STUDY PROTOCOL

REVISED Protocol for a prospective observational cohort study of cutaneous leishmaniasis in Ethiopia

[version 2; peer review: 2 approved, 1 approved with reservations]

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Abstract

Background

Cutaneous leishmaniasis (CL) is a skin neglected tropical disease, with an estimated 40,000 new cases each year in Ethiopia. CL causes ulcers, nodules, and plaques on the skin, and in some instances the destruction of the nasopharyngeal mucosa and cartilage. Some CL lesions may heal spontaneously, whilst other lesions may require therapies which are associated with discomfort, adverse effects,

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prolonged treatment, and a frequent lack of a complete response. Scarring, a sequela of CL, causes permanent disfigurement and is associated with stigma linked with a reduction in health-related quality of life.

The choice of treatment for CL is based upon factors including the causative species; the number, extent, size, and location of lesions; and the availability of treatments. The development of robust evidence for CL treatment is hindered by a lack of validated and appropriate outcome measures and few data to support hypothesis-generation and trial design. There is a paucity of prospective data with well-defined treatment outcomes for CL caused by *L. aethiopica*.

Aim

The overall aim of this study is to improve the understanding of the health and economic burden of CL.

Methods

We have designed an observational, multi-centre cohort study to examine treatment outcomes for CL in Ethiopia which includes clinical outcomes, laboratory outcomes, patient reported outcome measures, scar assessments and cost effectiveness. We aim to recruit up to 750 participants across two hospital sites. We present here the protocol for this cohort study with a 12-month follow up period for each participant.

Conclusions

These data will inform the design of randomized controlled trials to evaluate new treatment strategies, with appropriate economic evaluations. This will help improve evidence-based guidelines and support evidence-led policy decisions, not only in Ethiopia but also globally.

Keywords
Cutaneous leishmaniasis, L. aethiopica, clinical outcomes measure, scar assessment, cost effectiveness, patient reported outcome measures, Ethiopia

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REVISED **Amendments from Version 1**

The revised version includes changes following comments by our two reviewers in order to enhance the clarity and flow of the paper. Sentence construction in the introduction has been improved. Under methods, a clarification has been added under the headings "Study participants" and "Overview of the data collection", with an in-depth description of immunology samples as suggested by reviewers. The sample size section has been more clearly defined.

Any further responses from the reviewers can be found at the end of the article

Introduction

Up to a million new cases of cutaneous leishmaniasis (CL) occur each year, primarily in the world's poorest communities[1]. CL is the most common form of leishmaniasis, which is caused by infection with protozoa of the genus Leishmania, acquired via the bite of a female phlebotomine sandfly. Infection leads to the development of ulcers, nodules, and plaques on the skin. Depending on the infecting *Leishmania* species, spontaneous resolution of infection can take many months or years and medical therapies are associated with discomfort, adverse effects, prolonged treatment, and a frequent lack of complete response (World Health Organization). Lesions often heal with lifelong scarring or destruction of the nasopharyngeal mucosa causing permanent disfigurement, and associated stigma, and overall psychological morbidity, contributing to a reduction in health-related quality of life (HRQoL)[2]. These multidimensional morbidities and the often-arduous process of treatment-seeking also impose a substantial economic burden in terms of both costs of care and income loss, which can exacerbate the stress and social impact associated with CL and inhibit access to care[3–5].

The choice of treatment for CL is based upon several factors including the causative species; the number, extent, size and location of lesions; and the availability of treatments. Small lesions not located near a joint or facial structure may be allowed to self-heal or be treated with a topical agent, intra-lesional injection, cryotherapy, or heat therapy. Localised treatments for CL are associated with irritation, pain, discomfort, and in the case of cryotherapy, long-term skin changes[6]. Large or widespread lesions and those involving the mucosa require systemic treatment[7,8]. Despite the large number of cases of CL each year, the evidence for determining the best treatment modality remains limited.

In Ethiopia, CL is a growing public health problem, with an estimated 40,000 new cases each year. Several *Leishmania* species cause CL in Ethiopia; most infections are thought to be due to *L. aethiopica*[9], but *L. donovani*, *L. major* and *L. tropica* are also present. *L. aethiopica* is associated with three distinct clinical forms of CL; localised CL (LCL); mucocutaneous leishmaniasis (MCL); and diffuse CL (DCL), which is the most disfiguring and difficult to treat[9]. Lesions caused by *L. aethiopica* most commonly occur on the face[10]. The diverse range of clinical phenotypes and presentations makes early diagnosis by community-based health workers difficult. Standard CL treatment in Ethiopia is with intralesional or parenteral sodium stibogluconate (SSG), which may be combined with cryotherapy using liquid nitrogen. There is a particular paucity of prospective data with well-defined treatment outcomes for CL caused by *L. aethiopica*. A systematic review of treatment for CL caused by *L. aethiopica* identified two randomised trials with a total of 26 participants and two prospective, non-randomised studies[11]. All four studies were assessed to have methodological limitations by the authors. One of the small, randomised trials compared itraconazole with placebo (n=14 participants)[12] and the other compared rifampicin, isoniazid and amithiozone with pentamidine (n=12 participants)[13]. The much larger prospective study (n=123) reported improvement in various forms of CL: 103 (81%) of participants were cured with liquid nitrogen cryotherapy and 85% of the 20 participants treated with intramuscular SSG (20 mg/kg up to a maximum of 850mg daily) for 30 days were cured[14]. Another Ethiopian study reported that 21% of participants (n= 167) had "a positive outcome" with intralesional pentavalent antimonial drug meglumine antimoniate (MA) and 47% had "a positive outcome" with systemic MA (20 mg/kg)[15].

The development of robust evidence for CL treatment is hindered by a lack of validated and appropriate outcome measures and few data to support hypothesis-generation and trial design. A Cochrane review demonstrated that existing studies used heterogeneous outcome measures which mostly lacked validity, hindering comparison of study results[16]. A consensus document on "harmonized" clinical research methodologies for LCL (but not MCL or DCL) was published by an international group of experts following meetings organised by the World Health Organization (WHO) and the Drugs for Neglected Diseases initiative[17]. This publication highlighted the importance of laboratory confirmation with identification of *Leishmania* species in future trials and defined "initial" cure for non-American CL as more than 50% re-epithelialization (if an ulcer) or flattening (if a non-ulcerated lesion) at Day 42; and as 100% re-epithelialization or flattening at Day 90. "Final" cure was defined as 100% re-epithelialization or flattening at Day 180. More recently, open label studies, incorporating these expert-agreed outcomes, were conducted on treatment for CL due to *L. aethiopica*. One study reported physician-determined cure rates of 59.7% for intralesional SSG[18] at Day 90, whilst another study reported physician-determined cure rates of 12.5% and 72.7% for miltefosine[19] at two study sites at Day 90.

The "harmonized" clinical research methodologies for LCL provided a significant step forward but several gaps remain. No agreed methodologies are available for assessing flattening or re-epithelisation and it remains unclear if outcomes should be measured from the start or end of a course of treatment. The agreed outcome measures are entirely clinical and do not include measures to reflect the outcomes and experiences of affected individuals. The treatment preferences of individuals may be influenced by

the frequency of adverse events and the extent to which healing improves their health and psychosocial wellbeing. Appropriate patient reported outcome measures (PROMs) therefore need to be identified and collected alongside clinical measures. Finally, the consensus document recognised that scarring should be assessed as a long-term outcome but did not propose a method for doing so.

Given the scarcity of resources for health, and for the treatment of CL in particular, treatment recommendations should also be informed by an understanding of costs and efficiency. However, cost-effectiveness analyses of alternative treatment strategies for non-American CL have only been conducted in Asia[20–23], none have addressed infection with *L. aethiopica*, and only one has assessed effectiveness using a multidimensional outcome metric[20], which is essential for comparing the value of investments across intervention types and diseases. No studies of the economic burden of CL or treatment costs in Africa have been published.

The Ethiopian Federal Ministry of Health (FMOH) has identified an urgent need for the evaluation of treatments alternatives for all forms of CL. Such evaluation requires better understanding of outcomes, and cost-effectiveness data. We therefore designed an observational, multi-centre cohort study of CL in Ethiopia[24].

Aims & objectives

The overall aim of this study is to improve the understanding of the health, psychosocial and economic burden of CL, and to lay the groundwork for well-designed, randomized controlled trials and economic evaluations of alternative CL treatments in Ethiopia.

Objectives

1. To assess clinical outcomes in LCL, MCL and DCL in a prospective cohort
2. To assess clinical parameters in LCL, MCL and DCL in a prospective cohort
3. To assess the change in HRQoL associated with CL before and after treatment.
4. To develop a severity scale for CL
5. To understand the lived experiences of individuals with CL attending a referral centre by conducting in-depth interviews
6. To assess household costs of CL, health care provider and societal costs of treating CL
7. To characterise how cell-mediated immune responses associate with treatment responses in CL due to L. aethiopica and Human Leucocyte Antigen (HLA) variation correlate with different clinical phenotypes.

Methods

Patient and Public Involvement

Patients with CL were involved in the validation of the translations of PROMs through focus group discussions, cultural adaptation and piloting.

Study context

The study will enrol individuals receiving treatment for CL at two centres: ALERT Hospital, Addis Ababa and Boru Meda Hospital, South Wollo Zone, Amhara Region. ALERT Hospital serves as both a general hospital for patients in Addis Ababa and a regional and national specialized referral centre for individuals with CL and other skin conditions. The hospital has nine dermatologists and a 20-bed unit for the care of patients with CL and diagnoses approximately 1000 cases of CL managed on an inpatient or outpatient basis each year. Boru Meda serves as the general hospital and specialised referral service for all skin conditions in South Wollo zone. It has two dermatologists, and a 35-bed unit for the management of CL and diagnoses around 700 cases of CL, managed on an inpatient or outpatient basis each year.

Timelines

Study recruitment began in April 2022 and is expected to continue until the sample size is reached or until the cut-off of September 30th, 2023, whichever occurs first. Follow-up is therefore expected to be completed by September 30th, 2024.

Study participants and management

All adults and children diagnosed with CL and initiated on CL treatment at both study centres are eligible to be recruited in this study. Each participant will be followed for 360 days.

Inclusion criteria:

1. Individuals with parasitologically confirmed CL using microscopy or culture

2. Individuals receiving treatment at the study centres

Exclusion criteria:

1. Participants who have previously been treated with systemic anti-leishmania therapy

2. Participants who do not or are unable to give informed consent to participate in the study

Participants will be treated using the standard of care for CL at the centre where they are managed and in accordance with the Ethiopian national guideline[24] which will be agreed by two clinicians in consultation with the affected individual prior to enrolment. The treatment may be changed if deemed clinically appropriate. In general, if the size of the skin lesion is 4cm in diameter of smaller and not near a mucosal site the first line treatment is most frequently local therapy (cryotherapy and/or intralesional SSG). In general, if the

diameter of the skin lesion is greater than 4 cm and/or near or involving a mucosal surface the initial therapy is usually intramuscular SSG for 28 days with or without adjunctive cryotherapy. Participants will be assessed on day 42 and further local or systemic therapy may be offered depending on the degree of clinical response.

We will classify CL using the operational definitions shown in Box 1.

Box 1. Operational definitions of Cutaneous Leishmaniasis (CL)

- CL is diagnosed in a person with skin and/or mucosal lesions with evidence of *Leishmania* infection in the affected tissues characterised by the presence of *Leishmania* amastigotes on smear microscopy or growth of *Leishmania* promastigotes in culture or the detection of *Leishmania* DNA by polymerase chain reaction.
- Localized CL: A confirmed case of leishmaniasis, with no mucosal involvement, characterized by ten or fewer cutaneous papules and/or nodules and/or plaques with or without ulceration involving one body site
- Multi-regional localized CL: A confirmed case of leishmaniasis, with no mucosal involvement, characterized by ten or fewer cutaneous papules and/or nodules and/or plaques with or without ulceration involving two or more body sites
- Mucocutaneous leishmaniasis: A confirmed case of leishmaniasis characterized by ten or fewer papules and/or nodules and/or plaques with or without ulceration involving skin and an adjacent mucosal surface
- Mucosal leishmaniasis: A confirmed case of leishmaniasis characterized by papules and/or nodules and/or plaques with or without ulceration involving exclusively a mucosal surface
- Diffuse CL: A confirmed case of leishmaniasis characterized by eleven or more papules and/or nodules and/or plaques with or without mucosal involvement.

Body sites are classified as:

1. Head and neck
2. Torso - anterior (including genitalia)
3. Torso - posterior (including buttocks)
4. Right upper limb
5. Left upper limb
6. Right lower limb
7. Left lower limb

Overview of data collection

This prospective observational cohort will integrate different methodologies appropriate to the different "areas" under investigation, using qualitative and quantitative tools:

- Clinical: Observation of clinical features, treatments given, clinical outcomes as well as patient related outcome measure (quantitative)
- Psychosocial: serial patient related outcome measures (quantitative) and in-depth interviews (qualitative)
- Economic burden of CL: Serial economic evaluations
- Immunological investigations on skin and blood samples – quantitative laboratory research

Study visits for data collection are scheduled at day 1, 14, 28, 42, 90, 180 and 360 (Table 1). Treatment will be decided by the treating clinicians, who may or may not be one of the study dermatologists. All study specific data collection will be undertaken by a member of the study team. At the baseline visit, we will collect socio-demographic data, perform clinical assessment, and collect data on PROMs and costs. Follow-up data will be collected for all patients at specified time points, as detailed further below. In a subset of patients, additional biological samples will be collected for immunological and genetic studies. A further subset of participants will be selected for in-depth interviews.

Clinical data

At the baseline visit, we will record the clinical phenotype of CL including the number, size, morphology[25] and location of lesions. Lesion dimensions (defined as the largest diameter of the lesion) will be measured using a disposable tape measure. Individuals with more than one lesion will be asked to indicate the lesion causing greatest concern and this will be designated the "index" lesion. Individuals may indicate two or more lesions of equal concern. In those with mucosal involvement, mucosal signs and symptoms are recorded. Clinicians will then provide a subjective global assessment of CL severity using a 3-point Likert scale (mild, moderate, severe). Slit-skin smears will be collected at baseline for microscopy and species identification using polymerase chain reaction (PCR).

At each follow-up visit, we will assess lesions for the degree of re-epithelization or flattening. For those with DCL, the number of resolved lesions and percent of lesions flattened per region is recorded. Clinicians will provide a global assessment of the improvement or worsening of CL using a 5-point Likert scale (worse, no change, minor improvement, major improvement, cure). At each visit, we will record the treatment being administered and any changes since the last visit. The adverse reactions to CL treatments will be assessed including the nature and severity of the reaction, the action taken, the presumed cause and the outcome. The Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be used for describing adverse reactions and grading their severity. Slit-skin smear samples will be collected at day 90 for test of cure if the skin lesion is not healed.

PROMs

At baseline, Amharic or Oromiffa translations of several PROMs will be collected (Table 1): two general measures of HRQoL, the EQ-5D-5L and the SF-36, and two skin-specific measures of HRQoL, the Dermatology Life Quality Index (DLQI) and the Children's Dermatology Life Quality Index (CDLQI). Participants will complete the

Table 1. Timetable of planned reviews and activities. PSOAS-2: Patient and Observer Scar Assessment Scale version 2; DLQI: Dermatology Life Quality Index; CDLQI: Children's Dermatology Life Quality Index; CLIQ: Cutaneous Leishmaniasis Impact Questionnaire; HLA: Human Leucocyte Antigen; PCR: polymerase chain reaction.

	Day 1 (before 1st treatment)	Day 14	Day 28 (end of 1st cycle of systemic treatment)	Day 42	Day 90	Day 180	Day 360
Participant and physician assessment of change		X	X	X	X	X	
Adverse reaction monitoring (if on treatment)		X	X	X	X		
Pain assessment	X	X	X				
Test of cure (if not healed)					X		
Scar assessment (PSOAS-2)				X	X	X	X
DLQI/CDLQI	X	X	X	X	X	X	
EQ-5D-5L	X	X	X	X	X	X	X
SF36	X			X	X	X	X
CLIQ	X			X	X	X	
Household costs	X	X	X	X	X	X	X
Immunological studies blood sample (subset)	X		X		X		
HLA typing blood sample	X						
Slit-skin smear for PCR	X				X		
Skin biopsy (subset of adults only)	X				X		
Digital image	X	X	X	X	X	X	X

Cutaneous Leishmaniasis Impact Questionnaire (CLIQ), a CL-specific measure of HRQoL.

At the follow-up visits indicated in Table 1, we will collect PROMs again and ask participants for their global assessment of the improvement of their CL lesion(s) using a 5-point Likert scale (better, somewhat better, same, somewhat worse, worse). The Amharic or Afan Oromo translations of the Patient and Observer Scar Assessment Scale (POSAS) v2[26] will be completed by both the clinician and participant at the day 42 assessment and each subsequent visit.

Lived experience

A selection of participants will be recruited to participate in an in-depth interview to capture data on their lived experience of CL until data saturation has occurred. Initially, we aim to recruit a total of 30 adults and 10 children from both study centres. We will ensure representation of participants by key demographics including age, gender, and CL phenotype. Interviews will be conducted by members of the research team using a topic-guide covering key themes including the impact of the illness, the care-seeking pathways they have undertaken and their experience of traditional and allopathic medicine. Ethnographic observation will be used to enrich the data on treatment experiences in the health facilities.

Costs

At baseline data will be collected on costs incurred by the individuals and their household that can be attributed to a member having CL and/or seeking treatment for CL including costs incurred since onset of CL symptoms. We will also collect data on household income, expenditure, and asset possession to establish a month of income and expenditure data.

At each follow up visit, cost related to CL since the last visit/admission will be collected. These will include costs of medicines, hospitalisation, consultation, transportation, and opportunity costs related to having and treating CL (i.e., missed work or school). We will also collect information on how households cope with the economic burden of CL.

Data from the provider perspective will be collected. This will include costs of training health workers, providing services, medicines donated, and other service-related costs. These costs will be collected from the project's activity and financial records; the value of any donated items will be estimated based on market prices. Where necessary, government and donor sources will be consulted for relevant cost data.

Immunology samples

To interrogate the dynamics of immune responses in different clinical phenotypes of CL, blood and skin lesion samples will be collected at three time points. Participants will have blood samples and a skin biopsy (stored in RNAlater™) taken to evaluate subsets of the innate and adaptive immunity; pro-inflammatory (IFN-γ and TNF-α) and anti-inflammatory (IL-6, IL-27, IL-10, IL-4, IL-13, TGF-β, and hsp18) cytokines and interleukins (IL)-12, IL-2, IL-1, IL-18, IL-15, IL-8, IL-17A, and IL-22) using Luminex multiplex assay. (ii) The change in mRNA (Ribonucleic acid) expression level of Toll-like receptors (TLRs) and pro-inflammatory (IFN-γ, IL-12, IL-1β, IL-17A, and IL-12p70) and anti-inflammatory (IL-5, IL-6, IL-10, IL-4, TGF-β, and hsp18) cytokines will also be assessed before treatment (at day 0), during treatment (day 28) and after completion of treatment (day 90) using quantitative PCR (Real-time Polymerase Chain Reaction). In addition, the levels of chemokines (such as CXCL8, CXCL10, CCL5, CCL4, CCL3, and CCL2) will be determined in supernatants from the whole blood stimulation assays for the Luminex multiplex assay. HLA typing will be performed to assess if there is an association between HLA types (targeting probes for HLA-A, B, C, DR, DQ, and DP loci) and the clinical phenotypes of CL seen in our cohort.

Sample Size

The primary outcome is the proportion of individuals with a healed index lesion by study day 90. In line with the consensus document on clinical trial outcomes cure will be defined as 100% re-epithelialization or flattening of the index lesion. Pre-specified additional outcomes are the proportion healed at day 42 and 180. Secondary outcomes include the frequency of adverse events and measures of HRQoL life.

There are no pre-existing high-quality data on cure rates for CL caused by *L. aethiopica*. We calculated the sample size required to measure a range of cure-rates at 90 days with varying degrees of precision (Table 2). Based on this we aimed to recruit up to 250 participants with each CL phenotype (LCL, MCL, and DCL) across the two sites. We anticipate that mucosal leishmaniasis will be rare and will recruit as many individuals with this type of disease as possible.

Table 2. Sample sizes required to detect a range of cure rates with varying precision at day 90.

		Percentage cured at 90 days			
		50	60	70	80
Sample size	50	±13.1	±13.6	±12.7	±11.1
	100	±9.8	±9.6	±9.0	±7.8
	150	±8.0	±7.8	±7.3	±6.4
	200	±6.9	±6.8	±6.4	±5.5
	250	±6.2	±6.1	±5.7	±5.0

Data analysis

***Objective 1:* To assess clinical outcomes in LCL, MCL and DCL in a prospective cohort. & *Objective 2:* To assess clinical parameters in LCL, MCL and DCL in a prospective cohort.**

We will use descriptive statistics (means, medians, and proportions) to describe demographics and baseline characteristics of the cohort. In exploratory analysis, we will use univariable logistic regression to examine clinical and demographic variables associated with resolution of CL at Day 42, 90, and 180. Whilst not formally powered for these analyses, the findings will be viewed as hypothesis-generating and potentially form the basis for future studies.

***Objective 3:* To assess the change in HRQoL associated with CL before and after treatment.**

Changes in general, skin and CL-specific HRQoL will be assessed using the following PROMs: EQ-5D-5L, SF-36, DLQI/ CDLQI and CLIQ applied at the timepoints shown in Table 1, and compared with clinical outcomes to determine validity and responsiveness.

Validity and responsiveness of each PROM will be analysed using SPSS version 23.0 (IBM Corporation, Armonk, NY, USA). Descriptive statistics will be presented using frequencies, percentages, tables, and graphs.

Mean rank sum scores of each of sub-domains will be recorded using Kruskal Wallis rank sum test among as well as the different clinical (LCL, MCL and DCL) types of lesions to check known-group construct validity. Wilcoxon-signed rank test will be used to compare mean difference of scores before initiation of treatment (Day 0) and at Day 90 (date of initial cure assessment).

***Objective 4:* To develop a severity scale for CL.**

A round table CL expert discussion forum, informed by preliminary data, will be held, and consensus reached about potential items and scoring system for a severity scale. The prototype will be tested in individuals with CL at diagnosis.

Objective 5: ***To understand the lived experiences of individuals with CL.***
Recordings will be transcribed and translated verbatim, and interviewer's notes included for each interview. A codebook will be developed, and thematic analysis will be used. MAXQDA plus 2022 will be used to manage the in-depth interview data.

Objective 6: ***To assess household costs of CL, and health care provider, and societal costs of treating CL.***
We will assess costs from a disaggregated societal perspective, examining costs to providers (MOH, donors), households, and society as a whole (i.e., costs to providers and household combined). Incremental costs to providers will be estimated relative to a hypothetical counterfactual in which no treatment for CL is provided. Incremental costs to households will be estimated relative to a hypothetical counterfactual in which the CL infection has not occurred. We will estimate total costs of diagnostic and treatment strategies and average costs, including cost per case diagnosed and cost per case resolved, disaggregated by site, type of CL, and patient characteristics (where possible), with appropriate statistical measures of variation. We will explore the degree of impoverishing and catastrophic expenditure for families of individuals with CL.

Descriptive statistics frequencies, percentages, means and medians will be used as appropriate to estimate costs. Further analysis will be conducted to investigate catastrophic out-of-pocket health expenditure and poverty impact using a linear probability model. STATA version 17 will be used for data analysis

Objective 7: ***To characterise how cell-mediated immune responses associate with treatment responses in CL due to L. aethiopica and (HLA) variation correlate with different clinical phenotypes.***
Biopsies of affected skin and blood samples will be analysed to (i) evaluate secretion and quantification of selected host cytokines via the Luminex® platform, (ii) evaluate the expression of innate/adaptive, and pro-and anti-inflammatory biomarkers and by evaluating the change in mRNA expression level of TLRs and pro- and anti-inflammatory cytokines before treatment, during treatment, and after completion of treatment.

Statistical analyses of laboratory data will be performed using R (version 4.4.1, the R Foundation for Statistical Computing). Nonparametric Mann-Whitney statistics will be used to compare different groups. Differences in cytokine and chemokine concentrations and mRNA expressions among CL types, treatment outcomes, time points, and other variables will be compared by the Kruskal-Wallis and t-tests. Fisher's exact test will be employed to determine the association between HLA types and CL types.

Data management
Each participant will be assigned a unique study identification number and all data will be recorded and analysed with this unique identification number. Data will be electronically entered into a GCP-compliant, trial-specific study database [Research Electronic Data Capture (RedCap), Nashville, TN, USA]. Access to the database will be restricted to relevant researchers within the study team. Relevant documentation will be kept for a minimum of 10 years.

Ethics
Ethical approval was obtained from AHRI/ALERT Ethics Review Committee (Ref: PO/23/21 dated 15.07.2021), the National Research Ethics Review Committee (Ref: 7/2-506/m259/35 dated 17.12.2021), and the London School of Hygiene and Tropical Medicine (Ref: 26421 dated 11.10.2021). Written individual informed consent will be obtained from all participants in the study (see *Extended data*[27]). For study participants under 18 years of age, consent from a responsible adult (parent, relative, guardian, legal representative) will be obtained. In addition, assent will be obtained from study participants aged 12 to 17 years.

Discussion
CL represents a major public health, psychosocial and economic problem both in Ethiopia and in the tropical – subtropical regions. A key target of the 2021–2030 WHO Neglected Tropical Diseases roadmap is a significant increase in the proportion of individuals with CL who are diagnosed and receive appropriate treatment. The 2021–25 Ethiopian strategic plan for Neglected Tropical Diseases, aims to meet the 2030 global target by increasing CL treatment centres from 14 to 30, diagnostic centres from 25 to 170 and to be able to detect 85% of cases and treat 95% of these. A better understanding of strategies to improve accessibility and treatment outcomes is a priority action to achieve this goal[28].

There are currently few prospective data of well-defined treatment outcomes for individuals with CL caused by *L. aethiopica*, and no data on PROMs or costs. A large cohort of participants with CL will generate robust data on how outcomes are associated with key clinical characteristics such as lesion size, site and number. This will allow us to gain insights into expected clinical outcomes up to one year following treatment and accurately quantify the burden of adverse reactions associated with current treatments. There are new agents and combination therapies proposed for the treatment of leishmaniasis and our data will be valuable in helping design future trials of these approaches in individuals with *L. aethiopica*.

This prospective cohort study will generate the most comprehensive and holistic dataset of CL, combining clinician determined and patient reported outcomes alongside health economic and laboratory data to better capture the impact of CL for affected individuals, their families, and communities. These data will inform the design of randomised trials to evaluate new treatment strategies, help improve evidence-based guidelines and support evidence-led policy decisions.

Data availability

Underlying data

No underlying data are associated with this article.

Extended data

LSHTM Data Compass: Prospective observational cohort study of cutaneous leishmaniasis in Ethiopia - Informed consent form. https://doi.org/10.17037/DATA.00003618[27].

Acknowledgements

We wish to thank the individuals and communities for their participation in the work of the Skin Health Africa Research Programme.

SPONSOR: London School of Hygiene & Tropical Medicine

FUNDERS: UK National Institute of Health Research Grant Ref: NIHR200125

Additional members of the SHARP Collaboration

SHARP Steering Committee:

- Yaw Amoako - Kumasi Centre for Collaborative Research
- Richard Phillips - Kumasi Centre for Collaborative Research
- Rachel Pullan - LSHTM
- Dorothy Yeboah-Manu - Noguchi Memorial Institute for Medical Research

Named SHARP Applicants:

- Sinead Langan -LSHTM
- David Mabey -LSHTM
- Jennifer Palmer -LSHTM
- Prof Asrat Hailu- Addis Ababa University

SHARP collaboration members from LSHTM:

- Esther Amon
- Tara Mtuy
- Joseph Timothy
- Ruth Canter
- Maria Zuurmond
- Katherine Halliday

ETHIOPIA study staff involved in study running:

- Data team: Fikregabrail Aberra Kassa, Samuel Ayele, Mikiyas Gebremichael; Tedros Nigusse
- Nurses and runners: Almaz Bedye; Fire Nigusse; Atsede Tesfaye; Aberash Alena; Jawar Mohammed; Aziza Temesigen; Zelalem Tefera
- New medical member Dr Mulualem Degefa
- Economics team: Debisa Eshatu; Nebiyu Sherefa; Mosisa Bekele Degefa; Derese Bekele
- Study co-ordination: Aklilu Adane; Eyerusalem Tesfaye; Yematawork Kebede; Sagni Challi; Demeketch Damte
- Lab staff: Zemed Biyazen; Eyob Fekadu; Befekadu Debebe
- Social sciences contribution: Abay Woday; Kibur Engedawork

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Reference Source

Open Peer Review

Current Peer Review Status: ✔ ? ✔

Version 2

Reviewer Report 17 January 2025

https://doi.org/10.3310/nihropenres.15037.r34052

✔ **Sara M. Robledo**
Universidad de Antioquia, Medellín, Colombia

Thank you for addressing all the previous comments comprehensively. After reviewing the protocol, I found the document complete and well-structured. It includes all the essential components required for such a study, and the methodology is clearly outlined. The authors have adequately incorporated feedback from earlier reviews, ensuring clarity and rigor throughout.

I have no further suggestions for major revisions at this time. However, as a final refinement, I recommend a careful proofreading to ensure consistency in language, grammar, and formatting. This will enhance the overall readability and presentation of the protocol.

Is the rationale for, and objectives of, the study clearly described?
Yes

Is the study design appropriate for the research question?
Yes

Are sufficient details of the methods provided to allow replication by others?
Yes

Are the datasets clearly presented in a useable and accessible format?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Clinical trials, drug development

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 11 January 2025

https://doi.org/10.3310/nihropenres.15037.r33926

Najia Ahmed
[1] Bahria University of Health Sciences, Karachi, Pakistan
[2] Dermatology, Pakistan Naval Station Shifa Hospital, Karachi, Sindh, Pakistan

Thanks for re- review. Most of the observations have been explained except why using SPSS version 23 when even 30 is available. This version may be approved.

Is the rationale for, and objectives of, the study clearly described?
Yes

Is the study design appropriate for the research question?
Yes

Are sufficient details of the methods provided to allow replication by others?
Yes

Are the datasets clearly presented in a useable and accessible format?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Section of data analysis, specially the tests of significance to be used, should also be assessed by a statistician.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 23 November 2023

https://doi.org/10.3310/nihropenres.14569.r30757

? **Talal Alharazi**
Taiz University, Taiz, Yemen

Introduction
- Shorten the background/introduction to be more concise for a quantitative study

Methods
- Expand on the inclusion/exclusion criteria, especially related to prior treatments
- Specify which cytokines/biomarkers will be analyzed in the immunology studies
- Elaborate on the sample size calculations and precision expected
- State which statistical tests and analysis software will be used

Analysis Plan
- Specify proposed statistical tests and significance levels for objectives

Discussion
- Reduce repetition of content covered elsewhere

References
- Update older references, over 5 years old unless seminal works

Overall, add more methodological specifics in line with quantitative reporting guidelines to facilitate reproducibility and interpretation.

Is the rationale for, and objectives of, the study clearly described?
Yes

Is the study design appropriate for the research question?
Partly

Are sufficient details of the methods provided to allow replication by others?
Partly

Are the datasets clearly presented in a useable and accessible format?
Not applicable

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: My work primarily focuses on the epidemiology and control of malaria and neglected tropical diseases, including schistosomiasis, leishmaniasis, and other protozoan and helminth parasites .Also conducts research on the epidemiology of microorganisms and multidrug-resistant bacteria.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 01 Dec 2024

Saba Lambert

Dear Dr Alharazi,

We are grateful for your careful review of our protocol paper and your comments are important in helping us to improve it. We are addressing these one by one here.

Introduction

- Shorten the background/introduction to be more concise for a quantitative study

It is mixed method research.
This introduction reflects what is known about CL in Ethiopia and what data is available so far, bringing to the fore questions that need to be addressed. In view of the complex multi-disciplinary issues being addressed, and to address CL In Ethiopia holistically, we believe the introduction is as concise and inclusive as possible.

Methods

- Expand on the inclusion/exclusion criteria, especially related to prior treatments

Thank you, we have elaborated these criteria as follows:

"All adults and children diagnosed with CL and initiated on CL treatment at both study centers are eligible to be recruited in this study. Each participant will be followed for 360 days.
Inclusion criteria:

1. **Individuals with parasitologically confirmed CL using microscopy or culture**
2. **Individuals receiving treatment at the study centers**

Exclusion criteria:

1. **Participants who have previously been treated with systemic anti-leishmania therapy**
2. **Participants who do not or are unable to give informed consent to participate in the study"**

- Specify which cytokines/biomarkers will be analyzed in the immunology studies

We have amended the text to:

"To interrogate the dynamics of immune responses in different clinical phenotypes of CL, blood and skin lesion samples will be collected at three time points. Participants will have blood samples and a skin biopsy (stored in RNAlater™) taken to evaluate subsets of the innate and adaptive immunity; pro-inflammatory (IFN-γ and TNF-α) and anti-inflammatory (IL-6, IL-27, IL-10, IL-4, IL-13, TGF-β, and hsp18) cytokines and interleukins (IL)-12, IL-2, IL-1, IL-18, IL-15, IL-8, IL-17A, and IL-22) using Luminex multiplex assay. (ii) The change in mRNA (Ribonucleic acid) expression level of Toll-like receptors (TLRs) and pro-inflammatory (IFN-γ, IL-12, IL-1β, IL-17A, and IL-12p70) and anti-inflammatory (IL-5, IL-6, IL-10, IL-4, TGF-β, and hsp18) cytokines will also be

assessed before treatment (at day 0), during treatment (day 28) and after completion of treatment (day 90) using quantitative PCR (Real-time Polymerase Chain Reaction). In addition, the levels of chemokines (such as CXCL8, CXCL10, CCL5, CCL4, CCL3, and CCL2) will be determined in supernatants from the whole blood stimulation assays for the Luminex multiplex assay. HLA typing will be performed to assess if there is an association between HLA types (targeting probes for HLA-A, B, C, DR, DQ, and DP loci) and the clinical phenotypes of CL seen in our cohort."

- Elaborate on the sample size calculations and precision expected
  Table 2 details the sample sizes required for different cure rates.

- State which statistical tests and analysis software will be used

This has now been under in each section of the Data Analysis paragraph

Analysis Plan

- Specify proposed statistical tests and significance levels for objectives

This has now been under in each section of the Data Analysis paragraph

Discussion

- Reduce repetition of content covered elsewhere

We feel the brief discussion summarizes the important points presented previously.
References

- Update older references, over 5 years old unless seminal works

CL is a NTD and research in Ethiopia is limited. We believe we have provided a thorough literature review. There are a few more recent articles, but these were not available at the date of our protocol development. These will be reviewed when results are presented.

- Overall, add more methodological specifics in line with quantitative reporting guidelines to facilitate reproducibility and interpretation.

We believe our paper contains detailed methodological description

Thank you for helping us improve our paper

Competing Interests: none

Reviewer Report 01 November 2023

https://doi.org/10.3310/nihropenres.14569.r30538

Najia Ahmed
[1] Bahria University of Health Sciences, Karachi, Pakistan
[2] Dermatology, Pakistan Naval Station Shifa Hospital, Karachi, Sindh, Pakistan

- The study design has been stated as prospective observational cohort but it appears to be a mixed method study as it also involves patient interviews and their experiences.
- Introduction is too long if it is supposed to be quantitative research.
- Inclusion and exclusion criteria need to be elaborated more.
- In immunology samples, expressions of innate and adaptive immunity and pro and anti inflammatory cytokines to be measured, have not been specified.
- Explain in detail how sample size was calculated.
- Give details of software to be used for data analysis, statistical tests and significant values that will be used.
- Some of the abbreviations have also not been explained.
- Repetition of content in discussion.
- 19 of the 28 ref used are more than 5 years old.
- Ref 26, not in Vancouver style.
- No PMID or DOI for ref provided.

Is the rationale for, and objectives of, the study clearly described?
Yes

Is the study design appropriate for the research question?
Partly

Are sufficient details of the methods provided to allow replication by others?
Partly

Are the datasets clearly presented in a useable and accessible format?
Not applicable

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Section of data analysis, specially the tests of significance to be used, should also be assessed by a statistician.

I confirm that I have read this submission and believe that I have an appropriate level of

expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 01 Dec 2024

Saba Lambert

RESPONSE TO COMMENTS:

Dear Dr Ahmed

We are grateful for your careful review of our protocol paper and your comments are important in helping us to improve it. We are addressing these one by one here.

- The study design has been stated as prospective observational cohort but it appears to be a mixed method study as it also involves patient interviews and their experiences.

Indeed, this has been designed as a prospective observational cohort into which are integrated different methodologies appropriate to the different "areas" under investigation, using qualitative and quantitative tools:

- **Clinical: Observation of clinical features, treatments given, clinical outcomes as well as patient reported outcome measure (quantitative)**
- **Psychosocial: patient's treatment experience using ethnographic methods; qualitative (IDI)**
- **Immunological investigations on skin and blood samples – quantitative laboratory research**
- **Economic burden of CL: Serial economic evaluations**

We have added a brief clarification on page 6 under "Overview of Data Collection"

- Introduction is too long if it is supposed to be quantitative research.

We employ a variety of methods, and the length of the introduction reflects the broad scope of this cohort study.
This introduction reflects what is known about CL in Ethiopia and what data is available so far, bringing to the fore questions that need to be addressed. In view of the complex multi-disciplinary issues being addressed, and to address CL In Ethiopia holistically, we believe the introduction is as concise and inclusive as possible.

- Inclusion and exclusion criteria need to be elaborated more.

Thank you, we have elaborated these criteria as follows:

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- Explain in detail how sample size was calculated.

Table 2 details the sample sizes required for different cure rates.

- Give details of software to be used for data analysis, statistical tests and significant values that will be used.

The following have been expanded in each section
<u>Clinical:</u> Data will be analysed in STATA. Descriptive statistics will be used to describe the cohort. Odds ratio and logistic regression models will be used to identify clinical and demographic characteristics associated with healing.
<u>PROMS:</u> Validity and responsiveness of each PROM will be analysed using SPSS version 23.0 (IBM Corporation, Armonk, NY, USA). Descriptive statistics will be presented using frequencies, percentages, tables, and graphs.
Mean rank sum scores of each of sub-domains will be recorded using Kruskal Wallis rank sum test among as well as the different clinical (LCL, MCL and DCL) types of

lesions to check known-group construct validity. Wilcoxon-signed rank test will be used to compare mean difference of scores before initiation of treatment (Day 0) and at Day 90 (date of initial cure assessment).

Laboratory: Statistical analyses of laboratory data will be performed using R (version 4.4.1, the R Foundation for Statistical Computing). Nonparametric Mann-Whitney statistics will be used to compare different groups. Differences in cytokine and chemokine concentrations and mRNA expressions among CL types, treatment outcomes, time points, and other variables will be compared by the Kruskal-Wallis and t-tests. Fisher's exact test will be employed to determine the association between HLA types and CL types.

Economics: Descriptive statistics: frequencies, percentages, means and medians will be used as appropriate to estimate costs. Further analysis will be conducted to investigate catastrophic out-of-pocket health expenditure and poverty impact using a linear probability model. STATA version 17 will be used for data analysis

In-depth interviews: Recordings will be transcribed and translated verbatim, and interviewer's notes included for each interview. A codebook will be developed, and thematic analysis will be used. MAXQDA plus 2022 will be used to manage the in-depth interview data.

- Some of the abbreviations have also not been explained.

Thank you, this has been amended.

- Repetition of content in discussion.

We feel the brief discussion summarizes the important points presented previously.

- 19 of the 28 ref used are more than 5 years old.

CL is a NTD and research in Ethiopia is limited. We believe we have provided a thorough literature review. There are a few more recent articles, but these were not available at the date of our protocol development. These will be reviewed when results are presented.

- Ref 26, not in Vancouver style.
  We have checked referencing style. Thank you.
- No PMID or DOI for ref provided.

Apologies, this was provided but does not seem to have uploaded correctly. Will amend with editorial team

Competing Interests: none

Comments on this article

Version 1

Author Response 25 Oct 2024

Saba Lambert

STUDY PROTOCOL
Protocol for a prospective observational cohort study of cutaneous leishmaniasis in Ethiopia

RESPONSE TO COMMENTS:

Dear Dr Ahmed,

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Reviewer comment: Explain in detail how sample size was calculated.
Author response: Table 2 details the sample sizes required for different cure rates.

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PROMS: Validity and responsiveness of each PROM will be analysed using SPSS version 23.0 (IBM

Corporation, Armonk, NY, USA). Descriptive statistics will be presented using frequencies, percentages, tables, and graphs.
Mean rank sum scores of each of sub-domains will be recorded using Kruskal Wallis rank sum test among as well as the different clinical (LCL, MCL and DCL) types of lesions to check known-group construct validity. Wilcoxon-signed rank test will be used to compare mean difference of scores before initiation of treatment (Day 0) and at Day 90 (date of initial cure assessment).

Laboratory: Statistical analyses of laboratory data will be performed using R (version 4.4.1, the R Foundation for Statistical Computing). Nonparametric Mann-Whitney statistics will be used to compare different groups. Differences in cytokine and chemokine concentrations and mRNA expressions among CL types, treatment outcomes, time points, and other variables will be compared by the Kruskal-Wallis and t-tests. Fisher's exact test will be employed to determine the association between HLA types and CL types.

Economics: Descriptive statistics: frequencies, percentages, means and medians will be used as appropriate to estimate costs. Further analysis will be conducted to investigate catastrophic out-of-pocket health expenditure and poverty impact using a linear probability model. STATA version 17 will be used for data analysis

In-depth interviews: Recordings will be transcribed and translated verbatim, and interviewer's notes included for each interview. A codebook will be developed, and thematic analysis will be used. MAXQDA plus 2022 will be used to manage the in-depth interview data.

Reviewer comment: Some of the abbreviations have also not been explained.
Author response: Thank you, this has been amended.

Reviewer comment: Repetition of content in discussion.
Author response: We feel the brief discussion summarizes the important points presented previously.

Reviewer comment: 19 of the 28 ref used are more than 5 years old.
Author response: CL is a NTD and research in Ethiopia is limited. We believe we have provided a thorough literature review. There are a few more recent articles, but these were not available at the date of our protocol development. These will be reviewed when results are presented.

Reviewer comment: Ref 26, not in Vancouver style.
Author response: We have checked referencing style. Thank you.

Reviewer comment: No PMID or DOI for ref provided.
Author response: Apologies, this was provided but does not seem to have uploaded correctly. Will amend with editorial team

Introduction

Reviewer comment: Shorten the background/introduction to be more concise for a quantitative study
Author response: It is mixed method research.
This introduction reflects what is known about CL in Ethiopia and what data is available so far, bringing to the fore questions that need to be addressed. In view of the complex multi-disciplinary issues being addressed, and to address CL In Ethiopia holistically, we believe the introduction is as concise and inclusive as possible.

Methods

Reviewer comment: Expand on the inclusion/exclusion criteria, especially related to prior treatments
Author response: Thank you, we have elaborated these criteria as follows:

"All adults and children diagnosed with CL and initiated on CL treatment at both study centers are eligible to be recruited in this study. Each participant will be followed for 360 days.
Inclusion criteria:

1. Individuals with parasitologically confirmed CL using microscopy or culture
2. Individuals receiving treatment at the study centers

Exclusion criteria:

1. Participants who have previously been treated with systemic anti-leishmania therapy
2. Participants who do not or are unable to give informed consent to participate in the study"

Reviewer comment: Specify which cytokines/biomarkers will be analyzed in the immunology studies
Author response: We have amended the text to:

"To interrogate the dynamics of immune responses in different clinical phenotypes of CL, blood and skin lesion samples will be collected at three time points. Participants will have blood samples and a skin biopsy (stored in RNAlater™) taken to evaluate subsets of the innate and adaptive immunity; pro-inflammatory (IFN-γ and TNF-α) and anti-inflammatory (IL-6, IL-27, IL-10, IL-4, IL-13, TGF-β, and hsp18) cytokines and interleukins (IL)-12, IL-2, IL-1, IL-18, IL-15, IL-8, IL-17A, and IL-22) using Luminex multiplex assay. (ii) The change in mRNA (Ribonucleic acid) expression level of Toll-like receptors (TLRs) and pro-inflammatory (IFN-γ, IL-12, IL-1β, IL-17A, and IL-12p70) and anti-inflammatory (IL-5, IL-6, IL-10, IL-4, TGF-β, and hsp18) cytokines will also be assessed before treatment (at day 0), during treatment (day 28) and after completion of treatment (day 90) using quantitative PCR (Real-time Polymerase Chain Reaction). In addition, the levels of chemokines (such as CXCL8, CXCL10, CCL5, CCL4, CCL3, and CCL2) will be determined in supernatants from the whole blood stimulation assays for the Luminex multiplex assay. HLA typing will be performed to assess if there is an association between HLA types (targeting probes for HLA-A, B, C, DR, DQ, and DP loci) and the clinical phenotypes of CL seen in our cohort."

Reviewer comment: Elaborate on the sample size calculations and precision expected
Author response: Table 2 details the sample sizes required for different cure rates.

Reviewer comment: State which statistical tests and analysis software will be used
Author response: This has now been under in each section of the Data Analysis paragraph

Analysis Plan
Reviewer comment: Specify proposed statistical tests and significance levels for objectives **Author response:** This has now been under in each section of the Data Analysis paragraph

Discussion

Reviewer comment: Reduce repetition of content covered elsewhere
Author response: We feel the brief discussion summarizes the important points presented previously.

References

Reviewer comment: Update older references, over 5 years old unless seminal works
Author response: CL is a NTD and research in Ethiopia is limited. We believe we have provided a thorough literature review. There are a few more recent articles, but these were not available at the date of our protocol development. These will be reviewed when results are presented.

Reviewer comment: Overall, add more methodological specifics in line with quantitative reporting guidelines to facilitate reproducibility and interpretation.
Author response: We believe our paper contains detailed methodological description.

Competing Interests: No competing interests were disclosed.