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Evaluation of renal function profile in human visceral leishmaniasis (kala-azar) patients

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This is to certify that the thesis prepared by Kibrom Gerezgiher entitled " **Evaluation of renal function profile among patients with human visceral leishmaniasis (kala-azar) attending at Kahsay Abera and Mearg General Hospitals Western Tigray, Northern Ethiopia"** complies with the regulations of the university and meets the accepted standards concerning originality and quality.

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ABBREVIATIONS

AAP	Aminoantipyrine
AKI	Acute kidney injury
BMI	Body mass index
CDC	Center for disease control and prevention
CI	Confidence interval
GFR	Glomerular filtration rate
GLD	Glutamate dehydrogenase
LD	Leishmania donovani
LJ	Levy Jennings
NAD	Adenine dinucleotide
NADH	Nicotinamide adenine dinucleotide
RFT	Renal function test
RPM	Revolution per minute
SCr	Serum creatinine concentration
SD	Standard deviation
SOP	Standard operating procedure
SPSS	Statistical package for social science
TBHB	Tribromo-hydroxybenzoic acid
UCr	Urine creatinine concentration
VL	Visceral leishmaniasis
WHO	World Health Organization

ABSTRACT

Introduction: Leishmaniasis is a vector-borne protozoan infection which has a wide clinical spectrum in tropics and subtropics. Kidney damage is frequently associated with increased morbidity and mortality in visceral leishmaniasis patients. However, up to date, there is a very limited report on the effect of visceral leishmaniasis on kidney function profiling in Ethiopia.

Objective: To evaluate renal function profile in human visceral leishmaniasis (kala-azar) patients.

Method: Human blood was taken from VL patients (n= 100) and healthy controls (n= 100) attending at Kahsay Abera and Mearg Hospitals, Western Tigray of Ethiopia, from June – September 2019. Serum was separated according to the conventional protocol and kidney function profiling (creatinine, urea, and uric acid) were analyzed by Mindray 200E automated Chemistry analyzer. Estimated glomerular filtration rate (eGFR) was also assessed in this study. The obtained data were processed using SPSS Version 23.0. Descriptive statistics, independent-test, and bivariate correlation were used for data analysis. P values < 0.05 were considered as statistically significant at 95% confidence level.

Result: The mean level of serum creatinine was found significantly higher, while respective serum urea and eGFR were significantly lower in VL patients when compared to healthy controls. Specifically, from 100 VL cases, an increased level of serum creatinine, urea, and uric acid was found in 10%, 9% and 15% VL cases, respectively; meanwhile, a decreased serum urea and eGFR has been reported from 33% and 44% VL cases, respectively.

Conclusions: The finding of this study asserted that visceral leishmaniasis causes derangement in kidney activities characterized by alteration of renal function profile. This may indicate that VL is the determinant factor for developing kidney dysfunction**.** This study encourages researchers to engage in leishmaniasis and its effect on other organ function profiles in humans and identify potential markers for both prevention and intervention.

Keywords: Visceral leishmaniasis, renal function test, creatinine, urea, glomerular filtration rate.

1. INTRODUCTION

1.1. Background of the study

1.1.1. Overview of leishmaniasis

Leishmaniasis is a groups of parasitic diseases caused by more than 20 species of obligate intracellular protozoa of the genus leishmania that are transmitted between humans and other mammalian hosts by phlebotomine sand-flies (Aronson *et al.*, 2016). It occurs in three forms: visceral, cutaneous and mucocutaneous. Leishmania parasites have two basic life stages: promastigote and amastigote. The amastigotes exist in vertebrate hosts and promastigotes in an invertebrate host (Kobets *et al.*, 2012).

Among neglected tropical diseases, visceral leishmaniasis (VL) is one of the most fatal parasitic diseases that claim approximately 20,000 lives every year. Globally, thousands of deaths reported in many developing countries including Ethiopia (Mondiale de la Santé and WHO., 2017).

VL (kala-azar) is a chronic infectious disease caused by parasites of the Leishmania donovani complex in Ethiopia (Gelanew *et al.*, 2010). It is one of the most deadly infectious diseases that can cause various clinical manifestations ranging from irregular and recurrent fever to hepato-splenomegaly, anaemia, leukopenia and thrombocytopenia as well as hypergammaglobulinemia as a consequence of the intense parasitism of the reticular endothelial system by the Leishmania parasite (van Griensven and Diro, 2012; Tesfaye *et al.*, 2017).

In visceral leishmaniasis, the sandfly injects the infective stage (i.e., promastigotes) from its proboscis during the blood meal and infects the host's macrophages and other types of mononuclear phagocytic cells involved in the immune response of the patient. The promastigotes transform into amastigotes in the mononuclear phagocytic system, especially spleen, liver and bone marrow. The mononuclear phagocytic cells lyse and then the amastigotes infect other phagocytic cells (Varma and Naseem, 2010; Dawit *et al.*, 2013). Visceral leishmaniasis is diagnosed by serological tests like the rapid diagnostic tests (RDTs) of recombinant 39 amino acid antigen (rk39) and parasitological test like lymph node aspiration,bone mrrow aspiration, and spleen aspiration in combination with clinical signs and symptoms (Kumar and Nylén, 2012).

1.1.2. Visceral leishmaniasis and renal function

The kidneys are two bean-shaped organs found in vertebrates. They are located on both sides of the spinal column, in the posterior part of the abdomen. The kidney performs various activities such as maintaining fluid and electrolytes homeostasis in the body, urine formation, acid-base balance regulation, protein metabolism waste products excretion, hormonal function, and protein conservation. Glomerular filtration, tubular reabsorption, and tubular secretion are the three basic processes through which the kidneys perform their physiological functions (Ogedegbe, 2007).

The kidney is affected by a different disease like visceral leishmaniasis. Renal abnormalities caused by leishmania have been well documented in experimental animal studies and are comprised of interstitial and glomerular abnormalities. Immune complex deposition, T cells and adhesion molecules activation might be responsible for the renal involvement in visceral leishmaniasis (Silva Junior *et al.*, 2014).

Kidney function alteration and interstitial nephritis with glomerular changes can be seen from VL patients. Oxidative stress is a major determinant of various renal diseases and also contributes to the manifestation of glomerulonephritis during the pathogenesis of visceral leishmaniasis. Also, 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 (Kumar *et al*., 2017).

Measures of renal function are important and commonly used in most clinical investigations. Serum creatinine (SCr) and urea (U) concentrations, as well as electrolytes (E) as the most practical measure of renal function. Measuring the glomerular filtration rate (GFR) is most useful for assessing renal function. A useful and practical surrogate marker for the glomerular filtration rate is creatinine clearance. Creatinine clearance rate (CCr or CrCl) is the volume of blood plasma that is cleared of creatinine per unit time(Guyton and Hall, 2006). GFR can be calculated by measuring any chemical that has a steady level in the blood and is freely filtered but neither reabsorbed nor secreted by the kidneys. The GFR is typically recorded in units of volume per time, e.g., millilitres per minute (mL/min) (Barrett *et al.*, 2016; Scanlon and Sanders, 2018).

However, proximal tubular cell secretion of creatinine and endogenous production of creatinine have been identified as drawbacks when serum creatinine concentration (SCr) is used as estimates of GFR. Creatinine estimates of GFR also affected by race, body mass index, age, and sex (Jones *et al.*, 1998a; Rosner and Bolton, 2006).

1.2. Statement of the problem

Visceral leishmaniasis is a disease of major public health concern leading to severe mortality rate worldwide. The disease is endemic in several tropical and subtropical regions and the Mediterranean basin. The estimated annual global burden of VL is approximately 300, 000 new cases and more than 20, 000 deaths (WHO, 2018), of which 95% of new cases reported to World Health Organization (WHO) occurred in 10 countries: Bangladesh, Brazil, China, Ethiopia, India, Kenya, Nepal, Somalia, and South Sudan (Mondiale de la Santé and WHO., 2017).

In Ethiopia, VL has spread to become endemic in many parts of the country. The disease is prevalent mostly in lowland and arid areas, and the parasite involved is mainly the Leishmania donovani. Most endemic areas are Metema and Humera lowlands of Northwest of Ethiopia which accounts for 60% of the total burden (Hailu *et al.*, 2006), the Omo plains, and the Aba Roba focus and Weyto River Valley in Nationalities and Peoples' Regional State (SNNPR). The disease was also reported from the Moyale area and Genale river basin in the Oromia regional state, Afder, and Liban zones in Ethiopia's Somali region, and the Awash Valley in the afar regional state. The annual burden of VL in Ethiopia is estimated to be between 4,500 and 5,000 cases, and the population at risk is more than 3.2 million (Leta *et al.*, 2014).

The kidney is affected by a different disease like visceral leishmaniasis. Kidney involvement in chronic leishmaniasis is frequent and associated with increased mortality. It is endemic in southern Europe and tropical and subtropical areas of the globe with a worldwide incidence of approximately 0.5 million cases per year (Dantas-Torres and Brandão-Filho, 2006).

Kidney disease is closely inter-related with heart and blood vessel disease, with 7% of all cardiovascular deaths attributed to the reduced glomerular filtration rate (Eknoyan *et al.*, 2013). Therefore, Patient suspected for visceral leishmaniasis is better to assess for further kidney function profile and get early treatment to decrease mortality related to derangement of kidney activities.

Although different studies were performed on the renal profile of kala-azar patients in different parts of the world, up-to-date there is a very limited report on the effect of visceral leishmaniasis to kidney profiles in Ethiopia. Therefore, this study is intended to evaluate renal function profile in human visceral leishmaniasis.

1.3. Significance of the study

The finding of this study would have great significance primarily for patients, health professionals and health care providers, and policymakers to engage on implementing preventive and intervention approaches to a sequel of visceral leishmaniasis on kidney dysfunction, in particular. This finding will also use as a reference for the research community and insight to develop further research designs in the field.

2. LITERATURE REVIEW

2.1. Visceral leishmaniasis

Visceral leishmaniasis (VL), also known as kala-azar, is a chronic, lethal, vector-borne disease caused by the Leishmania parasite, an intracellular protozoan. It is a zoonosis typical of tropical areas (Moncaz *et al.*, 2012).

2.1.1. Etiology

There are about 30 species of phlebotomine sandflies known to transmit leishmaniases. Human infection is caused by about 21 of 30 species that infect mammals. In Ethiopia, Leishmania donovani complex (L. donovani, L. infantum, and L. chagasi) are the causative agents of VL (Alvar *et al.*, 2007; Kobets *et al.*, 2012).

2.1.2. Hosts

Human visceral leishmaniasis is a vector-borne disease. Man and dogs are the most commonly affected hosts, and they can also be a potential reservoir. Transmission of Leishmania parasites can be zoonotic (i.e., from animals such as dogs and rodents to humans) or anthroponotic (i.e., from infected humans to non-infected humans) (Yangzom *et al.*, 2012; Dawit et al., 2013).

2.1.3. The life cycle of visceral leishmaniasis

The life cycle of visceral leishmaniasis is completed within invertebrate and vertebrate hosts. Leishmania parasites have two basic life stages such as promastigote inside the invertebrate host and amastigote inside the vertebrate host. The life cycle of the visceral leishmaniasis starts when the sandfly ingesting infected cells during their blood meals. In sandflies, amastigotes transform into promastigotes, develop in the mid-gut and migrate to the proboscis, where they are injected by females into the human or vertebrate sylvatic mammalian hosts during a blood meal. At the site of the bite, promastigotes are phagocytized by macrophages and other types of phagocytic cells. The promastigotes then transform within these cells into amastigotes, which multiply by simple division and proceed to infect other mononuclear phagocytic cells (Figure 1).

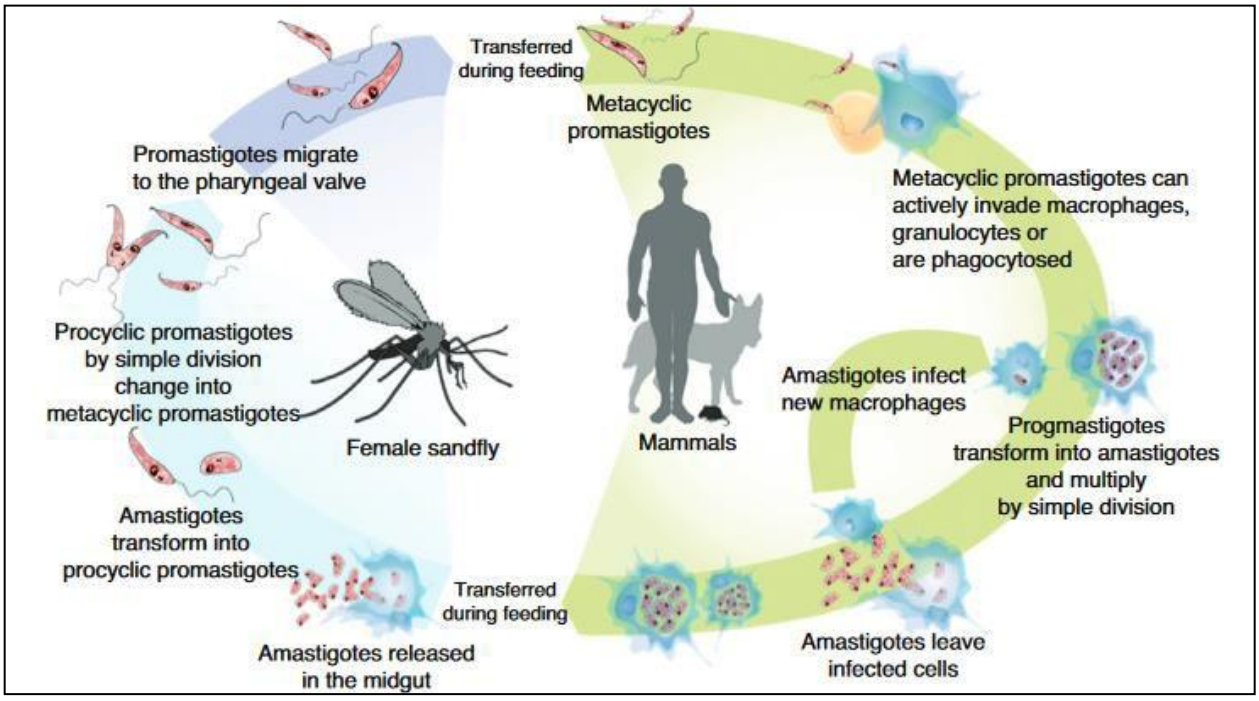

Figure 1: The life cycle of visceral leishmaniasis. Visceral leishmaniasis is a vector-borne zoonotic disease. The life cycle of leishmania exists in two forms: Promastigotes in the sandfly, amastigotes localized in macrophages in the mammalian host. After inoculation in the skin following a bite of an infected sandfly, a systemic infection can occur. Adopted from (Harhay *et al.*, 2011).

2.1.4. Clinical manifestation, diagnosis, and treatment

Visceral leishmaniasis is the most severe form of leishmaniasis caused by protozoan parasite, a potentially fatal parasitic disease of the viscera-particularly the spleen, liver and bone marrow due to infection by Leishmania donovani. It is characterized by fever, enlargement of liver and spleen, weight loss, pancytopenia and hypergammaglobulinemia. In rare circumstances, some patients will present with edema (dropsy), jaundice, vomiting, joint pains, abdominal pains, lymphadenopathy, and diarrhea (Varma and Naseem, 2010; Tesfaye *et al*., 2017).

Different laboratory diagnosis can be performed to diagnose visceral leishmaniasis. These include serological test, which detects antibodies against leishmania, like direct agglutination test (DAT) and rK39-based rapid diagnostic tests (RDTs). If serological tests results are negative or inconclusive then the diagnosis may be confirmed by demonstration of amastigotes in splenic/bone marrow aspirates or promastigotes in culture (Kumar and Nylén, 2012). Sodium stibogluconate and paromomycin sulphate combination therapy is recommended for the-

treatment of visceral leishmaniasis (Chappuis *et al.*, 2007). AmBisome® (amphotericin B) is currently used as the preferred medication for leishmaniasis (Saravolatz *et al.*, 2006).

2.2. Haematological, lipid, and liver profiles changes in visceral leishmaniasis

2.2.1. Haematological change in visceral leishmaniasis

Haematological abnormalities are a common complication of patients having visceral leishmaniasis. In this infection, the amastigote exists and proliferates in the mononuclear phagocytic system (MPS), especially spleen, liver and bone marrow. This leads to hyperplasia of the MPS with resultant disturbances in phagocyte bearing organs, producing haematological manifestations. Hence, this condition is of interest to hematopathologists, because the reticuloendothelial system is the target of parasitization. The spleen, in particular, becomes massively enlarged (Varma and Naseem, 2010).

Tesfaye and his colleagues from Ethiopia also reported that haematological abnormalities (anemia, leucopenia, and thrombocytopenia) were found in visceral leishmaniasis patients (Tesfaye *et al*., 2017). Among haematological abnormalities, anemia is the most common one (Dube *et al.*, 1995). The possible cause may be due to sequestration and destruction of red blood cells (RBCs) in the large spleen, immune mechanism and alteration in blood RBC membrane permeability (Pippard *et al.*, 1986).

Mechanical injury to red blood cells (RBCs) liberates haemoglobin to plasma and binds with haptoglobin forms haemoglobin-haptoglobin protein complex in the blood vessel. After saturation haemoglobin dissociates to heme and globin. Heme protein filtered by the kidney to proximal tubules. Heme protein has a cytotoxic effect to the nephron in the kidney (Qian *et al.*, 2010).

2.2.2. Lipid profile change in visceral leishmaniasis

Soares and his colleagues from Brazil reported that patients with VL had high triglycerides levels and low HDL (high-density lipoprotein), LDL (low- density lipoprotein) and total cholesterol levels. Due to high parasitic load in spleen patients with active VL experience dysfunctions in spleen, which is responsible for cholesterol biosynthesis and, therefore, increased the morbidity by the disease (Soares *et al.*, 2010). The same research group also confirmed low levels of HDL, LDL fraction, and apolipoprotein A1 in VL cases (Soares *et al.*,

2017). Another study conducted from the same country aimed at investigating the biochemical profile of VL patients showed that patients with active VL exhibited lower levels of total cholesterol, HDL, LDL and albumin and higher triglyceride levels (Gatto *et al.*, 2013).

The changes in the levels of lipoproteins may affect the immune response and the pathogenesis of the disease, as lipoproteins are related to the production of TNF-α, IL-6 and IL-10 (Soares et al., 2010).

Dyslipidemia (lower levels of HDL-C and higher levels of triglyceride) may cause kidney dysfunction. A growing body of evidence suggests that low HDL-C levels are associated with an increased risk of renal dysfunction in the general population (Schaeffner *et al.*, 2003; Fox *et al.*, 2004; Bowe *et al.*, 2016).

2. 2.3. Liver involvement during visceral leishmaniasis

Visceral leishmaniasis causes morphological and functional disturbance in the liver. Liver dysfunction may be caused directly by protozoa itself or indirectly to the effect related to the immune response of the parasite. Visceral leishmaniasis has direct effect in the mononuclear phagocytic system (MPS), especially spleen, liver and bone marrow. This leads to hyperplasia and decreases the production of the blood cells (Bates and Ekem, 2010). Reactive oxygen species (ROS) produced from the activated macrophage and elevated level of specific circulating cytokines during the parasite infection also possible cause for derangement of liver activities (Bankoti and Stäger, 2012; dos Santos *et al.*, 2016).

Renal dysfunction is a common sequela from liver diseases. The common pathway of renal dysfunction is the development of intense systemic arterial vasodilation, which follows an increased release of endogenous vasodilators, especially nitric oxide, which escapes from the splanchnic to the systemic circulation through portosystemic shunts. As the liver disease progresses, there is extreme vasoconstriction of the renal vascular bed that predisposes the kidneys to development of hepatorenal syndrome (Cárdenas, 2005).

2.3. Kidney involvement in visceral leishmaniasis

Kidney dysfunction in human visceral leishmaniasis has been reported in several studies (Clementi A *et al.*, 2011). This disease considered to affect the kidneys, in the form of haematuria, proteinuria or renal function impairment (Efstratiadis *et al.*, 2006). Renal abnormalities caused by leishmania

have been well documented in experimental animal studies and are comprised of interstitial and glomerular abnormalities (Salgado *et al.*, 2003). From 224 kala-azar patients, acute kidney injury (AKI) was observed in 33.9% of the case and 85.5% of these patients were males. Among the patients who developed AKI, 34.2% had serum creatinine greater than 1.4 mg/dL at the time of admission**.** Besides, AKI patients presented higher levels of urea, creatinine, potassium, direct bilirubin, and indirect bilirubin, as well as a higher number of WBCs. In contrast, the sodium, HCO_3^-, pCO_2, albumin levels, platelets count, and prothrombin time (PT) were lower when compared to non-AKI patients. Risk factors associated with AKI were male gender (odds ratio [OR] = 2.2; P = 0.03), advanced age (OR = 1.05; P < 0.001), and jaundice (OR = 2.9; P = 0.002).) (Oliveira *et al.*, 2010).

Glomerular filtration rate and serum urea were found significantly lower in kala-azar patients ($p < 0.05$) (Lima *et al*., 2007). VL patients presented macroscopic haematuria; develop acute nephritic syndrome; proteinuria, leukocyturia, and proximal tubulopathy (Salgado *et al*., 2003); blood urea above 40 mg/dL (Prasad *et al.*, 1992); increased protein excretion (Agenor *et al.*, 2009); lower serum uric acid and increased fractional urinary uric acid excretion (Verde *et al.*, 2010); rapidly progressive glomerulonephritis, AKI, mild to moderate creatinine increase and proteinuria (Alcântara *et al.*, 2018); hepatosplenomegaly and urea 184 mg/dL, serum creatinine 5 mg/dL, 2 g protein/24 h urine collection (Verde *et al*., 2010).

From a retrospective study conducted in Brazil, a total of 57 Patients with visceral leishmaniasis, AKI was observed in 26.3% of patients. The death occurred in three cases and their serum creatinine was greater than 114.92 μmol/L (Daher *et al.*, 2008).

A prospective study of thirty children VL patients were studied in Rajshahi Medical College Hospital, Bangladesh. 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 to1.2mg/dL (0.87±0.13 mg/dL), respectively (Haidary *et al.*, 2002).

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 was observed in 15 patients (26.3%) and death occurred in three cases and in all of them the serum creatinine was greater than 1.3 mg/dL (Clementi A *et al*., 2011).

2.3.1. Pathophysiology

Leishmania invades and replicates within host macrophages, evading innate and cell-mediated immune responses. Macrophage, neutrophils and other phagocytic cell are key components of antimicrobial and tumoricidal immune responses. In VL, the protozoan infects the fixed and circulating phagocytic cells involved in the immune response of the patient and phagocytosis of a foreign body by these cells occur. Once parasites are phagocytosed by macrophages, these cells produce reactive oxygen species (ROS) such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (^-OH) and nitric oxide (NO) as a host defense mechanism (Serarslan *et al.*, 2005).

Highly reactive oxygen free radicals have been indicated in the pathogenesis of various parasitic infections including visceral leishmaniasis. NAD(P)H oxidase in the plasma membrane is activated, transferring protons to molecular oxygen and forming highly reactive superoxide, hydrogen peroxide, and hydroxyl radicals at the site of phagocytosis, which interacts with pathogen phospholipid membranes. Lipid peroxidation caused by ROS results in the disarrangement and ultimately disruption of cell membranes, which leads to necrotic death and associated with kala-azar glomerular disease (Kocyigit *et al.*, 2003).

Leishmania parasites escape the humoral immune response of hosts by inducing the production of growth factor b, a macrophage-inhibiting cytokine, and interleukin-10, besides interfering in IFN-gamma signalling, all of which affect the cellular immune response and induce polyclonal B-cell activation that has been associated with kala-azar glomerular disease (Costa *et al.*, 2010).

Another macrophage defence mechanism is the acidification of the vesicle formed by the fusion of the phagosome and endosome by a proton ATPase. The acidic environment promotes protein denaturation that leaves the protein as well as DNA, RNA, and carbohydrates being susceptible to degradation by acid hydrolases (Cunningham, 2002).

T-helper cells also play a role in the immune response. The expansion of T-helper cells 1 clone protects during infection, while T-helper 2 cell expansion exacerbates the disease. IL-12 production by dendritic cells and macrophages causes naive T cells to differentiate into TH1 cells and induces the production of IFN-γ by T cells and natural killer (NK) cells. IFN-γ in conjunction with TNF-a, produced by the infected macrophages, activates the inducible nitric oxide synthetase (iNOS) gene, resulting in the production of nitric oxide (NO), which is toxic to the parasite(Kane

and Mosser, 2000). While T-helper 1 cell expansion is occurring, T-helper 2 cell expansion must be kept in check. IL-4 regulates TH2 cell differentiation, which confers susceptibility to leishmania by downregulating IL-12, IFN-γ production, and IL-12 receptor expression and inhibiting macrophage NO production (Jones *et al.*, 1998b; Kane and Mosser, 2000).

Antibodies produced in response to infection can be trapped in glomeruli by different mechanisms, such as immune complexes, in situ development of complexes or directly attached to glomerular antigens. Immune complex deposition, T cells and adhesion molecules activation have shown to be important mechanisms of injury in the glomerulonephritis occurring in visceral leishmaniasis. Oxidative stress is a major determinant of various renal diseases and also contributes to the manifestation of glomerulonephritis (Silva Junior *et al*., 2014)

2.3.2. Nephrotoxicity related to the treatment of the visceral leishmaniasis

Sodium stibogluconate and Meglumine antimoniate remain the most widely used anti-leishmanial agents (Den Boer and Davidson, 2006). Very few trials have reported renal effects of antimonial compounds. One of the first studies aimed to evaluate their nephrotoxicity was performed by Veiga and his colleagues in 1990 (Veiga, 1990).

Rodrigues and his colleagues reported a case of AKI due to acute tubular necrosis, demonstrated on kidney biopsy, in a 50- year-old male patient with generalized cutaneous leishmaniasis after meglumine antimoniate administration (Rodrigues *et al.*, 1999). Because of the increasing resistance to antimonial compounds in several countries, such as India (Bihar State) and Nepal, these agents have been progressively replaced by other drugs.

AmBisome® (amphotericin B) and its new formulations conventional amphotericin B deoxycholate have progressively substituted for pentavalent antimonial compounds in several countries due to increasing treatment failure rate (Sundar *et al.*, 2000). This drug possesses high anti-leishmanial efficacy but it is associated with a high risk of renal toxicity in addition to other side effects (rigour, fever, malaise, anorexia, thrombophlebitis, and bone marrow suppression) (Chappuis et al., 2007). Conventional AmBisome® (amphotericin B) may cause dose-dependent renal abnormalities, such as AKI, distal renal tubular acidosis, and nephrogenic diabetes insipidus (Goldman and Koren, 2004).

Oliveira and his colleagues have recently investigated the factors associated with AKI in 227 patients with visceral leishmaniasis. AKI was observed in 33.9% of cases and risk factors were male gender [odds ratio (OR) = 2.2; P = 0.03], advanced age (OR = 1.05; P < 0.001) and jaundice (OR = 2.9; P = 0.002). There was a strong association between AmBisome® use and AKI (OR = 18.4; P < 0.0001), whereas glucantime use was associated with lower incidence of AKI compared with amphotericin B use (OR = 0.05; P < 0.0001) (Oliveira *et al*., 2010).

In 2004, Aguado and his colleagues assessed the risk of haematological, renal and hepatic toxicity associated with AmBisome® (amphotericin B) lipid complex in a multicentre open-label study of 93 patients with suspected systemic fungal infection or leishmaniasis. They did not observe any difference between serum creatinine concentration before and after the study (Aguado *et al.*, 2004).

The new lipid formulations of AmBisome® (amphotericin B) (liposomal derivative and lipid complex formulations) have shown reduced nephrotoxicity when compared to the conventional form of the drug. In these formulations, deoxycholate has been replaced by other lipids that possess higher stability and higher affinity for fungal ergosterol, thus improving efficacy while reducing toxicity (Sundar *et al.*, 2000). AmBisome® (amphotericin B) showed lower rates of toxicity than conventional amphotericin B or amphotericin B lipid complex (Sundar *et al.*, 2004).

2.3. Conceptual framework

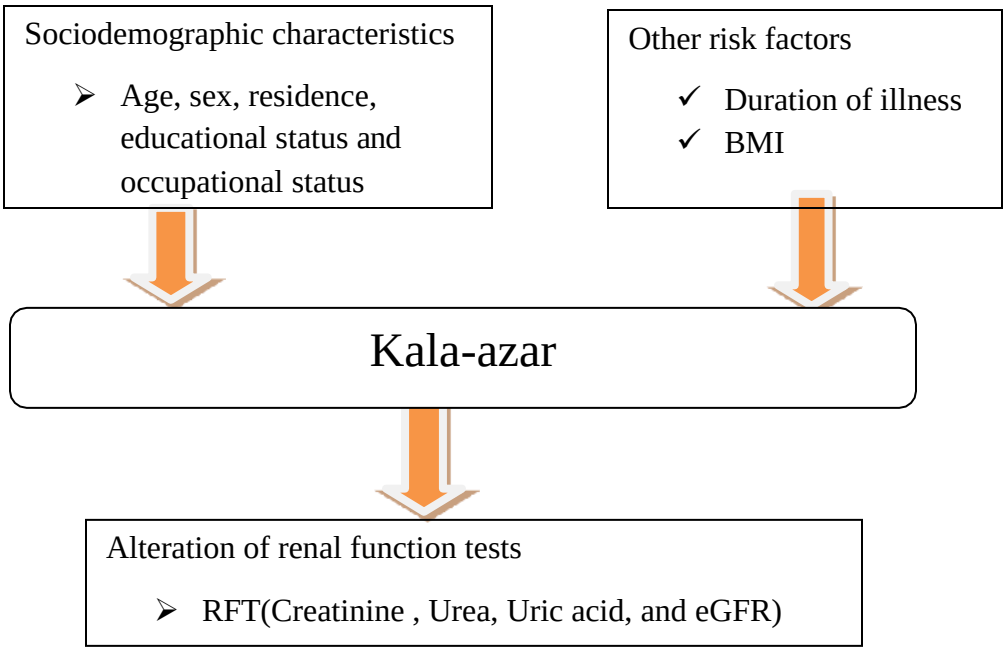

Figure 2: Conceptual framework of the study

3. OBJECTIVES

3.1. General objective

- Evaluation of renal function profiles in human visceral leishmaniasis (kala-azar) patients

3.2. Specific objectives

- To measure serum creatinine, urea, and a uric acid level of patients with human visceral leishmaniasis
- To determine estimated glomerular filtration (eGFR) of patients with human visceral leishmaniasis
- To determine the urea to creatinine ratio and uric acid to creatinine ratio of patients with human visceral leishmaniasis
- To compare renal function test results between VL patients and healthy groups
- To compare renal function parameters between VL patients with duration of illness
- To correlate renal function profile parameters (creatinine, urea, uric acid, and eGFR) with sociodemographic characteristics (age, sex, residence, educational status, and occupational status)

4. HYPOTHESIS

- **H_0=** Visceral leishmaniasis (kala-azar) has no significant effects on renal function profiles.

5. MATERIAL AND METHODS

5.1. Study area

This study was conducted in Kahsay Abera and Meareg Hospitals, Humera and Dansha towns, respectively, Western Tigray, Ethiopia. Humera is located in Western Tigray, Ethiopia, which is 984 km far from the capital city Addis Ababa. It is located at a longitude and latitude 14^{o}18'N 36 ^{o}37'E with an elevation of 585 meters above the sea level. Kahsay Abera Hospital is the district Hospitals found in kala-azar endemic region in Northern Ethiopia with 210 beds and an estimated 742,000 catchment population. Mearg hospital is the district hospital found in kala-azar 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, gynaecology, and paediatrics wards.

5.2. Study design and period

A comparative cross-sectional study design was employed from June to September 2019 G.C.

5.3. Study population and study participant

5.3.1. The source

population Case group

All VL patients confirmed at Kahsay Abera and Mearg Hospital laboratories from June to September 2019 G.C.

Control group

All those who were accompanied the patients at Kahsay Abera and Mearg Hospitals during the study period.

5.3.2. Study participant

Case group

VL patients confirmed at Kahsay Abera and Mearg Hospital laboratories during the study period who meets the inclusion criteria.

Control group

All healthy accompanied the patients at Kahsay Abera and Mearg Hospitals, who were matched 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 healthy accompanied the patients at Kahsay Abera and Mearg Hospitals, who were matched with cases in age and sex without having VL. Selection of controls was employed by the physician based on the WHO guideline 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 a 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 a habit of chronic alcohol drinking and smoking were also excluded from the study.

Control group

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

5.5. Study variables

5.5.1. Dependent variables

- Creatinine
- Urea

- Uric acid
- Glomerular filtration rate (eGFR)

5.5.2. Independent variables

- Socio-demographic factors (age, sex, residence, educational level and occupation)
- Duration of illness
- Body mass index (BMI)

5.6. Sample size determination and sampling methods

5.6.1. Sample size determination

The 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 – β. A 95% confidence level and 80% power were used to calculate the appropriate sample size. From the study conducted in Brazil mean value of serum creatinine for Kala-azar patients and controls was 0.89 ± 0.28 and 0.99 ± 0.14, respectively (Table 1).

Table 1: The levels of mean serum creatinine of kala-azar patients conducted in Brazil (used for calculating sample size by comparing two means)

Tests	Mean ± SD of VL cases	Mean ± SD of control
Serum creatinine, mg/dL	0.89 ± 0.28	0.99 ± 0.14

SD = Standard deviation

The sample size was calculated by using the mean value of creatinine from the study conducted in Brazil which gives the highest sample size using the following formula.

$n = (s_1^2+s_2^2)/d^2*(Z\alpha+Z\beta)^2$, where

n= desired sample size;

s1 (standard deviation of case group) =0.28;

s2 (standard deviation of control group) =0.14;

Zα=1.96, Zβ = power = 0.84;

Difference between two means (d) = $(0.89-0.99)^2 = 0.01$

Therefore, $n = \frac{(0.28)^2 + (0.14)^2 * (1.96+0.84)^2}{(0.1)^2} = 76.832$, round up to 77.

Considering a 10% nonresponse rate (= 0.1*77 = 8), the minimum sample size will be 77 + 8= 85 for each groups. Increasing sample size can give greater power to detect the difference between control and case group. Consequently, 100 case and 100 control groups' serum specimens were collected for the current study.

5.6.2. Sampling method

Convenient sampling technique was employed to select the 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 a clinical log sheet.

5.7.2. Laboratory analysis

5.7.2.1. Specimen Collection, processing, and transportation

About 5 mL venous blood sample was collected using serum separator tube from both VL patients and healthy controls. A serum sample was separated by centrifuge at 4000 rpm for 5 minutes and about 1.5-1.8 mL serum sample was stored at a temperature of -20^0C up to -30^0C in a deep freezer before laboratory analysis. The serum sample was transported to Adigrat General Hospital Laboratory department for clinical chemistry analysis.

5.7.2.2. 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 Biochemistry reagents and smart, versatile, easy Mindray 200 E automated chemistry analyzer (Germany) used to measure the concentration of creatinine, urea, and uric acid from serum. All the tests were performed according to the manufacture'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 Shre town to ensure clarity, length, logical sequence and skip patterns of the questions. A questionnaire was prepared in English and was translated to the Tigrigna and Amharic versions, which is easily understandable by the study participant. Experienced laboratory personnel participated for proper collection, processing, and transportation of the sample. Standard operating procedures (SOPs) were used strictly to ensure labelling with an identification number, proper sample container, and enough volume and test procedures. The collected sample was allowed to clot for 20-25 minute and centrifuged the specimen to separate serum for analysis. The standard working environment (optimized, instrument, high analytical grade reagent, and temperature,) were meet and sample hemolysis were checked before analysis.

5.8.2. Analytical

Before sample analysis, both normal and pathological quality controls were analyzed to ensure the proper function, validity and reliability of the instrument. Levy Jennings (LJ) chart was drawn for both controls and interpreted based on the west guard rule to reject or to accept controls. Levy Jennings is a graph that quality control data is plotted on to give visual indication whether a laboratory test is working well and west guard rule can be applied to see whether the results from the samples when the control was done can be released, or if they need re-run. 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 were interpreted by the principal investigator**.** The laboratory result was recorded in the logbook for rechecking. Clear and neat test results were reported to the investigator for analysis.

5.9. Data analysis and interpretation

The obtained data were entered into SPSS version 23 software that was released by international business machines (IBM) in 2019 and analyzed accordingly. Descriptive statistics were used and the generated data was expressed in number and percentage in the form of tables and figures. Independent student t-test was used to compare the mean difference between the VL cases and

control groups. Bivariate correlation analysis was used to check the significant correlation of associated factors with renal function tests of VL cases. The P-value less than 0.05 with corresponding 95% confidence interval was considered as a significant association.

5.10. Operational definition

Kala-azar: Visceral leishmaniasis (VL) is a chronic and vector-borne potentially fatal parasitic disease caused by the Leishmania (L.) *donovani* / L. *infantum* / L. *chagasi* complex**.**

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.

Acute kidney injury (AKI): An absolute increase in serum creatinine of more than or equal to 0.3 mg/dL or the percentage increase in serum creatinine of more than or equal to 50%.

Acute nephrotic syndrome: Is a group of symptoms that occur with some disorders that cause inflammation of the glomeruli in the kidney, or glomerulonephritis.

Glomerular filtration rate (GFR): Measures how well your kidneys are cleaning your blood and approximated by Creatinine clearance test which determines the overall GFR.

Case: Are individuals who have confirmed visceral leishmaniasis

Control: Are individuals who are 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 referenceranges

High: Is the value of the test lies above the established reference ranges

5.11. Ethical Consideration

Before starting data collection, ethical clearance letter with reference no. SOM/BCHM/121/011 was obtained from the Departmental Research and Ethics Review Committee, Department of Biochemistry, School of Medicine, College of Health Sciences, Addis Ababa University. A collaboration letter for data collection was also obtained from the Tigray Regional Health Bureau and Administrators of Kahsay Abera and Mearg hospitals. Samples and data were collected after written informed consent had been obtained from study participants and the confidentiality of any information obtained from each participant was coded for the samples and results. Further permission was also obtained from Adigrat General Hospital to perform the clinical chemistry tests.

6. SCHEMATIC WORK FLOW

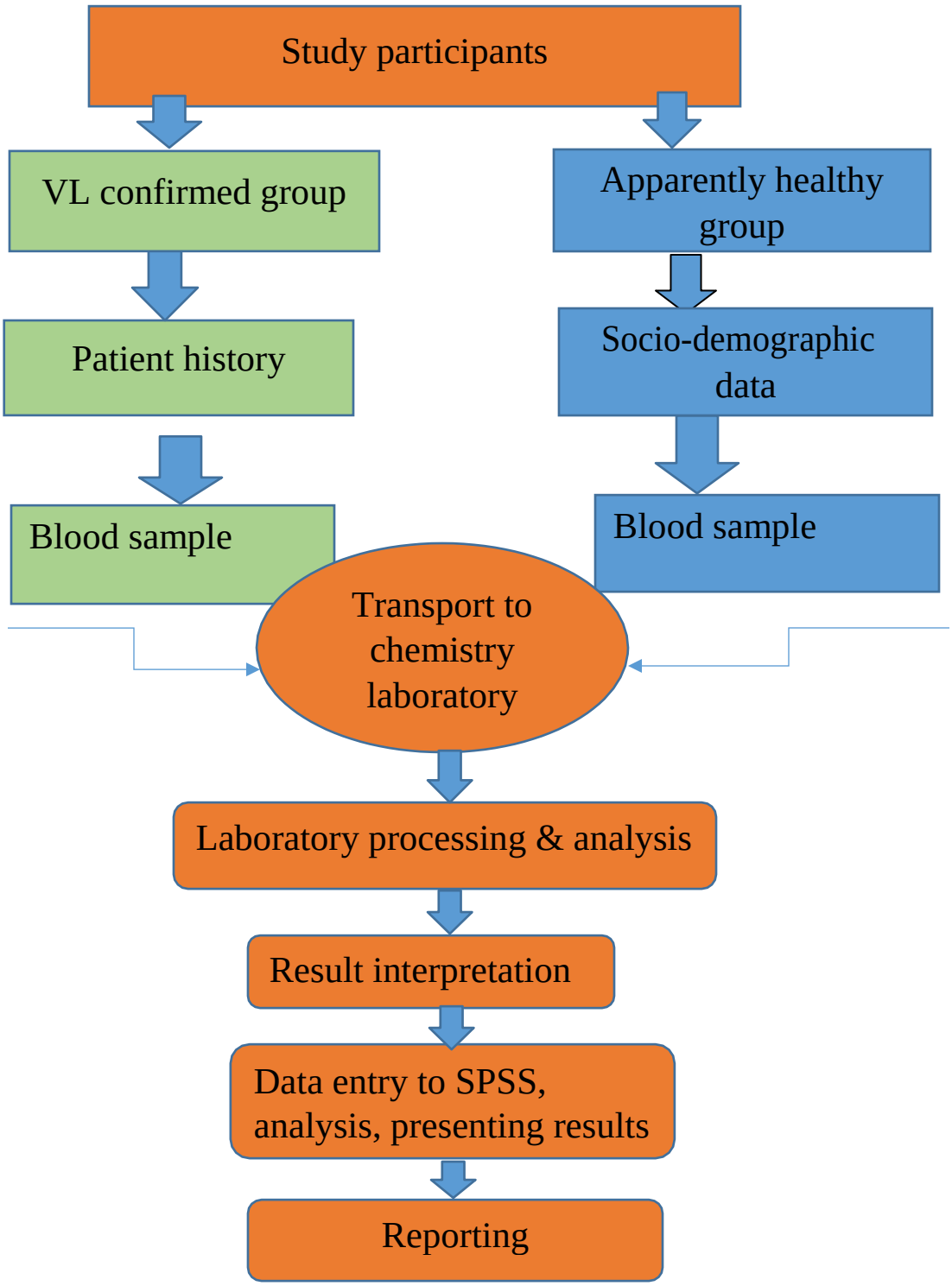

Figure 3: Schematic workflow of the study

7. RESULT

7.1. Socio-demographic characteristics of study participants

This study enrolled 100 VL confirmed patients (88 of them were males) and 100 healthy control people (90 of them were males). Without any further analysis, the infection was found highly pronounced in males; and the study was conducted almost between age-matched VL and control groups. The mean age of VL patients and healthy controls were 29.38 (± 9.72) years and 28.95 (± 10.00) years, respectively. Majority of the VL patients and healthy controls were within the age range of 15-30 years. There was no significant difference in the mean age of the VL patients and the healthy control subjects. About 44% of VL cases and 43% of healthy controls were single. Around 57% of VL cases and 71% of healthy controls were attended either primary or secondary schools. Besides, the majority of the patients (89%) were from the rural and kala-azar endemic areas (Table 2).

Table 2: Socio-demographic characteristics of VL patients (n=100) and healthy controls (n=100)

Sociodemography	VL cases (n=100)	Control groups (n=100)	p-value
Sex			
Male (%)	88	90	0.65
Female (%)	12	10	
Age in years			
15-30	63	60	
31-40	22	22	
>40	15	18	
Mean ± SD	29.38 ± 9.72	28.95±10	0.84
Marital status			
Single (%)	44	43	
Married (%)	54	57	
Divorced (%)	2	0	
Widowed (%)	0	0	
Educational status			
Illiterate (%)	20	1	
Primary (%)	57	71	
Secondary (%)	23	21	
Diploma and above (%)	0	1	
Occupational status			
Student (%)	29	34	
Housewife (%)	2	2	
Government employee (%)	0	1	
Private employee (%)	2	1	
Farmer (%)	22	15	
Merchant (%)	12	25	
Daily labor (%)	33	22	
Residence			
Urban (%)	11	13	
Rural (%)	89	87	

7.2. Renal function test results of visceral leishmaniasis patients deviated from established reference ranges

While the amount of serum urea and eGFR were found decreased in 33 and 44 percent of VL patients, serum creatinine and uric acid were found increased in 10 and 15 percent , respectively (Figure 4 and Figure 5).

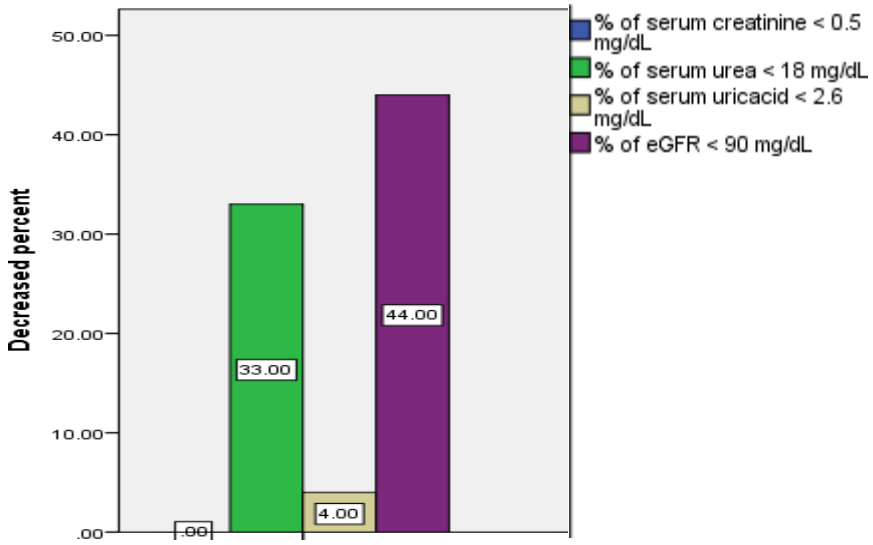

Figure 4: The percentage (absolute number) of VL patients having decreased serum analyte (creatinine, urea, uric acid) and eGFR level from lower limit normal (n=100)

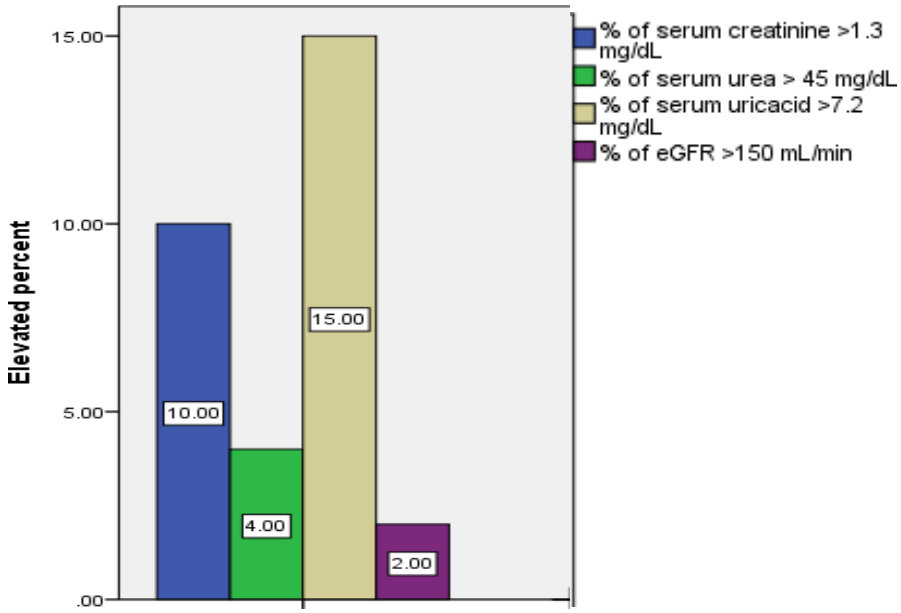

Figure 5: The percentage (absolute number) of VL patients having elevated serum analyte (creatinine, urea, and uric acid) and eGFR level from upper limit normal (n=100)

7.3. Comparison of renal function tests between VL and controls

The mean value of serum creatinine was significantly higher in VL patients (0.935±0.229 mg/dL) when compared to those of healthy controls (0.709±0.119 mg/dL) with p=0.001. Even though the mean value of serum uric acid shows slightly increased in VL patients than controls, there was no statistical significance between VL cases (5.16±2.25 mg/dL) and healthy controls (4.87±1.74 mg/dL) with p=0.31. The mean value of serum urea was significantly lower in VL patients (23.11±10.72 mg/dL) when compared to those of healthy controls (29.19 ± 9.32 mg/dL) with p=0.001. Similarly, the mean value of eGFR was significantly lower in VL patients (90.95±22.15 mg/dL) when compared to those of healthy controls (128.36±21.06 mL/min) with p=0.001); Figure 6).

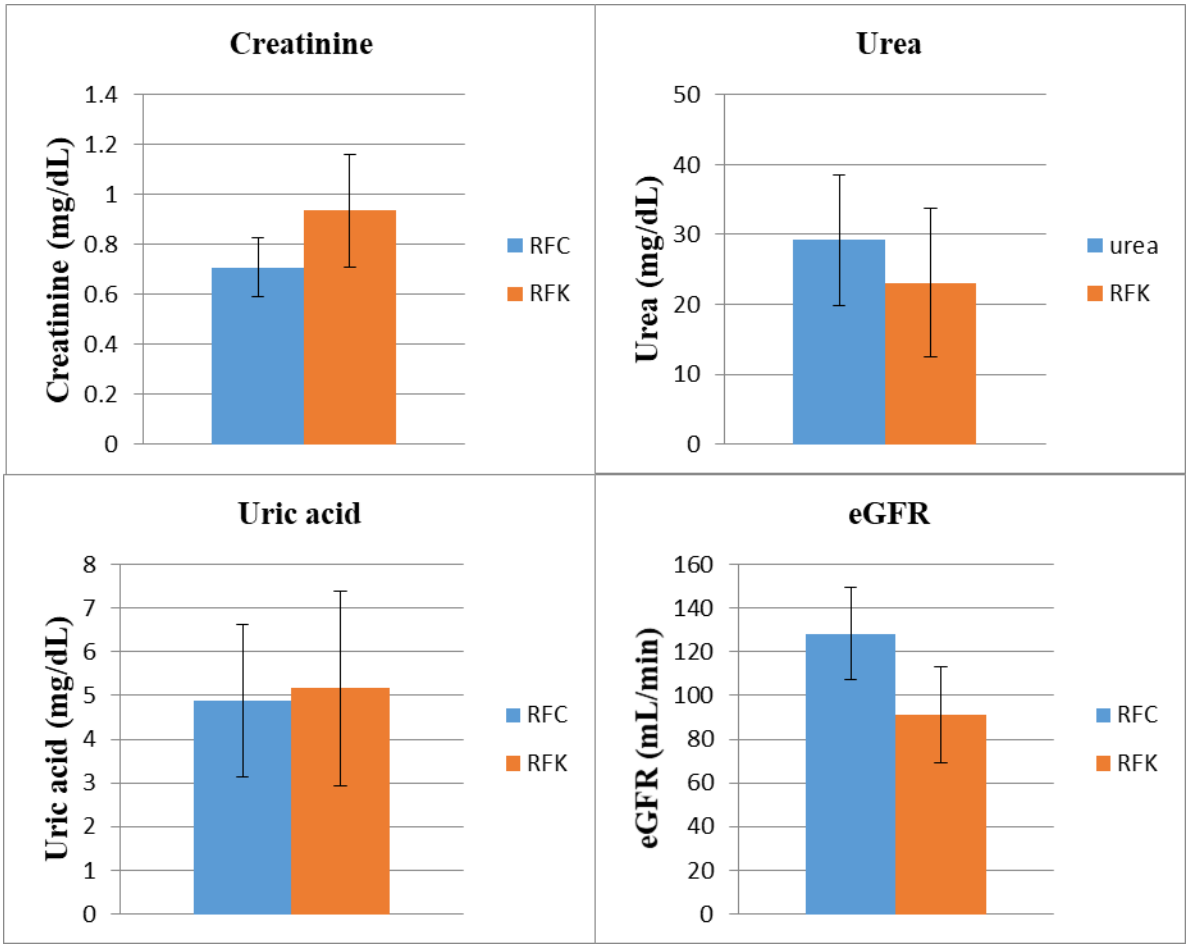

Figure 6: The amount of creatinine, uric acid, urea and eGFR of VL patients (n=100) compared to healthy controls (n=100). RFC = renal function test for control; RFK = renal function test for kala-azar.

7.4. Comparison of urea/creatinine and uric acid/creatinine ratios between VL patients and controls

Calculating the ratio of urea to creatinine and uric acid to creatinine levels is important to predict or indicate how well the kidneys functioning. The principle behind this ratio is the fact that both urea and creatinine are freely filtered by the glomerulus; however, urea reabsorbed by the tubules can be regulated whereas creatinine reabsorption remains the same. The ratio of the mean values of serum urea to creatinine (urea/creatinine) was significantly lower in VL patients (26.3± 15.2) when compared to those of healthy controls (42.1± 14.5.) with p=0.001. Similarly, serum uric acid to creatinine ratio was significantly lower in VL patients (5.8± 2.94) when compared to those of healthy controls (7.04 ±2.75) with p=0.002; Table 3).

Table 3: Comparison of urea/creatinine and uric acid/creatinine ratios between VL cases (n=100) and controls (n=100)

Parameters	Mean ±SD of controls	Mean± SD of cases	p-value
Urea/creatinine ratio	42.1± 14.5	26.3± 15.2	0.001
Uric acid/creatine ratio	7.04 ±2.75	5.8± 2.94	0.002

7.5. Comparison of renal function tests between VL cases with a duration of illness

From a total of 100 patients having visceral leishmaniasis, 51% of them were had a duration of illness for more than 4 weeks. The mean value of serum creatinine was significantly higher in VL patients with duration of illness more than 4 weeks (1.05±0.23 mg/dL) when compared to those of VL patients with duration of illness less than 4 weeks (0.82±0.16 mg/dL) with p= 0.001.

The mean value of eGFR was significantly lower in VL patients with duration of illness more than 4 weeks (79.35±17.3 mg/dL) when compared to those of VL patients with duration of illness 0-4 weeks (103.04±20.21 mg/dL) with p =0.001. Urea and uric acid levels were not shown any significant difference with the duration of illness (Figure 7).

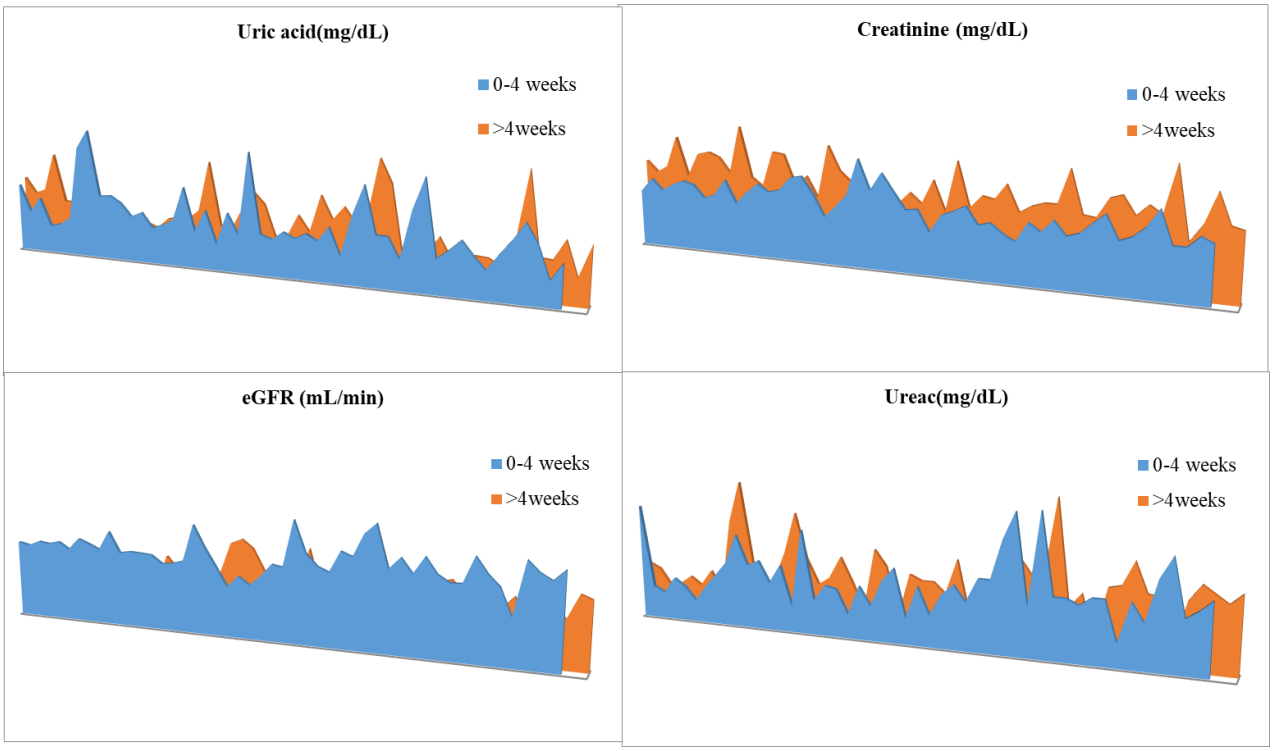

Figure 7: Duration of illness versus concentration of creatinine, uric acid, urea and eGFR among VL cases (n=100). More than four weeks (> 4 weeks) and less than four weeks (0-4 weeks).

7.6. Other factors and renal function test results of VL cases

Among visceral leishmaniasis patients, there was a statistically significant positive correlation between serum creatinine level and duration of illness (r_s=0.52, p=0.001). On the other hand, the eGFR level showed a statistically significant inverse association with duration of illness (r_s= -0.58, p=0.001) in VL patients. Correlation analyses also showed that there was a significant negative association between age and serum urea (r_s = -0.21* p=0.03). However, other factors or patients' history such as sex, age, educational level, residence, and BMI were not shown any significant correlation with renal function tests of VL cases as shown in Table 4.

Table 4: Correlation analysis between other factors and renal function test result of VL cases (n=100)

			Factors						
			Age	Sex	Marital status	Educational status	Residence	Occupational status	Duration of illness
Renal function test result of kala-azar patients	Creatinine	r_s	-0.12	-0.06	-0.13	0.19	0.08	0.25	0.52
		p-value	0.23	0.55	0.2	0.06	0.44	0.01	0.001
	Urea	r_s	-0.21*	0.15	-0.23*	-0.01	0.09	-0.26**	0.08
		p-value	0.03	0.13	0.02	0.99	0.93	0.01	0.44
	Uric acid	r_s	-0.098	0.03	-0.095	-0.05	-0.02	0.03	0.03
		p-value	0.33	0.75	0.34	0.64	0.81	0.76	0.78
	eGF	r_s	0.15	0.03	0.21*	-.018	0.07	-0.15	-0.58**

		p-value	0.14	0.78	0.04	0.08	0.46	0.13	0.001	0.3
	Urea/crea ratio	r_s	0.03	0.2	-0.12	0.12	-0.03	-0.35**	-0.12	0.06
		p-value	0.8	0.06	0.23	0.3	0.76	0.001	0.08	0.5
	Uric acid/crea	r_s	0.08	0.04	-0.05	-0.01	-0.12	-0.05	-0.2	-0.1
		p-value	0.4	0.7	0.6	0.9	0.2	0.6	0.02	0.3

- r_s = Spearman's rho correlation coefficient, where
- r_s = 0.00–0.10….Negligible correlation
- r_s = 0.10–0.39…. Weak correlation
- r_s = 0.40–0.69….. Moderate correlation
- r_s = 0.70–0.89….. Strong correlation
- r_s = 0.90–1.00……Very strong correlation
- (p-value)** - indicates correlation is significance at 0.01 level (2-tailed).
- Negative sign indicates as the values of variable 1 increases while the values of variable 2 decreases
- Postive sign indicates as the values of variable 1 increases while the values of variable 2 increases (Schober *et al.*, 2018).

8. DISCUSSION

The present study evaluates the effect of visceral leishmaniasis on the renal injuryprofiles (serum creatinine, uric acid, urea and eGFR) between VL patients and healthy controls. These parameters have been used as the most indicators for the renal damage (Guyton and Hall, 2006).

Our study showed that, while the mean value of serum creatinine was found significantly higher in VL patients, serum urea and eGFR were found significantly lower in VL patients, when compared to the healthy controls. However, the mean value of serum uric acid was not shown any statistical significance.

The result of this study revealed that the level of mean serum creatinine was found significantly increased in VL cases when compared to healthy controls. This finding is in corroboration with the previous studies conducted on renal function profile among VL cases, reported AKI and progressive glomerulonephritis found an increased in serum creatinine level (Daher *et al*., 2008; Clementi A *et al*., 2011; Alcântara *et al.*, 2018). This may be attributed to the level of immune complex deposition, T cells and adhesion molecules activation during the inflammatory processes observed in active VL disease result in renal dysfunction (Salgado *et al*., 2003; Efstratiadis G. *et al.*, 2006). The production of reactive oxygen species (ROSs) from the activated macrophage may also contributes to the alterations of renal function (Salgado *et al*., 2003; Reis AB *et al.*, 2009). The disease itself, and the hemodynamic disturbs in the context of the disease (anemia, hypotension, hypoalbuminemia) can be involved in renal damage (Clementi A *et al*., 2011). In contrast another study in which the level of serum creatinine did not show any significant difference between VL cases and controls (Lima *et al.*, 2007). This difference may be attributed to disease progression, diet effect, temperature, diurnal variation and sample size.

The level of mean serum urea was significantly lower in VL cases compared to the healthy controls. This finding is in agreement with study conducted in Brazil (Lima *et al.*, 2007). The decrease in urea level may be attributed to the low protein intake and reduced its production in the liver due to VL infction. VL causes morphological and functional disturbance in the liver and the dysfunction may be caused directly by protozoa itself or indirectly to the effect related to the immune response of the parasite (Bates and Ekem, 2010). However, the present study is not in-

congruous with studies conducted by Efstratiadis G. *et al*. (2006) and Alcântara *et al.* (2018) which reported a high level of serum urea for VL patients (Efstratiadis *et al*., 2006; Alcântara *et al*., 2018). The difference may be attributed to the duration of dehydration (with an environmental difference), diet and severity of infection (Higgins, 2016).

Even though 15% of VL cases showed an increased in uric acid, there was no significant difference in the mean uric acid levels between VL cases and healthy controls. In contrast, the study conducted in Brazil reported decreased uric acid levels in VL patients (Agenor *et al*., 2009; Verde *et al*., 2010). This difference may be due to genetic variabilities, diet effect, temperatures, and diurnal variation.

Approximately half of the patients with kala-azar were found decreased in eGFR. The mean value of eGFR was significantly lower in VL cases than healthy controls. In contrast another study in which the level of eGFR did not show any significant difference between VL cases and controls (Lima *et al*., 2007). This difference may be attributed to extrarenal fluid losses (diarrhea, vomiting, and sweating) in patients with kala-azar, disease progression, and means of estimating glomerular filtration rate.

The urea to creatinine ratio is one of the common laboratory tests used to distinguish pre-renal and acute tubular necrosis (Lieberthal and Nigam, 1998). High serum urea to creatinine ratio (UCR) is associated with the pre-renal injury. The hemodynamic instability which leads to reduced GFR accounts pre-renal injury (Macedo and Mehta, 2009). In our study, we found that the ratio of serum urea to creatinine and serum uric acid to creatinine was decreased in VL cases when compared to controls. Low urea to creatinine ratio suggests protein malnutrition and reduced urea synthesis as in advanced liver disease (Macedo and Mehta, 2009). However, different studies showed that urea to creatinine ratio (UCR) was not a reliable parameter to distinguish prerenal acute kidney injury from other forms of acute kidney injury (Vanmassenhove *et al.*, 2013; Manoeuvrier *et al.*, 2017). Extrarenal factors can affect the blood levels of these two markers.

Serum uric acid to creatinine ratio might be a better predictor of incident chronic kidney disease than serum UA alone (Gu *et al.*, 2017). According to our study, the mean value of serum uric acid to creatinine ratio is significantly lower in VL cases when compared to controls. This may b e attributed to significantly elevated mean serum creatinine level of VL patients, but the uric acid

the level did not show any significant difference. Besides, factors such as diet, genetic variance, and underlying medical condition can also affect the blood level of these two markers.

We have also assessed the association between duration of illness and renal function test results of VL patients. This study revealed that duration of illness had a moderate positive correlation with serum creatinine ($r_s = 0.52$; $p < 0.05$) in VL patients. Serum creatinine was found elevated in patients with more than 4 weeks of the duration of illness. This is in concordant with the previous study conducted by Silva Junior and his colleagues (Silva Junior *et al*., 2014). They reported that serum creatinine was found increased in patients with a longer time between symptom onset and hospital admission. Correlation analysis also showed that there was a significant moderate negative association between duration of illness and eGFR level ($r_s = -0.58$; $p<0.05$). VL patients with more than 4 weeks of illness were found decreased in eGFR compared to those with less than 4 weeks of illness. This may be attributed to as the disease progressing the occurrence of glomerulonephritis is inevitable resulting in decreased eGFR (De Brito T *et al.*, 1975; Weisinger JR *et al.*, 1978; Kaplan AA and OF., 1992).

Our study also went through assessing the association between patients' history (age, gender, education, marital status, occupation, and BMI) with and the renal function test results of VL patients, and found that the levels of serum creatinine has no significant association with gender and age. This finding is not in agreement with the study conducted in Australia, in which the serum creatinine concentration increased steadily with age (Tiao JY *et al.*, 2002). Similarly, eGFR was not shown a significant association with gender and age. This finding is not in agreement with the study conducted in Brazil and London. Both reports were shown that the level of GFR declined with age and the decrease in eGFR is less pronounced in male as they were getting aged (Micas- Nunez *et al.*, 2007; Glassock and Winearls, 2009; Oliveira et al., 2010). Correlation analysis also showed that age had a weak correlation with serum urea ($r_s = -0.21^*$ $p=0.03$). These results are in trajectory with a study done by Musch *et al* and Seki *et al.* Both researchers reported a direct correlation of age and urea (Musch *et al.*, 2006; Seki *et al.*, 2019). This discordant may be attributed to genetic variabilities, environmental factors (temperature, altitude and climatic changes), lifestyle (smoking, physical activities, and diet), and hormonal effect.

Malnutrition can affect the immune system of the host and increase the vulnerability or sensitivity for various infections (for example, VL infection). Prolonged situation eventually leads

underweight (BMI less than 18.5 kg/m^2) (Werneck *et al.*, 2011). In the present study, 59% of the VL patient's were found with low BMI. In our study, BMI was not shown any significant correlation with the level of the renal function tests in VL cases. However, unlike our results, previous studies conducted in Asian patients have shown that high BMI was found to be positively correlated with impairment in renal function. (Gelber *et al.*, 2005; Kramer *et al.*, 2005; Hallan *et al.*, 2006; Iseki, 2006). Liu and his colleagues also reported that the overall risk of AKI was significantly higher in underweight patients (Liu *et al.*, 2018). The reason for this discordant may be attributed to the lifestyle, nutrition, level of insulin sensitivity and muscle mass.

9. STRENGTH AND LIMITATIONS OF THE STUDY

9.1. Strength of the Study

As the first-ever research in Ethiopia, this study provides a piece of baseline information for further study and policymakers. Clinical investigation and rk39 test were used to screen healthy controls by physicians. All VL cases and healthy controls were first diagnosed with malaria.

9.2. Limitations of the Study

- In this study, different tests like electrolyte, protein, albumin and endocrine tests were not done due to limited budget.
- Research reports were found limited in this field that made short of discussion for our results.
- Furthermore, the cross-sectional nature of the study has also its limitation.

10. CONCLUSION

Visceral leishmaniasis (kala-azar) patients showed quite significant alterations of renal function profiles when compared to healthy controls. This may indicate that VL is the determinant factor for developing kidney dysfunction. Further investigation is required and will not be a layaway task for researchers particularly in this field of study and the scientific community.

11. RECOMMENDATIONS

Based on the study findings, the following important measures were recommended to implement appropriate preventive interventions on kidney damages associated with VL:

- It will be better if health professionals and stakeholders use clinical chemistry tests for the management of renal dysfunction of VL patients from endemic areas to prevent further complications of the disease.
- A cohort study is needed on leishmaniasis and its effect on the biomedical profiles in humans.

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13. ANNEX

13.1. Annexe -I -Participants' information sheet

A. English version

Title of the project: Evaluation of renal function profile in human visceral leishmaniasis (kala-azar) patients.

Principal investigator: Kibrom Gerezgiher Asfaw (BSc)

Introduction

Dear study participants you are invited to participate in the study on the renal profile of kala-azar patients in Kahsay Abera and Mearg Hospital, Tigray Region, Northern Ethiopia. This study is approved by the Department of Medical Biochemistry Research and Ethics Review Committee (DRERC), School of Medicine, College of Health Sciences, Addis Ababa University. You are voluntarily participating in this study and you have a full right to withdraw from participation if you have happened to feel something uncomfortable.

Purpose of the study. The main objective of the study is to evaluate renal profiles of kala-azar patients attending Kahsay Abera and Mearg Hospitals, Tigray Region, Northern Ethiopia.

Duration: The duration of this study depends on the availability of study subjects and it maytake 3-5 months.

The associated risk with the study: During sample collection from your vein, there is minor pain or discomfort. The sample is collected by the experienced Nurse and Medical laboratory technologist and the risk is very minimal.

The procedure of the study: If you are agreed to participate in this study, you will give about 5mL venous blood for clinical chemistry analysis.

Expected Benefit: Dear participants this study assess the renal function profile changes, and provides baseline information for health care providers and policymakers that are believed to contribute for appropriate preventive interventions on kidney damages associated with VL through early diagnosis and preventing complications resulted from such outcome. No payment is requested for the renal function tests.

Confidentiality:

The confidentiality of your information and laboratory results are respected strictly. A unique identification number is given to you and your name will be coded 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 are kindly requested to put your signature to indicate your agreement to participate in the study.

Participant name _____

Date_____signature_____

If you have any question please contact the following address:

Principal Investigator: Kbrom Gerezgiher Asfaw (BSc)

Mobile Phone: +251-967562388

Email: kibromgere14@gmail.com

B Amharic version

ለተሳታፊዎች መረጃ መስጫ ሰነድ

የጥናቱ ርእስ፡- የካላ ኣዛር በሽታ በኩላሊት ላይ ሊያመጣዉ የሚችለዉን ችግር በጥናታዊ ምርምር መፈተሽ።

የአጥኝው ስም:- ክብሮም ገረዝጊሄር ኣስፋው

የተቋሙ ስም:- ባዮኬሚስትሪ ት/ክፍል፣ የህክምና ት/ቤት፣ ጤና ሳይንስ ኮሌጅ፣ አዲስ አበባ ዩኒቨርሲቲ።

መግቢያ:- የተከበሩ የጥናቱ ተሳታፊ የካላ ኣዛር በሽታ በኩላሊት ላይ ሊያመጣዉ የሚችለዉን ችግር በጥናታዊ ምርምር መፈተሽ በሚል ጥናት ለመሳተፍ ተጋብዛዋል። ይህ ጥናት በ ባዮኬሚስትሪ ት/ክፍል፣ አዲስ አበባ ዩኒቨርሲቲ የጥናትና የሰነምግባር ኬሚቴ ያጸደቀዉ ጥናት መሆኑን መግለፅ እንወዳለን ። በዚህ ጥናት መሳተፍ ሙሉ በሙሉ በእርስዎ ፍቃደኝነት የተመሰረተ በመሆኑ በማንኛውም ሰአትና ቦታ የማቋረጥ ሙሉ መብትዎን የተጠበቀ ነው።

የጥናቱ ዋና አላማ፡- የካላ ኣዛር በሽታ በኩላሊት ላይ ሊያመጣዉ የሚችለዉን ችግር በጥናታዊ ምርምር መፈተሽ።

የጥናቱ ጊዜ:- የጥናቱ ግዜ በምናገኘዉ የካላኣዛር በሽተኞች ብዛት የሚወሰን ሲሆን ከ3 እስከ 4 ወር ሊወስድ ይችላል።

ከጥናቱ ጋር ተያይዞ የሚመጣ ጉዳት:- የደም ናሙና በሚሰጥበት ወቅት ምንም አይነት ችግር አያጋጥምዎትም ። ነገር ግን ደም ሲወስድ መጠነኛ የህመም ስሜት ሊያስከትል ይችላል ። ሆኖም ግን ናሙናዉን የሚሰበስብው ልምድ ባላቸዉ ባለሞያ ስለሆነ ና አስፈላጊውን ጥንቃቄ ስለሚወስድ የህመም ስሜት አይኖርም ወይንም እጅግ በጣም ዝቅተኛ ነዉ።

ከጥናቱ የሚያገኙት ጥቅም :- የተከበሩ የጥናቱ ተሳታፊ ያለ ምንም ክፍያ ኩላልት ምርመራ ያደርጋሉ። ሆኖም ግን በሰጡትም ደም ላይ የሚደረገው የምርመራ ውጤት ለጤና ፖሊሲ አውጪዎች ና ጤና ባለሞያ መረጃ ማድረስ ሲሆን ከዚህ ጥናት ለእርስዎ የሚሰጥ ጥቅም አይኖርም።

ከርስዎ የምናገኘዉ መረጃ እና ሚስጥራዊነቱ:- የእርሶ ስም በዚህ መጠይቅ ላይ አይጠቀስም። በተጨማሪም የሚሰጡት መረጃ ሚእና 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:- ክብሮም ገረዝጊሄር ኣስፋው ስልክ:- +251967562388 ኢሜል:- kibromgere14@gmail.com

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2) ዶ/ር ናና ሰከረን ኔቲን ኢሜል፣ ngsbio2@gmail.com

C. Tigrigna version

ናይ መፅናዓይ ሽም:- ክብሮም ገረዝጊሄር ኣስፋው

ናይ ትካል ሽም፡- አዲስ አበባ ዩኒቨርሲቲ ፣ ጥዕናን ሕክምና ሳይንስ ኮሌጅን፣ ባዮሜዲካል ትምህርቲ ክፍሊ

ናይቲ መፅናዕቲ ርእሲ፡- ሕማም ካላኣዛር ኣብ ኩላልት ከስዕቦ ዝክእል ሳዕቤን ብፅናዓታዊ ምርመራ ምፍታሽ

መእተዊ፡- ዝተከበሩ(ራ) ናይዚ ጽንዓት ተሳታፈ/ት ኣነ ናይ አድስ አበባ ዩኒቨርስቲ ጥዕናን ሕክምናን ሳይንስ ኮሌጅ ናይ ሕክምና ትምህርቲ ባዮሜዲካል ክፍሊ ብማስተርስ ድግሪ ተምሃራይ እየ።ንሶም ወይ ንሰን ኣብ ናይ ሕማም ካላኣዛር ኣብ ኩላልት ከስዕቦ ዝክእል ሳዕቤን ብፅናዓታዊ ምርመራ ምፍታሽ ኣብ ዝብል ናይ መመረቂ መፅናዕቲ ፅሑፍ ተሳታፊ ንክኾኑ ተዓድሞም አለዉ።

ናይቲ መፅናዕቲ ዋና ዓላማ፡- ሕማም ካላኣዛር ኣብ ኩላልት ከስዕቦ ዝክእል ሳዕቤን ብፅናዓታዊ ምርመራ ምፍታሽ።

እቲ ጽንዓት ዝካየደሉ 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 ተሳታፈ/ት እዞም ዝስዕቡ ናይዚ ጽንኣት ምስ ተረድኡ እና ፍቃደኛ እንድሕር ኮይኖም ነዚ ጽንዓት ዝከዉን ካብ 3 ክሳብ 5 ሚ.ሊ ዝከዉን ደም ክንወስደሎም ኢና።

ምሰቲ መፅናዕቲ ተታሒዙ ዝመፅእ ሳዕቤን፡- ንምርመራ ዝከዉን ደም አብ ዝሀብሉ እዋን ምንም ዓይነት ዝኸፍአ ፀገም አየጋጥሞምን። ነገር ግን ደም አብ ዝውሰደሉ እዋን ዝተወሰነ ናይ ምሕማም ስሚዕት ክህሉይ ኽእል እዩ። ይኹን ዳአምበር ደም ንምስብሳብ ልምዲ ብዘለዎም ባዓል ሞያታት ስለ ዝምደቡን አድላዪ ዝኾነ ጥንቃቄን ስለዝውሰድ ናይ ምሕማም ስምዕት አይህሉን።

ካብቲ መፅናዕቲ ዝረክብዎ ጥቅሚ፡- እዚ መፅናዕቲ ናይ ማስተርስ ድግሪ መመረቕ ፅሕፍ ዝውዕል እንትኮንአብዚ መፅናዕቲ ብምስታፎም ዝረኽብዎ ናይ ገንዘብ ጥቅሚ የለን። ካብቲ መፅናዕቲ ዝርከብ ውፅኢትን ፖሊሲ ጥዕና መውፃእቲን ባዓል ሞያ ጥዕና ዝጠቅም እዩ።

ናይ ሕክምና መረዳእታ ብምስጥር ምሕላዉ ዝምልከት፡-ኣብዚ ጽንዓት ስለ ናቶም ወይ ናተን ንእክቦ ዝኮነ ዓይነት መረዳእታ ብሚስጥር ከም ንሕዘለኩም ነፍልጥ። ነዚ መጽናዕቲ ኢልና ዘሎ ናቶም/ተን መንነት ዝገልጽ ኩሉ መረዳእታ ናብ ሚሸጥር ክንቕይሮ ኢና።ብተወሳኪ እቲ ትህቡና ደም ኮነ መረዳእታ ካብቲ ጽንዓት ወጻኢ ኣይንጥቀመሉን።

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

ንተወሳኺ ሓበሬታ ነዞም ዝስዕቡ ኣድራሻ ይጠቀሙ።

ናይ መፅናዓይ ሽም:- ክብሮም ገረዝጊሄር ኣስፋው ስልክ:- 0967562388 ኢሜል:- kibromgere14l@gmail.com

ኣማከርቲ:- 1) ዶ/ር ሰሎሙን ተበጀ ግዛው ስልክ:- +2519 11731148 ኢሜል:-solomon.tebeje@aau.edu.et

2) ዶ/ር ናና ሰከረን ስልክ:-------- ኢሜል፡ ngsbio2@gmail.com

13.2. Annex II consent form (adult study participants) –

A. English version

Principal investigator: Kibrom Gerezgiher Asfaw (BSC)

Research title: Evaluation of renal function profile in human visceral leishmaniasis (kala-azar) patients.

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_____

C. Amharic version

የፍቃደኝነት ማረጋገጫ ቅጽ

የጥናቱ ርእስ፡ በካሕሳይ ኣበራ ሆስፒታልና ማእርግ ሆስፒታል የሚገኙ የካላኣዛር ሕሙማን ላይ የካላ ኣዛር በሽታ በኩላሊት ላይ ሊያመጣዉ የሚችለዉን ችግር በጥናታዊ ምርምር መፈተሽ።

የአጥኝው ስም: ክብሮም ገረዝጊሄር ኣስፋው

የተቋሙ ስም: ባዮኬሚስትሪ ት/ክፍል፣ የህክምና ት/ቤት፣ ጤና ሳይንስ ኮሌጅ፣ አዲስ አበባ ዩኒቨርሲቲ

እኔ ከዚህ በታች የተገለጸዉ በዚህ ጥናት ተሳታፊ ልመሆን ስወሰን የጥናቱ ዓላማዎች ኣሳራሮችና ቅድመ ሁኔታዎች በግልጽ በመረዳትና እንዲሁም ከጥናቱ ተሳታፋይነት ፈቃደኝነቴን በማነኛዉም ግዜ የማስወገድ መብቴን በመራገጥ ነዉ።

ሰለዚህ በጥናቱ ተሳታፊ መሆኔን በፈርማየ እየራገጥኩ ይህንን ስውስን በጥናቱ ሊከሰቱ የሚችሉ ስጋቶችን በሚገባ የተረዳሁና ከጥናቱ በማነኛዉም ግዜ ራሴን ለምግለል ብወስን ተገቢ የሆኑ ህክምናዎችና እገዛዎች ሁሉ እንደማትነፈጉኝ በማመን ነው። እነዚህ መረጃዎች ሁሉ በሚገባ በምረዳዉ ቋንቋ የተገለጸልኝ መሆኑን በፈርማየ ኣጋግጣለሁ።

የተሳታፊው ስም ..... ፊርማ..... ቀን .....

የአጥኚው ስም ..... ፊርማ ..... ቀን.....

ስለ ትብብርዎ አመሰግናለሁ!

B. Tigrigna version

ናይ መፅናዓይ ሸም: ክብሮም ገረዝኒሄር ኣስፋው

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

ርእሲ ናይቲ መፅናዕቲ ፡- ሕማም ካላኣዛር ኣብ ኩላልት ከስዕቦ ዝክእል ሳዕቤን ብፅናዓታዊ ምርመራ ምፍታሽ።

ኣነ ኣብዚ ታሕቲ ተገሊጹ ዝኒሆ መጽናዕቲ ተሳታፋይ ንምኳን እንትዉስን እንተለኩ ናይዚ ጽንዓት ዘድልዩ ነገራት ብምርዳእ እና ካብዚ ጽንዓት እዚ ምስታፍ ኣብ ዝኮነ ይኩን እዋን ከም ዘግልል ብምርግጋጸይ እዩ።ስለዚ ኣብዚ ጸንዓት ተሳታፋይ ምኳነይ በፈረማይ የራጋግጽ።እዞም ኩሎም መረደእታታት ብዝግባእ ብዝሩድኡኒ ቋንቋ ዝተገለጹለይ ምኳኖም በፈረማይ የራጋግጽ።

ናይ ተሳታፊ/ፊት መለለዪ ቁፅሪ ..... 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 evaluation of renal function profile among patients with human visceral leishmaniasis (Kala-azar).

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 a 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 participate in this research study.

_____	_____	____/____/___
Name of parent	signature	Day/month/year
_____		____/____/___
Witness (Illiterate)		Day/month/year
_____		____/____/___
Name of the interviewer	Signature	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። የጥናቱርዕስ በካሕሳይ ኣበራ ሆስፒታል የሚገኙ የካላኣዛር ሕሙማን ላይ የካላ ኣዛር በሽታ በኩላሊት ላይ ሊያመጣዉ የሚችለዉን ችግር በጥናታዊ ምርምር መፈተሽ በሚል ጥናት ልጄ እንዲሳተፍ ምርጫው የእኔ መሆኑን ነግረውኛል። ናሙና መስጠት ምንም አይነት ጉዳት ልጄ ላይ እንደሌለዉ ተነግሮኛል። በጥናቱ ወቅትም የልጄ መረጀዎች በሚስጥር ስለሚያዝ በሌላ ሰዉ ዘንድ እንደማይታወቅ ተረድቻለሁ። ልጄ በግሉ የሚያገኘዉ ጥቅም እንደሌለ ተረድቻለሁ። ጥያቄ እንድጠይቅ ዕድል ተሰጥቶኝ ለጥያቄዎቼም በቂ ምላሽ አግኝቻለሁ። የልጄ በጥናቱ መሳተፍ በእኔ ፍላጎት ብቻ እንደሆነ እና በጥናቱም አለመሳተፍ ምንም አይነት ተፅዕኖ በልጄ ላይ እንደማያስከትል ተረድቻለሁ። በከዚህ ባሻገር የልጄ በጥናቱ ውስጥ ለመካተት የእኔ የወላጂ አሳዳጊ ፈቃድ እንደሚያስፈልግ ተረድቻለሁ። በእኔ ፍቃደኝነት ልጄ በጥናቱ እንደሚሳተፍ ከዚህ በታች በፊርማዬ አረጋግጣለሁ።

የተሳታፊ ስም…… ፊርማ…… ቀን …….

መረጃ የሰበሰበ ባለ ሞያ ስም …… ፊርማ …… ቀን……

የአጥኚው ስም …… ፊርማ …… ቀን……

ስለ ትብብርዎ አመሰግናለሁ!

B. Tigrigna version

ናይ ፍቓደኛነት መረጋገፂ ቅጥዒ

ኣነ---------- ናይ ዉላደይ መላዓሊ እንትከዉን ናይዚ ጽንዓት ዓላማ ተረዲአ ኣለኩ።ሕማም ካላኣዛር ኣብ ኩላልት ከምፆ ዝክእል ችግር ምፍታሽ ን ምጽናዕን ብዝብል ርእሲ ኣብ ካህሳይ ኣበራ ሆስፒታል እና ኣብ ማዓርግ ሆስፒታል ዝካየድ መፅናዕቲ ዉላድይ ንዝሳተፍ/ትሳተፍ እቲ ድልየት ናተይ ከም ዝኮነ ነጊሩኒ/ነጊራትኒ ኣሎ/ኣላ።ናሙና ምሃብ ኣብ ዉላደይ ምንም ዓይነት ሽግር ከም ዘይብሉ ተነጊሩኒ ኣሎ።ዝኮነ ዓይነት መረዳእታ ብሚስጥር ከም እትሕዝዎ እዉን ተረዲአ ኣለኩ። ካብዚ ዉጽኢት ወጻኢ ካሊእ ምንም ዓይነት ጥቅሚ ከም ዘይረክብ እዉን ተረዲአ ኣለኩ።ናይ ዉላደይ ኣብዚ ጽንዓት ምስታፍ ብናተይ ድልየት ዝዉሰን ከም ዝኮነና ኣብዚ ጽንዓት ብዘይምስታፉ/ፉ ምንም ዓይነት ተጽዕኖ ክም ዘየምጽእ ተርዲኣ ኣለኩ። ካብዚ ብተወሳኪ ናይ ውላዲ ድልየት ከም ዘድሊ ተረዲኣ ኣለኩ። ብናተይ ድልየት ዉላደይ ኣብዚ ጸንዓት ከም ዝሳተፍ ብፈርማይ የራጋግጽ።

ናይ ተሳታፊ/ፊት ሽም ..... ፊርማ ..... ዕለት.....

ሓበሬታ ዝኣክበ በዓል ሞያ ሽም..... ፊርማ.....ዕለት.....

ናይቲ መጽናዓይ ሽም.....ፊርማ..... ዕለት.....

ስለ ዝተሓባበሩኒ የቐንየለይ!

13.4. Annexe IV: Consent form for children aged 12-17 years

A. English version

I fully informed about the purpose of this study on Evaluation of renal function profile among patients with human visceral leishmaniasis (kala-azar).

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 a 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 allowed 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 to give their consent to give my blood for the study.

Name of participant signature Day/month/year

_____ _____ ____/____/___

Witness (Illiterate)

Parents phone number _____

Name of interviewer Signature Day/month/year

---------- ---------- ...../...../.....

Name of the researcher Signature Day/month/year

_____ _____ ____/____/___

B. Amharic version

የካላ ኣዛር በሽታ በኩላሊት ላይ ሊያመጣዉ የሚችለዉን ችግር በጥናታዊ ምርምር መፈተሽ በሚለው ርእስ ዋና ዓላማዉ ተነግሮኛል.ናሙና ብሰጥ ምንም ዓይነት ችግር እንድሌለዉም ተነግሮኛል። በጥናቱ ወቅትም የኔ መረጀዎች በሚስጥር ስለሚያዝ በሌላ ሰዉ ዘንድ እንደማይታወቅ ተረድቻለሁ። ልጄ በግሉ የገኘዉ ጥቅም እንደሌለ ተረድቻለሁ። ጥያቄ እንድጠይቅ ዕድል ተሰጥቶኝ ለጥያቄዎቼም በቂ ምላሽ አግኝቻለሁ። በዚህ ጥናቱ ለመሳተፍ የኔ ፍላጎት እንዳለ ሁኖ ወላጀቼ ከፈቀዱልኝ ተስማምቻለሁ።

የተሳታፊ ስም…… ፊርማ…… ቀን ……

መረጃ የሰበሰበ ባለ ሞያ ስም …… ፊርማ …… ቀን…….

የአጥኚው ስም ……ፊርማ …… ቀን ……

ስለ ትብብርዎ አመሰግናለሁ!

C. Tigrigna version

ሕማም ካላኣዛር ኣብ ኩላልት ከስዕቦ ዝክእል ሳዕቤን ብፅናዓታዊ ምርመራ ምፍታሽ ኣብ ዝብል ርእሲ ዋና ዓላምኡ ተነግሪኒ ኣሎ። ናሙና ምሃብ ምንም ዓይነት ሽግር ከም ዘየምጸአለይ ተነጊሩኒ ኣሎ።ዝኮነ ዓይነት መረዳእታ ብሚስጥር ከም እትሕዝዎ እዉን ተረዲአ ኣለኩ። ካብዚ ዉጽኢት ወጻኢ ካሊእ ምንም ዓይነት ጥቅሚ ከም ዘይረክብ እዉን ተረዲአ ኣለኩ። ኣብዚ ጸንዓት እዚ ምስታፍ ናተይ ድልየት ከም ዘሎ ኮይኑ ወለደይ እንድሕር ፈቂዶምለይ ንክሳተፍ ተስማዕሚዐ ኣለኩ።

ናይ ተሳታፊ/ፊት ሽም ..... ፈርማ ..... ዕለት.....

ሓበሬታ ዝኣክበ በዓል ሞያ ሽም.....ፈርማ.....ዕለት.....

ናይቲ መጽናዓይ ሽም.....ፈርማ..... ዕለት.....

ስለ ዝተሓባበሩኒ የቐንየለይ!

13.5. Annexe V: Structured Questionnaire for kala-azar patients and control group

A. English version

Identification no: ----------

Name of facility _____

Region_____Zone_____Woreda _____

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

No.	Variable	Response
1	Sex	1. Male 2. Female
2	Age	
3	Weight	
4	Height	
5	Marital Status	1. Single 2. Married 3. Divorced 4. Widowed
6	Educational status	1. Illiterate 2. Primary 3.Secondary 4.Diploma and above
7	Residence	1. Rural 2. Urban
8	Occupation status	1. Student 2. Housewife 3. Government employer 4. Private employers 5. Farmer 6. Merchant 7. Daily labour 8. Other
9	Duration of illness	1. 0-4 weeks 2. > 4 weeks
10	Do you have a history of hypertension?	1. Yes 2. No

11	Do you have a history of HIV/AIDS?	1. Yes 2. No
12	Do you have history tuberculosis for the past two years?	1. Yes 2. No
13	Do you have a history of DM?	1. Yes 2. No
14	Do you have a habit of smoking?	1. Yes 2. No
15	If yes to Q#14, how often do you smoke?	1. ≤ 1cigarate perd ay 2. 2-3 cigarate per day 3. >3 cigarate per day
16	Do you drink alcohol?	1. Yes 2. No
17	If yes to Q#16, how often do you take?	1 one beer bottle per day 2. Two or three beer bottle per week 3. More than four beer bottle per week 4. some times (holiday)
18	Do you take any medication within the last three months?	1. Yes 2. No
19	If yes to Q#18, what type of drug did you take?	1. Anti-protozoa 2. Anti-bacterial 3. Anti-TB 4. Others
20	Do you have a history of chronic liver disease?	1. Yes 2. No
21	Do you have a history of chronic kidney disease?	1. Yes 2. No

22	Do you have a history of cancer?	1. Yes 2. No
23	Do you have a history of chronic heart disease?	1. Yes 2. No
24	Creatinine level?	
25	Urea?	
26	Uric acid?	
27	eGFR?	

We highly appreciate your kind cooperation on behalf of the research group, the hospital, Addis Ababa and Adigrat Universities.

B. Amharic version

የሚስጥር ቁጥር:- _____

የተቋሙ ስም:- _____

ክልል_____ወረዳ_____ቀበሌ_____

ማሳሰብያ: እባክዎ ትክክለኛውን መልስ ያክብቡ ወይም ይፃፉ።

ተ.ቁ	ጥያቄዎች	መልስ
1	ጾታ	1. ወንድ 2. ሴት
2	እድሜ	
3	የክብደት መጠን	
4	ቁመት መጠን	
5	የጋብቻ ሁኔታ	1. ያላገባ/ች 2. በትዳር ላይ 3. የፈታ/ች 4. በሞት ምክንያት የተለያዩ
6	የትምህርት ደረጃ	1. ያልተማረ 2. ኣንደኛ ደረጃ(1-8) 3. ሁለተኛ ደረጃ (9-12) 4.ዲፕሎማ/ድግሪና ከዛ በላይ
7	መኖርያ	1.ገጠር 2.ከተማ

8	ስራ	1. ተማሪ 2. የቤት እመቤት 3. የግል ሰራተኛ 4. ገበሬ 5. .ነጋዴ 6. የቀን ሰራተኛ 7. ሌላ
9	የህመሙ ቆይታ?	1. 0-4 weeks 2. >4 weeks
10	በደሙ ዉስጥ ድም ግፈት ኣለበት ?	1. አዎ 2. የለኝም
11	በደሙ ዉስጥ የኤች ኣይቪ በሽታ ኣለበት ?	1. አዎ 2. የለኝም
12	የቲቢ በሽታ ታካሚ ኖት?	1. አዎ 2. የለኝም
13	በደሙ ዉስጥ የስኳር በሽታ ኣለበት?	1. አዎ 2. የለም
14	ሲጋራ የመጨሰ ልምድ ኣለበት ?	1. አዎ 2. የለኝም
15	ለተራ ቁጥር 14, መልስዎ አዎ ከሆነ ለምን ያክል ግዜ ያጨሳሉ?	1.በቀን ≤ 1 ስጋራ 2. በቀን 2-3 ስጋራ 3. >3ስጋራ
16	ኣልኮል የመጠጣት ልምድ ኣለበት ?	1. አዎ 2. የለም
17	ለተራ ቁጥር 16, መልስዎ አዎ ከሆነ ምን ያክል ይጠጣሉ?	1.በየቀኑ አንድ ቢራ 2. ሁለት ወይም ሰሶት ቢራ በሳምንት3. ኣንዳ ኣንድ ግዜ(በባኣል ግዜ)

18	በዚ ሶስት ወር ወስጥ መድሓኒት ወስዶ ያዉቓሉ?	1. አዎ 2. የለም
19	ለተራ ቁጥር 18, መልስዎ አዎ ከሆነ ለምን ኣይነት በሽታ?	1. ለ ፕሮቶዝዋ 2. ለባክቴሪያ 3. ለቲቪ 4.ሌላ
20	ለብዙ ግዜ የቆየ የጉበት በሽታ ኣለቦት?	1. አዎ 2. የለም
21	ለብዙ ግዜ የከላሊት በሽታ ኣለቦት ?	1. አዎ 2.የለም
22	የካንሰር በሽታ ኣለቦት ?	1. አዎ 2.የለም
23	ለብዙ ግዜ የቆየ የልብ በሽታ ኣለቦት?	1. አዎ 2. የለም
24	ክሪቲንን መጠን ስንት ነው?	
25	የደም ዩርያ መጠን ስንት ነው?	
26	ዩሪክ ኣሲድ መጠን ስንት ነው?	
27	ግሎሞርላር ፍልትሬሽን ሬት?	

ስለ ትብብርዎ አመሰግናለን!

C. Tigrigna version

ኮድ ቁጽሪ:_____

መለለዪ: ናይቲ ተቋም ሽም:_____

ክፍለ ከተማ_____ወረዳ_____ጣበያ_____

መተሓሳሰቢ: ትኽክለኛ ዝኾነ መልሲ የክብቡ ወይ ከዓ ይፅሓፉ።

ተ.ቑ	ሕቶታት	መልሲ
1	ፆታ	1.ተባዕታይ 2. አንስታይ
2	ዕድመ	
3	መጠን ክብደት	
4	መጠን ቁመት	
5	ኹነታት ሓዳር	1. ዘይተመርዓወ/ወት 2. በዓለ ሓዳር 3. ዝፈትሐ//ሐት 4. ብሞት ምኽንያት ዝተፈላለዩ
6	ናይትምህርቲ ደረጃ	1. ዘይተምሃረ 2.ቐዳማይ ደረጃ(1-8) 3. ካልኣይ ደረጃ (9-12) 4. ዲፕሎማ/ድግሪንልዕሉኡን
7	መንበሪ	1.ገጠር 2.ከተማ

8	ስራሕ	1.ተምሃራይ/ት 2.ናይ ገዛ ሰራሕተኛ 3.ናይዉልቀ ስራሕ 4.ሓረስታይ 5.ነጋዳይ 6.መዓልታዊ ሰራሕተኛ 7.ካሊእ
9	ናይቲ ሕማም ጸኒሒት	1. 0-4 weeks 2. >4 weeks
10	ኣብ ደሞም ዉሽጢ ጸቅጢ ደም ኣለዎም ዶ?	1. አወ 2. የለን
11	ኣብ ደሞም ዉሽጢ ኤች ኣይቪ ኣለዎም/ን?	1.አወ 2.የለን
12	ኣብ ደሞም ዉሽጢ ናይ ሽኮርያ ሕማም ኣለዎም/ን?	1.አወ 2.የለን
13	ናይ ቲቪ ሕማም ተሓካሚ ድዮም?	1.አወ 2.የለን
14	ሽጋራ ናይ ምትካክ ልምዲ ኣለዎም /ን?	1.አወ 2.የለን
15	ንተራ ቁጥር 14, መልሶም አዎ እንተኮይኑ?	1. አብ ማዕልቲ ≤1 ስጋራ 2. አብ ማዕልቲ 2-3 ስጋራ 3. አብ ማዕልቲ >3ስጋራ
16	ኣልኮል ናይ ምስታይ ልምዲ ኣለዎም /ን?	1.አወ 2.የለን
17	ንተራ ቁጥር 16, መልሶም አዎ እንተኮይኑ?	1.በቢማዓልቱ ሓደ ቢራ 2.ክልተ ወይ ሰለስተ ቢራ ኣብ ሰሙን 3.ካሊእ
18	ኣብዉሽጢሰለስተወርሒዝኮነዓይነትመድሓኒትንዝኮነዓይነ ትሕማምወሲዶምዶይፈልጡ ?	1.አወ 2.የለን
19	ንታራ ቁፅሪ 18, መልሶም እወ እንተኾይኑ ኣየናይዓይነት መድሓኒት እዮም ወሲዶም?	1. ጸረ-ፕረተዝዋ 2. ጸረ-ባክተርያ 3. ናይ ቲቢ 4. ካሊእ
20	ናይ ጸላም ከብዲ ሕማም ኣለዎም /ን ዶ?	1.አወ 2.የለን

21	ናይ ከላሊት ሕማም ኣለዎም /ን ዶ?	1.አወ 2.የለን
22	ናይ ካንሰር ሕማም ኣለዎም /ን ዶ?	1.አወ 2.የለን
23	ናይ ልቢ ሕማም ኣለዎም /ን ዶ?	1.አወ 2.የለን
24	መጠን ክሪቲንን ክንደይ እዩ?	
25	መጠን ናይ ደም ዩርያ ክንደይ እዩ ?	
26	መጠን ዩሪክ ኣሲድ ክንደይ እዩ ?	
27	ግሎሞርላር ፍልትሬሽን ሬት?	

ስለ ዝተሓባበሩኒ የቐንየለይ!

13.6. Annex VII. 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 make the patient a 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 in small outward circles with an 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

Renal Function Assay

Creatinine

Principle

This procedure is based upon a modification of the original picrate reaction (Jaffe). Creatinine under alkaline conditions reacts with picrate ions forming a reddish 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.

Creatinine + picric acid $\xrightarrow[37^0c]{PH>12}$ creatinine picrate complex

Procedure

1. Preincubate working reagent, samples and standard to reaction temperature (37ºC).
2. Set the photometer to 0 absorbance with distilled water.
3. Pipette into a cuvette**.**
   Working reagent: 1.0 ml
   Sample or standard: 100
   µL
4. Mix gently. Insert cuvette into the temperature-controlled instrument and start a stopwatch.
5. Record absorbance at 510 nm after 30 seconds (A1) and after 90 seconds (A2) of the sample or standard addition.

Calculations

$\frac{\text{A2 - A1) Sample}}{\text{(A2 - A1) Standard}} \times \text{C Standard} = \text{mg/dL creatinine}$

Samples with concentrations higher than 20 mg/dL should be diluted 1:4 with saline and assayed again. Multiply the results by 4. If results are to be expressed as SI units apply: mg/dL x 88.4 = µmol/L

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 (JOURILABS/biochemistry reagents, 2019).

Urea

Principle

The test is performed as a kinetic assay in which the initial rate of the reaction is linear for a limited time. 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 is oxidized for each mole of urea present. The initial rate of decrease in absorbance at 340 nm is proportional to the urea concentration in the sample

Result interpretations

Urea is the nitrogen-containing end product of protein catabolism. States associated with elevated levels of urea in the blood are referred to as hyperuricemia 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 (JOURILABS/biochemistry reagents, 2019).

Uric acid

Principle

Uric acid is oxidized to allantoin by uricase with the production of hydrogen peroxide (H2O2). The H2O2 reacts with 4-aminoantipyrine (4-AAP) and 2, 4, 6-tribromo-3-hydroxybenzoic acid (TBHB) in the presence of peroxidase to yield a quinoneimine dye. The resulting change in absorbance at 548 nm is proportional to the uric acid concentration in the sample.

Result interpretation

Uric acid is the final product of endogenic and exogenic (food origin) purine catabolism (adenosine and guanidine). This transformation takes place mainly in the liver. Approximatively 75% of uric acid is eliminated by kidneys; the rest is released in the gastro-intestinal tract where it will be degraded by the intestinal flora. Seric hypouricaemia is more unusual. This decrease can be observed in different cases as defect of renal elimination (Fanconi syndrome) (JOURILABS/biochemistry reagents, 2019)

Glomerular Filtration Rate (GFR)

It is the rate (volume per minute) at which the filtration of blood is performed by the glomerulus and transferred to Bowman's space. It is determined practically by measuring creatinine clearance. Creatinine clearance can be calculated without a 24-hour urine collection using Cockcroft equation which requires (serum creatinine concentration, sex, age, and weight)

Cockcroft equation:

- Creatinine clearance = $\frac{(140 - age) \times weight \times constant}{Serum\ creatinine}$
- Weight in Kg, in,
- If serum creatinine is measured in mg/dl:
- Serum creatinine must be multiplied by factor 72
- Constant = 1 for men, constant = 0.85 for women
- If serum creatinine is measured in µmol/l:
- Constant = 1.23 for men, constant = 1.04 for women (Cockcroft and Gault, 1976)

Adult Reference Ranges for RFT

Instrument used: Mindray 200E

S.no	Parameter	Reference		unit
		Men	Women	
1	Urea	18-45	18-45	mg/dL
2	Creatinine	0.6- 1.3	0.5 – 0.9	mg/dL
3	Uric acid	3.5 - 7.2	2.6 - 6	mg/dL

13.8. Anthropometrical measurements

The weight of VL patients was measured using a standard balance, and the height was measured using a height measuring device attached to the balance. BMI was then calculated from the body weight (kg) and height (m). Using the World Health Organization (WHO) classification, four categories of BMI can be identified as follows: underweight, <18.5 kg/m2; normal, >18.5–24.9 kg/m2; overweight, >25.0– 29.9 kg/m2; and obesity, >30 kg/m2. The nutritional status of the patients was also determined using body mass index (BMI). Using the World Health Organization (WHO) classification, the nutritional status was defined as severe, moderate,mild and normal when the individual had BMI of <16, ≥16- < 17, ≥17- < 18.5 and ≥18.5, respectively (WHO, 2004).

Declaration

I, the undersigned, declare that this MSc thesis is my original work and all sources of materials used for the thesis have been duly acknowledged.

Name of the principal investigator: Kibrom Gerezgiher Asfaw (BSc, MSc candidate)

Signature: _____

Date of submission: _____

Advisors;	Signature	Date
Dr. Solomon Tebeje Gizaw (PhD)	_____	_____
Dr. Natesan Gnanasekaran (PhD)	_____	_____

Place: Addis Ababa, Ethiopia