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Assessment of selected clinical chemistry test alterations and associated risk factors among visceral leishmaniasis patients attending at Kahsay Abera and Mearg Hospitals Western Tigray, Northern Ethiopia, 2019: a comparative study

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This is to certify that the thesis prepared by Brhane Tesfanchal, entitled: **Assessment of selected clinical chemistry test alterations and associated risk factors among visceral leishmaniasis patients attending at Kahsay Abera and Mearg Hospitals Western Tigray, Northern Ethiopia**, complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Abbreviations

VL	visceral leishmaniasis
LD	leishmania donovani
ALP	alkaline phosphate
ALT	Alanine Transeaminase
AST	Aspartate Transeaminase
BiD	Direct Bilirubin
BiT	Total Bilirubin
RFT	Renal function test
LFT	Liver function test
APC	Antigen presenting cells
LDL	Low density lipoprotein
HDL	High density lipoprotein
VLDL	Very low density lipoprotein
AKI	Acute kidney injury
GGT	Gammaglutamyltranspeptidase
LJ	Levy Jennings
PI	Principal Investigator
RPM	Revolution per Minute
SOP	Standard Operating Procedure
SPSS	Statistical package for social science
WHO	World health Organization
CI	Confidence interval
SD	Standard deviation

Abstract

Introduction: Visceral leishmaniasis causes alterations of lipid metabolism and associated with hypochlesterolemia and severe hypertriglyceridemia. Hepatic dysfunction and life threatening hepatitis is associated with visceral leishmaniasis. Kidney damage is frequently associated with increased morbidity and mortality in visceral leishmaniasis patients. However there is no published data on the effect of visceral leishmaniasis to kidney, liver, and lipid profiles in Ethiopia particularly in the study area.

Objective: To assess selected clinical chemistry test alterations and associated risk factors among visceral leishmaniasis patients attending at Kahsay Abera and Mearg Hospitals Western Tigray, Northern Ethiopia from November 2018 -March 2019.

Method: Institutional based comparative cross sectional study was conducted from November 2018 to March 2019 in Kahsay Abera and Mearg Hospitals, Western Tigray, Northern Ethiopia. A total of 100 visceral leishmaniasis patients and 100 control groups working in Kahsay Abera and Mearg Hospitals without visceral leishmaniasis were selected by using convenient sampling techniques. The samples were processed and analysed by Mindray 200E automated clinical chemistry analyzer. Data was entered and analyzed using SPSS Version 23. Descriptive statistics, independent-test, binary logistic regressions were used for data analysis. P values < 0.05 were considered as statistically significant at 95% confidence level.

Result: From 100 visceral leishmaniasis 9(9%), 10 (10%), 15(15%), 18(18%), 25(25%), 6(6%), 4(4%) and 16(16%) patients had increased level of aspartate amino transferase, alanine amino transferase, alkaline phosphatase direct bilirubin, total bilirubin, creatinine, urea and triglyceride respectively. The mean value serum aspartate amino transferase, alanine amino transferase, alkaline phosphatase, total bilirubin, triglyceride were significantly higher in visceral leishmaniasis patients than in healthy controls, but the mean value of serum urea and total cholesterol were significantly lower in visceral leishmaniasis patients than healthy controls.

Conclusions: The finding of this study concluded that visceral leishmaniasis causes significant alterations of clinical chemistry tests like liver and lipid profile tests. These alterations could be contributed to the immune complex formed during infection with visceral leishmaniasis.

Keywords: Kidney function Test, lipid profile test, Liver function Test, visceral leishmaniasis

1. INTRODUCTION

1.1. Background of the study

1.1.1. Lipid profile tests

Lipids are commonly referred to as fats and used as rich source of energy and an efficient way for the body to store excess calories. Lipids are also used as an integral part of cell membranes and play an important structural role in cells because of their unique physical properties. Lipids are transported by lipoproteins, namely triglycerides, phospholipids, cholesterol, and cholesteryl esters are also the principal lipids found in cells [1].

Cholesterol is the main component of eukaryotic membranes and necessary for binding and internalization of Leishmania parasite. It also plays an important role in the maintenance of membrane fluidity essential for coordination of signal transduction and proper antigen presenting cell (APC) function [2, 3]. The internalization of the *L. donovani* involves an interaction with the plasma membrane of the host cell [4]. Then the parasite enters to the cell and actively extracts cholesterol from lipid rafts, disrupting them and impairing multiple elements of immune response including T-cell stimulation [5].

The alterations of lipoprotein level in visceral leishmaniasis (VL) patients are related to the modulation of the immune response. The decrease in lipoproteins level may possibly affect the immune response and the pathogenesis of the disease, since lipoproteins are related to the production of cytokines like TNF- α , IL-6 and IL-10 [6]. Infection with VL alters the metabolism of lipids like triglycerides by blocking the lipoprotein lipase, an enzyme responsible for cleavage of triglyceride which leads to accumulation of triglyceride and decrease the formation of high density lipoprotein (HDL) and low density lipoprotein (LDL) from very low density lipoprotein (VLDL) [4].

1.1.2. Renal functions tests

Kidneys are bean shaped paired organ positioned in the posterior part of the abdomen on both sides of the spinal column. The physiological function of the kidney is performed by three basic processes like glomerular filtration, tubular reabsorption, and tubular secretion. Kidney function test includes urea, creatinine, and uric acid [7]. VL affects the kidney and causing alteration of glomerular and tubular functions. Immune complex deposition, T cells, and adhesion molecules activation have been shown to be important mechanisms of injury in the glomerulonephritis [8].

Leishmania donovani produce growth factor β and interleukin10 which affects cellular immune response and induce polyclonal B-cell activation which has been associated with kala-azar glomerular disease [9]. Oxidative stress is major determinant of various renal diseases and also contributes to the manifestation of glomerulonephritis [10].

1.1.3. Liver function tests

The liver is the largest internal organ of the human body and plays a critical biochemical role in the metabolism, digestion, detoxification, and elimination of substances from the body. The liver is unique in the sense that it is a relatively resilient organ that can regenerate cells that have been destroyed by some short term injury or disease. However, if the liver is damaged repeatedly over a long period of time, it may undergo irreversible changes that permanently interfere with its essential functions [1].

Liver Function Tests (LFTs) are one of the most commonly requested screening blood tests for the investigation of suspected liver disease, monitoring of disease activity, or simply as ‘routine’ blood analysis, it can provides information on a range of disease processes. Liver function tests include Aspartate amino transferase (AST), Alanine amino transferase (ALT), alkaline phosphatase (ALP), gamma glutamyltranspeptidase (GGT), and bilirubin [11].

During infection with VL the promastigotes invade the resident macrophages, Kupffer cells and dendritic cells of the liver where they become amastigotes and replicates. During acute infection parasite growth is uncontrolled, due to elevated transforming growth factor ($TGF\beta$) levels and ineffective Th1 response. The resolution of the infection in the liver is attributed to the generation of reactive nitrogen and oxygen intermediates. The development of a Th1-dominated granulomatous response characterized by high $IFN\gamma$ production by CD4 T cells is associated with VL. The parasite invade the immune system and excessive production granuloma and reactive nitrogen and oxygen intermediates may affect the hepatic tissues [12, 13].

1.1.4. Overview of leishmaniasis

Leishmaniasis is vector-borne protozoan infections which have a wide clinical spectrum. There are more than 21 *Leishmania* species known to infect humans and they are transmitted by approximately 30 species of phlebotomine sand flies. *Leishmania* parasites have two basic life stages such as promastigote inside the invertebrate host and amastigote inside the vertebrate

host[14]. Most of Leishmania infections are zoonotic and rodents and canids are appropriate reservoir host. From the leshimania species only two species like *L. donovani* and *L. tropica* can maintain anthroponotic human-human cycle [15]. VL was first described in 1903, by Leishman and Donovan. It is a protozoan systemic infection which is chronic and vector-borne potentially fatal parasitic disease caused by the Leishmania (*L.*) *donovani* / *L. infantum* / *L. chagasi* complex [16].

In VL, the protozoan infects the fixed and circulating phagocytic cells (macrophages and monocytes) involved in the immune response of the patient. The flagellate promastigote form is transformed into the non flagellate amastigote form after phagocytosis by the macrophages of the liver, spleen, and bone marrow. The intracellular amastigote form also called the Leishman–Donovan (LD) body is oval, 2–5 mm in length and can be identified on microscopy by the presence of a nucleus and the extranuclear densely staining kinetoplas [17]. VL is diagnosed by Serological tests like (rk39) in combination with clinical sign and symptoms. However, the gold standard for diagnosis of VL is demonstration of amastigote in a tissue biopsy [18].

1.2. Statement of the problem

Visceral leishmaniasis is a severe disease which leads to 100% mortality if left untreated [19, 20]. The onset of symptoms is usually fever, general weakness, anorexia, and weight loss. It is also manifested by hepato-splenomegaly, anemia, pallor, leukopenia, and thrombocytopenia as well as hypergammaglobulinaemia. Later symptoms include cachexia, hepatic dysfunction with jaundice, hypo albuminemia, and oedema [8, 21]. It is a common disease in many tropical and subtropical regions. Annually about 200,000 to 400,000 new cases of VL were reported worldwide and more than 90% of these VL cases were reported from six countries like Bangladesh, Brazil, Ethiopia, India, South Sudan, and Sudan. The disease is also endemic in Eritrea, Kenya, Somalia, and Uganda. In Ethiopia 4,000 VL cases were reported annually and 60% of cases were reported from the main endemic areas of Humera and Metema plains in the northwest [19].

Visceral leishmaniasis causes alterations of liver function and rarely patients manifested a severe life-threatening hepatitis [22]. VL also causes hepatic dysfunction, such as coagulation defects, increased serum concentrations of several liver specific enzymes, and changes in the cholesterol biosynthesis [23]. VL patients presented with enlarged liver and shows higher value of ALT, AST and ALP [24], and the disease is also characterized by severe hypertriglyceridemia with reduced levels of total cholesterol, LDL - cholesterol, and HDL- cholesterol in adult patient [4]. In VL cases serum concentrations of cholesterol are inversely correlated with the density of amastigotes in the spleen and cholesterol is significantly low in high hepatic and splenic parasite load [25] .

The kidney is also affected by VL and kidney involvement in chronic leishmaniasis is frequently associated with increased morbidity and mortality. Acute kidney injury was found in a significant proportion from VL patients. Loss of kidney function and interstitial nephritis with glomerular changes can be seen from VL patients. Antibodies produced in response to infection can be trapped in glomeruli by different mechanisms, such as immune complexes and leads to cause damage to the glomerulus of the kidney [9]. Even though kala-azar nephropathy is still poorly understood, kidney dysfunction in this disease has been reported in several studies[8].

Even though different studies were performed in clinical chemistry alterations of VL patients in different parts of the world; there is no published study in Ethiopia particularly in the study area.

Therefore, this study was aimed to assess the selected clinical chemistry test alterations among VL patients attending at Kahsay Abera and Mearg Hospitals.

1.3. Significance of the study

The finding of this study would have great significance to policy maker's and health administrators to implement appropriate preventive interventions on kidney and liver damages associated with visceral leishmaniasis. This finding would also have great significance to health professionals and community, since it can assist in the clinical management of liver dysfunctions and lipid disorder of visceral leishmaniasis patients. This finding will also use as reference for other researchers.

2. LITERATURE REVIEW

2.1. Kidney function tests in visceral leishmaniasis

From a case control study conducted in Brazil, A total of 224 VL patients were included. The main signs and symptoms at admission were fever (90.6%), splenomegaly (87.1%), hepatomegaly (77.2%), asthenia (61.6%), anorexia (58.5%), coughing (41.5%), headache (34.8%), abdominal pain (34.8%), diarrhea (28.6%), dyspnea (21.4%), jaundice (19.6%), and edema (18.8%). AKI was observed in 76 Kalzar patients (33.9%) and 85.5% of these patients were males. Out of 76 patients who developed AKI, 26 (34.2%) had serum creatinine > 1.4 mg/dL at admission. AKI patients presented higher levels of urea, creatinine, direct bilirubins, and indirect bilirubins, as well as a higher number of WBCs. Risk factors associated with AKI were male gender (OR = 2.2; P = 0.03), advanced age (OR = 1.05; P < 0.001), and jaundice (OR = 2.9; P = 0.002) [26].

A cross sectional study was conducted on 50 VL patients in Brazil. At admission, all VL patients presented with daily fever, intense sweating, diarrhea, frequent vomiting, weight loss and mild edema of lower limbs. The mean value of serum urea was significantly lower in VL cases than healthy controls ($p < 0.05$). Whereas the mean value of creatinine was no statistically significant difference among VL cases and healthy controls. There was no significant difference with regard to age and gender between VL patients and healthy controls [27].

A prospective study of thirty children VL patients were studied in Rajshahi Medical College Hospital, Bangladeshi. The patients' were diagnosed clinically as well as by laboratory findings. All of the patients have fever and splenoegally and hepatomegaly was reported from 24 patients. Serum urea and serum creatinine were found to be 18.0 to 35.0 mg/dl (24.7 ± 13.9 mg/dl) and 0.6 to 1.2 mg/dl (0.87 ± 0.13 mg/dl), respectively [28].

In Italy, one study reviewed 57 VL patients who presented initially with fever (97%), splenomegaly (96.4%), weight loss (95.5%), pallor (93.6%), cough (89.7%), hepatomegaly (87.2%), asthenia (83.3%), anorexia (82.9%) and vomiting (73.9%). Acute kidney injury (AKI) was observed in 15 patients (26.3%) and death occurred in three cases and in all of them serum creatinine was >1.3 mg/dl [8].

2.2. Liver function tests in visceral leishmaniasis

A case report conducted in Italy, a 36-year-old Italian man had fever for 15 consecutive days, spontaneously remitting in the absence of other signs or symptoms. Two months later the patient was hospitalized in a local hospital because of jaundice, asthenia, anorexia and over abundant night sweats. Liver and spleen were enlarged and the following abnormalities were recorded: AST 1339 IU/mL, ALT 951 IU/mL; total bilirubin 8.6 mg/dL (conjugated 6.3 mg/dL), serum albumin 3.3 g/dL, serum γ -globulin 4.4 g/dL with a polyclonal peak, protrombin activity 51%. Visceral leishmaniasis was hypothesized and a 1:1024 antibody title was found. The diagnosis was confirmed by the presence of numerous *Leishmaniae* in the bone marrow [29].

A cross sectional study was carried out for serological detection and liver functions of pediatric visceral leishmaniasis (VL) in Baghdad hospitals, Iraq, Liver enzymes like serum ALT, AST and ALP were significantly higher in VL patients ($P < 0.01$) than in healthy controls, which its means was (58.43 IU/L $\pm$ 50.303; 20.02 $\pm$ 7.055), (87.374 IU/L $\pm$ 53.993; 32.304 $\pm$ 8.135) and (238.14 IU/L $\pm$ 157.778; 132.94 $\pm$ 48.433) respectively [24].

From the study conducted in Khulna Medical College, Bangladesh a total of eleven kalaazar Pediatric patients were studied. Among them 6 (55%) 4 girls and 2 boys presented with jaundice. The patients presenting symptoms of fever, yellow discoloration of eye & urine, loss of weight, pain in the abdomen was found in 10(91%), 6(55%), 3(27%), 3(27%) respectively. Regarding the clinical signs, anaemia of different degree and splenomegaly was observed in 11(100%), Hepatomegaly in 10(91%). Raised serum bilirubin was found in all the patients and SGPT was high in 5(83%) cases [30].

From the prospective study conducted by the Department of Microbiology at India Institute of Medical Sciences, New Delhi, a total of 114 confirmed VL cases were included in the study. From those 112 patients had fever; 112 (98%) had splenomegaly and 107 (94%) had hepatomegaly. Of these, 15 (13%) had jaundice at the time of presentation, 58 (51%), 37 (32%), 27 (24%), and 24 (21%) had increased levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and bilirubin, respectively. A statistically significantly higher number of fatal cases of VL had an increased level of AST ($P = .049$), alkaline phosphatase ($P = .0091$), and bilirubin ($P = .0049$) as compared with nonfatal cases. However, the difference in the case of ALT was not significant ($P = .183$) [22].

A case report conducted in New Delhi, India, the patient clinical examination showed icterus and hepato-splenomegaly but no lymphadenopathy. The spleen tip was just palpable. The laboratory parameters were as follows: hemoglobin 10.6 g/dL, WBC $6.5 \times 10^3/\text{mL}$, platelets $100 \times 10^3/\text{mL}$, total bilirubin 2.7 mg/dL, direct bilirubin 1.8 mg/dL, AST 194 IU/L, ALT 81 IU/L, ALP 356 IU/L. The case is confirmed by the demonstration of LD body in spleen [31].

From a study conducted in Bihar, India a total of 100 VL confirmed patients and 25 healthy controls among medical students, staffs were studied. The mean value of serum bilirubin, AST, ALT and ALP were significantly higher in VL cases than healthy controls with $p < 0.001$ [32].

A case report conducted in Adana, Turkey, the physical examination of the patient revealed poor general condition and hepatomegaly and splenomegaly. In admission, laboratory values revealed pancytopenia with hemoglobin concentration of 5.8 g/dL, white blood cell count $2.8 \times 10^3/\text{mm}^3$, platelet count $39 \times 10^3/\text{mm}^3$; elevated liver function tests with aspartate aminotransferase 518 IU/L, alanine aminotransferase 247 IU/L, gamma glutamyltransferase 51 mg/dL and elevated bilirubin levels [33].

From other study conducted by University of Khartoum, Sudan, inflammatory changes were seen in all liver biopsy specimens both in the portal tracts and intralobularly. The portal tracts were enlarged and contained large numbers of macrophages, lymphocytes, and occasional plasma cells. The level of ALP, ALT, AST, GGT and lactate dehydrogenase values were significantly higher in VL patients than healthy controls ($p < 0.05$) [34].

2.3. Lipid profile tests in visceral leishmaniasis

From the study conducted in University of Baghdad, Iraq VL patients showed highly significant $p < 0.05$ increase in both triglyceride and very low density lipoprotein VLDL levels (253.55) and (50.58) respectively in comparisons to control group (60.6) and (12) respectively. On the other hand, patients with VL revealed a significant decrease $p < 0.05$ of cholesterol, low density lipoprotein (LDL) and high density lipoprotein (HDL), (86.14), (34.33) and (25.16) respectively in comparison to control groups (120), (63) and (47) respectively [35].

From other study conducted in Brazil, 13 pre-treatment VL patients were studied and showed significantly lower levels of total cholesterol, HDL and LDL compared to control subjects (p -value <0.05). Prior to treatment, patients exhibited significantly higher levels of triglycerides compared to the control subjects [4].

The study conducted in Brazil aimed at investigating the lipid profile of VL patients. Lipids were quantified in 26 VL patients and 26 healthy controls from a VL endemic area. The patient serum had significantly higher triglyceride and VLDL levels, and significantly lower total cholesterol, LDL and HDL levels than the serum of healthy controls ($P<0.05$) [6].

From the study conducted in Brazil 15 VL confirmed patients and healthy controls were studied and Low levels of HDL, LDL fraction, and apolipoprotein A1 were found in VL patients' sera. The mean level of HDL and LDL were significantly lower in VL cases than healthy controls ($p<0.05$). On the other hand the mean level of VLDL was significantly higher in VL patients than healthy controls ($p<0.05$) [36].

From the case report conducted in Greece, a 36-years-old stockman with an unremarkable medical history was admitted to clinic because of fever of 6 month duration. The patient appeared cachectic and physical examination revealed marked hepato-splenomegaly. The patient's diagnosis of VL was established by bone marrow aspiration and demonstration of LD body in the spleen. The patient is presented with marked hypocholesterolemia, mild hypertriglyceridemia, severely decreased serum levels of HDL-cholesterol, LDL cholesterol [37].

2.4. Conceptual frame work

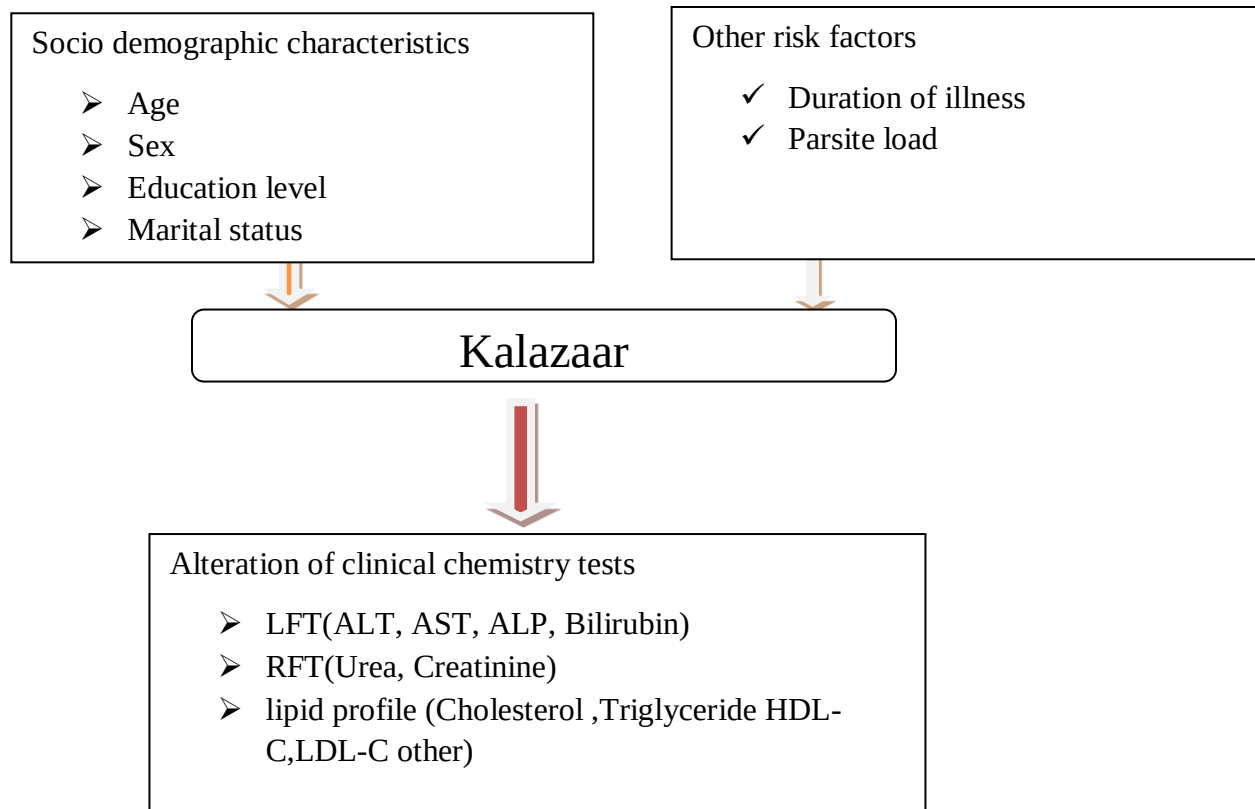


Figure: 1 Conceptual frame work

3. OBJECTIVES

3.1.General objective

- To assess selected clinical chemistry alterations and associated risk factors among visceral leishmaniasis patients attending at Kahsay Abera and Mearg Hospitals Western Tigray, Northern Ethiopia from November 2018 - March 2019.

3.2.Specific Objective

- To assess renal function alterations among visceral leishmaniasis patients versus controls.
- To assess liver functions alterations among visceral leishmaniasis patients versus controls.
- To assess lipid profiles alterations among visceral leishmaniasis patients versus controls.
- To identify risk factors associated with clinical chemistry test alterations among visceral leishmaniasis patients.

4. HYPOTHESIS

H₀= There is no significance difference of clinical chemistry tests between VL patients and control groups

5. MATERIALS AND METHODS

5.1. Study area

This study was conducted in Kahsay Abera Hospital, Humera town and Mearg Hospital, Dansha town, western Tigray, Northern Ethiopia. Western zone of Tigray is the biggest in terms of geographic and territorial possessions that stretches along the border of Sudan in the North West, the Amhara Adminstrative region in the south and the Eritrea border in the north where people live in clusters over a wide range of areas. It is one of the agriculture surplus areas in Ethiopia known for producing sorghum and exportable products like sesame which makes it a net contributor to the national economy. Humera is the capital city of Western Tigray located in north western Ethiopia 984 km far from the capital city Addis Ababa. It is located at longitude and latitude 14°18'N 36 °37'E with an elevation of 585 meters above the sea level [38].

Kahsay Abera Hospital is the district Hospitals found in Kalaazar endemic region in north Ethiopia with close to 210 beds and an estimated 742,000 catchment population. Mearg hospital is the district hospital found in kalaazar endemic area in Tsegede Wereda, Western Tigray which have an estimated 299,594 catchment population including migrant population and have 134 beds at emergency, medical, surgical, gynecology, and pediatrics wards [39].

5.2. Study Design and period

An institutional based comparative cross sectional study was employed from November 2018 to March 2019 G.C.

5.3. Source population and study participant

5.3.1. Source Population

Case group

All VL suspected patients who were visiting Kahsay Abera and Mearg Hospitals from November 2018 to March 2019 G.C.

Control group

All staff members of Kahsay Abera and Mearg Hospitals who were available during the study period.

5.3.2. Study participant

Case group

All VL patients confirmed at Kahsay Abera and Mearg Hospital laboratories during the study period.

Control group

All apparently healthy staff members of Kahsay Abera and Mearg Hospitals, who were match with cases in age and sex without having VL.

5.4. Inclusion and exclusion criteria

5.4.1. Inclusion criteria

Case group

All VL patients confirmed at Kahsay Abera and Mearg hospital laboratories during the study period.

Control group

All apparently healthy staff members of Kahsay Abera and Mearg Hospitals, who were match with cases in age and sex without having VL. Selection of controls was employed by physician based on the WHO guide line for the diagnosis of VL and rk39 was used to screen the healthy controls.

5.4.2. Exclusion criteria

Case group

VL patients who have history of any other chronic disease (kidney disease, liver disease, cancer, HIV/AIDS, DM, hypertension TB, and malaria). Patients under treatment of anti kala-azar were excluded from the study. Patients with habit of chronic alcohol drinking and smoking were also excluded from the study.

Control group

Individuals who have history of any chronic disease (kidney disease, liver disease, cancer, HIV/AIDS, DM, hypertension TB, and malaria). Individuals with habit of chronic alcohol drinking and smoking were also excluded from the study.

5.5. Study Variables

5.5.1. Dependent variable

- Clinical chemistry profiles
 - Level of Liver function tests(ALT, AST, ALP, direct andTotal bilirubin)
 - Level of Renal function tests (Urea, creatinine)
 - Levels of lipid profile tests (total cholesterol, HDL-c, LDL-c and triglycerides)

5.5.2. Independent variable

- Socio demography characteristics
- Parasite load
- Duration of illness

5.6. Sample Size Determination and sampling methods

5.6.1. Sample size determination

From comparative studies conducted in Brazil and Sudan, the mean value of different clinical chemistry tests were compared between cases and controls. The sample size was calculated by using the mean value of creatinine from the study conducted in Brazil which gives the highest sample size.

Table: 1 Mean and SD of kalaazar patients and control groups from Brazil and Sudan

Tests	Mean ± SD of VL cases	Mean ± SD of controls	N(sample size)
Urea	28±10	35±2.8	20
Creatinine	0.89±0.28	0.99±0.14	77
From the study conducted in sudan			
AST	159± 121	34± 11	8
ALT	57± 50	12± 6	11
ALP	234± 221	74± 29	10

Sample size needed for comparing the means of two normally distributed samples is calculated by using a two-sided test with significance level α and power $1 - \beta$ and we use 95% confidence level and 80% power to calculate appropriate sample size. From the study conducted in Brazil mean value of creatinine among VL patients and healthy controls (0.89 ± 0.28 ; 0.99 ± 0.14) was used to calculate sample size using the following formula:

$$n = (s_1^2 + s_2^2) / d^2 * (Z\alpha + Z\beta)^2 \quad \text{where } n = \text{desired sample size, } s_1 = \text{standard deviation of case group} \\ = 0.28, s_2 = \text{standard deviation of control group} = 0.14 \text{ from previous study, } Z\alpha = 1.96, Z\beta = \text{power} \\ = 0.84, d = \text{difference between two means} = 0.89 - 0.99 = -0.01 \text{ from previous study conducted in} \\ \text{Brazil.} \quad n = \frac{(0.28)^2 + (0.14)^2 * (1.96 + 0.84)^2}{(0.01)^2} = 76.8 \text{ round up to } 77$$

10% nonresponse rate = $0.1 * 77 = 7.7$ therefore the minimum sample size will be $77 + 7.7 = 85$ for each groups. Increasing sample size can give greater power to detect difference between control and case groups. Therefore 100 case groups and 100 control groups were studied in this study depending on time and cost.

5.6.2. Sampling method

Convenient sampling technique was employed to select study participants.

5.7. Measurement and Data collection

5.7.1. Data Collection Procedure

A semi-structured pretested and translated questionnaire was used to collect socio demographic characteristics. Data collectors fill the questionnaire by direct interview of the study participants. Information concerning the clinical history was obtained from clinical log sheet.

5.7.2. Laboratory Analysis

5.7.2.1. Specimen Collection, processing, and transportation

About 3-5 ml venous blood sample was collected using serum separator tube from both VL patients and healthy controls. Serum sample was separated by centrifuge at 4000 rpm for 5 minute and the sample was stored at temperature of -20 up to -30°C in deep freezer prior to laboratory analysis. The serum sample was transported to Adigrat general Hospital laboratory department for clinical chemistry analysis.

5.7.2.2. Liver Function Tests Analysis

Liver function test includes measurement of the concentration of alanine transaminase, aspartate transaminase, alkaline phosphatase (ALP), direct bilirubin and total bilirubin in the serum. The analysis was done by the principle of spectrophotometry for measuring the absorption spectrum of the analyte at each wavelength. The JOURI LABS reagent and Mindray 200 E automated chemistry analyzer was used. All the tests were performed based on the manufacturer's protocol.

5.7.2.3. Renal Function Tests Analysis

Renal function test includes measurement of urea, creatinine, and uric acid. The analysis was done by the principle of spectrophotometry for measuring the absorption spectrum of the analyte at each wavelength. The JOURI LABS reagent and Mindray 200 E automated chemistry analyzer was used to measure concentration of urea and creatinine from serum. All the tests were performed based on the manufacturer's protocol.

5.7.2.4. Lipid profile analysis

Lipid profile or lipid panel is a panel of blood tests that serves as an initial screening tool for abnormalities in lipids, such as cholesterol, triglycerides, HDL and LDL. The analysis was done by the principle of spectrophotometry for measuring the absorption spectrum of the analyte at each wavelength. The JOURI LABS reagent and biosystem reagents and Mindray 200 E automated chemistry analyzer was used to measure concentration of total cholesterol, triglyceride HDL and LDL and creatinine from serum. All the tests were performed based on the manufacturer's protocol.

5.8. Data Quality Assurance

5.8.1. Pre analytical

The questionnaires were pre tested on 5% of the study population one week before the actual data collection in Sheraro town to ensure clarity, length, logical sequence and skip patterns of the questions. Questionnaire was prepared in English and it was translated to the Tigrigna/Amharic which is easily understandable by the study participant. Experienced laboratory personnel were participated for proper collection, processing, and transportation of the sample. SOPs were used strictly to ensure labeling with identification number, proper sample container, sufficient volume and test procedures. The collected sample was allowed to clot for 20-25 minute and centrifuge

the specimen to separate serum for analysis. The quality of the instrument, reagent expiration, reagent volume, room temperature and sample hemolysis were checked before analysis.

5.8.2. Analytical

Both normal and pathological quality controls were analyzed before the sample analysis to ensure the proper function, validity and reliability of the instrument. LJ chart was drawn for both controls and interpreted based on the west guard rule to reject or to accept controls. The samples were analyzed after both controls were accepted.

5.8.3. Post Analytical

The result was properly recorded based on sample identification number and the data is properly handled and interpreted by the principal investigator. The laboratory result was recorded in the log book for rechecking. A clear and neat test result was reported to investigator for analysis.

5.9. Data Analysis and Interpretation

SPSS (Statistical package for social science) software was used to analyze the obtained data. The data was entered in to SPSS version 23 software and analyzed accordingly. Descriptive statistics was used and the descriptive data was expressed in number and percentage in the form of tables and figures. Binary logistic regression were used to check the significant association of clinical features and risk factors with abnormal values of clinical chemistry tests. Independent student t-test was used to compare the mean difference of VL cases and control groups. The P value < 0.05 with corresponding 95% confidence interval was considered as significant association.

5.10. Operational Definition

Liver function Tests- are groups of blood tests that useful in the evaluation and management of patients with hepatic dysfunction. Commonly used tests to check liver function are the alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), albumin, and bilirubin tests.

Renal Function Tests- are groups of blood tests that useful in the evaluation and management of patients with kidney dysfunction. Some of blood tests are urea, creatinine, and uric acid

Lipid profile test- are group of tests include total cholesterol, triglyceride, HDL-C, LDL-C, VLDL-C and other Apo lipoproteins.

Clinical chemistry alterations- it is the alteration of different tests includes RFT, LFT and Lipid profiles)

Cases - are individuals who have visceral leishmaniasis

Controls - are individuals who are apparently healthy and unlikely to share visceral leishmaniasis.

Chronic disease – is a human health condition or disease that is long lasting in its effect which includes hypertension, heart disease, kidney disease, liver disease, HIV/AIDS, cancer, diabetic mellitus e.t.c.

Normal- is the test value lies with in the established reference ranges

Low – is the value of the test lies below the established reference ranges

High – is the value of the test lies above the established reference ranges

5.11. Ethical Consideration

Ethical clearance was obtained from research and ethics committee of department of Medical Laboratory Science, Addis Ababa University. Further permission was also obtained from Tigray Regional Health Bureau and from Administrators of Kahsay Abera and Mearg hospitals. Before starting data and specimen collection, an informed written consent and assent was obtained from study participant by explaining the purpose of the study. Participants were respected for voluntary participation with adequate information and the confidentiality of any information obtained from participant was respected by using unique codes for the samples and results. Cooperation letter was obtained from Adigrat General Hospital to perform the clinical chemistry tests.

6. WORK FLOW

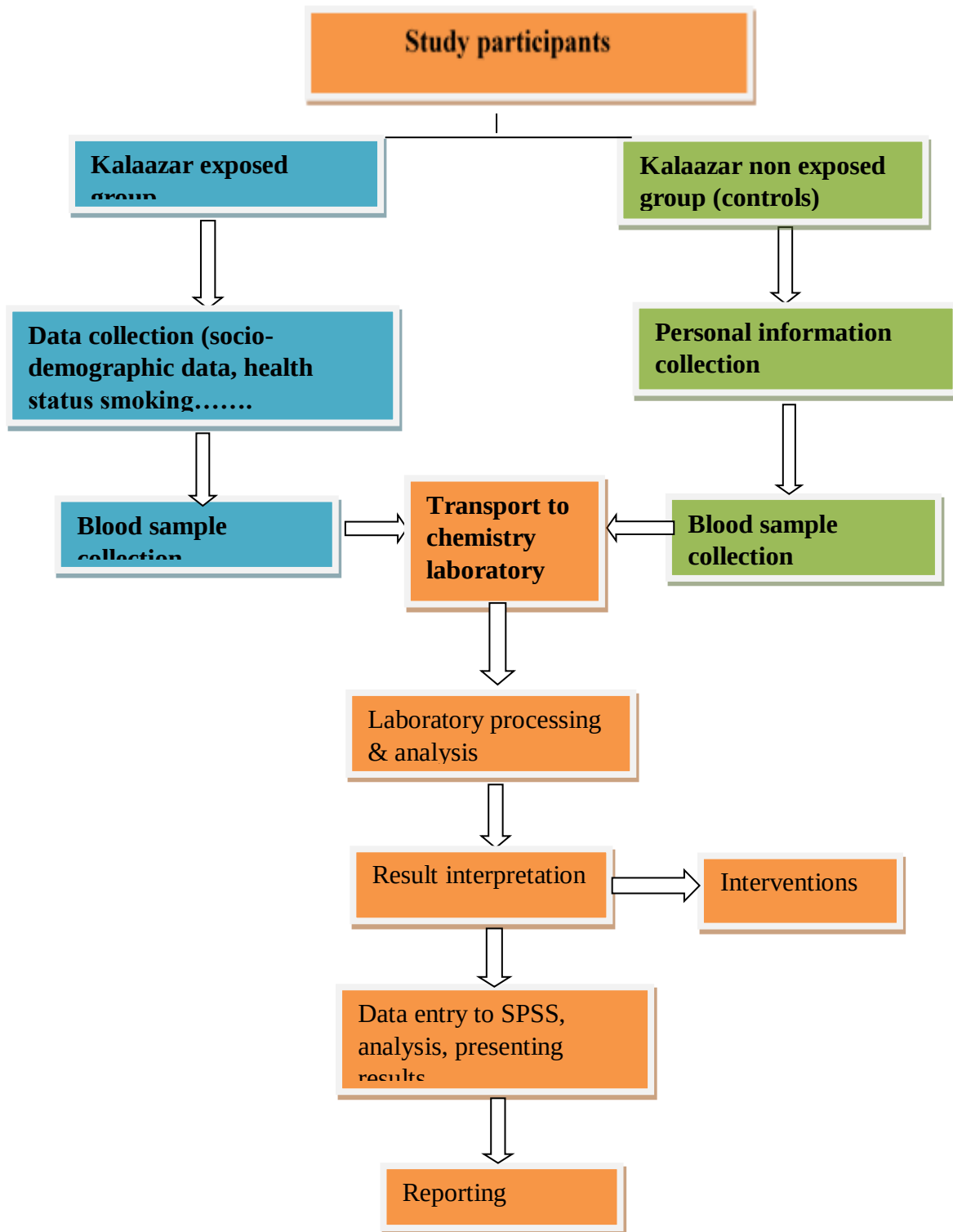


Figure: 2 Work flow of the study

7. RESULT

7.1. Socio demographic Characteristics of study participants

This study included 100 VL confirmed patients (91 of them were males) and 100 healthy control groups (90 of them were males). The mean age $\pm$ SD of VL patients and healthy controls were 27.98 $\pm$ 9.634 years (range 15-54 years) and 27.64 $\pm$ 4.758 years (range 17-41 years) respectively. About 49% of VL cases and 55% of healthy controls were single. Around 51% of VL cases were primary school and 85% of healthy controls were diploma and above (Table 2).

Table 2: Socio-demographic characteristics of visceral leishmaniasis patients and healthy controls in Western Tigray, Northern Ethiopia November 2018 to April 2019 (n=200)

Socio demography	VL cases (n=100)	Control groups (n=100)	P- value
Gender			
Male n (%)	91(91%)	90(90%)	0.809
Female n (%)	9(9%)	10(10%)	
Age in year n (%)			
15-20	28	4	
21-25	20	31	
26-30	23	42	
31-35	9	15	
>35	20	8	
Mean $\pm$ SD	27.98 $\pm$ 9.634	27.64 $\pm$ 4.758	0.752
Marital status			
Single n (%)	49	55	
Married n (%)	37	42	
Divorced n (%)	13	3	
Widowed n (%)	1	0	
Educational status			
Illiterate	29	0	
Primary	51	0	
Secondary	20	15	
Diploma and above	0	85	
Occupation			
Student	15	0	
Farmer	24	0	
Daily labour	60	0	
Merchant	1	0	
Government employee	0	100	
Residence			
Urban	6	100	
Rural	94	0	

7.2. Clinical features of visceral leishmaniasis patients

The main clinical symptoms and signs presented at the initial evaluation of VL cases were: fever (100%), splenomegaly (100%), general weakness (85%), skin mucosal pallor (72%), bleeding (67%), weight loss (65%), anorexia (52%) and hepatomegaly (36%) (Table 3).

Table 3: The frequency of clinical features of visceral leishmaniasis patients in Western Tigray, Northern Ethiopia November 2018 to April 2019 (n=100)

Sign and symptom	No (%)
Splenomegaly	100
Hepatomegaly	36
Fever	100
Weight loss	65
Jaundice	21
Skin mucosal pallor	72
Diarrhea	15
Anorexia	52
Abdominal pain	17
General weakness	85
Bleeding	67

7.2.1. Abnormal clinical chemistry test result of visceral leishmaniasis patients

From 100 VL cases an increased level of serum AST, ALT, ALP, direct bilirubin, total bilirubin, creatinine, urea and triglyceride was found in 9(9%), 10 (10%), 15(15%), 18(18%), 25(25%), 6(6%), 4(4%) and 16(16%) VL cases respectively; meanwhile a decreased serum urea, cholesterol and HDL-C has been reported from 33(33%), 35(35%) and 20(20%) VL cases respectively. LDL-C was lies with in the refrence ranges in all VL patients.

7.2.2. Clinical features of VL and abnormal clinical chemistry test results

An increased value of AST was significantly associated with hepatomegaly (AOR=10.7) and jaundice (AOR=5.4) with $p<0.05$. VL patients with hepatomegaly would have 10.7 times more likely to have higher AST. VL patients with jaundice would have 5.4 times more likely to have higher AST. The increased value of total bilirubin was significantly associated with jaundice with (AOR= 12.2, $p<0.001$). VL patients with jaundice would have 12.2 times more likely to have higher total bilirubin. Similarly the increased value of triglyceride was singnificantly associated with skin mucosal pallor with (AOR=0.2, $p<0.05$). VL patients with skin pallar would

have 0.8 times less likely to have higher triglyceride. However there was no statistical significant association of AST, total bilirubin, and triglyceride with gender, age, parasite load and duration of illness with $p>0.05$ (Table 4-6).

Table 4: The association of AST with clinical features of VL in Western Tigray, Northern Ethiopia November 2018 to April 2019 (n=100)

Factors		Aspartate aminotransferase				
		N(n)	H(n)	P-value	COR(95% CI)	AOR(95% CI)
Hepatomegaly	YES	28	8	0.035	0.17(0.4-0.74)	10.7(1.2-19.72)
	No	63	1	1	1	1
Jaundice	Yes	15	6	0.002	10.1(0.99-45)	5.44(1.81-29)
	No	76	3	1	1	1
Diarrhea	Yes	11	4	0.307	0.17(0.4-0.74)	4.9(0.23-102)
	No	80	5	1	1	1
Abdominal pain	Yes	13	4	0.74	0.2(0.049-0.88)	0.59(0.27-13.3)
	No	78	5	1	1	1
Weight loss	Yes	59	6	0.36	0.91(0.22-3.9)	0.31(0.03-3.8)
	No	32	3	1	1	1
Skin pallor	Yes	66	6	0.234	1.3(0.3-5.6)	0.14(0.006-3.5)
	No	25	3	1	1	1
Bleeding	Yes	60	7	0.47	0.55(0.1-2.8)	3.5(0.09-13.7)
	No	31	2	1	1	1
Gender	Male	83	8	0.8	1.3(0.14-11.7)	1.18(0.06-23.1)
	female	8	1	1	1	1
Age category	15-20	24	4	1	1	1
	21-25	18	2	0.37	0.66(.11-4.0)	0.27(0.02-4.6)
	26-30	21	2	0.54	0.57(0.09-3.4)	0.55(0.05-6.4)
	31-35	9	0	0.42	1.3(0.6-4.2)	0.27(0.01-6.3)
	>35	19	1	0.3	0.31(0.03-3.0)	2.1(0.76-8.9)
P. load	+1	12	1	1	1	1
	+2	41	3	0.9	0.8(0.08-9.2)	1.02(0.01-8.8)
	+3	35	5	0.601	1.7(0.18-16)	1.45(0.02-11.8)
	>+4	3	0	0.9	2.1(0.24-8.5)	2.21(0.34-9.3)

AST- Aspartate amino transaminase, P.load- parasite load COR- crude odds ratio, AOR-adjusted odds ratio, N-normal value, H- high value, P- p-value

Table 5: The association of total bilirubin with clinical features of VL in Western Tigray, Northern Ethiopia November 2018 to April 2019 (n=100)

Factors		Total bilirubin				
		N(n)	H(n)	P-value	COR(95% CI)	AOR(95% CI)
Hepatomegaly	YES	28	8	0.53	0.79(0.3-2)	0.68(0.005-1.0)
	No	47	17	1	1	1
Jaundice	Yes	7	14	0.001	0.08(0.03-0.24)	12.2(3.89-38)
	No	68	11	1	1	1
Diarrhea	Yes	11	4	0.185	0.9(0.26-3.10)	16.4(0.26-10.37)
	No	64	21	1	1	1
Abdominal pain	Yes	13	4	0.10	1.1(0.3-3.7)	0.035(0.001-1.96)
	No	62	21	1	1	1
Weight loss	Yes	49	16	0.80	1.0(0.4-2.7)	0.82(0.18-3.7)
	No	26	9	1	1	1
Skin pallor	Yes	52	20	0.3	0.56(0.19-1.7)	0.81(0.17-3.8)
	No	23	5	1	1	1
Bleeding	Yes	48	19	0.36	0.5(0.2-1.5)	1.9(0.4-9.6)
	No	27	6	1	1	1
Gender	Male	69	22	0.37	1.5(0.3-6.8)	2.75(0.3-25)
	female	6	3	1	1	1
Age category	15-20	17	11	1	1	1
	21-25	19	1	0.07	1.5(0.4-5.1)	0.67(0.71-2.9)
	26-30	19	4	0.8	0.12(0.02-1.1)	0.18(0.03-1.3)
	31-35	6	3	0.70	0.5(1.1-2.1)	1.0(0.1-10)
	>35	14	6	0.5	1.2(0.2-6.3)	0.52(0.08-3.5)
P. load	+1	11	2	1	1	1
	+2	34	10	0.78	2.9(.22-9.7)	0.73(0.085-6.3)
	+3	27	13	0.54	4.7(.13-5.8)	1.67(0.78-12.1)
	>+4	3	0	0.65	7.7(.8-8.7)	1.41(0.42-6.3)

P.load- parasite load, COR- crude odds ratio, AOR- adjusted odds ratio, CI- confidence interval

N-normal value, H- high value, P- p-value

Table 6: The association of triglyceride with clinical features of VL in Western Tigray, Northern Ethiopia November 2018 to April 2019 (n=100)

Factors		Triglyceride				
		N(n)	H(n)	P-value	COR(95% CI)	AOR(95% CI)
Hepatomegaly	YES	32	4	0.32	0.54(0.16-1.6)	1.0(0.19-5.2)
	No	52	12	1	1	1
Jaundice	Yes	20	1	0.14	4.6(0.6-37.1)	0.105(0.005-2.4)
	No	64	15	1	1	1
Diarrhea	Yes	15	0	0.9	3.7(0.1-7.6)	2.1(0.74-3.4)
	No	69	16	1	1	1
Abdominal pain	Yes	16	1	0.23	3.5(0.4-28)	5.6(0.15-20.8)
	No	68	15	1	1	1
Weight loss	Yes	54	11	0.70	0.8(0.26-2.6)	1.0(0.25-4.1)
	No	30	5	1	1	1
Skin pallor	Yes	64	8	0.03	3.2(1-9.6)	0.2(0.041-0.97)
	No	20	8	1	1	1
Bleeding	Yes	56	11	0.22	0.9(0.2-2.8)	2.7(0.6-12.5)
	No	28	5	1	1	1
Gender	Male	76	15	0.88	0.63(0.07-5.4)	0.83(0.077-9)
	female	8	1	1	1	1
Age category	15-20	25	3	1	1	1
	21-25	18	2	0.85	0.36(0.07-1.7)	0.8(0.09-7.5)
	26-30	18	5	0.9	0.33(0.05-1.9)	1.1(0.17-6.8)
	31-35	8	1	0.9	0.8(0.2-3.4)	1.2(0.07-19.3)
	>35	15	5	0.54	0.37(.04-3.8)	1.8(0.26-13)
P. load	+1	10	3	1	1	1
	+2	38	6	0.15	4.8(0.7-8.6)	0.25(0.04-1.6)
	+3	33	7	0.77	2.5(0.47-6.4)	0.75(0.1-5.1)
	>+4	3	0	0.45	3.4(0.26-7.4)	0.88(0.11-5.6)

P.load - parasite load, *COR*- crude odds ratio, *AOR*- adjusted odds ratio, *CI* -confidence interval, *N*- normal value, *H*- high value, *P*- p-value

7.3. Comparison of clinical chemistry tests among VL cases and healthy controls

Liver enzymes like ALT, AST, ALP and total bilirubin were significantly higher in VL patients than in healthy controls with means $\pm$ SD was (25.3 ± 19.3 versus 17.58 ± 6.38 , $p < 0.001$), (28.34 ± 29.17 versus 19.09 ± 7.94 , $p = 0.003$), (95.3 ± 60 versus 76.02 ± 19.22 , $p = 0.002$) and (0.759 ± 0.413 versus 0.62 ± 0.23 , $p = 0.004$) respectively. Similarly the mean value of serum triglyceride was significantly higher among VL patients compared to healthy controls (146.36 ± 63.64 versus 111.46 ± 33.3 , $p < 0.001$).

The mean value of serum urea and total cholesterol was significantly lower in VL patients than healthy controls (23.11 ± 10.72 versus 29.19 ± 9.32 , $p < 0.001$) and (130.37 ± 36.69 versus 145.1 ± 20.07 , $p = 0.001$) respectively (Table 7).

Table 7: Comparison of clinical chemistry tests among VL cases and healthy controls in Western Tigray, Northern Ethiopia, November 2018 to April, 2019 (n=200)

Parameter	VL cases(n=100) Mean $\pm$ SD	Healthy Controls(n=100) Mean $\pm$ SD	p-value
ALT U/L	25.3 $\pm$ 19.3	17.58 $\pm$ 6.38	0.001
AST U/L	28.34 $\pm$ 29.17	19.09 $\pm$ 7.94	0.003
ALP U/L	95.3 $\pm$ 60	76.02 $\pm$ 19.22	0.002
Total Bilir (md/dL)	0.759 $\pm$ 0.413	0.62 $\pm$ 0.23	0.004
Direct Bilir md/dL)	0.19 $\pm$ 0.079	0.186 $\pm$ 0.056	0.592
Urea md/dL	23.11 $\pm$ 10.72	29.19 $\pm$ 9.32	0.001
Creatinine mg/dL	0.806 $\pm$ 0.25	0.82 $\pm$ 0.18	0.651
Total CHO	130.37 $\pm$ 36.69	145.1 $\pm$ 20.07	0.001
Triglyceride	146.36 $\pm$ 63.64	111.46 $\pm$ 33.3	0.001
HDL	45.97 $\pm$ 10.24	48.11 $\pm$ 6.2	0.075
LDL	78.14 $\pm$ 18.24	75.21 $\pm$ 13.82	0.202

SD- Standard deviation, ALT-Alanine amino transaminase, AST- Aspartate amino transaminase, ALP- Alkaline phosphatase, Bilir- bilirubin, CHO- cholesterol, HDL- high density lipoprotein and LDL- low density lipoprotein.

- Independent-t –test was used for comparison and P- value ≤ 0.05 is considered as statistical significant.

8. DISCUSSIONS

Visceral leishmaniasis causes derangement of liver functions and rarely VL patients also can present with severe life-threatening hepatitis [22]. *L. donovani* causes lipid profile changes and affect the kidney which leads to alteration of glomerular and tubular functions [4, 8].

According to this study visceral leishmaniasis has significant effect on the function of liver and lipid profiles changes in VL patients in comparison to healthy controls. The mean value of ALT, AST, ALP, total bilirubin and triglyceride were significantly higher in leishmaniasis patients than in healthy controls. The mean value of serum urea and total cholesterol was significantly lower in VL patients than healthy controls. However there was no statistical significant effect of kalaazar on the mean of direct bilirubin, creatinine, HDL and LDL.

In the present study the mean of serum ALT, AST and ALP were significantly higher in VL cases than healthy controls. This finding is in line with the previous study conducted in Sudan, Iraq and India [22, 24, 32, 34] which reported significantly higher value of AST, ALT and ALP in VL patients. Furthermore, different case reports support the present study and reported liver dysfunction with raised value of serum AST, ALT and bilirubin from VL patients [29, 31, 33, 40-42]. Similarly a studies conducted in India and Bangladesh reported abnormally high liver inflammatory enzymes like ALT, AST, ALP in VL patients [22, 30]. This may be attributed to the levels of particular circulating cytokines during the inflammatory processes observed in active VL disease could be a determinant and consequence of severe liver disease [42]. The liver function alteration may be due to hepatomegaly and the generation of immune complex in VL patients. The production of reactive oxygen species from the activated macrophage may also contributes to the alterations of liver function [13].

The mean value of direct bilirubin and serum total bilirubin showed slight increment in VL cases than controls. However, only mean value of total bilirubin was significantly higher in VL patients compared to healthy controls. This finding is in line with the study conducted in Bihar, India [32] which shows that serum bilirubin was significantly higher in VL cases than in healthy controls. In addition different case reports conducted in India, Turkey and also a cohort study conducted in Brazil showed that elevated total and direct bilirubin in VL patients [31, 33, 43]. This may attribute to hemolysis of red blood cells and sequestration and destruction of red blood cells (RBC) in enlarged spleen of VL patients [17].

The mean of Serum Triglyceride was significantly higher and serum total cholesterol was significantly lower among VL patients compared to healthy controls. These results are in agreement with studies conducted in Iraq, Brazil and Greece [4, 6, 35]. A case report conducted in Greece and other study conducted in California were reported decreased serum cholesterol levels, severe hypocholesterolemia, mildhypertriglyceridemia from VL patients [3, 37]. This lipid alteration may be due to extraction of membrane cholesterol by *L. donovani* and the changes in the levels of lipoproteins may be directly related to the modulation of the immune response [5]. Endothelial lipoprotein lipase, an enzyme responsible for cleavage of triglyceride may be impaired in patients with VL. This can be decreasing the metabolism of triglyceride which leads to increased triglyceride and decrease the formation of HDL and LDL from VLDL [4, 6].

There is no significant difference on the mean of HDL and LDL among VL cases and healthy controls. In contrast different studies conducted in Iraq, Greece and Brazil reported significantly low value of serum HDL and LDL among VL patients than controls [4-6, 35-37]. This difference may be due to genetic variabilities, diet effect, temperatures, seasonal variation.

The mean value of serum creatinine has no significant difference among VL patients and healthy controls. This finding is in line with another study conducted in Brazil which reported no significant difference of creatinine level between VL cases and controls [27]. Whereas serum urea was significantly lower in VL cases compared to the healthy controls. This finding is in agreement with study conducted in Brazil [27]. This significantly decreased urea may be attributed to low protein diet and reduced production in the liver.

In the present study assessed the effect of gender, age, and parasite load on clinical chemistry test alterations of VL patients. The abnormal value of AST, total bilirubin, and triglyceride has no significant association with gender, age, parasite load in the present study. This finding is not in agreement with the study conducted in Baghdad, Iraq which reported significant association of gender, and age with AST and serum bilirubin [32]. This discordant may be attributed to genetic variabilities and environmental factors like temperature, diet, altitude and climatic changes.

The present study also assessed the association of clinical features of VL with the alteration of clinical chemistry tests. The value of AST was significantly associated with hepatomegaly and jaundice. This may be attributed to the over growth of the parasite in the liver which results abnormal morphology and altered functions [34]. The value of total bilirubin was also significantly associated with Jaundice. This may be attributed to immune complex mediated RBC hemolysis and sequestration of spleen [17].

9. STRENGTHENS AND LIMITATIONS OF THE STUDY

9.1.Strengthens of the Study

Up to the knowledge of principal investigator and advisors this study is the first in Ethiopia and it provides baseline information for further study and policy makers. Control individuals were screened clinically by physician and by rK39 test kit in the laboratory. All VL cases and healthy controls were diagnosed for malaria.

9.2.Limitations of the Study

Different tests like electrolyte, GFR, uric acid, protein, albumin and endocrine tests were not done in this study due to lack of budget and time.

The literatures for this study were limited and this makes difficult to discuss our result with other findings.

10. CONCLUSIONS

Visceral leishmaniasis patients showed significant alterations of liver function tests and lipid profile tests compared to healthy controls. This may indicate that VL is the determinant factor for developing liver and lipid disorders. Even though the renal function tests show slightly increased in VL patients than controls, there was no statistical difference between VL cases and healthy controls. The clinical chemistry tests like AST, total bilirubin, and triglyceride were significantly associated with the main clinical features like hepatomegaly and jaundice.

11. RECOMMENDATIONS

- It will be better if health professionals and stakeholders use clinical chemistry tests for management of liver dysfunction and lipid disorders of VL patients from endemic areas to prevent the complications of the disease.
- Large scale Cohort study is recommended to study the effect of visceral leishmaniasis on different organs of the body to build a comprehensive picture on the risk of VL.

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13. ANNEXS

13.1. Annex I: Participants' information sheet

A. English version

Title of the project: Assessment of potential clinical chemistry biomarker for the diagnosis of Kalazaar in Kahsay Abera and Mearg Hospital, Western Tigray, North Ethiopia

Principal investigator: Brhane Tesfanchal G/gergis (BSC, MSC candidate)

Introduction

Dear study participants you are invited to participate in the study on the clinical and chemistry profile of kalazaar patients in Kahsay Abera Hospital, Tigray Region, Northern Ethiopia. This study is approved by Addis Ababa University Department of Medical Laboratory Science research ethics committee. You are voluntarily participating in this study and you have a full right to stop participation if you have something uncomfortable.

Purpose of the study. The main objective of the study is to assess clinical and chemistry profile of kalazaar patients attending Kahsay Abera Hospital, Tigray Region, Northern Ethiopia.

Duration: the duration of this study depend on the availability of study subjects and it may takes 3-5 months.

Associated risk with the study: during sample collection from your vein, there is minor pain or discomfort. The sample is collected by the experienced laboratory personnel and the risk is minimizing as well.

Procedure of the study

If you are agreed to participate in this study, you will give about 3-4 venous blood for clinical chemistry analysis.

Expected Benefit: Dear participants you will have a good benefit from this study because this study assess your liver function, kidney function and lipid profile changes. No payment is requested for the clinical chemistry tests and you will know your health status early. If your result shows any abnormalities of clinical chemistry tests, you will manage and treated early.

Confidentiality:

The confidentiality of your information and laboratory results are respected strictly. A unique identification number is given to you and your name will not be written in the form and the result of laboratory tests could only be accessed by the researcher.

Agreement

Dear participant, you have read all the information described above and you will request to put your signature to indicate your agreement to participate in the study.

Participant name _____

Date _____ sign _____

If you have any question please contact the following address:

Principal Investigator: Brhane Tesfanchal Gebregergis (BSc, MSc candidate)

Mobile Phone: +251949604754

Email: birhanetesfanchal@gmail.com

B. Amharic version

ለተሳታፊዎች መረጃ መስጫ ሰነድ

የጥናቱ ርዕስ: በካሕሳይ አበራ ሆስፒታል የሚገኙ የካላክዘር ሕሙማን ላይ የክሊኒካል ኬሚስትሪ ምርመራና ጥናት

የአጥኝው ስም: ብርሃነ ተስፋነችኤል ገብረገርግስ

የተቋሙ ስም: አዲስ አበባ ዩኒቨርሲቲ የህክምናና ጤና ሳይንስ ኮሌጅ የሕክምና ላቦራቶሪ ትምህርት ክፍል

መግቢያ: የተከበሩ የጥናቱ ተሳታፊ በካሕሳይ አበራ ሆስፒታል የሚገኙ የካላክዘር ሕሙማን ላይ የሚደረገው የክሊኒካል ኬሚስትሪ ምርመራ ጥናት ለመሳተፍ ተጋብዞል ::ይህ ጥናት በ አዲስ አበባ ዩኒቨርሲቲ ላቦራቶሪ ትምህርት ክፍል የጥናትና የሰነድ ምርመራ ኬሚስት የጸደቀው ጥናተ መሆኑን መገለጽ እንወዳለን :: በዚህ ጥናት መሳተፍ ሙሉ በሙሉ በእርስዎ ፍቃደኝነት የተመሰረተ በመሆኑ በማንኛውም ሰአትና በታ የማቋረጥ ሙሉ መብትዎን የተጠበቀ ነው::

የጥናቱ ዋና አላማ:- በካሕሳይ አበራ ሆስፒታል የሚገኙ የካላክዘር ሕሙማን ላይ የክሊኒካል ኬሚስትሪ ምርመራ ማድረግና ማጥናት ::

የጥናቱ ጊዜ: የጥናቱ ጊዜ በምናገኘው የካላክዘር በሽተኞች ብዛት የሚወሰን ሲሆን ከ3 እስከ 4 ወር ሊወስድ ይችላል::

ከጥናቱ ጋር ተያይዞ የሚመጣ ጉዳት: የደም ናሙና በሚሰጥበት ወቅት ምንም አይነት የከፋ ችግር አያጋጥምዎትም :: ነገር ግን ደም ሲወስድ መጠነኛ የህመም ስሜት ልያስከትል ይችላል :: ሆኖም ግን

ናሙናውን ለመሰብሰብ ልምድ ባላቸው ባለሙያ ስለሚመደብና አስፈላጊውን ጥንቃቄ እርምጃ ስለሚወስድ የህመም ስሜት አይኖርም።

ከጥናቱ የሚያገኙት ጥቅም፡ ያለ ምንም ክፍያ የጉበት ና ኩላልት ምርመራ ያደርጋሉ። ችግር ካለ በፍጥነት ህክምና ይሰጣቸዋል።

ከርስዎ የምናገኘው መረጃ እና ሚስጥራዊነቱ፡ የእርሶ ስም በዚህ መጠይቅ ላይ አይጠቀስም። በተጨማሪም የሚሰጡት መረጃ እና በሰጡትም ደም ላይ የሚደረገው የምርመራ ውጤት ከተባለለት ጉዳይ ውጪ እንደማይውል እና ሚስጥራዊነቱ የተጠበቀ እንደሚሆን አረጋግጣለሁኝ።

በዚህ ጥናት ላይ ያለዎትን ጥያቄ በሚከተሉት አድራሻ በማንኛውም መጠቀም ይችላሉ።

የአጥኝው ስም፡ ብርሃነ ተስፋነችኤል ገብረገርግስ ስልክ፡ +251924327946 Email: birhanetesfanchal@gmail.com

አማካሪዎች 1) ዶ/ር ሚስጥረ ወልዴ ስልክ፡ +251-911699710 Email: mistire08@gmail.com

C. Tigrigna version

ናይ መፅናዓይ ሸም፡ ብርሃነ ተስፋነችኤል ገብረገርግስ

ናይ ትካል ሸም፡ አዲስ አበባ ዩኒቨርሲቲ ጥዕናን ሕክምና ሳይንስን ኮሌጅ ናይ ሕክምና ላቦራቶሪ ትምህርቲ ክፍሊ

ናይቲ መፅናዕቲ ርእሲ፡- ኣብ ካህሳይ ኣበራ ሆስፒታል ዝርከቡ ናይ ካላኣዛር ሕሙማን ናይ ኬሚስትሪ ምርመራ ምግባርን ምጽናዕን

መእተዊ፡ ዝተከበሩ(ራ) ናይዚ ጽንዓት ተሳታፊ/ት ኣነ ናይ አድስ አበባ ዩኒቨርስቲ ጥዕናን ሕክምናን ሳይንስ ኮሌጅ ናይ ሕክምና ላቦራቶሪ ትምህርቲ ክፍሊ ብማስተርስ ድግሪ ተምሃራይ እዮ። ንሶም ወይ ንሶን ኣብ ካህሳይ ኣበራ ሆስፒታል ዝርከቡ ናይ ካላኣዛር ሕሙማን ናይ ኬሚስትሪ ምርመራ ምግባርን ምጽናዕን ኣብ ዝብል ናይ መመረቂ መፅናዕቲ ፅሑፍ ተሳታፊ ንክኹ ተዓድሞም አለዉ።

ናይቲ መፅናዕቲ ዋና ዓላማ፡- ኣብ ካህሳይ ኣበራ ሆስፒታል ዝርከቡ ናይ ካላኣዛር ሕሙማን ናይ ኬሚስትሪ ምርመራ ምግባርን ምፍላጥን።

እቲ ጽንዓት ዝካየድሉ እዋን : ናይቲ ጽንዓት እዋን ዝዉሰን በቶም ንረክቦም ናይ ካላኣዛር ሕሙማት እንትከዉን ካብ 3 ክሳብ 4 ወርሒ ክወስድ ይክእል እዩ።

ቐደም ሳዓብ እቲ ጽንዓት: ዝተከበሩ (ራ) ናይዚ ጽንዓት ተሳታፊ/ት እዞም ዝስዕቡ ናይዚ ጽንዓት ምስ ተረድኡ እና ፍቓደኛ እንድሕር ኮይኖም ነዚ ጽንዓት ዝከዉን ካብ 3 ክሳብ 5 ሚ.ሊ ዝከዉን ደም ክንወስድሎም ኢና።

ምሰቲ መፅናዕቲ ተታሒዙ ዝመፅእ ሳዕቤን:- ንምርመራ ዝከዉን ደም ኣብ ዝህብሉ እዋን ምንም ዓይነት ዝኸፍኦ ፀገም ኣየጋጥሞምን። ነገር ግን ደም ኣብ ዝውሰድሉ እዋን ዝተወሰነ ናይ ምሕማም ስሚዕት ክህሉይ ኽእል እዩ። ይኹን ዳኣምበር ደም ንምስብሳብ ልምዲ ብዘለዎም ባዓል ሞያታት ስለ ዝምደቡን ኣድላዪ ዝኾነ ጥንቃቄን ስለዝውሰድ ናይ ምሕማም ስምዕት ኣይህሉን።

ካብቲ መፅናዕቲ ዝረክብዎ ጥቕሚ: እዚ መፅናዕቲ ናይ ማስተርስ ድግሪ መመረቕ ፅሕፍ ከም ምኻኑ መጠን ኣብዚ መፅናዕቲ ብምስታፎም ዝረኽብዎ ናይ ገንዘብ ጥቕሚ ዋላኳ እንተዘይሃለዎ ካብቲ መፅናዕቲ ብዝርከብ ውፅኢት ግን ተጠቓሚ እዮም። ነቲ መፅናዕቲ ካብ ዝተወሰደ ደም ዝርከብ ውፅኢት ብነፃ ይረኽቡ እዮም። ብተወሳኺ ኣብቲ ናይ ደም ውፅኢት ለውጢ እንተሃልይዎ ምስ ሓኻይም ንክራኽቡን ንኽምርመሩን ይግበር እዩ።

ናይ ሕክምና መረዳእታ ብምስጥር ምሕላዉ ዝምልከት : ኣብዚ ጽንዓት ስለ ናቶም ወይ ናተን ንእክበ ዝኮነ ዓይነት መረዳእታ ብሚስጥር ከም ንሕዘለኩም ነፍልጥ። ነዚ መጽናዕቲ ኢልና ዘሎ ናቶም/ተን መንነት ዝገልጽ ኩሉ መረዳእታ ናብ ሚሽጥር ክንቐይሮ ኢና።ብተወሳኺ እቲ ትህቡና ደም ኮነ መረዳእታ ካብቲ ጽንዓት ወጻኢ ኣይንጥቀመሉን።

ካብቲ መፅናዕቲ ስለምቁራፅ:- ኣብቲ መፅናዕቲ ምስታፍ ብናቶም/ተን ፍቓደኝነት ዝተመስረተ ኮይኑ ኣብ ማእከል ምቕራፅን ዘይደለይዎ ሕቶ ዘይምምላስ ይኽእሉ/ላ እዮም/የን። ኣብዚ መፅናዕቲ ዘለዎም/ወን ሕቶን/ ርኢቶን ኣብ ዝኾነ ይኹን ግዜ ክሓቱ/ታ ይኽእሉ/ላ።

ንተወሳኺ ሓበሬታ ነዞም ዝስዕቡ ኣድራሻ ይጠቀሙ።

ናይ መፅናዕቲ ሽም: ብርሃነ ተስፋነችኤል ገብረገርግስ ሞባይል: 0949604754 ኢሜል
birhanetesfanchal@gmail.com

ኣማከርቲ 1) ዶ/ር ሚስጥረ ወልዴ ስልክ: +251-911699710 Email: mistire08@gmail.com

13.2. Annex II: Consent Form (adult study participants) –

A. English version

Principal investigator: Brhane Tesfanchal (BSC, MSC candidate)

Research title: Assessment of potential clinical chemistry biomarker for the diagnosis of Kalazaar in Kahsay Abera and Mearg Hospital Western Tigray, Northern Ethiopia.

I have read, or have had this document read to me in a language that I understand, and I understand the purposes, procedures and risks of this research project as described within it. I understand that at any time I may withdraw from this study without giving a reason. I know that no special payment for being participating in the study. I freely agree to participate in this study, as described. I understand that I was given a signed copy of this document to keep.

Name of participant. _____ Age _____ Address _____ Signature _____ Date _____

Interviewer's name _____ Signature _____

Principal investigator Name _____ Signature _____

B. Amharic version

የፍቃደኝነት ማረጋገጫ ቅጽ

የጥናቱ ርዕስ: በካሕሳይ አበራ ሆስፒታል የሚገኙ የካላኣዛር ሕመማን ላይ የክሊኒካል ኬሚስትሪ ምርመራና ጥናት

የአጥኝው ስም: ብርሃነ ተስፋነችኤል ገብረገርግስ

የተቋሙ ስም: አዲስ አበባ ዩኒቨርሲቲ የህክምናና ጤና ሳይንስ ኮሌጅ የሕክምና ላቦራቶሪ ትምህርት ክፍል

እኔ ከዚህ በታች የተገለጸው በዚህ ጥናት ተሳታፊ ልመሆን ስወሰን የጥናቱ ዓላማዎች አሳራጮችና ችግሮች ሁኔታዎች በግልጽ በመረዳትና እንዲሁም ከጥናቱ ተሳታፊነት ፈቃደኝነቴን በማነፃፅም ግዜ የማስወገድ መብቴን በመረጋገጥ ነዉ።

ስለዚህ በጥናቱ ተሳታፊ መሆኔን በፈርማዬ እየረጋገጥኩ ይህንን ስወስን በጥናቱ ሊከሰቱ የሚችሉ ስጋቶችን በሚገባ የተረዳሁና ከጥናቱ በማነፃፅም ግዜ ራሴን ለምግለል ብወስን ተገቢ የሆኑ ህክምናዎችና

እገዛዎች ሁሉ እንደማትነፈጉኝ በማመን ነው። እነዚህ መረጃዎች ሁሉ በሚገባ በምረዳዉ ቋንቋ የተገለጸልኝ መሆኑን በፈረማየ አጋግጣለሁ።

የተሳታፊው ስም
 ፊርማ..... ቀን
 የአጥኚው ስም ፊርማ ቀን

ስለ ትብብርዎ አመሰግናለሁ!

C. Tigrigna version

ናይ መፅናዓይ ሸም: ብርሃነ ተስፋነችኤል ገብረገርግስ

ናይ ትካል ሸም: አዲስ አበባ ዩኒቨርሲቲ ጥዕናን ሕክምና ሳይንስን ኮሌጅ ናይ ሕክምና ላቦራቶሪ ትምህርቲ ክፍሊ

ናይቲ መፅናዕቲ ርእሲ:- አብ ካህሳይ አበራ ሆስፒታል ዝርከቡ ናይ ካላኣዛር ሕሙማን ናይ ኬሚስትሪ ምርመራ ምግባርን ምጽናዕን

ኣነ አብዚ ታሕቲ ተገሊጹ ዝኒሆ መጽናዕቲ ተሳታፊ ንምኒን እንትዉስን እንተለኩ ናይዚ ጽንዓት ዘድልዩ ነገራት ብምርዳእ እና ካብዚ ጽንዓት እዚ ምስታፍ አብ ዝኮነ ይኹን እዋን ከም ዘግልል ብምርግጋጾይ እዩ።ስለዚ አብዚ ጽንዓት ተሳታፊ ምኒንይ በፈረማይ የራጋግጽ።እዞም ኩሎም መረደእታታት ብዝግባእ ብዝፍድኡኒ ቋንቋ ዝተገለጹለይ ምኒኖም በፈረማይ የራጋግጽ።

ናይ ተሳታፊ/ፊት መለለዪ ቐፅሪ ----- ፊርማ ----- ዕለት-----

ሓበሬታ ዝኣክበ በዓል ሞያ ሸም ----- ፊርማ ----- ዕለት-----

ስለ ዝተሓባበሩኒ የቐንየለይ!

13.3. Annex III: Parental consent form

A. English version

I _____ parent, after being fully informed about the purpose of this study on the assessment of potential clinical chemistry biomarker for the diagnosis of Kalazaar in Kahsay Abera and Mearg Hospital Western Tigray Northern Ethiopia. I, the undersigned, have been told about this research. I have been informed there is no harm related to giving specimen. I have been informed that other people will not know my child results as it coded with number rather than writing name. I understand that there may be no benefit to me personally apart from clinical service I get from these results. I have been encouraged to ask questions and have had my questions answered. I have been told that participation in this study is voluntary and I may refuse to be in the study. I know my participation will also be approved by my child. By signing below I agree to let my child to participate in this research study.

_____	_____	____/____/____
Name of parent	signature	Day/month/year
_____	_____	____/____/____
Witness (Illiterate)		Day/month/year
_____	_____	____/____/____
Name of the researcher	Signature	Day/month/year

B. Amharic version

የስምምነት መጠየቂያ ቅጽ

እኔ-----የልጄ አስታማሚ ስሆን የዚህን ጥናት አላማ በዉል ተረድቻለሁ።
የጥናቱ ርዕስ በካሕሳይ አበራ ሆስፒታል የሚገኙ የካላአዛር ሕሙማን ላይ የክሊኒካል ኬሚስትሪ ምርመራ ና
ጥናትበጥናቱ ልጄ እንዲሳተፍ ምርጫው የእኔ መሆኑን ነግረውኛል። ናሙና መስጠት ምንም አይነት
ጉዳት ልጄ ላይ እንደሌለው ተነግሮኛል። በጥናቱ ወቅትም የልጄ መረጃዎች በሚስጥር ስለሚያዝ በሌላ
ሰው ዘንድ እንደማይታወቅ ተረድቻለሁ። በውጤቱ ከሚገኘው የህክምና አገልግሎት በቀር ሌላ ልጄ በግሉ
የሚያገኘው ጥቅም እንደሌለ ተረድቻለሁ። ጥያቄ እንደጠይቅ ዕድል ተሰጥቶኝ ለጥያቄዎቼም በቂ ምላሽ
አግኝቻለሁ። የልጄ በጥናቱ መሳተፍ በእኔ ፍላጎት ብቻ እንደሆነ እና በጥናቱም አለመሳተፍ ምንም አይነት
ተፅዕኖ በልጄ ላይ እንደማያስከትል ተረድቻለሁ። በከዚህ በሻገር የልጄ በጥናቱ ውስጥ ለመካተት የእኔ

የወላጁ አሳዳጊ ፈቃድ እንደሚያስፈልግ ተረድቻለሁ። በእኔ ፍቃደኝነት ልጄ በጥናቱ እንደሚሳተፍ ከዚህ በታች በፈርማዬ አረጋግጣለሁ።

የተሳታፊ ስም.....

ፊርማ..... ቀን

የአጥኚው ስም ፊርማ

..... ቀን

ስለ ትብብርዎ አመሰግናለሁ!

C. Tigrigna version

ናይ ፍቓደኝነት መረጋገጫ ቅጥዒ

አነ----- ናይ ዉላደይ መላዓሊ እንትከዉን ናይዚ ጽንዓት ዓላማ ተረዲአ ኣለኩ። ኣብ ካህሳይ ኣበራ ሆስፒታል እና ኣብ ማዓርግ ሆስፒታል ዝርከቡ ናይ ካላኣዘር ሕሙማት ናይ ክሊኒካል ኬሚስትሪ ምርመራ ምፍላጥን ምጽናዕን ብዝተባህለ ርእሲ ዉላድይ ከም ዝሳተፍ/ትሳተፍ እቲ ድልየት ናተይ ከም ዝኮነ ነጊሩ/ነጊራትኒ ኣሎ/ኣለ።ናሙና ምሃብ ኣብ ዉላደይ ምንም ዓይነት ሽግር ከም ዘይብሉ ተነጊሩ ኣሎ።ዝኮነ ዓይነት መረዳእታ ብሚስጥር ከም እትሕዝዎ እዉን ተረዲአ ኣለኩ። ካብዚ ዉጽኢት ወጻኢ ካሊእ ምንም ዓይነት ጥቕሚ ከም ዘይረክብ እዉን ተረዲአ ኣለኩ።ናይ ዉላደይ ኣብዚ ጽንዓት ምስታፍ ብናተይ ድልየት ዝዉሰን ከም ዝኮነና ኣብዚ ጽንዓት ብዘይምስታፋ/ፋ ምንም ዓይነት ተጽዕኖ ከም ዘየምጽእ ተረዲአ ኣለኩ።ካብዚ ብተወሳኪ ናይ ዉላዲ ድልየት ከም ዘድሊ ተረዲአ ኣለኩ። ብናተይ ድልየት ዉላደይ ኣብዚ ጽንዓት ከም ዝሳተፍ ብፈርማይ የራጋግጽ።

ናይ ተሳታፊ/ፊት ሽም ----- ፊርማ ----- ዕለት-----

ሓበሬታ ዝኣክበ በዓል ሞያ ሽም ----- ፊርማ -----ዕለት-----

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ናይቲ መጽናዓይ ሽም----- ፊርማ -----ዕለት-----

ስለ ዝተሓበበሩኒ የቐንየለይ!

13.4. Annex IV: Assent form for children aged 12-17 years

A. English version

I fully informed about the purpose of this study on assessment of potential clinical chemistry biomarker for the diagnosis of Kalazaar in Kahsay Abera and Mearg Hospital Western Tigray Northern Ethiopia. I have been informed there is no harm related to giving specimen. I have been informed that other people will not know my test results as it coded with number rather than writing name. I understand that there may be no benefit to me personally apart from clinical service I get from these results. I have been given the opportunity to ask questions and my questions have been answered to my satisfaction. I voluntarily assent that I would participate in this study provided my parents/guardians give their consent to give my blood for the study.

Name of participant _____ signature _____ Day/month/year ____/____/____
Witness (Illiterate) _____

Parents phone number _____

Name of the researcher _____ Signature _____ Day/month/year ____/____/____

B. Amharic version

በካሕሳይ አበራ ሆስፒታል እና በ ማዓርግ ሆስፒታል የሚገኙ በካሕሳይ አበራ ሆስፒታል የሚገኙ የካላክረ ሕሙማን ላይ የክሊኒካል ኬሚስትሪ ምርመራ ና ጥናት በሚለው ርእስ ዋና ዓላማው ተነግሮታል፡፡ናሙና ብስጥ ምንም ዓይነት ችግር እንድሌለውም ተነግሮታል፡፡ በጥናቱ ወቅትም የኔ መረጃዎች በሚስጥር ስለሚያዝ በሌላ ሰው ዘንድ እንደማይታወቅ ተረድቻለሁ፡፡ በውጤቱ ከሚገኘው የህክምና አገልግሎት በቀር ሌላ ልጄ በግሉ የገኘው ጥቅም እንደሌለ ተረድቻለሁ፡፡ ጥያቄ እንድጠይቅ ዕድል ተሰጥቶኝ ለጥያቄዎቼም በቂ ምላሽ አግኝቻለሁ፡፡ በዚህ ጥናቱ ለመሳተፍ የኔ ፍላጎት እንዳለ ሁኖ ወላጆቼ ከፈቀዱልኝ ተስማምቻለሁ፡፡

የተሳታፊ ስም.....
ፊርማ..... ቀን

የአጥኚው ስም

ፊርማ ቀን

ስለ ትብብርዎ አመሰግናለሁ!

C. Tigrigna version

ኣብ ካህሳይ ኣበራ ሆስፒታል እና ኣብ ማዓርግ ሆስፒታል ዝርከቡ ናይ ኣብ ካህሳይ ኣበራ ሆስፒታል እና ኣብ ማዓርግ ሆስፒታል ዝርከቡ ናይ ካላኣዛር ሕሙማት ናይ ክሊኒካል ኬሚስትሪ ምርመራ ምፍላጥን ምጽናዕን ብዝተባህለ ርእሲ ኣብ ዝተባህለ ርእሲ ዋና ዓላምኡ ተነግሪኒ ኣሎ። ናሙና ምሃብ ምንም ዓይነት ሽግር ከም ዘየምጸእለይ ተነገሩኒ ኣሎ። ዝኮነ ዓይነት መረዳእታ ብሚስጥር ከም እትሕዝዎ እዉን ተረዲኦ ኣለኩ። ካብዚ ዉጽኢት ወጻኢ ካሊእ ምንም ዓይነት ጥቅሚ ከም ዘይረክብ እዉን ተረዲኦ ኣለኩ። ኣብዚ ጸንዓት እዚ ምስታፍ ናተይ ድልዩት ከም ዘሎ ኮይኑ ወለደይ እንድሕር ፈቂዶምለይ ንክሳተፍ ተስማዕሚዐ ኣለኩ።

ናይ ተሳታፊ/ፊት ሽም ----- ፊርማ ----- ዕለት-----

ሓበሬታ ዝኣክበ በዓል ሞያ ሽም ----- ፊርማ ----- ዕለት-----

-

ናይቲ መጽናዓይ ሽም----- ፊርማ ----- ዕለት-----

ስለ ዝተሓበበሩኒ የቐንየለይ!

13.5. Annex V: Structured Questionnaire for visceral leishmaniasis patients and control groups

A. English version

Identification: Name of facility _____

Region _____ Zone _____ Woreda _____ Tel: -----

Note: Please Encircle or Write the appropriate answer on the provided space.

No.	Variable	Response
1	Sex	1. Male 2. Female
2	Age	_____
3	Marital Status	1. Single 2. Married 3. Divorced 4. Widowed
4	Education level	1. Illiterate 2. Primary 3.Secondary 4.Diploma and above
5	Residence	1. Rural 2. Urban
6.	Occupation	1. Student 2. House wife 3. Government employer 4. Private employers 5. Farmer 6. Merchant 7. Daily labor 8. Other
7	Do you have history of hypertension	1. yes 2. No
8	Do you have history of HIV/AIDS?	1. Yes 2. No
9	Do you have history tuberculosis for the past two years?	1.Yes 2. No
10	Do you have history of DM?	1. Yes 2.No
11	Do you have habit of smoking?	1. Yes 2. No
12	If yes to Q # 11, how often do you smoke?	1. every day 2. two or three times per week 3. Other
13	Do you have alcohol drinking habit	1. yes 2. No
14	If yes to Q#13, how often do you take?	1. one per day 2. Two or three per day 2. More than four per week 4. some times(holiday)

15	Do you take any medication within three months?	1. Yes 2. No
16	If your answer is yes for Q#15, what type of drug do use?	1. Anti-protozoa 2. Anti-bacterial 3. Anti TB 4. other
17	Do you have history of chronic liver disease?	1. Yes 2. No
18	Do you have history of chronic kidney disease?	1. Yes 2. No
19	Do you have history of cancer?	1. Yes 2. No
20	Do you have history of chronic heart disease?	1. Yes 2. No

I thank you for your cooperation

B. Amharic version

መለያ: የተቋሙ ስም _____

ክልል _____ ወረዳ _____ ቀበሌ _____

ስልክ: -----

ማሳሰቢያ: እባክዎ ትክክለኛውን መልስ ያክብቡ ወይም ይፃፉ።

ተ.ቁ	ጥያቄዎች	መልስ
1	ጾታ	1. ወንድ 2. ሴት
2	እድሜ	
3	የጋብቻ ሁኔታ	1. ያለገባ/ች 2. በትዳር ላይ 3. የፈታ/ች 4. በሞት ምክንያት የተለያዩ
4	የትምህርት ደረጃ	1. ያልተማረ 2. አንደኛ ደረጃ(1-8) 3. ሁለተኛ ደረጃ (9-12) 4. ዲፕሎማ/ድግሪና ከዛ በላይ
5	መነረያ	1.ገጠር 2.ከተማ
6	ስራ	1.ተማሪ 2.የቤት እመቤት 3.የግል ሰራተኛ 4.ገበሬ 5.ነጋዴ 6.የቀን ሰራተኛ 7.ሌላ
7	በደሙ ውስጥ የድም ግፈት አለበት	1. አዎ 2. የለኝም

8	በደሙ ውስጥ የኤች ኦይቪ በሽታ አለበት	1. አዎ 2. የለኝም
9	የቲቪ በሽታ ታካሚ ኖት	አዎ 2. የለኝም
10	በደሙ ውስጥ የስኳር በሽታ አለበት	1. አዎ 2. የለም
11	ሲፖራ የመጨሰ ልምድ አለበት	1. አዎ 2. የለኝም
12	ለተራ ቁጥር 11 መልስዎ አዎ ከሆነ ለምን ያክል ግዜ ያጨሳሉ	1.በየቀኑ 2.ሁለት ወይም ሰባት ግዜ በሳምንት 3.ሌላ
13	አልኮል የመጠጣት ልምድ አለበት	1. አዎ 2. የለም
14	ለተራ ቁጥር 13 መልስዎ አዎ ከሆነ ምን ያክል ይጠጣሉ	1. 1.በየቀኑ 2. ሁለት ወይም ሰባት ግዜ በሳምንት 3.አንድ አንድ ግዜ(በባህል ግዜ)
15	በዚ ሶስት ወር ውስጥ መድሐኒት ወስዶ ያዉቃሉ	1. አዎ 2. የለም
16	ለተራ ቁጥር 15 መልስዎ አዎ ለምን አይነት በሽታ	1.ለ ፕሮቶዝዋ 2. ለባክቴሪያ 3.ለቲቪ 4.ሌላ
17	ለብዙ ግዜ የቆየ የጉበት በሽታ አለበት	1. አዎ 2. የለም
18	ለብዙ ግዜ የከላሊት በሽታ አለበት	1. አዎ 2. የለም
19	የካንሰር በሽታ አለበት	1. አዎ 2. የለም
20	ለብዙ ግዜ የቆየ የልብ በሽታ አለበት	1. አዎ 2. የለም

ስለ ትብብርዎ አመሰግናለሁ!

C. Tigrigna version

መለለዪ: ናይቲ ተቋም ሽም:_____ ናይቲ ተቋም ኮድ:_____

ክፍለ ከተማ_____ ወረዳ_____ ጣቢያ_____ ስልክ: -----

መተሳሰቢ: ትክክለኛ ዝኾነ መልሲ የክብቡ ወይ ከዓ ይፀሓፉ።

ተ. ቁ	ሕቶታት	መልሲ
1	ፆታ	1. ተባዕታይ 2. አንስታይ
2	ዕድሜ	
3	ኹነታት ሓዳር	1. ዘይተመርዓው/ወት 2. በዓለ ሓዳር

		3. ዝፈትሉ//ሐት 4. ብሞት ምኽንያት ዝተፈላለዩ
4	ናይ ትምህርቲ ደረጃ	1. ዘይተምሃረ 2. ቐዳማይ ደረጃ(1-8) 3. ካልኣይ ደረጃ (9-12) 4. ዲፕሎማ/ድግሪንልዕሉኡን
5	መንበሪ	1. ገጠር 2. ከተማ
6	ስራሕ	1. ተምሃራይ/ት 2. ናይ ገዛ ሰራሕተኛ 3. ናይዉልቀ ስራሕ 4. ሓረስታይ 5. ነጋዳይ 6. መዓልታዊ ሰራሕተኛ 7. ካሊእ
7	ኣብ ደመም ዉሽጢ ኣለዎም ዶ?	1. እዉ 2. የለን
8	ኣብ ደመም ዉሽጢ ኤች ኣይቪ ኣለዎም/ን?	1. እዉ 2. የብለይን
9	ናይ ቲቪ ሕማም ተሓካሚ ድዮም?	1. እዉ 2. የብለይን
10	ኣብ ደመም ዉሽጢ ናይ ሽኮርያ ሕማም ኣለዎም/ን?	1. እዉ 2. የብለይን
11	ሽጋራ ናይ ምትካክ ልምዲ ኣለዎም /ን?	1. እዉ 2. የብለይን
12	ንተራ ቁጥር 11 መልሶም ኣዎ እንተኮይኑ?	1. በቢማዓልቱ 2. ክልተ ወይ ሰለስተ ግዜ ኣብ ሰሙን 3. ካሊእ
13	ኣልኮል ናይ ምስታይ ልምዲ ኣለዎም /ን?	1. እዉ 2. የብለይን
14	ንተራ ቁጥር 14 መልሶም ኣዎ እንተኮይኑ?	1. በቢማዓልቱ 2. ክልተ ወይ ሰለስተ ግዜ ኣብ ሰሙን 3. ካሊእ
15	ኣብዉሽጢ ሰለስተወርሒ ዝኮነዓይነት መድሓኒትን ዝኮነዓይነት ሕማም ወሲዶም ዶይፈልጡ?	1. እዉ 2. የለን
16	ንታራቁፅሪ 15 መልሶም እዉ እንተኾይኑ ኣየናይዓይነት መድሓኒት እዮም ወሲዶም?	1. ፀረ-ፕሮቶዝዋ 2. ፀረ-ባክቴሪያ 3. ፀረ-ቲቢ 4. ካሊእ
17	ናይ ጸላም ከብዲ ሕማም ኣለዎም /ን ዶ?	1. ኣዉ 2. የለን
18	ናይ ከላሊት ሕማም ኣለዎም /ን ዶ?	1. ኣዉ 2. የለን
19	ናይ ካንሰር ሕማም ኣለዎም /ን ዶ?	1. ኣዉ 2. የለን
20	ናይ ልቢ ሕማም ኣለዎም /ን ዶ?	

ሰለ ዝተሓበበሩና ነመስግን

13.6. Annex VI: Standard Operating Procedure

SOP for Blood Collection

Equipment

- 21 gauge needle for each participant
- Blood collection tubes (serum separator tube)
- Tourniquet
- Box of nitrile/vinyl gloves
- 70% alcohol
- Cotton

Laboratory Blood sample collection procedure and processing

1. Assemble blood collection materials.
2. Identify and prepare the patient
3. Label tubes with the client's name/identification number.
4. Wear the rubber gloves and prepare the patient on comfortable position
5. Tie the tourniquet around the arm of the patient just above the bend in the elbow. The tourniquet should be positioned 7.5cm to 10cm above the puncture site.
6. using the tip of the index finger examine the phlebotomy site, feel the vein, and decide exactly where to place the puncture
7. Disinfect the phlebotomy site by swabbing the skin with alcohol swab.
8. Insert the needle directly into the vein and withdraw peripheral blood of approximately 3ml in serum separator tube
9. Withdraw the needle from the vein and cover the puncture site cotton swab and hold pressure at the puncture site for 3 minutes.
10. Properly discard the used materials in a safe container.
11. Leave for 30 to 45 min. to clot the blood
12. Centrifuge at 4000 rpm for 5 minutes and Serum will separate

13.7. Annex VII: Principles of clinical chemistry tests

A. Liver function tests

1. Alanine amino transeaminase

Principle of the test

Alanine aminotransferase (ALT/GPT) catalyzes the transfer of the amino group from alanine to oxoglutarate with the formation of glutamate and pyruvate. The latter is reduced to lactate by lactate dehydrogenase (LDH) in the presence of reduced nicotinamide adenine dinucleotide (NADH). The reaction is monitored kinetically at 340 nm by the rate of decrease in absorbance resulting from the oxidation of NADH to NAD⁺, proportional to the activity of ALT present in the sample.



Result interpretation

The liver is specially rich in ALT, being the enzyme measurement used primarily as a test for infectious and toxic hepatitis, although high levels of both ALT and AST may also be found in cases of liver cell damage and acute pancreatitis, suggesting that the obstruction of the biliary tree by the edematous pancreas and the presence of associate hepatic disease may contribute to elevated AST levels in these patients. Slight or moderate elevations of AST and ALT activities may be observed after intake of alcohol and after administration of various drugs, such as salicylates, opiates and ampicillin.

Aspartate amino transeaminase

Principle

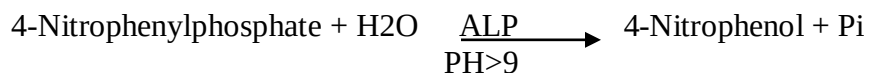
Aspartate aminotransferase (AST/GOT) catalyzes the transfer of the amino group from aspartate to oxoglutarate with the formation of glutamate and oxalacetate. The latter is reduced to malate by malate dehydrogenase (MDH) in the presence of reduced nicotinamide adenine dinucleotide (NADH). The reaction is monitored kinetically at 340 nm by the rate of decrease in absorbance resulting from the oxidation of NADH to NAD⁺, proportional to the activity of AST present in the sample.



Alkaline phosphatase

Principle of the test

Alkaline phosphatase (ALP) catalyzes the hydrolysis of 4- nitrophenylphosphate (4-NPP) with the formation of free 4- nitrophenol and inorganic phosphate, acting the alkaline buffer as a phosphate-group acceptor. The reaction is monitored kinetically at 405 nm by the rate of formation of 4-nitrophenol, proportional to the activity of ALP present in the sample.



Interpretation of result

Serum ALP measurements are of particular interest in the investigation of two groups of conditions: bone disease and hepatobiliary disease. Physiological bone growth elevates ALP in serum of growing children and a transient elevation may be found during healing of bone fractures. The response to the liver to any form of biliary tree obstruction is to synthesize more ALP. Intrahepatic obstruction of the bile flow by invading cancer or drugs raises serum ALP. Any drug that is hepatotoxic or induces cholestasis will greatly increase serum ALP.

Bilirubin direct

Principle

Bilirubin is converted to coloured azobilirubin by diazotized sulfanilic acid and is measured photometrically. Of the two bilirubin fractions in serum –bilirubin-glucuronide and free bilirubin which is bound to albumin– only the former reacts directly, while free albumin reacts after being displaced from protein by an accelerator. The difference of two measurements total bilirubin (with accelerator) and direct bilirubin (without accelerator) enables the calculation of indirect bilirubin. The terms «direct» and «indirect» bilirubin refers exclusively to the reaction characteristics in the presence or absence of an accelerator or solubilizer and are only approximate equivalents of the two bilirubin fractions.

Result interpretation

Conjugated or unconjugated) in plasma is an indication of a disturbance in bilirubin metabolism. This condition is caused either by an overproduction of bilirubin or by an impairment in the metabolic pathway. The increase in bilirubin production is usually caused by a rapid destruction of erythrocytes, resulting from blood diseases such as haemolytic anemia. In newborns the increase in bilirubin may be caused by Rh, ABO, or other blood group incompatibility, by sepsis,

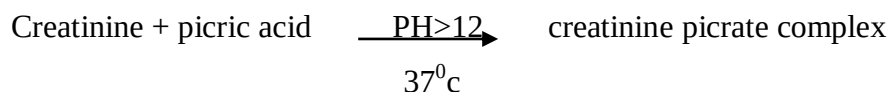
hepatic immaturity, or by a variety of hereditary defects in bilirubin conjugation. Impairment in the bilirubin metabolism is caused either by an enzyme deficiency or by a physical obstruction in bilirubin flow such as biliary (bile duct) obstruction. The hyperbilirubinemia leads to kernicterus (deposition of unconjugated bilirubin in brain and nerve cells) or jaundice (discoloration of mucous membranes, sclera and skin caused by the deposition of bilirubin pigment) [44].

Renal Function Assay

Creatinine

Principle

Creatinine under alkaline conditions reacts with picrate ions forming a red color complex. The formation rate of the complex measured through the increase of absorbance in a prefixed interval of time is proportional to the concentration of creatinine in the sample.



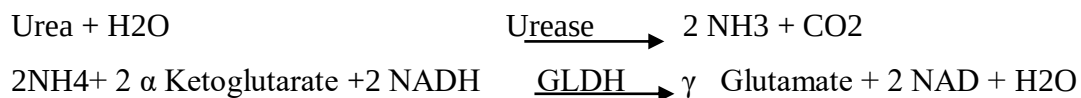
Result interpretations

Creatinine is synthesized in the body at a fairly constant rate from creatine, which is produced during muscle contractions from creatine phosphate. Creatinine in the blood is then removed by filtration through the glomeruli of the kidney for excretion in the urine. Since the excretion of creatinine in healthy individuals is independent of diet and thus relatively constant, the creatinine Clearance (CC) test is one of the most sensitive tests to diagnose renal function especially the glomerular filtration rate (GFR) the concentration of creatinine in serum being dependent almost entirely upon its rate of excretion by the kidney. Elevated levels of creatinine in serum are usually associated with renal diseases, especially those related to GFR such as glomerular nephritis [44].

Urea

Principle

Urea in the sample is hydrolyzed by urease to ammonia and carbon dioxide. The second reaction, catalyzed by glutamate dehydrogenase (GLD) converts ammonia and α -ketoglutarate to glutamate and water with the concurrent oxidation of reduced nicotinamide adenine dinucleotide (NADH) to nicotinamide adenine dinucleotide (NAD). Two moles of NADH are oxidized for each mole of urea present. The rate of decrease in absorbance at 340 nm is measured and proportional to the concentration urea in the sample



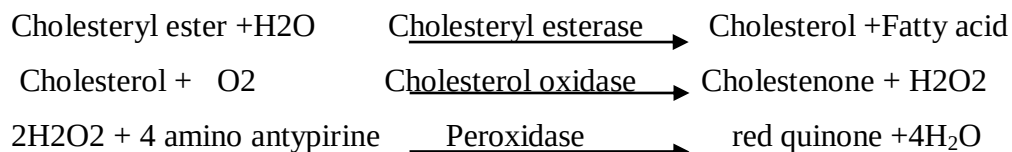
Result interpretations

Urea is the nitrogen-containing end product of protein catabolism. States associated with elevated levels of urea in blood are referred to as hyperuremia or azotemia. Parallel determination of urea and creatinine is performed to differentiate between pre-renal and post-renal azotemia. Pre-renal azotemia, caused by e.g. dehydration, increased protein catabolism, cortisol treatment or decreased renal perfusion, leads to increased urea levels, while creatinine values remain within the reference range. In post-renal azotemias, caused by the obstruction of the urinary tract, both urea and creatinine levels rise, but creatinine in a smaller extent [44].

Lipid profile tests

Total cholesterol

The measurement of total cholesterol in serum involves three enzymes: cholesterol esterase (CE), cholesterol oxidase (CO) and peroxidase (POD). In the presence of the former the mixture of phenol and 4-aminoantipyrine (4-AA) are condensed by hydrogen peroxide to form a quinoneimine dye proportional to the concentration of cholesterol in the sample.



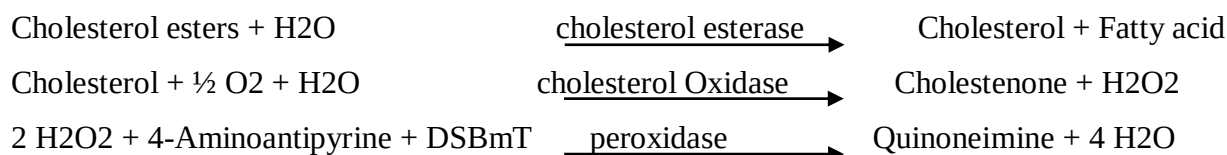
Interpretation of result

Cholesterol exists in the human blood as a free sterol and in an esterified form. The knowledge of the plasma level of lipids (cholesterol and triglycerides) together with lipoproteins of high and low density (HDL and LDL) aids in the detection of many conditions bound to metabolic disorders of high risk. The imbalance in the level of lipoproteins in plasma leads to hyperlipoproteinemias, a group of disorders that affects lipid levels in serum, causing coronary heart disease (CHD) and atherosclerosis, conditions in which the cholesterol levels are important tools in their diagnosis and classification. Jaundice of the obstructive type usually is accompanied by elevated total serum cholesterol with a normal ester fraction. Diabetes, hypothyroidism, and certain types of kidney disease are other disorders that may exhibit the same cholesterol disturbance. Low total cholesterol values with normal ester fractions are noted mainly in hyperthyroidism and malnutrition [44].

HDL-cholesterol direct

Principle

The cholesterol from low density lipoproteins (LDL), very low density lipoproteins (VLDL) and Chylomicrons, is broken down by the cholesterol oxidase in an enzymatic accelerated non -color forming reaction. The detergent present in the reagent B, solubilizes cholesterol from high density lipoproteins (HDL) in the sample. The HDL cholesterol is then spectrophotometrically measured by means of the coupled reactions described below



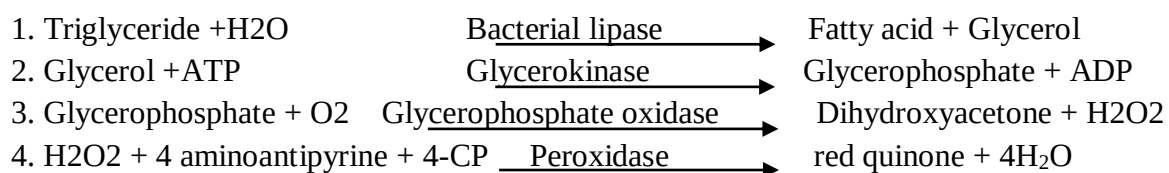
Result interpretation

HDL play an important part in the removal of cholesterol from tissues and its transportation to the liver for removal as bile acids. Decreased plasma HDL-cholesterol concentrations are positively correlated with the incidence of atherosclerotic diseases, basis of myocardial infarction and cerebrovascular accidents. There are several disease states or environmental influences associated with reduced levels of HDL: acute or chronic hepatocellular diseases, intravenous hyperalimentation, severe malnutrition, diabetes, chronic anemia, myeloproliferative disorders, Tangier disease, analphalipoproteinemia, acute stress, some drugs and smoking [45].

Triglycerides

Principle

Triglycerides are enzymatically hydrolyzed by lipase to free fatty acids and glycerol. The glycerol is phosphorylated by adenosine triphosphate (ATP) with glycerol kinase (GK) to produce glycerol-3-phosphate and adenosine diphosphate (ADP). Glycerol-3-phosphate is oxidized to dihydroxyacetone phosphate (DAP) by glycerol phosphate oxidase (GPO) producing hydrogen peroxide (H₂O₂). In a color reaction catalyzed by peroxidase, the H₂O₂ reacts with 4-aminoantipyrine (4-AAP) and 4-chlorophenol (4-CP) to produce a red colored dye. The absorbance of this dye is proportional to the concentration of triglyceride present in the sample [44].



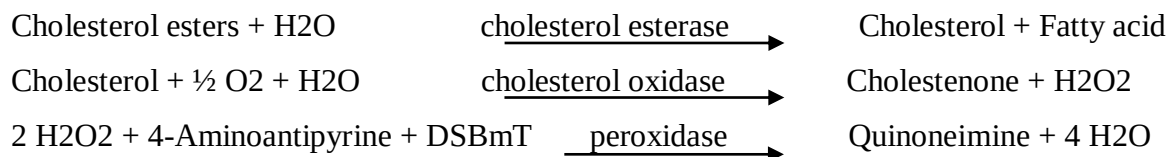
Interpretations of the test

Triglycerides are a family of lipids absorbed from the diet and produced endogenously from carbohydrates and fatty acids. Measurement of triglyceride is important in the diagnosis and management of hyperlipidemia. These diseases can be genetic or secondary to other disorders including nephrosis, diabetes mellitus, and endocrine disturbances. The National Cholesterol Education Program (NCEP) cites evidence that triglycerides are an independent risk factor for atherosclerosis. Individuals with hypertension, obesity, and/or diabetes are at greater risk than are those without these conditions [45].

LDL- cholesterol

Direct measurement

A specific detergent solubilizes the cholesterol from high density lipoproteins (HDL), very low density lipoproteins (VLDL) and chylomicrons. The cholesterol esters are broken down by cholesterol esterase and cholesterol oxidase in a non-color forming reaction. The second detergent, present in the reagent B, solubilizes cholesterol from low density lipoproteins (LDL) in the sample. The LDL cholesterol is then spectrophotometrically measured by means of the coupled reactions described below.



Interpretations of result

LDL is the main lipoprotein transporting cholesterol from liver to tissues. Increased plasma LDL-cholesterol concentrations are positively correlated with the incidence of atherosclerotic diseases, basis of myocardial infarction and cerebrovascular accidents. There are several disease states or environmental influences associated with increased levels of LDL-cholesterol: nephrosis, diabetes, obesity, some drugs and smoking. Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data [45].

Adult Reference Ranges for LFT &RFT

Instrument used: Mindray 200E

S.no	Parameter	Reference		Unit
		Men	Women	
1	ALT	≤ 45 (37°C)	≤ 34 (37°C)	U/L
2	AST	< 35 (37°C)	< 31 (37°C)	U/L
3	ALP	Adult:- 40-129	Adult:- 35-104	U/L
4	Total Bilirubin	0.2 - 1.0	0.1 - 1.0	mg/dL
5	Direct bilirubin	0-0.25	0-0.25	mg/dL
6	Urea	18-45	18-45	mg/dL
7	Creatinine	0.6- 1.3	0.5 – 0.9	mg/Dl
8	Cholesterol	118-200	118-200	mg/dL
9	HDL	39.4-60.7	47-76.3	mg/dL
10	LDL	<130	<130	mg/dL
11	Triglyceride	<150	<150	mg/dL

Declaration

I, the undersigned, declare that this MSc thesis is my original work, it was not presented anywhere. I also declare that all sources of materials used for the thesis have been duly acknowledged.

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