Addis Ababa University

College of Health Sciences

Centre for Drug Development and Therapeutic Trials for Africa

Efficacy and Safety of Combination of Sodium Stibogluconate and Paromomycin for Treatment of Visceral Leishmaniasis and Factors Associated with Poor Treatment Outcomes. A Retrospective Study, North West Ethiopia

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Research Thesis Signature Page

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Acronyms

ABCD Amphotericin B cholesterol dispersion

ABLC Amphotericin B – lipid complex

ALT Alanine transaminase

AST Aspartate transaminase

CI Confidence interval

DAT Direct agglutination test

DNDi Drugs for Neglected Disease initiative

FDA Food and Drug Authority

GCP Good Clinical Practice

HIV Human Immunodeficiency Virus

ICT Immunochromatographic test

IFAT Indirect fluorescent antibody test

IRB Institutional Review Board

L-AMP B Liposomal amphotericin B

LRTC Leishmaniasis Research and Treatment Center

MSF Medicines sans Frontieres'

PCR Polymerase chain reaction

PKDL Post kala-azar dermal leishmaniasis

PM Paromomycin

SSG Sodium Stibogluconate

SSG/PM Combination of Sodium Stibogluconate and Paromomycin

ToC Test of cure

UoGH University of Gondar Hospital

VL Visceral leishmaniasis

VL- HIV Visceral leishmanisis and human immunodeficiency virus

WHO World Health Organization

X^2 Chi-square

Table of Contents

List of Tables and Figures

Abstract

Background: Visceral leishmaniasis is among the most neglected tropical infectious disease caused by the protozoan parasite of the genius lesihmania. The incidence of VL is estimated to be 0.2 to 0.4 million cases /year worldwide. It is fatal if left untreated.

Objective: The objective of this study was to assess efficacy and safety of combination of SSG (20mg/kg/day) and paromomycin (15mg/kg/day) and associated factors for poor treatment outcomes.

Methods: A retrospective cohort study design was used. Medical record review of VL patients treated with combination of sodium stibogluconate and Paromomycin between 2012 and 2019 was conducted.

Results: In total, 1000 VL patients were included. The overall initial cure was achieved in 924 (92.4%). Treatment failure, treatment interruptions, default and deaths respectively were noted in 30(3%), 25 (2.5%), 13 (1.3%) and 8(0.8%). The efficacy of SSG/PM (186/208, 89.4%) Vs (738/792, 93.2%) was not different respectively for patients with weight ≤ 42.5kg and >42.5kg (p=0.07). The most common adverse events were raised liver transaminases 351(35.1%), injection site pain 291(29.1%) and increased in serum alpha-amylase 291 (29.1%). The frequency of cardiac arrhythmia 6(0.6%) and clinical pancreatitis 21(2.1%) were low. Factors associated with poor treatment outcomes were sepsis (OR= 7.6, 95% CI: 1.86 - 31.03, P = 0.005), clinical pancreatitis (OR= 4, 95% CI: 1.21 – 13.43, P= 0.02), and cardiac arrhythmia (OR=13, 95% CI: 2.30 – 84.34, P: 0.004). Patients' body weight had no effect on poor treatment outcome (OR = 1.6, 95% CI: 0.89 – 3.06). Of patients who attended six-month visit, cure was achieved in 259/276 (93.8%). Post kala-azar dermal leishmaniasis was 13 (1.3%).

Conclusion and Recommendations. The efficacy of SSG at 20mg/kg with upper maximum dose limit (850mg/day) and PM at 15mg/kg was 92.4%. Our data affirms effectiveness of SSG/PM, as presently used in Eastern African countries, and no issues of concern have been identified. The continued use of the combination therapy is warranted. Similar studies are recommended in the countries affected by the disease. Sepsis, pancreatitis and cardiac arrhythmias should be identified and managed as per VL management protocol to prevent poor treatment outcomes .

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1. Introduction

1.1. Background

Visceral leishmaniasis (VL) is among the neglected tropical diseases caused by the protozoan parasite of the genus Leishmania (*Leishmania donovani* and *Leishmania infantum)*(1). Transmission occurs through the bite of phlebotomine sand flies. Other routes of transmission such as; blood transfusion, intravenous drug use, organ transplantation, and congenital and laboratory accidents has also been implicated(2). In the Old World (East Africa and Indian sub-continent) where *L. donavani* is the cause of infection, transmission is anthroponotic (human to human). In contrast, in Mediterranean basin and Latin America where L. infantum infection is common, transmission is zoonotic(1). The typical clinical features of VL includes fever, weight loss, loss of appetite, splenomegaly, weakness, leucopemia, thrombocytopenia and anemia(3). Globally, the annual incidence of VL is approximately 0.2 to 0.4 million. Over 90% of global VL cases occur in six countries: India, Bangladesh, Sudan, South Sudan, Ethiopia and Brazil(4).

In Ethiopia, since its first report in the Omo river valley in 1942(5), it has been spreading and imposing series public health problem. It commonly occurs in the low land arid and semi-arid areas(6). The disease has rapidly spread to highland areas as exemplified by epidemics of VL in 2005 in the district of Libo Kemkem in Amhara Regional State where the first epidemics of VL occurred(7). The information on the epidemiology of visceral leishmaniasis in Ethiopia is incomplete as no surveillance system has been in place. However, reports of VL endemicity date back to the 1940s. The six areas of endemic foci are; northwestern Ethiopia (Metema, Humera, Wolkayit, and Libo/Fogera), northeastern Ethiopia (Awash Valley and Ethio-Djibouti border), south and southwest Ethiopia (Dawa, Genale, Gelana, Segen, Woito, Konso, and Omo River Valley), the Kenyan border and the Gambella-Sudan border(8). Of the endemic regions, the northwest region particularly the Metema-Humera foci accounts for 90% of VL case burden (unpublished data from KalaCore Ethiopia).

In Ethiopia its incidence is estimated to be between 3700 – 7400 cases per year(4). The diagnosis of visceral leishmaniasis is misleading as its presentation mimics other infectious disease like malaria. Hence, appropriate diagnostic method should be used for confirmation of the disease. The golden approach for VL diagnosis is parasite detection in tissue aspirates of spleen, bone marrow or lymph node(9). The sensitivity of this method varies with site of aspiration; while tissue aspiration from the bone marrow has sensitivity of 60-85%, the sensitivity from splenic aspirate is 93-99% and it ranges from 52 to 58% for lymph node(10, 11).

Serological diagnosis of VL in endemic areas is the usual practice especially in areas where resources are limited. The serologic test by rk-39 immunochromatographic test (ICT) is the most popular in most VL endemic regions. In primary suspected VL patients with history of fever and splenomegaly, the overall sensitivity and specificity of rK39 ICT is 91.9% and 92.4%, respectively. However, the sensitivity in East Africa (84.3%) is lower than sensitivity in the Indian subcontinent (97.0%) with no geographical variation in specificity(12). Serologic diagnostic test evaluation conducted in leishmania epidemic foci of Ethiopia showed that the sensitivity in 35 PCR confirmed VL cases was 94.3%, 91.4%, 91.4%, and 100%, for Detect®, DiaMed-IT Leish®, DAT, and IFAT respectively and the specificities were 98.5%, 94%, 98.5%, and 98.5%, respectively(13).

The diagnosis of VL using PCR is the most sensitive and specific diagnostic modality. Conventional PCR protocol for VL has a sensitivity and specificity of 100%. However, the procedure needs more sophisticated machine and needs well-trained professionals. Moreover, it is more expensive, tedious, and not affordable in endemic areas(14).

The cornerstone of VL treatment has been pentavalent antimonials [sodium stibogucoante (SSG) or glucantime 85mg/ml] injections for 30 days. Its use has been continued in many endemic countries except where resistance to pentavalent antimonials has been reported in the state of Bihar and Nepal(15,16).

Currently, combination of sodium stibogluconate at 20mg/kg and paromomycin at 15mg/kg is the first line drug for VL in Eastern Africa including Yemen(6). It is necessary to do pharmacovigilance and postmarketing studies of new drugs after recommended for use in the health system. It helps to monitor the effectiveness (safety, lack of response and drug resistance). Such studies are downstream of the pathway in the drug development and translation.

1.2. Statement of the Problem

Visceral leishmaniasis is among the most neglected tropical disease. It almost exclusively affects people with poor socioeconomic status(17). As a consequence of poor socioeconomic status, persons at risk of VL suffer from nutritional deficiencies (protein, energy, vitamins A, Zinc and iron), which weakens the host immune system. Furthermore, poor housing and poor sanitation condition has been implicated as a conducive environment for sandy fly (vector for VL) breeding, increasing transmission of the disease(5). The disease is fatal if left untreated, indicating the need for aggressive management with effective and safe drugs.

In Ethiopia, VL has been spreading rapidly since its first report in the Omo river valley(5). More than 90% of the VL burden occurs in the northwest (Metema, Humera, Wolkayit and Libo/Fogera) foci (unpublished report from KalaCore Ethiopia). In these foci, the disease commonly affects the youngsters who migrate to large farmlands in the Metema Humera platforms where they work as daily laborers.

In Eastern African countries (Ethiopia and Sudan) where the causative agent for VL (*L. donovani)* are highly virulent, drug unresponsiveness had been reported(18,19,20)

A multicenter non-inferiority study conducted in East Africa comparing three regimens of VL drugs (SSG/PM, SSG, and PM) showed that efficacy of PM injections of 21 days was generally low in Ethiopia and Sudan suggesting geographical variation in treatment response(21). Therefore, there is no guarantee that *L. donovani* parasite in the region continues to be sensitive to the current drug and needs continuous assessment.

Even though combination treatment (SSG/PM) improved the safety profiles by reducing the duration of treatment from 30 to 17 days, it still has serious adverse effect as compared to the second-line drugs such as L-AMPB(22). Thus, continuous surveillance of safety is necessary as a part of pharmacovigilance activity.

Despite the World Health Organization (WHO) recommendation of SSG use at a dose of 20mg/kg without upper maximum dose limit (850mg/day), UoGH LRTC had been using SSG at 20mg/kg /day taking 850mg as upper maximum dose limit for various reasons; clinical trial experience, fear of toxicity as patients being treated are wasted and non-tolerant to drugs, and death of patients at a dose recommended by WHO).

Treatment failure and relapse of VL cases had been reported during the clinical management of VL with SSG/PM. Moreover, factors associated with poor treatment outcomes had not been studied in VL patients being treated with SSG/PM. Hence, the objective of this study was to assess the efficacy and safety of SSG/PM and associated factors for poor treatment outcomes.

1.3. Literature Review

1.3.1. Treatment of Visceral Leishmaniasis

1.3.1.1. Pentavalent Antimonials

The pentavalent antimonials [sodium stibogluconate (SSG) and meglumine antimoniate (MA)] at the dose of 20 mg/kg body weight had been the standard treatment for VL since 1940s. It is still effective in many VL endemic countries except in the Indian subcontinent where high failure rate was reported(23,24). Despite its effectiveness, the drug is associated with adverse effects to visceral organs (cardiac, pancreas, liver, and kidney) that threaten life if not regularly monitored. The cure rate of SSG in Eastern Africa was reported to be 95% in treatment naïve VL patients without HIV-co-infection(25). In Ethiopian VL-HIV co-infected patients, high parasitological failure with an initial cure rate of 34% was reported(26). Currently, it is used as a second line drug for treatment of VL patients with and without HIV co-infection in the event of shortage of the

standard treatment (SSG and PM combination for VL without HIV and AmBisome for VL patients with HIV)(6).

1.3.1.2. Liposomal amphotercin B

Liposomal amphotericin B (L-AMP B) is the lipid formulation of amphotericin B. It has shown proven efficacy against leishmania parasite and approved for VL treatment in 1997(27). Amphotericin B deoxycholate is effective at 0.75 - 1mg/kg for Indian visceral leishmaniasis with more than 90% cure rate (28). However, it is associated with toxicities like nephrotoxicity, hypokalemia and infusion related reactions(29). Lipid formulations of amphotericin B have been tested extensively for VL treatment. The lipid formulation is less toxic and highly concentrated in organs like liver, spleen and bone marrow easily reaching to the site where parasites are concentrated(30).Three lipid formulations have been evaluated in VL. Liposomal amphotericin B (AmBisome; Gilead Sciences; L-AMP B), amphotericin B lipid complex (ABLC; Abelcet_, Enzon pharmaceuticals) and amphotericin B cholesterol dispersion (ABCD; Amphotec, InterMune Corp). Of the three formulations liposomal amphotericin B was approved by FDA for treatment of VL in 1997(27). A multi-center clinical trial involving 203 patients in India; 7.5mg/kg of liposomal Amphotericin B showed a cure rate of 96% (95% CI, 92–98) (31). An open label study in India involving 412 patients randomly assigned to either single dose L-AMP B (10mg/kg) or conventional amphotericin B (1mg/kg/day, 15 doses in 29 days); The final cure at 6 month was 95.7% versus 96% for L-AMP B and conventional amphotericin B respectively(32).

Liposomal amphotericin B has not been well studied in East Africa. An open label study conducted in Sudan evaluated 3 different regimens (3-5mg/kg/day for 3 days, 6 days and 4 -5mg/kg for 4 days) to treat complicated kala-azar patients; based on this study the optimal regimen was 4mg/kg for 6 days with a cure rate of 88%(33). Similar study in 64 VL patients (52 relapse and 12 new cases) using L-AMP B total dose of 15-49mg/kg in Sudan, cure (both clinical and parasitological) was achieved in 35 (55%) indicating the need of high total dose of L-AMP B in this region(20). In Kenya a cohort of 10 patients were

treated with three regimens; 14mg/kg, 10mg/kg, and 6mg/kg; all patients were cured with regimen 1, and 90% in regimen 2 and 20% with regimen 3. Concordant study in India showed 100% cure with the three regimens. But in Brazil regimen one was only partially curative; 5 of 13 patients (62%) were cured indicating geographical variation in treatment response(34).

In Ethiopia, the use of liposomal amphotericin B was first introduced by MSF treating VL patients in remote areas in 2006(18). A study conducted in a routine setting in Ethiopia where high VL-HIV co-infection rate (15 -40%) was reported, high dose L-AMP B (30mg/kg total dose) had limited effectiveness against Visceral leishmaniasis in HIV co-infected patients 195 (60%). However, initial cure rate was achieved in 74 (93%) HIV negative VL patients. Treatment failures in VL-HIV patients were rescued by SSG at 20mg/kg for 28 days increasing the cure rate from 60% to 83% (18).

1.3.1.3. Paromomycin

Paromomycin is a broad-spectrum aminoglycosidic aminocyclitol. It was isolated for the first time in 1956, and is produced by Streptomyces riomosus var. Paromomycinus. It is effective against a wide range of bacteria and protozoa(35). In Phase III clinical trial in India involving 667 VL patients, 502 patients on PM at 15mg/kg (11mg base) IM for 21 days and 165 patients on amphotercin B at 1mg/kg IV 15 doses in 30 days, the efficacy of PM was 94.6%(36). In the same country, the efficacy of PM was 94.2% in a large phase IV trial in VL patients(37). However, the efficacy of PM alone was inferior to SSG (63.8% versus 92.2%) in East Africa (Sudan, Ethiopia, and Kenya). Efficacy varied among centers and was significantly lower in Sudan compared to Kenya and Ethiopia(33). However, another multicentric study from East Africa that have used a dose of 20 mg/kg for 21 days, had significantly lower cure rate (CR) compared to SSG (84.3 Versus 94.1%)(38). The adverse effects related to PM was reported to be mild injection site pain, mild elevation in liver transaminases and reversible hearing loss(28).

1.3.1.4. Combination Treatment (SSG/PM)

Combination drugs are the current strategy for treatment of VL in endemic regions with the aim of increasing therapeutic effect by the synergistic effect of one drug with the other, decreased toxicity related to shortening of hospitalization, decreased cost and decreased emergence of resistance(39). The combination treatment (pentavalent antimonials and paromomycin injections) was first evaluated in clinical setting in Kenya in 1990(40). A retrospective cohort study conducted in southern Sudan reported that the initial cure rate among patients treated with SSG/PM was 97% compared with 92.4% among patients treated with SSG monotherapy(41). In randomized comparative trial in eastern Africa (in Sudan, Kenya and Ethiopia), the efficacy of SSG & PM was comparable to SSG (91.4% versus 93.9%, difference = 2.5%, 95% CI: -1.3 to 6.3%, p= 0.198)(38). Another observational study from eastern Sudan reported efficacy of 86% at 6 month(42). Currently, SSG/PM is extensively used in east Africa as first line drug following the 2010 WHO recommendation (6).

1.3.1.5. Miltefosine

Miltefosine is the only approved oral treatment for VL in endemic areas especially in India (43). It is extensively studied in India with different efficacies over time. A large study in India using 100-150 mg and 2.5 mg/kg/day of miltefosine for adult and pediatric VL respectively, miltefosine had equal cure rate (94%) in both adults and pediatrics. After this study, miltefosine became the first line anti-leishmanial drug for VL in India(44). Shorter course of miltefosine (50mg twice daily for 14 and 21 days) achieved cure rate of 89 – 100% with good safety end points(45). Similar study in Nepal reported 95.8% initial cure in treatment naïve VL patients and relapse of 10% and 20% in VL patients previously treated with miltefosine at 6 and 12 months respectively(16). Clinical trial conducted in Ethiopia reported initial cure rate of 88% in immunocompetent VL patients but cure rate decreased to 60% with relapse rate of 10%(25). Miltefosine monotherapy efficacy data in east Africa suffers from scarcity for VL treatment. However, its compassionate use in combination with liposomal amphotericin B has been common practice by Medicines Sans Fronteer's (MSF)

in HIV patients with VL relapse (personnel communication of expertise from Leishmaniasis Research and Treatment Center in University of Gondar).

1.3.2. Factors associated with Poor Treatment Outcomes in VL Patients

In visceral leishmaniasis endemic areas, early knowledge of factors associated with poor treatment outcomes is important for proper management of VL. In a study conducted in the State of Minas Gerais in Brazil involving 250 children under 12 years of age; age < 18 months of age, abnormal respiratory finding on physical examination at admission and platelet < 85,000cells/mm^3 were reported as risk factors for poor outcomes(46). Similar study in same area and population indicated that parasite load had no association with poor outcomes(47). Another historical cohort study conducted in Brazil in patients treated with liposomal amphotericin B showed that age >35 years, jaundice, renal disease, presence of co-morbidities, edema, platelet count below 50,000/mm3, AST higher than 100 U/L were poor prognostic factors for death(48). A meta-analysis conducted in America reported that jaundice, thrombocytopenia, hemorrhage, HIV co-infection, diarrhea, age < 5 and age > 40 years, severe neutropenia, dyspnoea and bacterial infections were strong predictors for poor adverse prognosis and death (49).

In East Africa, where VL burden is high, risk factors for poor treatment outcomes had been reported. A retrospective study conducted in Amudat hospital (Eastern Uganda); age < 6 years and > 45years, co-infection with tuberculosis, hepatopathy, and drug-related adverse event were identified as risk factors for death(50). In Ethiopia, late diagnosis, severe illness at admission, co-infection with HIV and tuberculosis were identified as risk factors for poor treatment outcomes(51,52).

1.4. Significance of the Study

The issue of use of SSG at upper maximum dose limit had been a debating issue since 1982(expert consultation). No consensus has been reached among expertise. Moreover, no similar study had been conducted under routine condition to assess the drug's effect and safety profile over prolonged use.

Hence, the study assessed whether or not SSG/PM was effective while SSG dose was being used at upper maximum dose limit of 850mg /day.

The finding of this study would be beneficial to various parties working in the areas of visceral leishmaniasis. VL patients can get better standard of care related to VL management. For clinicians taking care of VL patients, it enables to choose the appropriate drugs to be used. Moreover, it enables them to know factors associated with poor treatment outcomes and manage patients accordingly. For a researcher or researchers, it can be used as reference and explore gaps to be further studied. Furthermore, the findings can be utilized by the policy makers who are responsible for decisions related to VL management.

2. Objectives of the study

2.1. General Objective

To assess the efficacy and safety of combination of SSG at 20mg/kg/day with upper maximum dose limit of 850mg/day and Paromomycin sulfate 15mg/kg/day (11mg base) for 17 days, and to identify factors associated with poor treatment outcome

2.2. Specific Objectives

- To evaluate initial treatment outcomes of VL patients at the end of treatment
- To determine final treatment outcomes of VL patients at 6 months post end of treatment
- To document common adverse events
- To identify factors associated with poor treatment outcomes
- To evaluate the effect of weight (≤42.5kg and > 42.5kg) on treatment outcomes of VL patients

3. Materials and Methods

3.1. Study Design

We used a retrospective cohort study design to address the objectives. Data were extracted from medical records of VL patients specifically, medical records of VL patients admitted to University of Gondar Hospital Leishmaniasis Research

and Treatment Center (UoGH LRTC) between January1, 2012 to June 30, 2019 were reviewed from January to end of February 2020.

3.2. Study Setting

Gondar Leishmaniasis Research and Treatment Center (LRTC) is located in North West Ethiopia and is 738km away from the capital city, Addis Ababa. The Drugs for Neglected Disease initiative (DNDi) in collaboration with University of Gondar Hospital established the LRTC in May 2005. Since then the treatment center has been conducting various clinical trials in search of safe and efficacious treatment for visceral leishmaniasis. Moreover, it has been serving the community by providing diagnostic and treatment services for the affected individuals. The treatment center has a ward with 24 beds, a separate laboratory and pharmacy unit. The health professionals are well trained on Good Clinical Practice (GCP) to provide standard care to VL patients.

The center also has HIV testing and counseling services. Tests are done based on the national HIV counseling and testing algorism applicable in Ethiopia.

3.3. Source Population

Source population was all VL suspected cases screened at UoGH LRTC from 2012 to 2019.

3.4. Study Population (Patients)

Patients coming to the treatment center were either self-presenters or referred from other health facilities. VL suspected patients were evaluated by well-experienced health professional.

VL diagnoses were made based on Ethiopian guideline for diagnosis of VL. Individuals who fulfilled the clinical VL case definitions of WHO (fever of greater than 2 weeks, splenomegaly and/or lymphadenopathy, or either loss of weight, anemia or leucopenia) would further undergo additional tests (serological examination using rk-39 rapid diagnostic test. Individuals who tested either positive or negative for rk-39 had undergone the confirmatory test procedures (parasitological examination) using splenic or bone marrow aspirations unless contraindicated. Parasite load in splenic or bone marrow aspiration was graded on logarithmic scale which ranges from 0 (0 parasites/1000 fields to +6 (>100

parasites/field). Patients were eligible for treatment if aspiration results turned positive, and in those negative but with clinical findings suggestive of VL.

3.5. Treatment Protocols

Patients eligible for VL treatment and with no VL complications and concomitant diseases (absence of edema, severe disease, deranged hepatic and renal function tests, malnutrition and HIV positive) received combination of SSG at 20mg/kg/day IM/IV with upper maximum dose limit (850mg/day) and Paromomycin sulphate 15mg/kg (11mg base) for 17 days. The age for pediatric patients to receive combination of SSG and PM was 4 years and above. Hence, most pediatric VL patients under 4 years of age were treated with the alternative treatment (liposomal amphotericin B) for safety reasons. Moreover, for patients older than 60 years of age the drug was considered unsafe.

VL patients who either became parasite positive and/or judged to have had poor clinical improvement at the end of treatment, were given additional 13 injections of SSG alone to add up to 30 doses or shifted to the second-line treatment (Liposomal amphotericin B at 5mg/kg /day, 6 doses) based on clinician decision. Liposomal amphotericin B was usually reserved for severely ill and VL-HIV co-infected patients. L-AMP B was given at 30mg/kg total dose (5mg/kg/day 6 doses in 30 days by infusion) for immunocompetent patients.

For almost all VL patients, weekly laboratory investigations (hematology and biochemical tests to monitor liver and renal functions) were performed. Clinical and laboratory profiles were documented until treatment completion. At the end of treatment, initial treatment was evaluated either by performing test of cure (microscopic examination of the parasite in spleen or bone marrow aspirate) or by clinical evaluation. Patients were appointed to come at 3 month in case they noticed symptoms of VL and 6 months (mandatory appointment) after the end of treatment to assess for final cure.

3.6. Sample size Determination

We used a convenient sampling method. Medical records of VL patients treated with SSG and PM combination admitted to UoGH LRTC from January 1, 2012 to June 30, 2019 provided that the inclusion criteria were met.

3.7. Variables of the study

3.7.1. Dependent Variables

The primary efficacy end point was initial cure, defined as parasite negative from spleen or bone marrow aspirate and/or clinical cure defined as improvement in clinical and laboratory parameters (absence of fever, weight gain, spleen size regression and improved in hematological profiles) at the end of treatment. The secondary efficacy end point was cure at six month.

The safety end point was incidence of drug related adverse events (AEs) during the course of treatment. Abnormal biochemical laboratory findings from the baseline and self-reported drug related adverse events were used to capture AEs.

3.7.2. Independent variables

The socio-demographic characteristics such as age, gender, and migration status; clinical characteristics which include, medical history and physical examination data; laboratory characteristics that encompasses hematologic profiles such as white blood cell count, hemoglobin, and platelet count; biochemical assessments; alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total bilirubin for liver function tests and blood urea nitrogen (BUN) and creatinine for renal function test and alpha amylase for pancreatic function test; clinical characteristics, which include fever, weight loss, splenomegaly, abdominal swelling, bleeding, lymphadenopathy, loss of appetite and cough; concomitant disease (pneumonia, TB, sepsis, diarrheal disease, otitis media, and malaria and others to be specified). Weight in kilograms, height in centimeters (cm) were captured from patients' medical records.

3.8. Operational Definitions

Initial cure: clinical cure or negative aspiration result at the end of treatment.

Definitive cure: clinical cure or negative aspiration result at six month.

Clinical cure: absence of clinical signs and symptoms and improvement in hematological profiles either at the end of treatment or 6 months after VL treatment

Treatment failure: presence of signs and symptoms of VL and/or parasite in spleen or bone marrow aspirate at the end of treatment and one month post end of treatment.

Defaulters: A patient who took less than 14 doses of SSG/PM due to the patient leaving the hospital.

Treatment interruption due to drug toxicity: Treatment discontinuation due to SSG/PM toxicity.

Poor treatment outcome: The initial treatment outcome defined as treatment failures, deaths and treatment interruptions due to drug toxicity. Furthermore, "yes" was label as "1"and "no was labeled as "0" for VL patients with and without poor treatment outcome respectively.

Adverse event: abnormal biochemical laboratory results from the baseline and documented self-reported patient complaints related to drugs.

Clinical pancreatitis: presence of sign and symptoms of pancreatitis and raised amylase which leads to treatment interruptions.

Non-clinical pancreatitis: raised amylase with no clinical sign and symptoms of clinical pancreatitis.

3.9. Inclusion and exclusion criteria

The inclusion criteria were age 4 – 60 years, VL diagnosis confirmed parasitologically and/or clinically, admission to UoGH LRTC in the period between January 1, 2012 and June 30 ,2019 and VL patients treated with combination of SSG and paromomycin.

Patients who had VL treatment history in the past and HIV positives were excluded from the study.

3.10. Data Collection and Measurements

Data collection was done by four trained clinical staffs who had experience of visceral leishmaniasis or health professional oriented to clinical aspects of the disease. Patients' chart was used to demonstrate on how to review and collect different variables. A standard format (checklist) with little modification to address the objectives described above was used to collect the data. Variables, which included the demographics of the patient (age, gender, and residency),

baseline clinical characteristics (physical examination and symptoms of VL), biochemical and hematological profiles, adverse events and treatment outcome profiles were captured.

3.11. Data Quality Assurance

Data collectors were trained for two days and supervisor for this study was the investigator. The training focused on the standard checklist with minor modification prepared for data collection. The checklist was pretested on the medical record of VL patients not included in the study. Completeness, accuracy and consistency of the collected data were checked during data collection by the principal investigator. Data were coded before data entry. Completeness and consistency of variables were also checked using frequency distributions.

3.12. Data Process and Analysis

The data was collected using a standard format (checklist) then entered into Epi-data version 3.1 and exported to STATA software version 14.2 and SPSS version 20 for analysis. Descriptive statistical analysis was done by STATA and analytical statistics were done by SPSS. Chi-square test and Fischer Exact test were used to compare proportions of categorical variables. A bivariate logistic regression was used to identify factors associated with poor treatment outcomes. Furthermore, multivariate logistic regression model was used to control confounding and generate adjusted measures of effect. Confidence intervals which did not include one or p-value less than 0.05 were considered as indicators for statistical significance.

3.13. Ethical Consideration

Ethical clearance was obtained from Scientific and Ethics Review Committee (SERC) of Center for Innovative Drug Development and Therapeutics Trials for Africa (CDT-Africa) - Addis Ababa University - College of Health Sciences; and from University of Gondar Institutional Review Board (IRB). The confidentiality of patients' medical record was maintained throughout. As the study involved a retrospective data, informed consent was impossible to obtain. To ensure anonymity, patients' medical registration number and codes were used to identify medical records. Patient unique identifiers of individual subjects (e.g.,

name, address, email address, phone number, etc.) were not captured during data collection.

4. Results

A total of 2429 VL patients were treated between January 1, 2012 and June 30, 2019. Of the total, 1000 patients were eligible for SSG/PM and the remaining 1429 patients were excluded from the study based on eligibility criteria. Table 1 shows the socio-demographic characteristics of VL patients. The median interquartile range (IQR) of age of VL patients was 23 (20- 27). Almost all patients (n=996, 99.6%) were male and the remaining 4 were females. Regarding the residency, nearly 85% of them were migrant workers and the remainders were local residents in endemic areas.

Table 1 Sociodemographic characteristics of VL patients treated with SSG/PM from 2012 to 2019 at UoGH LRTC (N= 1000)

Variables	n (%)
Age Median (IQR)	23 (20- 27)
Sex	
Male	996 (99.6)
Female	4 (0.4)
Residency	
Migrant	849 (84.9)
Resident	151 (15.1)

Table 2 shows the baseline clinical characteristics of VL patients treated from January 1, 2012 to June 30, 2019. The median IQR of duration of illness before admission was 4 (3 - 8) weeks. On admission, almost all 995(99.5%) patients had fever. The remaining 5 patients had no fever from the outset. Nearly, 95% and 93% of them had history of weight loss and loss of appetite at admission respectively. The majority 964 (96.4%) of patients had splenomegaly. Almost half 485(48.5%) of them had cough. The occurrence of other clinical conditions such as edema, jaundice, bleeding and lymphadenopathy were 279(27.9%), 870 (87%), 238(23.8%) and 20 (2%) respectively.

The diagnosis of VL was made based on the Ethiopian national guideline for treatment and diagnosis of leishmaniasis. For the majority of patients (n = 874; 87.4%), VL diagnosis was made by parasitological method which is by direct examination of *Leishmania donovani* parasite in smears of tissue aspirates from spleen or bone marrow. For the remaining patients (n=126, 12.6%), diagnosis of VL was made based on clinical presentations and /or by serological examination.

Table 3 shows the baseline laboratory characteristics of VL patients. Nearly, half of the study population had moderate anemia at admission and had platelet count less than 100,000 cells/μl. Biochemical tests were almost in normal range for 50% of patients enrolled to SSG and PM at the start.

Table 2 Baseline Clinical Characteristics of VL patients Treated with SSG/PM at UoGH-LRTC from January 1, 2012 to June 30, 2019 North West Ethiopia (N=1000)

Variables	N= 1000 n (%)
Fever	
Yes	995 (99.5)
No	5 (0.5)
Weight loss	
Yes	945 (94.5)
No	55 (5.5)
Loss of appetite	
Yes	928 (92.8)
No	72 (7.2)
Splenomegaly	
Yes	964 (96.4)
No	36 (3.6)
Hepatomegaly	
Yes	289 (28.9)
No	711 (71.1)
Presence of cough	
Yes	485 (48.5)
No	515 (51.5)
Presence edema	
Yes	279 (27.9)
No	721 (72.1)
Jaundice	
Yes	130 (13)
No	870 (87)
Bleeding	
Yes	238 (23.8)
No	762 (76.2)
Lymphadenopathy	
Yes	20 (2)
No	980 (98)
Spleen size at admission [Mean (±SD)] in cm	9.4 (4.5)
Method of diagnosis	
Parasitological	874 (87.4)
Clinical and/or serological	126 (12.6)

Table 3 Baseline Laboratory Characteristics of VL patients treated with SSG/PM from January 1, 2012 to June 30, 2019

Variables	Median (IQR)
White blood cell count (WBC) x 10^3 cells/ μl	1.8 (1.3 -2.5)
Hemoglobin in mg/dl	8.5 (7 – 9.9)
Platelet x 10^3 cells/ μl	68 (44 - 105)
Alanine Transaminase (ALT) in Unit/L	39 (23 - 68)
Aspartate Transaminase (AST) in Unit/L	76.2 (44 - 134)
Blood Urea Nitrogen (BUN) in mg/dl	11.4 (8.9 -14.6)
Creatinine in mg/dl	0.8 (0.7 – 0.91)
Alpha-amylase in Unit/L	177.5 (124.8 -254.3)

Table 4 shows the initial treatment outcomes of VL patients. Of the total 1000 VL patients treated with SSG and PM, cure was achieved in 924 (92.4%). Treatment failure, treatment interruptions due to drug toxicity, default and death respectively were noted in 30 (3.0%), 25(2.5%), 13(1.3%) and 8(0.8%) during the initial course of treatment. Of VL patients who discontinued drug due to toxicity, seven patients died while on rescue treatment.

Table 4 Over all initial Treatment outcomes of VL patients treated with SSG and PM combination from January 1, 2012 to June 30, 2019 (N = 1000)

Initial Treatment outcome	Frequency	Percent (%)
Cured	924	92.4
Failed	30	3
Treatment interruption due to drug toxicity	25*	2.5
Defaulted	13	1.3
Died	8	0.8

NB: *: Seven deaths were from those patients who discontinued treatment due to drug toxicity while on rescue treatment

Table 5 shows initial treatment outcomes of VL patients stratified by patients' body weight at admission (≤ 42.5kg and > 42.5kg). There was no statistically significant difference in the efficacy of SSG/PM between VL patients who had weight ≤ 42.5kg and > 42.5kg (P = 0.07).

Table 5. Initial Treatment Outcomes of VL patients stratified by body weight at admission (≤ 42.5kg and > 42.5kg), UoGH-LRTC January 1, 2012 - June 30, 2019, N=1000

Initial Treatment Outcome	Weight ≤ 42.5kg n (%)	Weight > 42.5kg n (%)	P- value
Cured	186(89.4)	738 (93.2)	0.07 [a]
Failed	5 (2.4)	25 (3.2)	0.57 [a]
Treatment discontinuation due to drug toxicity	7(3.4)	18 (2.3)	0.36 [a]
Defaulter	6 (2.9)	7 (0.9)	0.03[a]
Died	4 (1.9)	4 (0.5)	0.06[b]

NB: [a] P values are calculated by chi-square, [b] P values are based on Fischer Exact test

Table 6. The final treatment outcomes of VL patients treated with SSG/PM from January 1, 2012 to June 30, 2019, North West Ethiopia (N = 972)

Final Treatment outcome	Frequency	Percent (%)
Cured	259	26.6
Relapsed	4	0.4
PKDL	13	1.3
Lost follow-up	696	71.6
Total	972	100

NB: PKDL: Post kala-azar dermal leishmaniasis. Twenty-Eight cases including 13 defaulters and 15 deaths during the initial treatment period were not included in this analysis.

Table 6 depicts the final treatment outcomes of VL patients. In total 972 patients were appointed to come for six month follow up visit. Of these cure was achieved in 259 (26.6%) and relapse and post-Kala-azar leishmaniasis (PKDL) were noted in 4(0.4%) and 13(1.3) respectively. The remaining 696 (71.6%) were lost to follow-up. Among those who attended six month follow up visit (N= 276), cure was achieved in 259 (93.8%). Similarly, relapse and PKDL were noted in (4)1.4% and 13 (4.7%) respectively.

Table 7 shows incidence of adverse events (AEs) during the course of treatment. Overall, adverse event occurred in 725 (72.5%) during the initial treatment period. Among AEs, the incidence of raised transaminase was 351 (35.1%), injection site pain and raised in serum amylase 291 (29.1%) each, and increased in serum creatinine 88 (8.8%) were the most common AEs. The occurrence of potentially life-threatening adverse events such as cardiac toxicity and clinical pancreatitis were 6 (0.6%) and 21 (2.1%) respectively.

Table 7. Adverse events of VL patients treated with SSG/PM from January 1, 2012 to June 30, 2019 at UoGH-LRTC, North West Ethiopia (N=1000)

Adverse events	N= 1000
	n (%)
Vomiting	
Yes	37 (3.7)
No	963 (96.3)
Nausea	
Yes	8 (0.8)
No	992 (99.2)
Arthlargia	
Yes	3 (0.3)
No	997 (99.7)
Injection site pain	
Yes	291 (29.1)
No	709 (70.9)
Increased serum Transaminases	
Yes	351 (35.1)
No	649 (64.9)
Clinical Pancreatitis	
Yes	21 (2.1)
No	979 (97.9)
Increased serum creatinine	
Yes	88 (8.8)
No	912 (91.2)
Increased serum Amylase	
Yes	291 (29.1)
No	709 (70.9)
Cardiac arrhythmia	
Yes	6 (0.6)
No	994(99.4)
Thrombophlebitis	
Yes	3 (0.3)
No	997 (99.7)
Dyspepsia	
Yes	17 (1.7)
No	983 (98.3)
Hearing loss	
Yes	9(0.9)
No	991 (99.1)

Table 8 Bivarate logistic regression for factors Associated with Poor Treatment Outcomes (N=987)

Variables	Poor treatment outcome		COR	95% CI	P- value
	No	Yes			
Age					0.96
< 18 years	157	6	1		
18 – 45 years	771	39	1.1	0.53 – 2.30	0.8
> 45 years	14	0	0.001		0.9
Migration status					
Migrant	800	36	0.96	0.46 – 2.9	0.9
Resident	142	9	1		
Body mass Index					0.74
< 16	398	16	1.01	0.40 – 2.54	0.97
16 -16.99	188	13	1.13	0.42 – 3.04	0.8
17 -18.49	265	12	0.74	0.27 – 2.02	0.56
18.5 – 24.99	91	4	1		
Duration of illness in weeks					0.27
< 6 weeks	566	26	1		
6 – 12 weeks	289	12	0.9	0.45– 1.82	0.77
>12 weeks	87	7	1.7	0.74 – 4.16	0.2
Hepatomegaly					0.57
Yes	672	13	1	0.52 -1.90	0.9
No	270	32	1		
Cough					
Yes	452	27	1.4	0.88 – 3	0.12
No	490	18	1		
Edema					
Yes	257	29	1.4	0.78 – 2.75	0.23
No	685	16	1		
Jaundice					
Yes	120	7	1.2	0.55 – 2.80	0.58
No	822	38	1		
Bleeding					
Yes	223	14	1.4	0.76 -2.78	0.26
No	719	31	1		
Spleen size					
< 14cm	816	36	1.6	0.76-3.44	0.21
≥14 cm	126	9			
Body Weight at admission					
≤ 42.5	190	12	1		
>42.5	752	33	0.69	0.35 – 1.37	0.29

Table 8 Continued

Variables	Poor treatment outcome		COR	95% CI	P- Value
Concomitant disease	No	Yes			
Pneumonia					
Yes	168	12	1.6	0.85– 3.31	0.14
No	774	33	1		
Malaria					
Yes	73	4	1.2	0.41 – 3.33	0.78
No	869	41	1		
Sepsis					
Yes	7	3	9.5	2.38 – 38.2	0.001
No	935	42	1		
Adverse events					
Vomiting					
Yes	32	5	3.5	1.32 – 9.61	0.01
No	910	40	1		
Raised transaminase					
Yes	328	17	1.1	0.61-2.11	0.68
No	614	28	1		
Clinical pancreatitis					
Yes	17	4	5.3	1.71 – 16.48	0.004
No	925	41			
Increased serum creatinine					
Yes	78	6	2.31	0.70– 4.15	0.24
No	864	39	1		
[@]Increased serum amylase					
Yes	277	10	0.69	0.34 -1.40	0.3
No	665	35	1		
Cardiac arrhythmia					
Yes	3	3	22	4.38 – 114	< 0.001
No	939	42	1		

Note:[@] increased serum amylase without clinical symptoms of clinical pancreatitis , COR: Crude odds ratio

The proportion of poor treatment outcomes of VL patients treated with combination of SSG and PM was 45 (4.5%). Out of 20 variables fitted into bivarate logistic regression model (Table 8), sepsis, cardiac arrhythmia and clinical pancreatitis were significantly and independently associated with poor treatment outcomes. Body weight at admission had no effect on poor treatment outcome (OR 1.6, 95%CI; 0.89 – 3.06, P=0.11) (Table 9).

VL patients who had sepsis at admission were nearly 8 times more likely to have poor treatment outcome than those without sepsis (OR: 7.6, 95% CI: 1.86-31.03, P = 0.005). VL patients who developed clinical pancreatitis were more prone to have poor treatment outcomes than those without the condition (OR: 4, 95% CI: 1.21-13.43, P = 0.02). Patients who had cardiac toxicity during treatment were 13 times more likely to have poor treatment outcome than those without (OR: 13, 95% CI: 2.30 – 84.34, P=0.004).

Table 9 Multivariate logistic regression for factors associated with Poor Treatment outcomes, N=987

Variables	Poor treatment outcome		AOR	95% CI	P- value
	No	Yes			
Cough					
Yes	452	33	1.6	0.83 – 3.12	0.16
No	490	12	1		
Edema					
Yes	257	29	1.2	0.67– 2.51	0.49
No	685	16	1		
Pneumonia					
Yes	168	12	1.3	0.69 – 2.56	0.38
No	774	33	1		
Sepsis					
Yes	7	3	7.6	1.86-31.03	0.005
No	935	42	1		
Spleen size					0.14
< 14cm	816	36	1		
≥14cm	126	9	1.6	0.73 – 3.49	0.24
[#]Admission weight					
≤ 42.5	190	12	1		
>42.5	752	33	1.6	0.89– 3.06	0.11
Vomiting					
Yes	32	5	1.5	0.48– 4.43	0.49
No	910	40	1		
Clinical Pancreatitis					
Yes	17	4	4	1.21-13.43	0.02
No	925	41	1		
Cardiac arrhythmia					
Yes	3	3	13	2.30- 84.34	0.004
No	939	42	1		

Note: [#]: Weight at admission category is based on the Sodium stibogluconate upper maximum dose cut point (42.5 kg). AOR: Adjusted odds ratio, CI: Confidence interval.

5. Discussion

This retrospective cohort study involved medical record review of VL patients treated with SSG/PM for 17 days. The study assessed the efficacy and safety of these combination treatments under routine condition in Ethiopia. In the study area, males were more affected than females. This difference could be due to the working habit of males. Males usually work in large farmlands and forests where the disease transmitting vectors (sandflies) are abundantly found. The vast majority were presented with typical clinical pictures of VL (fever, weight loss, loss of appetite and splenomegaly) at admission (Table 2). Small number 20(2%) of VL patients presented with lymphadenopathy as compared to results from Sudan where 84% of VL patients had generalized lymphadenopathy(53). This could be partly attributed to genetic variation and the type of host immune response.

The overall initial efficacy of SSG and PM combination was 92.4%. In the analysis that compared VL patients who received SSG with and without upper maximum dose (weight ≤ 42.5kg and weight > 42.5kg) combined with PM, the efficacy of SSG/PM (89.4% Vs. 93.2%, P=0.07) was not statistically significant. The results obtained in both analysis were comparable with results of similar studies conducted in East Africa(38,42).

Efficacy of SSG and PM at six months was affected by lost follow-ups. This could be due to the usual habit that patients who come any time before 6 month do not attend the final visit. The practice of appointing patients to come before six month should be changed. Limiting follow up time point to one (six month only) unless indicated in exceptional medical condition, may improve follow up visit.

In the overall analysis of final treatment outcome (Table 6), 28 cases (13 defaulters and 15 deaths) during the initial treatment period were not included. The analysis of efficacy at six month (26.6%) was significantly affected by lost follow up. Hence, we used analyses results obtained from those who attended the six month follow up visit to draw conclusions. The analysis of efficacy among

those who attended the six-month follow up visit (N =275), cure was achieved in 259 (93.8%) which was comparable with similar study reported by Musa(38) The efficacy result that excluded lost follow up might overestimate the result and should be carefully interpreted. Among those who attended the final visit, 13 (1.3%) developed PKDL. The occurrence of PKDL was small as compared to similar study in Sudan and India where it had been commonly reported(53). PKDL patients had been reported as the best reservoir especially in patients with HIV co-infection with high parasitemea (55). Hence, aggressive management of severe grade II and III cases of PKDL cases is required to control further spread of the disease.

The use of SSG at upper maximum dose limit at 850mg has several advantages; decreases toxicity, decreases the need for high volume of SSG and hence decrease injection site pain and is more convenient for administration. Its use at the upper maximum dose with PM had been reported to have acceptable efficacy profile as reported in several studies (38, 55). However, WHO still recommends its use at 20mg/kg disregarding upper maximum dose limit in all VL endemic regions, its use with upper maximum dose limit can effectively be used in East African countries.

At least one adverse event occurred in 725 (72.5%) of VL patients. Increase in liver transaminase enzymes was among the most common AEs that occurred in 35% of patients. The overlapping toxicity of both SSG and PM on the liver could explain higher number of patients to have increased in liver enzymes. Injection site pain was also more common as reported in results of similar studies(56). Of note, this AE had been the common complaint by patients at the study area during the course of treatment. The large volume of SSG injection (maximum, 8.5ml) coupled with burning type of pain by PM could explain the high rate of injection site pain. Clinical pancreatitis, even though not very common, resulted in drug interruptions and treatment change. The frequency of raised amylase level with no sign and symptoms of clinical pancreatitis was relatively high. In our study, cardiac toxicity was not common (only 0.6%) and was comparable

with study conducted in south Sudan(41). However, it resulted in either death or treatment interruptions.

Information related to factors associated with poor treatment outcomes is pivotal in the management of VL. The proportion of poor treatment outcome (4.5%) was comparable with previous study reported from Ethiopia (5.8%)(51).Socio-demographic factors such as age has been reported as a risk factors for death as poor treatment outcome (51). In contrast, our study did not show these factors to have association with poor treatment outcome. Sepsis was significantly associated with poor treatment outcome. A similar study reported, bacterial infection as risk factor associated with the outcome variable(49). In contrary to studies from Brazil and Uganda that reported edema as risk factor for death(48, 49) our study did not show significant association with the outcome variable. Even though, it is common practice that VL patients with high parasite load end up with treatment failure, we could not undertake the analysis as some patients lacked results of parasite load and of course, as some of them were treated clinically.

Body weight at admission was not associated with poor treatment outcomes. This finding has implication that the use of SSG at upper maximum dose limit with PM cab be used. Potential factors such as edema, jaundice and malnutrition (wasting based on BMI), were not associated with poor treatment outcome. This could be explained by the fact that patients enrolled to SSG/PM regimen were mostly those without complications such as edema, severe malnutrition, severe anemia and jaundice).

Even though SSG/PM injections of 17 days showed decreased toxicity as compared to SSG injections of 30 days, it has a potential to induce serious AEs such as cardiac toxicity, pancreatitis, liver toxicity and renal toxicity. Adverse events had been reported as risk factors for VL death from Uganda(50). In contrary to study from Brazil that reported liver enzyme elevation as a prognostic factor for death, our study did not show its associated with poor treatment outcomes (48). Among the AEs, pancreatitis and cardiac arrhythmia showed

significant association with poor treatment outcome. The study is probably the first to report these factors to have association with poor treatment outcomes.

6. Strength Limitations of the Study

Strength of the Study

- The study setting follows GCP principles and documentations were good
- The treatment center had laboratory facility to run laboratory investigations including biochemical test for safety monitoring
- Data collectors were staffs from LRTC with experience in the areas of visceral leishmaniasis

Limitations of the Study

- Missing of data due to the retrospective nature of the data
- There was high lost follow-ups that resulted in attrition bias
- Procedures such as electro-cardiography and audiometric examinations were not performed to monitor cardiac and ear safety respectively during treatment

7. Conclusion and Recommendation

The efficacy of combination of SSG at 20mg/kg with upper maximum dose limit (850mg/day) and PM at 15mg/kg was 92.4%. Most AEs related to SSG/PM were reversible. Raised serum liver transaminase, injection site pain and raised creatinine were the most common AEs during initial treatment period. The occurrence of serious AEs such as cardiac toxicity and clinical pancreatitis were very few. The use of SSG above the upper maximum dose limit may further worsen AEs when combined with PM. The continued use of the combination therapy is warranted. Similar studies are recommended in the countries affected by the disease. Sepsis, adverse events like pancreatitis and cardiac arrhythmias should be identified and managed as per VL management protocol to prevent poor treatment outcomes. Since there is consensus that this combination is not the ideal treatment for VL, especially considering the need for VL elimination, the search for new treatments that can do away the use of two injectable drugs is recommended.

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Annex

Annex I Data collection Form

I. .Socio-demographic data

Patient ID: _____

101. Age : _____ weight _____ Height _____ BMI _____

102. Sex:

- Male
- Female

103. Residency

- Migrant
- Resident

104. Duration of illness in weeks _____

II. Baseline Clinical and laboratory characteristics.

201. Base line clinical characteristics(tick all that apply)

- Fever
- Weight loss
- Loss of appetite
- Splenomegaly
- Hepatomegaly
- Cough
- Edema
- Jaundice
- Bleeding
- Lymphadenopathy

202. Diagnosis of VL made by

- Parasitological
- Clinical and/or serological

203. If parasite + parasite load _____

204. If parasitological method were used for diagnosis, site of aspiration used

- Spleen
- Bone marrow
- Lymph node

205. Concomitant Disease at admission (Tick all that apply)

- Pneumonia
- Tuberculosis (PTB and EPTB)
- Malaria

- ☐ Diarrheal disease
- ☐ Sepsis
- ☐ Otitis media
- ☐ Others (Specify) _____

206. Baseline and the subsequent laboratory and physical examination characteristics during the initial treatment period

Type of lab test	**Day - 0**	**End of Treatment**
Hematological test		
WBC in cells/µl		
Hgb in g/dl		
Platelet cells/µl		
Blood Chemistry		
SGPT in U/L		
SGOT in U/L		
BUN in mg/dl		
Creatinine in mg/dl		
Amylase		
Physical Examination		
Weight		
Spleen size		

III. Questions related to Outcome, Treatment and adverse events

301. Was treatment interrupted?
- ☐ Yes
- ☐ No

302. Initial treatment outcome
- ☐ Cured
- ☐ Failed
- ☐ Treatment interruption due to drug toxicity
- ☐ Defaulted
- ☐ Died

303. Type of drug used as rescue, dose and duration (applied only for initial treatment failure cases) _____

304. Final treatment outcome (6month)

- Cured
- Relapsed
- PKDL
- Lost follow up

305. Adverse events (self –reported or laboratory test result abnormality) (Tick all that apply)

- Vomiting
- Nausea
- Arthralgia
- Injection site pain
- Clinical Pancreatitis
- Raised transaminases
- Raised creatinine
- Raised amylase
- Cardiac arrhythmia
- Hearing loss
- Other (specify) _____

Annex II: Ethical Approval Letters

Center for Innovative Drug Development and Therapeutic Trials for Africa
College of Health Sciences, Addis Ababa University

Date 2/12/19
Ref No. CDT/1897/19

To Aschalew Tamiru Hailemariam

Re: **Waver of Informed Consent of the proposal "Efficacy and safety of combination of sodium stibogluconate and paromomycine for treatment of visceral leishmaniasis and associated factors for poor treatment outcomes: A retrospective study in north-west Ethiopia"**

Dear Aschalew,

The Scientific and Ethics Review Committee of the Center for Innovative Drug Development and Therapeutic Trials for Africa (CDT-Africa), College of Health Sciences, Addis Ababa University, has reviewed and provided the above-referenced proposal a Waiver of Informed Consent. Progress report shall be submitted every six months. Any change to the proposal must be reviewed and approved by the Committee through an amendment process prior to its implementation.

With regards,

Eyasu Makonnen, PhD
Professor of Pharmacology
Chair, Scientific and Ethics Review Committee, Center for Innovative Drug Development and Therapeutic Trials for Africa (CDT-Africa)
Deputy Head, CDT-Africa
College of Health Sciences
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"An African Solution for a Global Problem"

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University of Gondar
Vice President Research & Community Service
Gondar, Ethiopia

R. No.- V/P/RCS/05/ 543 /2019

Date:- 2 January 2019

To:- Mr. Aschalew Tamiru
University of Gondar

Subject: - Ethical Clearance

Your research project proposal entitled **"Efficacy and safety of combination of sodium stibogluconate and paromomycine for treatment of visceral leishmaniasis and associated factors for poor treatment outcomes. A retrospective study, northwest Ethiopia."** has been reviewed by the Institutional Ethical Review Board of University of Gondar for its Ethical soundness, and it is found to be ethically acceptable.

Thus, the Research and Community Service Vice President Office has awarded this Ethical Clearance for the above stated study to be carried out by **Mr. Aschalew Tamiru** as Principal Investigator and **Professor Asrat Hailu, Dr. Girmay Medhin,** as Co- investigator as of **December 19th, 2019**.

These investigators are expected to submit their research progress report to the Vice President for Research and Community Service Office of the University of Gondar.

Best Regards

Meresha Chunie Asfede (Professor)
Vice President Research and
Community Service

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Annex III: Normal values for Hematology and Biochemical Tests

Box 1. Normal values for Hematology and Biochemical Tests				
		Lower Limit	**Upper Limit**	**Unit**
Hematology				
White Blood Cell (WBC) x 10^3		3.2	8.8	Cells/ml
Hemoglobin	Male	11.5	18	g/dl
	Female	11	16.7	g/dl
Platelet x 10^3		128	432	Cells/ml
Clinical chemistry				
Aspartate Transaminase (AST)		1	37	Unit/Liter (U/L)
Alanin Transmainase (ALT)		0	42	U/L
Bilirubin total		0	1.1	mg/dl
Blood Urea Nitrogine (BUN)		4.7	23.4	mg/dl
Creatinine		0.6	1.1	mg/dl
Alpha Amylase		0	220	U/L
Adopted from University of Gondar- Leishmaniasis Research and Treatment Centre Laboratory normal laboratory test values, 2018.				

Annex IV : Authors Declaration

I hereby declare that I am the sole author of this MSc thesis entitled **"Efficacy and Safety of Combination of Sodium Stibogluconate and Paromomycin for Treatment of Visceral Leishmaniasis and Factors Associated with Poor Treatment Outcomes. A Retrospective Study, North West Ethiopia"**. I further declare that I have not submitted this thesis to any other institution in order to obtain a degree.

Name: _____

Signature: _____

Date: _____