CHANGE IN NUTRITIONAL STATUS AND ASSOCIATED FACTORS AMONG CHILDREN TREATED FOR VISCERAL LEISHMANIASIS IN ARBAMINCH HOSPITAL, ETHIOPIA: PROSPECTIVE COHORT STUDY. ARBAMINCH HOSPITAL, ETHIOPIA, 2014/15

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A RESEARCH PROPOSAL TO BE SUBMITTED TO JIMMA UNIVERSITY , COLLEGE OF MEDICINE AND HEALTH SCIENCES, SCHOOL OF GRADUATE STUDIES, DEPARTMENT OF PEDIATRICS AND CHILD HEALTH FOR THE PREPARATION OF A SENIOR PAPER IN PARTIAL FULFILLMENT FOR THE REQUIREMENT OF SPECIALTY CERTIFICATE IN PEDIATRICS AND CHILD HEALTH

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ABSTRACT

Back ground; Sever acute malnutrition (SAM) and visceral leishmaniasis (VL) are important public health problems which affect millions of people worldwide. Both are major problems of the pediatric population in developing nations including Ethiopia.

Nevertheless, there is paucity of studies regarding the nutritional outcome after the treatment of pediatric visceral leishmaniasis in Ethiopia

Objective; the overall purpose of this study is to assess the nutritional status of VL treated patients and associated factors in the treatment of pediatric visceral leishmaniasis (VL) at Arba Minch Hospital (AMH), Leishmania Research and Treatment Center (LRTC)

Methods and material; A five years retrospective study, with source population of pediatric VL patients aged 15 years and less was included in the study. Structured questionnaire was employed to collect data from medical records of VL cases at leishmania research and treatment center, Arba Minch General Hospital. The data was entered to EpiData (version 3.5.1) and the SPSS (version 20.0) data bases. Data was analyzed and presented the results in frequencies, percentages and tables. Association of variables was done using chi-square, logistic regression accordingly with p-value <0.05 was used as significant for associated variables.

The parameters analyzed were VL disease category; duration of illness; demographic characteristics of VL patients; co-morbidities, and nutritional status.

Results: A total of 234 patients were included in the study with mean and median of 113.5 & 120 months respectively with standard deviation of + 45.9 months (ranged 24 to 180 months), male accounted for 75.6 % with a male: female to sex ratio of 3.1.

The majority of patients (99.6%) were primary VL. The majority of patients presented 1-6 months since the onset of illness. Patients were assessed for presence or absence of co morbidities with VL. As shown in table 2 one quarter of patients had one or more concomitant infection(s)

Anemia was diagnosed in 99.6 % of patients; half of them had hemoglobin level below 7 g/dl with mean and median of 7 ± 1.6 g/dl

The nutritional statuses of patients were assessed at admission and end of treatment using BAZ, HAZ and WAZ. As shown in table 3, the mean difference in the nutritional status between the two groups has a value less than the level of significance i.e. patients showed statistically significantly greater mean BAZ

score and WAZ score ($p < 0.001$) following treatment for VL than their pretreatment condition. The 95% confidence interval for the difference is (-.69,-.49 and -.53,-.33 respectively).

By using multivariate regression analysis, three variables; age, base line BAZ score and WAZ score are factors associated with change in BAZ score after treatment for VL.

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ABBREVIATIONS

AAU – Addis Ababa University

AIDS – Acquired Immunodeficiency Syndrome

AMH – Arbaminch Hospital

BMI – Body Mass Index

BAZ – BMI-for-age z-score

CL – Cutaneous Leishmaniasis

DNDi – Drug for neglected disease initiative

DACA – Drug authority and control agency

HAZ – Height-for-age z-score

WAZ – Weight-for-age z-score

HIV – Human immunodeficiency virus

HMIS – Health Management Information System

LD – Leishmania Donavan

ML – Mucocutaneous Leishmaniasis

MOH – Ministry of Health

NL – Normal

PEM – Protein Energy Malnutrition

SAM – Sever Acute Malnutrition

SNNPR – Southern Nations and Nationality People Republic

VL – Visceral Leishmaniasis

VLTC – Visceral Leishmaniasis and Treatment Center

WFH – Weight for Height

CHAPTER ONE: INTRODUCTION

Background

Leishmaniasis is a neglected tropical disease (NTD) caused by protozoan parasites of the Leishmania genus, and transmitted by sandfly bites from about 30 species that are proven vectors. There are three forms of leishmaniasis, visceral (VL), cutaneous (CL), and mucosal (ML). Of the three forms, VL is the most prevalent in eastern Africa, followed by CL and ML. (1)

According to the report of a meeting from the WHO Expert Committee on the control of Leishmaniasis, Geneva, 22-26 March 2010, the geographic distribution of Visceral leishmaniasis in East Africa and the south-west Arabian peninsula caused by L. donovani and L. infantum include: Eritrea, Ethiopia (Metema-Humera in the northwestern lowlands; Libo Kemkem and Fogera districts in Amhara regional State and north of Lake Turkana; in the south, the Segen and Woito valleys, the Genale and Gelana river basins and west Moyale at the border with Kenya), Djibouti, Kenya (Machacos, Kitui, West Pokot, Masinga, Meru, Baringo, Turkana), Saudi Arabia (south-west region: Jazan, Assir), Somalia, the Sudan (North: Gadaref, Blue Nile, White Nile, Sinnar, South Kordofan and West Darfur states; South: Upper Nile, Jonglei, Unity States, Eastern Equatoria), Uganda (northeastern focus: Pokot Department) and Yemen (Taiz, Lahj and Ibb governorates). (2)

Ethiopia is one of the six countries (Bangladesh, Brazil, Ethiopia, India, Nepal and Sudan) in which more than 90% of global Visceral Leishmaniasis (VL) cases occur and one of the ten countries with the highest estimated case counts, which together account for 70 to 75% of global estimated VL incidence. Both Cutaneous Leishmaniasis (CL) and VL are growing health problems in Ethiopia, with endemic areas that are continually spreading. Geographically, VL is found in Tigray, Amhara, Oromia, Afar, Somali and SNNPR, whereas CL is prevalent in Tigray, Amhara, SNNPR, Addis Ababa and Oromia regions. (3)

In the poorest communities of these endemic countries, VL is a leading cause of illness and economic distress to families. VL is often associated with malnutrition, which is also a symptom of more severe infection and a major risk factor for poorer clinical and treatment outcomes. The precise mechanisms of the interaction between VL and malnutrition are not well-understood, partly because of limited epidemiological data on patient populations. This lack of basic information on VL, particularly demographic and anthropometric data, hinders the ability of health providers to properly prepare for patient management, informed drug procurement for disease control, and the design of clinical trials and development of new drug therapies in the DIFFERENT endemic areas. When available, data often represents single health clinics or small populations. (4)

Historically the first case of VL in Ethiopia was identified in 1942 in southern Ethiopia. Every year, an estimated 3700–7400 cases occur in Ethiopia. (3)

VL is the most severe form of leishmaniasis, almost always fatal if untreated. Over 90% of the estimated annual incidence of 500,000 VL cases worldwide occurs in just six countries: Bangladesh, India, Nepal, Sudan, Ethiopia and Brazil. (1)

Clinical features of established visceral leishmaniasis include fever, abdominal pain, weight loss, splenomegaly, hepatomegaly, and lymphadenopathy. It should be noted that infection can remain subclinical or develop into clinical disease. The latter displays fatality rates of 100% if untreated. Risk factors to develop clinical disease include malnutrition and immune suppression, and are often linked to the overarching factor of poverty. Visceral leishmaniasis is also an important infection associated with HIV/AIDS. (5)

It well known that the seriousness of VL does not correspond only to its high incidence and amplitude of distribution, but also to the possibility to assume serious and lethal forms when associated to nutritional deficiencies and concomitant infections. (6)

In the case of tropical disease, data obtained on leishmaniasis in endemic regions of Ethiopia show that 67% of cases of cutaneous leishmaniasis (CL) occur in younger people, and even more striking, 85% of cases of VL occur in children. (6)

There is no study done on nutritional outcome in the treatment of pediatric visceral leishmaniasis at AMH (LRTC).This study will give us important clues on change in nutritional status based on anthropometric indices before and after treatment visceral leishmaniasis. Moreover, this research can be used as a reference for future studies.

Statement of the problem

The protein-energy malnutrition (PEM) and visceral leishmaniasis (VL) are serious problems of public health. When analyzed together, the epidemiologic indexes of VL and the prevalence of PEM are responsible for millions of deaths worldwide. (6)

Visceral leishmaniasis is ranked second in mortality and fourth in morbidity among tropical diseases, with 20,000 to 40,000 deaths per year and over 2 million DALYs (disability-adjusted life years) lost.(7)

East Africa is one of the world's main endemic areas for VL, which over the last 20 years has seen a dramatic increase in the number of VL cases, due to a complexity of factors. Several studies have convincingly shown that malnutrition, HIV and genetic susceptibility are individual risk factors for VL. (8)

In East Africa, the annual number of cases of VL is estimated at 30,000 and related deaths at 4000. Especially in Sudan, Ethiopia and Kenya, VL is associated with high mortality and morbidity, exacerbated by poor nutritional status and the remote location of VL endemic areas.(9)

Visceral leishmaniasis (VL), the fatal form of the disease, is endemic in five regions of the country (Tigray, Amhara, Oromia, SNNPR and Somali). The number of VL cases treated in the last four years in the country is increasing (1936 cases in 2008, 1083 in 2009, 1936 in 2010 and 2032 in 2011). The VL/HIV co-infection rate ranges from 18.5% to 40% in Humera/Tigray and 15%to18% in Libo/Amhara, making the control programme challenging. (10)

The lowlands of Kafta-Humera and Metema in north-western Ethiopia and the Konso district and Segen Valley in southern Ethiopia are established foci for VL, with the disease spreading. It is one of the emerging infectious diseases which are rapidly increasing in incidence over an expanded geographical range. It affects populations or communities where the disease did not previously occur.(11)

According to the Ministry of Health (MoH) of Ethiopia, the burden of VL in Ethiopia is between 4 500 and 5 000 annually. It is reported from more than 40 different localities or administrative areas, affecting five of the nine administrative regions with different degrees of endemicity.

VL often exists in areas that are either remote or inaccessible and where health facilities are barely available or, if available, are inadequate. Those most likely to be infected are people who are poor, living in villages far from roads and health facilities. VL leads to death before medical advice is sought. (11)

In areas where leishmaniasis has been endemic for a long time, children are at greatest risk. At their young age they have not yet developed immunity to the disease; many adults in endemic communities are reservoirs of infection, facilitating continuing disease transmission to those without immunity. (1)

Conditions such as malnutrition and HIV have also been shown to increase the risk of developing VL and exacerbating the severity of the disease.(1)

According to Pearson et al. and Gomes et al., the nutritional status of individuals infected with Leishmania ssp. has a significant role in the clinical evolution of VL, especially in children under 5 years. In the case of tropical disease, data obtained on leishmaniasis in endemic regions of Ethiopia show that 67% of cases of cutaneous leishmaniasis (CL) occur in younger people, and even more striking, 85% of cases of VL occur in children. Thus, among humans, the majority of the clinical cases appear to occur in younger individuals. (6)

Wasting is a common clinical presentation for VL as 30% of patients present with malnutrition in Ethiopia. Wasting is also a common presentation for VL in Sudan, Brazil and India. Fever, weight loss and splenomegaly are the most common VL features in Ethiopia.(11)

Nutritional evaluations revealed the disease has wide range of clinical variation, demonstrated by the presence of patients within the normal weight percentiles (63% of the patients), while 25.9 % of patients were severely malnourished. It should be noted that a majority of the patients had suffered from the disease for less than 60 days; a period, which may well, not be sufficient for chronic nutritional problems to develop and which may explain the presence of well- nourished patients. (12)

Due to the sub-species variation in the parasite, host immune responses and the choice of antileishmania drugs, there are some differences in clinical manifestations and treatment responses of VL patients in different geographic localities.(11)

In a study in Sudan, risk factors for death in VL patients revealed extremes of age, namely from younger than two years to older than 45 years, malnutrition, anemia, bleeding, diarrhoea and vomiting (Collin et al 2004:618). Moreover, VL relapse cases and HIV/VL co-infected individuals have a poor treatment outcome.(11)

However, the impact of PEM specifically on immune response against infection with Leishmania is not totally understood yet and the nutritional status is many times neglected. In Latin America, VL is still a disease of childhood with 60% of the cases occurring in children under 10 years old, an age group that has

shown several other morbidities such as diarrhoea (27) and PEM contributes to the development of VL. (13)

PEM and VL provoke disastrous consequences for the growth, development and survival of children, mainly those with age between 1 and 4 years old. PEM in individuals with VL has been associated with increased risk of in-hospital morbidity and mortality and increased period of stay, cost and use of healthcare resources.(6)

At present there is no standardised national reporting system for leishmaniasis in Ethiopia, but a HMIS for the collection of national health statistics has recently been introduced by the MoH and includes leishmaniasis in its surveillance categories.(1)

Many factors can contribute to high rates of PEM, ranging from those as fundamental as political instability and slow economic growth, to highly specific ones such as the frequency of infectious diseases and the lack of education. Moreover, PEM is also simply caused by famine. It is of little value to assess child growth if the assessment is not followed-up by political action to improve the health and nutritional status of children. Political actions are likely to have the greatest impact on child malnutrition if they are directed at the early stages of child development (i.e. prenatal, infancy and early childhood). A good start in life will pay off, both in terms of human capital and economic development. Interventions that improve the physical growth and mental development of children will not only decrease the prevalence of underweight, but also prevent its negative functional consequences throughout life. Reducing malnutrition, therefore, does not only benefit child health and development in the short term, but also promotes the future, long-term growth and economic progress of the nation.(6)

With increasing epidemiology of VL in areas that were non-endemic before and with increasing world population, coupled with the trend in rising food prices (conditions which may increase the poverty of the population and the prevalence of malnutrition), it is necessary to investigate the relationship between these diseases. Both the VL and PEM are common problems in poor areas and should not be neglected by science or by society.(14)

Significance of the study

The study will assess the role of nutritional status and related factors on the treatment outcome of pediatric VL patients, which should benefit the Programme planners, service providers and health care professionals on improving outcome of patients. Above all, future VL patient shall benefit from the study. Moreover, the research can be used as a baseline data for further studies

CHAPTER TWO: LITERATURE REVIEW

In 1968, the World Health Organization published "Interactions of Nutrition and Infection," which suggested that the relationship between infection and malnutrition was a synergistic one.(15)

In its synergy with infection, under nutrition contributes to approximately 50% of childhood deaths worldwide. Apart from PEM, deficiencies in single nutrients, such as vitamins, fatty acids, amino acids and trace elements also alter immune function and increase the risk of infection.(16)

One of the infections whose risk is increased by malnutrition is visceral leishmaniasis (VL), caused by the intracellular protozoan parasites of the Leishmania donovani complex (L.infantum (L.chagasi) and L. donovani).(16)

The impact of malnutrition in the world is staggering. Malnutrition is thought to directly or indirectly contribute to more than half of all childhood deaths, most of them related to heightened susceptibility to infection. Visceral leishmaniasis (VL), caused by the intracellular protozoan Leishmania donovani, is a progressive, potentially fatal infection found in many resource-poor regions of the world. Most people who get infected with this parasite have only an asymptomatic latent infection; however, people who are malnourished have a greatly increased risk of developing severe VL.(16)

The precise mechanism of the association between malnutrition and development of symptomatic visceral leishmaniasis has not yet been clarified; however, both a leishmania-induced effect and a more chronic course reflecting economic deprivation of the host appears to exist. Control of asymptomatic infection is mainly accomplished through Tcell–mediated immune responses, and malnutrition has a negative impact on this component of immunity.(13)

According to a study by Mengistu, HIV infection, severe malnutrition and bleeding tendency are factors predicting death in VL patients. Seife, Nohelly, Argaw, Mulugeta, Herrero, Nieto, Chicharro, Cañavate and Bern found a treatment outcome of 4% mortality rate with age above 45 years, HIV infection, severe malnutrition, pneumonia; tuberculosis and vomiting were associated with increased risk of death.(11)

Malnutrition was also found to be a common clinical presentation in VL patients because 38.5% (n=114) of the study population had severe malnutrition (z score < -3SD) at admission for VL treatment; 42.5% and 37.0% of the VL patients had severe malnutrition in Humera and Addis Zemen, respectively. Severe malnutrition was found to be significantly higher in relapse VL cases compared to the primary ones (64.7% vs 37.0% respectively). Hence, management of severe malnutrition should be part and parcel of the VL treatment to speed up patients' recovery.(11)

Experiences such as these suggested that a dual attack on nutrition and infection was needed for an optimal response.(17)

Epidemiologic studies have documented an increased risk for VL in the malnourished host and children with moderate to severe PEM were found to have about a nine-fold increased risk of developing VL. Malnutrition was identified as a risk factor for severe disease and death from VL in both children (WFH, 60%; OR 5.0) and adults (BMI, 13; OR 11.0). Malnutrition-related VL is particularly evident in displaced and impoverished populations.(16)

A case control study also demonstrated a positive correlation between good nutritional status and immune response to infection with L. chagasi. In this study, mice were divided in two groups receiving either the control or the hypoproteic diet and water ad libitum (control or malnutrition group). Control diet contained 14% casein and hypoproteic diet contained 3% casein. Total body weight was analyzed weekly and, after malnutrition was established, mice were inoculated intravenously with promastigotes of L. chagasi. The data show that 4 and 6 weeks later infected malnourished mice presented a significant reduction in body weight (36.1% reduction, $P < 0.01$) and a reduction in total protein (18.5% reduction, $P < 0.05$),albumin (26.5% reduction, $P < 0.05$), glucose (12.3% reduction, $P < 0.05$) and globulin seric concentration(15.2% reduction, $P < 0.05$). In addition, at 6 weeks of infection, parasite load in liver was greater in infected malnutrition group if compared to infected control group. In relation to the spleen, we observed an increased parasite load at 4 and 6 weeks of infection in malnutrition group compared to control group, suggesting that PEM alters immune response from BALB⁄c mice to L. chagasi.(6)

Epidemiologic studies have documented an increased risk for VL in the malnourished host. In the recent study of Maciel et al., for example, the authors assessed whether nutrition influenced the outcome of Leishmania infection by comparing relatives of children with VL with either self-resolving Leishmania spp. Infection or apparently uninfected households. The authors observed a decrease in body mass index ($P < 0.0005$) and mid-upper arm circumference by age ($P = 0.022$) z-scores for children with VL. Levels of vitamin A were lower in active children with VL as measured by serum retinol ($P = 0.035$) and the modified-relative-dose-response test ($P = 0.009$). Higher birth weight ($P = 0.047$) and albumin concentrations ($P = 0.040$) protected against disease. Increased breastfeeding time ($P = 0.036$) was associated with asymptomatic infection. The results indicate that modifiable nutritional aspects are associated to the outcome of Leishmania spp. infection in humans.(6)

A prospective study was carried out to study the outcome of patients with visceral leishmaniasis and to determine factors associated with poor outcome. The study included 132 in-patient children who were admitted to Basrah Maternity and Children Hospital and Basrah General Hospital during one year (from

the first of November 2004 till the end of October 2005), with visceral leishmaniasis confirmed by bone marrow examination and direct agglutination test. Nutritional assessment was done for each patient. All patients with confirmed visceral leishmaniasis were sent for complete blood count. The final diagnosis and the outcome of the patients with visceral leishmaniasis were recorded also.(18)

Results: Among 132 sero-positive cases, 78 (59.0%) cases were males, and 54(40.9%) were females. The age of patients ranged between 2 months to 12 years. Sixty five (49.2%) of the cases were improved, 31.8% were discharged on the family responsibility, and 15 cases died. The commonest cause of death was bleeding in 6 cases (40%), followed by hepatic failure in 4 (26.6%), other causes of death were bronchopneumonia, renal and heart failure.(18)

Nutritional status and duration of illness were significant determinants of the outcome of visceral leishmaniasis patients. A significantly higher number of malnourished patients didn't improve compared to well nourished patients, and a significantly higher percent of patients who presented late have died compared to those who presented earlier especially in the first 2 weeks of illness, $P<0.05$. By using multivariate regression analysis, six variables; low hemoglobin value, low platelet count, male sex, young age, high Direct Agglutination Test titer and malnutrition were found to be significant predictors of death and relapse.(18)

Conclusion: Male sex, anemia, thrombocytopenia, high DAT titer and malnutrition are poor prognostic factors in addition to late presentation.(18)

In addition to the above study, data obtained from 3365 patients with kala-azar (KA) or post-KA dermal leishmaniasis (PKDL) treated by Me´decins Sans Frontie`res–Holland in south Sudan from October 1998–May 2002. Patients were malnourished (median body mass index [BMI], 15.5; median weight for height [WFH], 75.5%) and anemic (median hemoglobin (Hb) level, 8.5 g/dL). Risk factors for death among adults were age ≥45 years (odds ratio [OR], 4.6), malnutrition (BMI, 13; OR, 11.0), anemia (Hb level, 8 g/dL; OR, 4.0), and duration of illness (duration, ≥5 months; OR, 2.3). Risk factors for death among children and adolescents were age 2 years (OR, 5.4,), malnutrition (WFH, 60%; OR, 5.0), anemia (Hb level, 6 g/dL; OR, 3.7), and splenomegaly (OR, 2.9). A higher risk of death was associated with episodes of diarrhea (OR, 1.4), vomiting (OR, 2.7), and bleeding (OR, 2.9). Relapse and PKDL occurred in 3.9% and 10.0% of cases, respectively.(19)

CHAPTER THREE: OBJECTIVES

General objectives

Assess change in nutritional status among children treated for VL

Specific Objective

Assess change in nutritional status among children treated for VL

Identify factors affecting change in nutritional status of children treated for VL.

CHAPTER FOUR: METHODS AND MATERIALS

Study area and period

The study was conducted at leishmania research and treatment center, which was opened in 2006 by Drug for Neglected Disease Initiatives (DNDi) within Arbaminch Hospital, Arba Minch, SNNPR, Ethiopia, covering the period from the first of January 2009 till the end of January 2014 G.C. AMH is situated at Gamo Gofa zone, an area located at south – western part of Ethiopia, about 500 kilo meters from the capital city, Addis Ababa.

LRTC is Africa's first research facility focusing on VL and is part of the DNDi supported LEAP, implemented in collaboration with the MoH, Addis Ababa University (AAU) and DACA. A clinical trial testing the efficacy of a 17-day short-course of SSG/Paromomycin combination therapy compared to each drug used alone.

Study design

Retrospective cohort study design will be carried out (from the first of January 2009 till the end of January 2014) to study the nutritional outcome of VL treated pediatric cases and to determine factors associated with poor nutritional outcome.

Population

4.3.1 Source population

All children age < 15 years, infected with leishmaniasis and treated at AMH within the study period, fulfilling the inclusion criteria will be included. DNDi diagnose clinically suspected cases with rK39 RDT at outreach health centers, and refer cases for confirmation to its treatment centers. VL cases will be admitted only after diagnosis is confirmed by microscopy of splenic or bone marrow aspirate examination.

4.3.2 Study population

All the source population that met the inclusion criteria and were found to be admitted during the study period would be the study population for this study.

Inclusion criteria

Children < 15 years of age

Confirmed diagnosis of VL

VL Treatment initiated and completed

Exclusion Criteria

Children with unknown treatment outcome

Sample size and sampling technique

All VL cases who were admitted and treated in the period between Jan. 2009 and 2014 G.C; in AMH LRTC, and those who fulfill the above inclusion criteria were included in the study.

Data collection technique and Measurement

4.5.1 Measurements

Sociodemographic profile; patient's gender, age, birth order, address educational status,

Anthropometric profile before and after treatment for VL: (weight, height (length)

Co-morbidities with VL; diarrheal disease, Tb, pneumonia, malaria, anemia

Patient's change in nutritional outcome for VL treatment; Type of chemotherapy, duration of illness, side effect

Dependant variable

Nutritional outcome at the end of treatment for VL

Independent variables

Socio-demographic profile of VL patient;
Anthropometric profile of VL patient at beginning of treatment
Patient's co-morbidities with VL

4.5.2 Data collection technique

The data was be collected by structured questionnaires, from medical record specifically developed for this study.

Two Nurses employed to collect data after a day of training on the structured questionnaires and collection techniques. A physician assigned at the study area supervising the data collectors and structured questionnaires will be used for data collection.

Before beginning of the data collection 10 % of the data pretested to determine whether it can answer the objective of the study and appropriate for the setting, then the final questionnaire was developed and implemented. The pretested questionnaires were excluded.

Data quality control

Before data collection one day training was conducted for data collectors. Data collectors and supervisor were blinded for the outcome of interest during the entire period. During data collection, a physician assigned monitored whether all information being recorded and collected correctly. All collected data checked for completeness, accuracy and clarity by the principal investigator, at the end of the day. Pretest for 10% of data collection tool was used.

Data processing and analysis

Anthropometric indices were calculated using Anthroplus 3.02 software (CDC, Atlanta, GA, USA), which uses the U.S. National Centre for Health Statistics (NCHS) reference values.(20) The anthropometric indices— BMI-for-age (BAZ)—were expressed as differences from the median in standard deviation units or z-scores. Individuals were classified as wasted if BAZ were 2 SD below the NCHS median.(21)

Anemia was defined as a haemoglobin level<130g/l for men, <120g/l for children aged 12–13 years, <115g/l for children aged 5–11 years and <110g/l for children aged <5 years. Severe anemia was defined as a haemoglobin level <70g/l.(22)

Continuous variables were re coded into pre- defined categories to allow for comparison with previous studies (e.g. age, duration of illness and anemia). Nutritional status was based on nutritional z-score of BMI-for-age, Height for age and Weight for age.

First, a paired t test analysis was used to assess the change in the mean nutritional status before and after treatment for VL. Anthropometric variables with a mean difference above the level of significance in the treatment of VL are excluded from the final model. Multivariate analysis was used to explore the association between the explanatory variables and the change in the nutritional outcome (nutritional status).

In this study we have included all variables that are significant in the bivariate analysis at the 25 percent level to fit the initial multivariable model. Variables that appear to be important from step1 are then fitted together in a multivariable model. Variables, that were not important on their own, and so were not under consideration in step 2, may become important in the presence of others. These variables are therefore added to the model from step 2, with forward selection method

Frequency tables , paired t test analyses and when appropriate a multivariate regression was used to see the association between different variables, and a P- value of less than 0.05 is used as significant.

Ethical consideration

Permission for the study was obtained from department of pediatrics and child health and from ethical and research committee of Jimma University. Confidentiality was insured and unauthorized person would not have access to the collected data. A unique code number was assigned to identify each subject

Dissemination plan

After data has been collected and analyzed, the final result will be disseminated to the advisors, Jimma University research committee and to other respective bodies for possible evaluation and scientific publication of the paper.

Limitation of the study

Because of resource constraints and the retrospective nature of the study, the researcher used the anthropometric measurement and clinical edema as a method to assess the nutritional status of VL patients.

The study was conducted in one selected clinic in one highly VL endemic region in the south-western part of Ethiopia. The study is therefore limited by resource constraints, including time, human resources and finance to cover more VL clinics.

Despite these limitations, the data available in the selected study center is large and well organized; the researcher regards the findings as avaluable evidence and guidance for further studies

Operational definition

Nutritional status – was assessed based on the anthropometric indices BMI-for- age. Anthropometric indices were calculated using Anthroplus 3.02 software (CDC, Atlanta, GA, USA), which uses the U.S. National Centre for Health Statistics (NCHS) reference values.The anthropometric indices— BMI-for-age (BAZ)—were expressed as differences from the median in standard deviation units or z-scores.
Individuals were classified as wasted if BAZ were 2 SD below the NCHS median. Categorization of nutritional status was based on nutritional z-score of BMI-for-age (BAZ) for children (BAZ <-3SD: severe wasting, BAZ -3 to -2SD: moderate wasting, BAZ >=-2SD: No malnutrition).

Confirmed VL case – refers to a suspected VL case plus parasitological confirmation for leishmania donovani (LD) bodies (or the leishmania parasite from a tissue slide or culture taken from blood, splenic or bone marrow aspirate).

Suspected VL case – refers to a person who presents with non-malaria fever of more than two weeks and splenomegaly or lympadenophathy with or without the following clinical features: weight loss, wasting, enlarged liver or pallor.

Outcome measures:

Nutritional outcome

Nutritional status based on anthropometry, which is BMI-for-age, Height for age and Weight for age at the end of treatment, was used as the nutritional outcome of the VL treated patient.

Microbiologic parameters

The primary end point is cure (i.e., efficacy) at 6 months after treatment, based on the absence of leishmania parasites in tissue aspirates.

Any patient who died from VL, received rescue medication during the treatment period or had parasites visualized on microscopy at the 6-month assessment is regarded as a treatment failure in primary efficacy analyses.

The secondary endpoint is initial cure at the end of treatment (i.e. at day 22 for PM, at day 31 for SSG, and at day 18 for the combination).

Any patient who died from VL, received rescue medication during the period of the trial or had parasites visualized on microscopy at the end of treatment was regarded as a treatment failure in the secondary efficacy analyses

Patients for whom trial medication had to be stopped due to lack of response or a serious adverse event (SAE) were given rescue medication (liposomal amphotericin B, manufactured as Ambisome H by Gilead, USA) according to national VL guidelines from participating countries

Treatment cure:

In this study we used the initial cure, which is parasitological examination from tissue specimen at the end of treatment, as treatment cure from the patient record.

Primary VL and Relapse:

In brief, patients with VL who had no previous history of treatment were termed “patients with primary VL,” and patients with VL who reported a history of previous treatment for VL were termed “patients with relapsed VL.”

We used these terms as it is from the patient record.

RESULT

We studied 234 patients with VL. The age of patients ranged between 2 years and 15 years. Ten year was the median of the children (range: 13 years). The majority of patients were males (75.6%), with a male: female sex ratio of 3.1. Overall, 15.4% of the VL patients in this study were under the age of five and 54.7% of the total VL cases were 10 years and above, table 1.

Table 1 Socio-demographic characteristics of study population, Arbaminch referral Hospital, SNNPR, Ethiopia, 2014/15

Socio-demographic Variable		Frequency (%)
Age of the child (Months)	< 5	15.4
	5-9	29.9
	>=10	54.7
Sex of the child	Male	75.6
	Female	24.4
Care giver relation to the patient	Mother	5.1
	Father	88.9
	Guardian	5.1
Educational status of the care giver	Illiterate	82.1
	Primary	16.2
	Secondary and higher	1.7
Religion	Orthodox	43.6
	Muslim	18.4
	Protestant	26.1
	Other	12
Duration of illness (days)	≤ 1 month	12.4
	1-6 months	85.5
	≥ 6 months	2.1

One hundred two (43.6%) of the caregivers were orthodox, 61 (26.1%) protestant and 43 (18.4%) were Muslims. The majority (82.1%) of the care givers did not attend formal education.

Of the cases, 12.4 reported that they had noticed signs of VL 1month before admission. The rest (85.5% to 62.1%) had waited for 1 month or more to report for diagnosis and treatment at the hospital.

The majority of patients (99.6%) were primary VL. Adverse events, reported in 2.1% of the cases. Overall a cure rate of 99.1 % was achieved with Antileishmania drugs: (50.4%) SSG, (31.6%) SSG plus Paromomycin, (9.8%) liposomal amphotericin B and the rest received rescue medication

Table 2 Co-morbidities associated with VL in the study population, Arbaminch referral Hospital, SNNPR, Ethiopia, 2014/15

Comorbidity		Frequency (%)
Malaria	Yes	5.1
	No	94
	Unknown	0.9
Tb	Yes	1.3
	No	97.4
	Unknown	1.3
Pneumonia	Yes	13.7
	No	84.6
	Unknown	1.7
Diarrheal disease	Yes	4.3
	No	94.4
Hemoglobin (g/dl)	Sever anemia	50
	Moderate anemia	45.3
	Mild anemia	4.3
	No anemia	0.4

VL disease could be complicated or presented with various co-morbidities. As shown in table 2 one quarter of patients had one or more concomitant infection(s) such as pneumonia (13.7%), malaria (5.1%), diarrhea (4.3%) and tuberculosis (2.1%) during hospitalization.

Severe anemia with haemoglobin level below 7 g/dl was found in half of VL patients at admission; with a mean haemoglobin level of 7.05 ± 1.59.

Table 3.Nutrtional status at baseline and after treatment for VL, Arbaminch referral Hospital, SNNPR, Ethiopia, 2014/15

	Baseline	After treatment for VL	95% CI	P value
	mean ± SD	mean ± SD		
BAZ	-2.3449 ±1.33738	-1.7513±1.29115	-.69873, -.48836	<0.001
HAZ	-1.2772±1.37364	-1.2778±1.37293	-.00054, .00166	0.318
WAZ	-2.1530±1.15130	-1.7198±1.09494	-.53530, -.33117	<0.001

Data on nutritional status was available for 234 cases. The nutritional statuses of patients were assessed at admission and end of treatment using BAZ, HAZ and WAZ. At admission the means of patients BAZ-score, HAZ score and WAZ score were 2.3 (±1.3), 1.2 (±1.4) and 2.1 (±1.1) respectively; after completion of treatment the means of patients BAZ-score, HAZ score and WAZ score were 1.7 (±1.3), 1.2 (±1.4) and 1.7 (±1.1) respectively, table 3.

A paired t-test analysis used to identify (compare) the difference in the means of nutritional status before and after treatment for VL shows a value below the level of significance ($p < 0.05$) for BAZ and WAZ.

Bivariate analysis was performed to see the association between the explanatory variables and the change in the nutritional outcome, table 4. Factors included were age, sex of patents, care taker educational status, religion, relationship of care giver, co morbidities (pneumonia, malaria, tuberculosis, anemia and diarrheal disease) and baseline nutritional status as measured by BAZ, HAZ and WAZ

Table 4 Bivariate analyses of factors with nutritional status after treatment, Arbaminch referral Hospital, SNNPR, Ethiopia, 2014/15

	BMI z P-value, 95% CI for B	WFA z P-value, 95% CI for B	HFA z P-value, 95% CI for B
Age	0.006(-.009, -.001)	.251(-.003, .010)	0.314(-.006, .002)
Sex	0.937(-.373, .404)	.392(-.563, .222)	0.260(-.176, .647)
Duration of illness	0.883(-.124, .106)	0.311(-.196,.063)	0.227(-.197, .047)
Weight for age baseline	<0.001(.178, .546)	<0.001(.732, .899)	0.00(.403, .593)
Height for age baseline	0.096(-.223, .018)	<0.001(.686, 1.012)	0.00(.999, 1.000)
BMI for age baseline	<0.001(706, .853)	<0.001(.164, .414)	0.279(-.205, .060)
Malaria	0.28(-1.070, 311)	0.251(-1.401,.369)	0.867(-.798, .673)
TB	0.18(-1.932, .364)	0.956(-1.072 1.134)	0.352(-.645, 1.802)
Pneumonia	0.052(-.882, 003)	0.163(-.135,.791)	0.023(.075, 1.013)
Hemoglobin	0.256(-.164, .044)	0.033(.011,.239)	0.002(.064, .281)
Diarrhea	0.73(-.838, .588)	0.034(.054,1.301)	0.03(.083,1.583)

Multivariate analysis

The cohort study analyzed using multivariate logistic regression to identify factors associated with the change in nutritional outcome. Variables associated with the outcome in the bivariate analysis at a 25 % level of significance were included in the multivariate analysis.

Table 5 Multivariate analyses of factors with nutritional status, BAZ score of VL patient, after treatment (N =234)

Variable	Beta	T	P value	95.0% CI for B
AGE (months)	-.117	-2.291	.024	-.006, .000
Baseline BAZ	.896	14.740	.000	.749, .981
Baseline WAZ	-.164	-2.684	.008	-.320, -.048
Tuberculosis	.004	.087	.931	-.831, .908
Pneumonia	.003	.058	.954	-.331, .351

Dependent Variable: BAZ score after treatment for VL

Three variables (age of patient, base line BAZ score and WAZ score) were found to be significantly associated for the change in BAZ score in the VL treated population, $P < 0.05$ see table 5.

Similarly a multivariate logistic regression is used to identify factors associated with change in WAZ score. After adjusting for other variables in the model, baseline BAZ score and WAZ score were found to be the associated factors for the change in WAZ score following treatment for VL; $p < 0.05$, table 6

Table 6 Multivariate analyses of factors with nutritional status, WAZ score of VL patient, after treatment (N = 136)

Variable	Beta	T	P value	95 % CI for B
Duration of illness	-.048	-1.087	.279	-.101, .029
Baseline BAZ	-.163	-3.048	.003	-.220, -.047
Baseline WAZ	.958	17.289	.000	.807, 1.016
Hemoglobin level	-.062	-1.356	.177	-.105, .020
Pneumonia	.053	1.188	.237	-.104, .416
Diarrhea	.003	.068	.946	-.395, .423

Dependent Variable: WAZ score after treatment for VL

DISCUSSION

Anthropometric nutritional assessment has several public health and development uses, such as overall population assessment, identification of target groups or areas for intervention, continuous nutritional surveillance as a tool for development planning, monitoring nutritional status to determine trends of particular health importance, evaluating the impact of programmes, selecting persons in need of immediate attention in emergency situations, studying the effects of seasonal changes in food supply or disease prevalence, and individual nutritional assessment, including use of sequential measurements to determine if growth is proceeding properly.(21)

This paper highlights some important points about affected population in one of the VL-endemic area of the country

Males were found to be predominantly affected by VL compared to females (M:F 3.1:1).. Severe malnutrition was observed across all categories, although the percentage varied considerably. This finding is also in agreement with other studies, done in Brazil, East Africa, and South Asia, where wasting (acute malnutrition) is present across all ages.(4)

Nutritional and micronutrient status have long been known to influence the risk of infectious diseases. Although it is known that nutritional status might influence the outcome of different infections, there are few data available on how nutrition influences the outcome of *Leishmania* spp. infection. The interaction of nutritional history and basic health care measures might be long-term determinants that result in increased risk of disease development. .

Malnutrition was found to be a common clinical presentation in VL patients because 32.9% (n=77) of the study population had severe malnutrition at admission for VL treatment, (table 4). These findings are comparable with previous studies.(11)

In this study, though nutritional improvement (BAZE > -3 SD) was seen in 55. % of VL treated patients; severe wasting at admission contributed the majority (82.9%) of the group at the end of treatment. Our finding complements the previous studies where: children who recovered from VL continue to have lower levels of vitamin A, even after a year of treatment, when inflammation has disappeared, which was shown by average C-reactive protein and alpha-1-acid glycoprotein concentrations. The diminished vitamin A status may have preceded active VL, as observed in a recent prospective study conducted in Bangladesh. These results reinforce the hypothesis that malnutrition precedes infection.(23)

Since our Paired Samples Statistics for the three variables revealed a statistically significant mean improvement in BAZ score ($p < 0.001$, CI =-.69, -.49) and WAZ score ($p < 0.001$, CI =-.53, -.33)

following treatment for VL; we can conclude that, as in study done in south Sudan a significant nutritional recovery was also observed after treatment of VL.(19)

CONCLUSION AND RECOMMENDATION

We were not able to determine whether the low anthropometric measures seen were caused by infection *per se* or were a contributing factor. From the Paired Samples Statistics shown in table 4, we can conclude that there is a statistically significant difference between the mean nutritional statuses (BAZ and WAZ) before and after treatment of VL. Two nutritional variables BAZ and WAZ score are significantly associated with nutritional outcome following VL treatment, $P<0.05$.. In addition age is a significant association with change in BAZ score.

Though our finding suggests a significant improvement in the mean BAZ and WAZ following treatment for VL; further case control study is recommended.

Reference

1. Malaria Consortium.Leishmaniasis control in eastern Africa: Past and present efforts and future needs. Situation and Gap Analysis2010.
2. WHO. Report of a meeting of the WHO Expert Committee on the Control of Leishmaniases. (WHO technical report series ; no 949)2010. p. 22-6.
3. Kebede Deribe KM, Teshome Gebre, Asrat Hailu, Ahmed Ali, Abraham Aseffa and Gail Davey. The burden of neglected tropical diseases in Ethiopia, and opportunities for integrated control and elimination. Parasites & Vectors2012;5(240).
4. Michael O. Harhay PLO, Michel Vaillant , François Chappuis , María Angeles Lima , Koert Ritmeijer , Carlos Henrique Costa , Dorcas Lamounier Costa , Suman Rijal , Shyam Sundar , and Manica Balasegaram. Who Is a Typical Patient with Visceral Leishmaniasis? Characterizing the Demographic and Nutritional Profile of Patients in Brazil, East Africa, and South Asia. *Am J Trop Med Hyg*2011;4(84):543-50.
5. Poonam Salotra RS, and Karin Seifert. Disease Burden, Current Treatments, Medical Needs, and Strategic Approaches. Trypanosomatid Diseases: Molecular Routes to Drug Discovery2013.
6. MALAFAIA G. Protein-energy malnutrition as a risk factor for visceral leishmaniasis. Parasite Immunology. [Review Article]. 2009(31): 587–96.
7. Laura-Isobel McCall W-WZ, Greg Matlashewski. Determinants for the Development of Visceral Leishmaniasis Disease. PLoS Pathog2013;9(1).
8. Jan H Kolaczinski RR, Dagemlidet T Worku, Andrew Ocheng, John Kasimiro, Narcis Kabatereine and Simon Brooker. Risk factors of visceral leishmaniasis in East Africa: a case-control study in Pokot territory of Kenya and Uganda. *International Journal of Epidemiology*2008(37):344-52.
9. Hailu A MA WM, Balasegaram M, Yifru S, et al. Geographical Variation in the Response of Visceral Leishmaniasis to Paromomycin in East Africa: A Multicentre, Open-Label, Randomized Trial. PLoS Negl Trop Diseases 2010;4(10).
10. WHO. WHO Country Cooperation Strategy WHO Country 2012-2015 Ethiopia. 2013.
11. BESHAH AM. Clinical Manifestations and Anthropometric Profiles of Visceral Leishmaniasis in selected centres in Ethiopia [Mph Thesis.]: University of South Africa; 2011.
12. Mitiku K. A neglected old disease in a new focus: Epidemic of visceral Leishmaniasis at Libo-kemkem Woreda, North West Ethiopia. *Ethiopian Journal of Pediatrics and Child Health*2012;8(8).

13. H. C. Maltezou CS, M. Mavrikou, P. Spyridis,C. Stavrinadis,Th. Karpathios, and D. A. Kafetzis. Visceral Leishmaniasis during Childhood in Southern Greece. Clinical Infectious Diseases 2000(31):1139-43.

14. MALAFAIA G. Importance of Encouragement of studies on effects of Protein-Energy Malnutrition on Vaccination Against Visceral Leishmaniasis. *Saude & Amb Rev* jan-jun 2009;4(1):47-53.

15. Katona-Apte PKaJ. The Interaction between Nutrition and Infection. Clinical Infectious Diseases2008(46):1582-8.

16. Ibrahim MK BJ, Anstead GM, Jimenez F, Travi BL, et al. The Malnutrition-Related Increase in Early Visceralization of Leishmania donovani Is Associated with a Reduced Number of Lymph Node Phagocytes and Altered Conduit System Flow. PLoS Negl Trop Dis 2013;7(8).

17. GT K. The History of Nutrition: Malnutrition, Infection and Immunity. J Nutrition 2003(133):336s-40s.

18. Zainab H. Gani MKHA-MHJ. Outcome of Hospitalized Children with Visceral Leishmaniasis in Basrah, Southern Iraq. *The Medical Journal Of Basrah University* 2008;26(2).

19. Simon Collin RD, Koert Ritmeijer, Kees Keus, Yosef Melaku, Sammy Kipngetich, and Clive Davies. Conflict and Kala-Azar: Determinants of Adverse Outcomes of Kala-Azaramong Patients in Southern Sudan. Clinical Infectious Diseases2004 1 March(38):612–9.

20. WHO. AnthroPlus Software for assessing growth of the worlds children and adolescents, Geneva. 2009.

21. Analyses R. Use and interpretation of anthropometric indicators of nutritional status. Bulletin of the World Health Organization1986;6(64):929-41.

22. WHO. Iron deficiency anaemia assessment, prevention, and control: a guide for programme managers. Geneva: world health organization2001.

23. Bruna L. Lima Maciel HGL, José W. Queiroz, Juliana Galvão, Núbia N. Pontes, Roberto Dimenstein, Stephen E. McGowan, Lúcia F. C. Pedrosa, and Selma M. B. Jerônimo. Association of Nutritional Status with the Response to Infection with Leishmania chagasi. Am J Trop Med Hyg2008;4(79):591-8.

Annexes

Information sheet and consent form

The researcher requested and obtained permission from the University of Jimma (JUSH), Department of Pediatrics and Child Health.

The letter from the Jimma University Department of Pediatrics and Child Health was provided to the board of the VL clinics to obtain permission to access the data and to conduct the study at a specified time and place.

Requested and obtained permission to do the study

Data collection format

Jimma University

College of medicine and health sciences

School of Medicine

Questionnaire

A structured questionnaire designed and adopted to collect data for the study of nutritional status and clinical outcome of visceral leishmaniasis in pediatric patients at Leishmania research center, Arbaminch referral Hospital, SNNPR, Ethiopia, 2006

The purpose of this questionnaire is to collect data which will assess nutritional status and clinical outcome of visceral leishmaniasis in pediatric patients

The information collected will be confidential and will not be available to any unauthorised individuals who were not directly involved in the study. Medical records will not get removed, copied or take any part of the patient records from the designated area.

Your genuine response will be valuable for the valid and effective completion of the research.

Thank You!

Anwar Abdella (MD)

Part I. Sociodemographic characteristics	
1. Medical record Number	_____
2. Age of the child (Months/or Years)	a. In months b. In years
3. Sex of the child	1. Male 2. Female
4. Care giver relation to the patient	1. Mother 2. Father 3. Guardian (specify) _____
5. Family size (Number)	_____
6. Birth order of the VL patient	_____
7. Educational status of the care giver	1. Illiterate 2. Primary 3. Secondary 4. Higher
8. Religion	1. Orthodox 2. Muslim 3. Protestant 4. Catholic 5. Others (Specify)_____

Part II. Nutritional status of the VL patient (Anthropometry)	
1. Weight (in kg)	1. Beginning of Rx _____ 2. End of Rx _____
2. Height (in cm)	1. Beginning of Rx _____ 2. End of Rx _____
3. Presence of oedema	1. Beginning of Rx i. yes (Specify) _____ ii. No 2. End of Rx i. Yes (Specify)_____ ii. NO
4. Any Nutritional intervention provided?	1. Yes, Specify _____ 2. No

Part III. Co-morbid conditions	
1. Was there malaria diagnosed and treated during the period of VL treatment?	1. Yes (Specify)_____ 2. No
2. Was there Tuberculosis diagnosed and treated during the period of VL treatment?	1. Yes (Specify)_____ 2. No
3. Was there Pneumonia diagnosed and treated during the period of VL treatment?	1. Yes (Specify)_____ 2. No
4. What is the HIV status of the child?	1. Positive 2. Negative 3. Unknown
5. Jump to Q no 7 if the answer for Q #3 is 2 or 3	
4. If the child is positive for HIV/AIDS, what is the CD4 value	_____
5. If the child is positive for HIV/AIDS, what intervention is the patient being provided? (Specify)	_____
6. Was there Diarrheal disease diagnosed and treated during the period of VL treatment?	1. Yes (specify)_____ 2. No
7. What is the hemoglobin status of the patient?(in number)	1. Before Rx _____ 2. After Rx _____
8. Was the child vaccinated?	1. Yes 2. No
9. If yes, was the child vaccination complete for his age?	1. Yes 2. No
10. Duration of illness at admission (days or weeks)	

Part IV. VL disease category	
1. What was the diagnosis type of visceral leishmaniasis?	1. Primary 2. Relapse 3. Unknown

Part V. Treatment of the VL patient	
1. Date of start of the VL treatment(dd/mm/yy)	
2. Date of completion of VL treatment(dd/mm/yy)	
3. Type of Drugs used for the treatment of VL patient?	1. Liposaomal amphotericin B 2. Paromomycine 3. SSG 4. Combination therapy: pentavalent antimonials and paromomycine
4. Total duration of stay in the hospital (in days)	
5. Treatment outcome	1. Cured 2. Died 3. Rx failure 4. Others (Specify) _____
6. Serology and /or microscopy	1. End of Rx 2. After 6 month
7. Side effects of VL treatment?	1. Yes 2. No
8. If yes to Q. # 6 specify	1. Vomiting 2. Diarrhoea 3. Bleeding 4. Pancreatitis 5. Cardiac toxicity (EKG) 6. Jaundice 7. other
9. If the answer for Q # 7 is 4 specify	1. Clinical or 2. Amylase level