45 (old adult)	67(73.63)	24(26.37)	0.341	1.55 (0.62, 3.82)	
Residence	Rural	148(69.48)	65(30.52)	0.807	1.06(0.63, 1.79)	NA
	Urban	64(68.09)	30(31.91)		1	
Occupation	Farmer	126(71.18)	51(28.81)	0.382	1.52(0.59, 3.88)	
	Housewife	25(75.75)	8(24.25)	0.280	1.92(0.58, 6.30)	
	Merchant	13(56.53)	10(43.47)	0.717	0.8(0.23, 2.67)	
	Private employee	13(68.42)	6(31.58)	0.666	1.33(0.36, 4.93)	NA
	Other	22(64.70)	12(35.30)	0.834	1.12(0.36, 3.48)	
	Government employee	13(61.90)	8(38.10)		1	
	No education	96(67.13)	47(32.87)	0.426	1.48(0.56, 3.93)	1.40(0.42, 4.62)

	No education	96(67.13)	47(32.87)	0.426	1.46(0.56, 3.93)	1.40(0.42, 4.62)
Educational level	Primary school	87(73.10)	32(26.90)	0.180	1.97(0.72, 5.35)	2.11(0.63, 7.06)
	High school	18(69.23)	8(30.77)	0.434	1.63(0.47, 5.62)	1.50(0.35, 6.29)
	University& College	11(57.90)	8(42.10)		1	1
Marital status	Unmarried	121(66.85)	60(33.15)	0.317	1.12(0.78, 2.12)	NA
	Married	91(72.22)	35(27.78)		1	
Use of medication other than liver disease	Yes	60(69.77)	26(30.23)	0.866	1.04(0.60,1.79)	
	No	152(68.78)	69(31.22)		1	
Presence of anemia	Yes	41(80.40)	10(19.60)	0.059	2.03(0.97, 4.26)	2.11(0.96, 4.61)
	No	171(66.80)	85(32.20)		1	1
Presence of DM	Yes	11(84.61)	2(15.39)	0.230	2.54(0.55,11.71)	NA
	No	201(68.36)	93(31.64)		1	
Presence of cardiac disease	Yes	23(74.20)	8(25.80)	0.515	1.32(0.56, 3.07)	NA
	No	189(68.47)	87(31.52)		1	
History of blood transfusion	Yes	21(53.85)	18(46.15)	0.030	0.47(0.23,0.93)	0.45(0.21 0.95) *
	No	191(71.27)	77(28.73)		1	1
Presence of hypertension	Yes	17(80.95)	4(19.05)	0.230	1.98(0.64, 6.06)	NA
	No	195(68.18)	91(31.82)		1	
Tea or coffee drinking habit	No	189(70.00)	81(30.00)	0.335	0.70(0.34, 1.43)	NA
	Yes	23(62.16)	14(37.84)		1	
Meat feeding habit	Yes	195(68.66)	89(31.34)	0.601	0.77(0.29, 2.02)	NA
	No	17(73.91)	6(26.09)		1	
Vegetable feeding habit	No	155(65.40)	82(34.60)	0.012	2.31(1.19, 4.48)	2.73(1.34, 5.59) *
	Yes	57(81.43)	13(18.57)		1	1
Alcohol drinking habit	Yes	146(71.22)	59 (28.78)	0.246	1.34(0.81, 2.23)	NA
	No	66(64.70)	36(35.30)		1	
Cigarette smoking habit	Yes	7(63.64)	4(36.36)	0.693	0.77(0.22, 2.71)	NA
	No	205(69.26	91(30.74)		1	
Physical exercise habit	No	22(48.89)	23(51.11)	0.002	2.75(1.44, 5.25)	2.97(1.44, 6.11) *
	Yes	190(72.52)	72(27.48)		1	1
Type of liver disease	CLD	95(60.50)	62(39.50)	0.428	0.73(0.44, 1.57)	0.83(0.36, 1.89)
	ALD	25(96.15)	1(3.85)	0.021	11.99(1.44, 99.37)	17.09(1.96, 148.98) *
	Viral hepatitis	67(77.01)	20(22.99)	0.274	1.60(0.68, 3.76)	1.75(0.70, 4.38)
	Acute liver disease	25(67.57)	12(32.43)		1	1
Presence of virus hepatitis (HBV and HCV)	Yes	55(63.22)	32(36.78)	0.165	0.68(0.40, 1.16)	0.74(0.41, 1.33)
	No	157(71.36)	63(28.64)		1	1

Abbreviation: **= Significant Variable, **ALD**: Alcoholic Liver Disease, **CLD:** Chronic Liver Disease **DM**=Diabetes millets, **AOR**= Adjusted odds ratio, **COR**=Crude Odd Ratio, **CI**=Confidence Interval, **1**= the reference group, **other**= includes student and have no jobs, **NA**=not applicable.*

In bivariable logistic regression analysis revealed associations between total bilirubin elevation and independent factors such as lack of education, anemia, vegetable eating habits, and physical activity. After multivariable logistic regression analysis, being anemic (AOR=3.33; 95%CI: 1.58-7.08), lack of vegetable feeding habit (AOR=1.98; 95%CI: 1.06-3.69), and not doing physical exercise (AOR=4.03; 95%CI: 1.90- 8.57) remained significantly associated (Table 5).

Table 5. Bivariable and multivariable logistic regression of abnormal Total bilirubin among liver disease patients attending at the UoG-CSH, Northwest, Ethiopia (n=307)

Variable	Category	Total Bilirubin		Bivariable analysis		Multivariable analysis
		Abnormal (%)	Normal (%)	P-value	COR (95%CI)	AOR (95%CI)
Sex	Male	122(55.45)	98(44.55)	0.682	1.10 (0.67, 1.82)	NA
	Female	46(52.87)	41(47.13)		1	
Age	<18 (children)	14(50.00)	14(50.00)		1	
	18-45 (young adult)	106(56.38)	82(47.62)	0.527	1.29(0.58, 2.86)	NA
	>45 (old adult)	48(52.75)	43(47.25)	0.799	1.11 (0.47, 2.60)	
Residence	Rural	117(54.93)	96(45.07)	0.913	1.02(0.63, 1.67)	NA
	Urban	51(54.26)	43(45.74)		1	
Occupation	Farmer	98(55.37)	79(44.63)	0.280	1.65(0.66, 4.12)	0.44(0.07, 2.74)
	Housewife	16(48.48)	17(51.52)	0.686	1.25(0.41, 3.77)	0.28(0.04, 2.01)
	Merchant	12(52.17)	11(47.83)	0.537	1.45(0.44, 4.78)	0.45(0.64, 3.22)
	Private employee	14(73.68)	5(26.32)	0.054	3.73(0.97, 14.22)	1.33(0.16, 10.94)
	Other	19(55.88)	15(44.12)	0.349	1.68(0.56, 5.06)	0.71(0.10, 4.75)
	Government employee	9(42.86)	12(57.14)		1	1
Educational level	No education	82(57.34)	61(42.66)	0.040	2.91(1.04, 8.09)	4.91(0.72, 33.55)
	Primary school	64(53.78)	55(46.22)	0.079	2.52(0.89, 7.07)	4.53(0.68, 30.11)
	High school	16(61.54)	10(38.46)	0.051	3.46(0.99, 12.08)	5.42(0.74, 39.68)
	University& College	6(31.58)	13(68.42)		1	1
Marital status	Unmarried	95(52.49)	86(47.51)	0.346	1.24(0.78, 1.97)	NA
	Married	73(57.94)	53(42.06)		1	
Use of medication other than liver disease	Yes	44(51.16)	42(48.84)	0.435	0.81(0.49,1.35)	
	No	124(56.10)	97(43.90)		1	
Presence of anemia	Yes	38(74.50)	13(25.50)	0.003	2.83(1.44, 5.56)	3.33(1.58, 7.08) *
	No	130(50.78)	126(49.22)		1	1
Presence of DM	Yes	7(58.33)	5(41.67)	0.528	0.69(0.22, 2.12)	NA
	No	161(54.58)	134(45.42)		1	
Presence of cardiac disease	Yes	16(51.61)	15(48.39)	0.714	0.87(0.41, 1.82)	NA
	No	152(55.07)	124(44.93)		1	
History of blood transfusion	Yes	17(43.59)	22(56.41)	0.138	0.59(0.30, 1.17)	0.62(0.29, 1.29)
	No	151(56.34)	117(43.66)		1	1
Presence of hypertension	Yes	10(47.62)	11(52.38)	0.499	0.73(0.30, 1.78)	NA
	No	158(55.24)	128(44.76)		1	

	No	158(55.24)	128(44.76)		1	
Tea or coffee drinking habit	No	149(55.19)	121(44.81)	0.661	0.85(0.43, 1.70)	NA
	Yes	19(51.35)	18(48.65)		1	
Meat feeding habit	Yes	155(54.58)	129(45.42)	0.857	0.92(0.39, 2.17)	NA
	No	13(56.52)	10(43.48)		1	
Vegetable feeding habit	No	122(51.48)	115(48.52)	0.037	1.80(1.03, 3.14)	1.98(1.06, 3.69) *
	Yes	46(65.71)	24(34.29)		1	1
Alcohol drinking habit	Yes	119(58.05)	86(41.95)	0.098	1.49(0.92, 2.41)	1.21(0.71, 2.07)
	No	49(48.04)	53(51.96)		1	1
Cigarette smoking habit	Yes	4(36.36)	7(63.64)	0.223	0.45(0.13, 1.60)	NA
	No	164(55.40)	132(44.60)		1	
Physical exercise habit	No	13(28.89)	32(71.11)	0.000	3.56(1.78, 7.10)	4.03(1.90, 8.57) *
	Yes	155(59.16)	107(40.84)		1	1
Type of liver disease	CLD	80(50.96)	77(49.04)	0.801	1.09(0.53, 2.24)	1.42(0.58, 3.48)
	ALD	18(69.23)	8(30.77)	0.107	2.37(0.82, 6.80)	3.21(0.90,11.36)
	Viral hepatitis	52(61.90)	32(38.10)	0.255	1.56(0.72, 3.40)	1.88(0.72, 4.93)
	Acute liver disease	18(48.65)	19(51.35)		1	1
Presence of virus hepatitis (HBV and HCV)	Yes	44(50.57)	43(49.43)	0.359	0.79(0.48, 1.30)	NA
	No	124(56.36)	96(43.64)		1	

Abbreviation: **= Significant Variable, **ALD**: Alcoholic Liver Disease, **CLD**: Chronic Liver Disease **DM**=Diabetes millets, **AOR**= Adjusted odds ratio, **COR**=Crude Odd Ratio, **CI**=Confidence Interval, **1**= the reference group, **other**= includes student and have no jobs, **NA**=not applicable.*

Discussion

Liver disease is a global public health issue that causes mortality and morbidity. It is one of the most common causes of coagulopathy and impaired liver function in both developed and developing countries. This study investigated on the extent and associated factors influencing liver function abnormalities in liver disease patients visiting UoG-CSH.

The findings of this study showed that the overall prevalence of abnormal liver function test results was 38.11%, 69.06%, 54.72%, of the study participants had abnormal ALT, AST, and total bilirubin respectively. Furthermore,25.40% of study participants had all liver function abnormalities. When liver cells are damaged, they release ALT, AST, and increase bilirubin into the bloodstream. ALT is preferably specific indicator than AST for active hepatic cell damage due to its location. The rising of blood AST indicates the occurrence of other events besides liver damage [21].

In this study Patient's sex and hepatic virus were showed statistically significant association with rising of ALT. The likely hood of patient's ALT abnormality occurrence being male was reduced by 83%, which contradicts with the study done in China [22], Taiwan [23]. This could be associated with BMI mostly males had good BMI which is significantly associated with less liver function test abnormality, the previous study showed Males have greater risk of abnormal liver function by

controlling hepatitis B, obesity. Being patents infected by hepatic virus(s) the odds of developing ALT abnormality is 3.85 (95% CI: 1.23- 12.02) times likely than other type hepatic disease similar with the previous study in China [22][24], Serbia [6], and Korea [25]. The virus can multiply in the host and more tissue are damaged in short time leads to release more ALT enzyme [26].

Patient's status of blood transfusion history, vegetable dietary habit, and regular physical exercise, and ALD were showed statistically significant association with rising of AST. Patients having blood transfusion history less likely to happened AST abnormality by 55% (95%CI: 0.21- 0.95) than non-transfused which similar with the study conducted in USA [27]. Liver disease patients who lacked of vegetable feeding habit had makes AST abnormality by 2.73 (95%CI: 1.34- 5.59) times than study participant has habit of feeding vegetables, it similar with the study done in Iran [28]. Vegetables contain antioxidants may reduce the value of AST. The odds of developing AST rising were 2.97 (95%CI: 1.44- 6.11) more likely among patients lack of doing regular exercise than doing. Diet and weight loss are essential things in enhancing liver enzymes, with antioxidants provided by vegetables [29]. Exercise decreases stress on the liver, fats, increases energy levels and helps to prevent obesity which is risk factor for liver disease[30]. Lastly ALD patients were 17.09 (95%CI: 1.96-148.98) times more likely to rise AST the others similar with study in Sweden [31]. Because vitamin B6 is used as coenzyme for liver activities, but alcoholism reduces the level of vitamin B6 [32].

Patient's status of anemia, vegetable dietary habit, physical exercises and blood transfusion were showed statistically significant association with rising total bilirubin. The odds of rising total bilirubin were 3.33 (95%CI: 1.58- 7.08) times more likely in anemic patients than non-anemic. Bilirubin is formed when hemoglobin is broken down. High bilirubin levels in the circulation may indicate hemolytic anemia. High amounts of this chemical are also associated with certain liver and gallbladder illnesses. Erythrocyte abnormalities were clinically important and frequent findings in patients with chronic disease [33]. In this study, patients who lack vegetable in dietary habit 1.98 (95%CI: 1.06- 3.69) times more likely to rising total bilirubin which may markers associated with cardiovascular problems. The controversial ideas between vegetables and bilirubin. When vegetables and balanced date is abnormal the red cells and immunity is affected causes to cellular damage, infection, and protein abnormalities. Therefore, balanced diet and vegetarians has good prognosis among liver disease patients [34]. Lack of physical exercise was raising the total bilirubin 4.03 (95%CI: 1.90- 8.57) times than its counterpart which is contradict with study done in Columbia [35].

Strength and Limitation of the study

This study has its strengths and limitations. The strength of this study is that it is the first study on the determination of the magnitude and associated factors of liver function abnormalities in liver disease patients in Ethiopia. However, the limitation of this study was that we could not perform parasitic infection screening, which may interfere with the finding of the study. In addition, detailed liver function tests such as total protein, albumin, urea and other proteins were not performed due to budget scarcity.

Conclusion and recommendation

In liver diseased patients' majority of patients rise AST followed by bilirubin. About 1/4th of the patients indicates all liver enzymes abnormalities. Females, having hepatic virus, blood transfusion history, lack vegetable dietary habit, and lack regular physical exercise, and ALD, anemic risk factors for liver function tests. We recommended, healthcare providers should monitor and consider those factors for better improving and drug of choice.

List of abbreviations

ALD: Alcoholic Liver Disease, **ALT**: Alanine Transaminase, **AST**: Aspartate Transaminase, **CLD**: Chronic Liver Disease, **HBV**: Hepatitis B virus, **HCV**: Hepatitis C virus, **LFT**: Liver function test, **SPSS:** Statistical Package for the Social Sciences, **UoG-CSH**: University of Gondar Comprehensive Specialized Hospital.

Statements and Declarations

Ethical approval and consent to participate

This research was carried out by the Helsinki Declaration[36]. An ethical clearance was obtained from the Ethics Review Committee of the School of Biomedical and Laboratory Science, College of Medicine and Health Science, University of Gondar Research, with reference number SBMLS/384/2022. Then, a permission letter was secured from the UoG-CSH medical director. Before starting data collection, the data collectors were informed to ask permission, explain the purpose of the study and its importance and benefits, and offer to answer all the participants' questions to confirm their willingness. Then, written informed consent, or assent, was obtained. Participation in the study and refusals were possible. To ensure confidentiality of data, study participants were identified using codes, and unauthorized persons had no access to the data. All abnormal laboratory findings were linked to health professionals for proper management and treatment of patients.

Conflict of interest

The authors declare that there is no conflict of interest related to the publication of this manuscript.

Authors' contributions

AM: data collection, feeding, performing the statistical analysis, drafting the manuscript, YM: Data analysis, drafting and editing the manuscript, AY, ML, BM, GA, AA, AA: Review and editing the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All the data supporting these findings are contained within the manuscript.

References

1. ^*Monroe D, Hoffman M, Roberts H. Williams hematology. New York NY: McGraw-Hill Professional Publishing; 2010. p. 2191.*
2. a, b*Cheemerla S, Balakrishnan M. Global Epidemiology of Chronic Liver Disease. Clinical liver disease. 2021;17(5):365-70.*
3. a, b*Krishnan S. Liver diseases-an overview. World journal of pharmacy and pharmaceutical sciences. 2019;8:1385-95.*
4. ^*Lopes D SH. Hepatic failure. [Updated 2022 Jul 18] In: StatPearls [Internet] Treasure Island (FL): StatPearls Publishing; 2022 Jan. 2022.*
5. ^*Rahmioglu N, Andrew T, Cherkas L, Surdulescu G, Swaminathan R, Spector T, et al. Epidemiology and genetic epidemiology of the liver function test proteins. PloS one. 2009;4:e4435.*
6. a, b*Mijač D, Krstić MN, Marković AP, Popović DD, Krstić JM, Milosavljević T. Abnormal Liver blood tests: Primary care approach. Digestive Diseases. 2022;40(2):215-22.*
7. ^*Mcclatchey KD. Clinical laboratory medicine: Lippincott Williams & Wilkins; 2002.*
8. ^*Devarbhavi H, Asrani SK, Arab JP, Nartey YA, Pose E, Kamath PS. Global burden of liver disease: 2023 update. J Hepatol. 2023;79(2):516-37.*
9. ^*Spearman CW. The burden of chronic liver disease in west Africa: a time for action. The Lancet Global Health. 2023;11(9):e1319-e20.*
10. ^*Tesfaye BT, Feyissa TM, Workneh AB, Gudina EK, Yizengaw MA. Chronic Liver Disease in Ethiopia with a Particular Focus on the Etiological Spectrums: A Systematic Review and Meta-Analysis of Observational Studies. Can J Gastroenterol Hepatol. 2021;2021:8740157.*
11. ^*Atsede D. Tegegne MAN MKD, Kumela G. Nedessa, Hone M. Belaye https://mdl.donau-uni.ac.at/ses/pluginfile.php/314/mod_page/content/4/CITY%20PROFILE%20GONDAR.pdf. City profile Gondar {29 October 2022).*
12. ^*Woldekidan NA, Mohammed AS. Clinical Knowledge and Practice of "Ketofol" at University of Gondar Comprehensive Specialized Hospital. Frontiers in Medicine. 2021:1518.*

13. ^Dong X-X, Wang R-R, Liu J-Y, Ma Q-H, Pan C-W. Habitual tea consumption and 5-year incident metabolic syndrome among older adults: a community-based cohort study. BMC Geriatrics. 2021;21(1):728.
14. ^Snetselaar LG, de Jesus JM, DeSilva DM, Stoody EE. Dietary guidelines for Americans, 2020–2025: understanding the scientific process, guidelines, and key recommendations. Nutrition today. 2021;56(6):287-95.
15. a, b, c Rifai N. Tietz textbook of clinical chemistry and molecular diagnostics. 7 ed: Elsevier Health Sciences; 2017. 1637 p.
16. ^Diaz KM, Shimbo D. Physical activity and the prevention of hypertension. Curr Hypertens Rep. 2013;15(6):659-68.
17. ^Pem D, Jeewon R. Fruit and Vegetable Intake: Benefits and Progress of Nutrition Education Interventions- Narrative Review Article. Iran J Public Health. 2015;44(10):1309-21.
18. ^Lofgren PA. Meat, Poultry, and Meat Products: Nutritional Value. In: Caballero B, editor. Encyclopedia of Human Nutrition (Third Edition). Waltham: Academic Press; 2013. p. 160-7.
19. ^Collaborative Laboratory Services LLC. Laboratory Procedure Manual, Total Bilirubin. Synchron BUD, editor2012.
20. a, b Arneson WL, Brickell JM. Clinical Chemistry: a laboratory perspective: FA Davis; 2007. 245-6 p.
21. ^Rifai N. Tietz textbook of clinical chemistry and molecular diagnostics: Elsevier Health Sciences; 2017.
22. a, b Zhang H, He SM, Sun J, Wang C, Jiang YF, Gu Q, et al. Prevalence and etiology of abnormal liver tests in an adult population in Jilin, China. International journal of medical sciences. 2011;8(3):254-62.
23. ^Association for the advancement of blood biotherapies. Whole blood and red blood cell components 2022 [cited 2022 /28/10]. Available from: https://www.aabb.org/regulatory-and-advocacy/regulatory-affairs/regulatory-for-blood/whole-blood-and-red-blood-cell-components.
24. ^Sarin SK, Kumar M. Should chronic HBV infected patients with normal ALT treated: debate. Hepatology international. 2008;2(2):179-84.
25. ^Oh JS, Choi JS, Lee YH, Ko KO, Lim JW, Cheon EJ, et al. The Relationships between Respiratory Virus Infection and Aminotransferase in Children. Pediatric gastroenterology, hepatology & nutrition. 2016;19(4):243-50.
26. ^Liu B, Yang X, Zhao D, Mao X. Viral Hepatitis. In: Li H, editor. Radiology of Infectious Diseases: Volume 1. Dordrecht: Springer Netherlands; 2015. p. 589-653.
27. ^Nachnani JS, Hamid F, Pandya P, Clarkston W, Alba LM. Transfusion-related acute hepatic enzyme elevation: a new disease entity? European Journal of Gastroenterology & Hepatology. 2010;22(3):378.
28. ^Abazarfard Z, Eslamian G, Salehi M, Keshavarzi S. A Randomized Controlled Trial of the Effects of an Almond-enriched, Hypocaloric Diet on Liver Function Tests in Overweight/Obese Women. Iranian Red Crescent medical journal. 2016;18(3):e23628.
29. ^Rafiq N, Younossi ZM. Effects of weight loss on nonalcoholic fatty liver disease. Seminars in liver disease. 2008;28(4):427-33.
30. ^van der Windt DJ, Sud V, Zhang H, Tsung A, Huang H. The Effects of Physical Exercise on Fatty Liver Disease. Gene Expr. 2018;18(2):89-101.
31. ^Nyblom H, Berggren U, Balldin J, Olsson R. High AST/ALT ratio may indicate advanced alcoholic liver disease rather than heavy drinking. Alcohol and alcoholism (Oxford, Oxfordshire). 2004;39(4):336-9.
32. ^Vech RL, Lumeng L, Li TK. Vitamin B6 metabolism in chronic alcohol abuse The effect of ethanol oxidation on hepatic

pyridoxal 5'-phosphate metabolism. J Clin Invest. 1975;55(5):1026-32.

33. ^*Ueda T, Kawakami R, Horii M, Sugawara Y, Matsumoto T, Okada S, et al. High mean corpuscular volume is a new indicator of prognosis in acute decompensated heart failure. Circulation Journal. 2013;77(11):2766-71.*
34. ^*Loprinzi PD, Mahoney SE. Association between flavonoid-rich fruit and vegetable consumption and total serum bilirubin. Angiology. 2015;66(3):286-90.*
35. ^*Swift DL, Johannsen NM, Earnest CP, Blair SN, Church TS. Effect of different doses of aerobic exercise training on total bilirubin levels. Medicine and science in sports and exercise. 2012;44(4):569-74.*
36. ^*World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. The Journal of the American College of Dentists. 2014;81(3):14-8.*