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Clinical, haematological and biochemical profiling of podoconiosis lymphoedema patients prior to their involvement into a clinical trial in the North West Region of Cameroon

Bertrand Lontum Ndzeshang\textsuperscript{1,2}, Randy Tchachoua Mbiakop\textsuperscript{1,2}, Gordon Takop Nchanji \textsuperscript{1,2}, Chi Anizette Kien\textsuperscript{1,2}, Glory Ngongeh Amanbo\textsuperscript{1,2,}\textsuperscript{2}, Raphael Awah Abong\textsuperscript{1,2}, Timothy Yuyun\textsuperscript{3}, Amuam Andrew Beng\textsuperscript{1,2}, John Bonekeh, Manuel Ritter\textsuperscript{4}, Mathias Eyong Esum\textsuperscript{1,2}, Jerome Fru Cho\textsuperscript{1,2}, Abdel Jelil Njouendou\textsuperscript{1,2}, Ignatius Nde Ndifor\textsuperscript{5}, Kebede Deribe\textsuperscript{6,7}, Fanny Fri. Fombad\textsuperscript{1,2}, Peter Enyong\textsuperscript{1,2}, Ute Klarmann-Schulz\textsuperscript{4,8}, Achim Hoerauf\textsuperscript{4,8}, Samuel Wanji\textsuperscript{1,2*}

\textsuperscript{1}Department of Microbiology and Parasitology, University of Buea, P.O. Box 63, Buea, Cameroon.
\textsuperscript{2}Research Foundation in Tropical Diseases and Environment, P.O. Box 474, Buea, Cameroon.
\textsuperscript{3}Regional Hospital Bamenda, P.O. 818, Bamenda Cameroon
\textsuperscript{4}Institute for Medical Microbiology, Immunology and Parasitology (IMMIP), University Hospital Bonn (UKB), Bonn, Germany
\textsuperscript{5}Ndop District Hospital, P.O. 07, Ndop, Cameroon
\textsuperscript{6}Global Health and Infection Department, Brighton and Sussex Medical School, Brighton, BN1 9PX, United Kingdom.
\textsuperscript{7}School of Public Health, Addis Ababa University, Ethiopia
\textsuperscript{8}German Center for Infection Research (DZIF), partner-site Bonn-Cologne, Germany

\textsuperscript{*}Corresponding author:
Tel: +237 694 727 715
E-mail: swanji@yahoo.fr
Abstract

Background: Prior to carrying out clinical trials, it is important to assess the health status of the study participants to be able to interpret subsequent changes that may be related to the effects of the treatments during patient follow up. This study presents the clinical, haematological, and biochemical profiles of podoconiosis patients prior to their involvement in the PodoLEDoxy clinical trial.

Methods: All lower limbs lymphoedema patients visiting the centre were screened and podoconiosis diagnosis was based on clinical manifestation and detailed medical history. Patients that satisfied the eligibility criteria were enrolled in to the study and demographic data, vital signs and medical history collected followed by biochemical and haematological examinations.

Results: Of the 222 participants enrolled into the study, 55.4 % and 41.4 % had stages 3 and 2 as their highest stages respectively. On physical examination, gastritis (46 %) and poor vision (2.7 %) were most prevalent health issues identified. Majority of haematological and biochemical values were within normal ranges except for MPV (47.7 %), PCT (58.1 %), PDW (66.2 %), MCV (67.6 %) and RDWSD (79.3 %), where more than 40 % of the study participants had values out of the normal.

Conclusion: The clinical, haematological and biochemical profile of the study participants were within normal range except for certain haematological parameters that might be worth investigating.

Keywords: Lymphodema, Podoconiosis, Clinical, Haematological, Biochemical, Profiling
Introduction
Lower limb lymphoedema is caused by several neglected tropical diseases such as podoconiosis, lymphatic filariasis (LF), and leprosy. While the latter two conditions are caused by a parasite and bacterium respectively, podoconiosis however is associated with exposure of bare feet to irritant alkaline red clay soils (Korevar and Visser, 2012; Wanji et al., 2008).

Podoconiosis locally known as “mossy foot” causes massive swelling of the lower legs and feet resulting to heavy aching discomfort which limits a person’s ability to use his or her legs. It increases the risk to certain infections, causes emotional distress, depression and stigmatization in the affected person. This imposes huge burdens on affected individuals, their families and communities, resulting to loss in economic productivity (Semrau et al., 2019; Hofstraat and Brakel, 2016).

It is a non-infectious disease that arises mostly in barefoot subsistence farmers after long term exposure to irritant red clay soils (Davey et al., 2007). Podoconiosis occurs on the lower limbs and has an ascending progression, starting in the foot and progressing to the knee. It has been classified into five stages based on the severity with stage 5 being the most severe stage (Tekola et al., 2008).

Podoconiosis was identified as a neglected tropical disease by WHO in 2011 (WHO, 2018). Approximately 20 million people worldwide are thought to be affected by lymphoedema of which 4 million are due to podoconiosis (Tekola et al., 2012). It is a major health problem in developing countries and has been reported in over 32 countries in Africa, Latin America and Southeast Asia (Davey et al., 2007; Tekola et al., 2012). It affects people of all ages, but is more prevalent among older age groups. However, females are more affected due to the nature of their activities such as farming (Wanji et al., 2008). The recent nationwide mapping of podoconiosis in Cameroon reported a 0.5 % prevalence of lower limb lymphoedema, of which 62.7 % were podoconiosis
cases. This also highlighted the North West Region of Cameroon as an endemic area recording the highest prevalence (1.7 %) among all 10 regions (Deribe et al., 2018a).

Up to date, there is no specific diagnostic tool for defining podoconiosis, the diagnosis is made solely on the basis of medical history and clinical examination, coupled with the exclusion of other causes of lower limb lymphoedema. Although there has been a significant improvement over the past few years in the management of podoconiosis cases, much is still to be known to improve on the diagnosis and treatment. Knowledge on the clinical and laboratory pattern of people with lower limbs lymphoedema due to podoconiosis is of interest as it presents opportunities for diagnosis of podoconiosis, but interestingly, so far no studies were performed to investigate the clinical and laboratory features of podoconiosis patients. There is therefore a need to understand the clinical, haematological, and biochemical profiles among patients suffering from lymphoedema of non-filarial origin (podoconiosis) in view to identify patterns unique to the disease. In addition, some of the measurements can also be indicative of the burden of other non-communicable diseases among podoconiosis patients, which will have clinical and programmatic implication. These patterns can open up avenues that might serve as biomarkers to discriminate podoconiosis from other diseases that cause lymphoedema.

In summary, the aim of this study is to describe the clinical, haematological, and biochemical profiles of podoconiosis patients of the North West Region of Cameroon screened prior to their involvement in the TAKeOFF PodoLEDox (Doxycycline for treatment of non-filarial lymphoedema due to podoconiosis) clinical trial. This is a randomized double-blind placebo-controlled phase two trial to determine if a doxycycline treatment regimen of 6-weeks will improve lymphoedema due to podoconiosis in patients with stage 2 and 3.
Materials and methods

Study area
This study was conducted at a lymphoedema clinical trial centre (PodoLedoxy clinical trial center) situated at Foncha Street Nkwen (with coordinates; 5.9811374, 10.1706479) in Bamenda, the headquarters of the North West Region of Cameroon. It is located 366 kilometres (227 mil) northwest of the capital, Yaoundé. Participants for the study came from 11 of 19 health districts in the region which has the highest prevalence of podoconiosis in Cameroon by region standing at 1.7 % (Deribe et al., 2018a). The health districts were Bafut, Bali, Bamenda, Batibo, Fundong, Kumbo, Ndop, Ndu, Nkambe, Santa and Tubah and have an estimated population of ~1548926 people.

Study design and period
A cross-sectional study was conducted from April to December, 2019. Figure 1 presents the flow chart of recruitment and the number of individuals who were excluded and eligible for the clinical trial.

Study population
All lymphoedema patients clinically suspected for podoconiosis visiting the lymphoedema clinical trial centre within the study period.

Inclusion and exclusion criteria
The following inclusion criteria were used: people with podoconiosis of stage 2 or 3 at least in one leg, age between 18 and 60 years, body weight 40 kg or above, individuals who reside in podoconiosis endemic districts for 2 years or more, and those who tested negative using Filarial Test Strip (FTS) test for lymphatic filariasis (LF). The following exclusion criteria were used;
individuals who do not fulfil the inclusion criteria, pregnant women and breast feeding mothers, lymphoedema due to other causes, HIV, podoconiosis patients with alanine aminotransferase (ALT) > 80 U/L, aspartate aminotransferase (AST) > 80 U/L, gamma-glutamyl transferase (γ-GT) >100 U/L, serum creatinine > 2.8 mg/dL, neutrophil < 1100 /mm³, Haemoglobin < 8 g/dL, platelets < 100,000 /mm³.

**Figure 1**: Flow chart for activities

**Study variable**

**The following key variables were measured in the study:**

- Socio-demographic variables: sex, age, occupation, duration in the community (endemicity).
- Clinical variables: blood pressure (BP), pulse rate, temperature, weight, physical examination and medical history.
- Haematological variables: White blood cell (WBC), red blood cell (RBC), haemoglobin (HGB), haematocrit (HCT) mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), red cell distribution width (RDW), platelet count (PLT), mean platelet volume (MPV), plateletcrit (PCT), platelet distribution width (PDW).
- Biochemical variables: ALT, AST, γ-GT), serum creatinine. Urinalysis: specific gravity, protein, blood, glucose, nitrite, pH, ketone, bilirubin, urobilinogen and leucocytes.

**Case identification**

A total of 222 podoconiosis patients were screened for enrollment into the clinical trial after obtaining their informed consent. All lower limbs lymphoedema patients that visited the centre were screened for podoconiosis by 2 trained personnel with experience in podoconiosis diagnosis.
Diagnosis was based on clinical manifestation and detailed medical history, followed by staging using the 5 point scale of Tekola et al., 2008. Confirmed podoconiosis participants that satisfied the eligibility criteria were retained and enrolled in the study. Patient’s demographic data was recorded, vital signs measured and medical history recorded.

**Sample collection, processing and transportation**

Samples (blood and urine) were collected in the laboratory unit of the centre and properly labelled with the date of collection and participant code. In the centre, 50-60 mL of mid-stream urine was collected in sterile urine cups for urinalysis. Urinalysis dip stick, (URIT 11G: Urit Medical Electronic Co., Ltd, Guangxi) was done on the spot using the urine sample collected to assess the following parameters; pH, glucose, protein, blood, nitrite, ketone, bilirubin, urobilinogen, specific gravity and leucocytes.

A total of 6 mL of venous blood was collected through venepuncture from each study participant. Following, 4 mL blood was collected into an EDTA tube for biochemical analysis and 2 mL into a clot activator tube (BD Vacutainer, UK) for haematological analysis. The laboratory request form was filled and the time of sample collection noted. Whole blood and serum samples were transported to the Bamenda Regional Hospital laboratory for analysis. The 2 mL blood samples collected in the clot activator tubes were centrifuged at a speed of 2000 xg for 10 minutes and 500 µL of serum was extracted and aliquoted into labelled Eppendorf tubes. To exclude LF as possible cause of lymphoedema in lymphoedema individuals, 75 µL of capillary blood was collected to perform the Alere® (Alere Scarborough, inc. USA) filarial test according to manufacturer’s instructions.
Laboratory examination

The EDTA tube containing 4 mL whole blood and Eppendorf tube containing 500 µL were packaged into iceboxes (4 °C) and transported within 4 – 5 hours to the Bamenda Regional Hospital Laboratory, situated approximately 6.2 km from the centre. The samples were received and documented by the laboratory reception alongside the test request form upon arrival. The samples were forwarded to the various laboratory units (biochemistry and haematology) and analyses were done immediately. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (γ-GT) and serum creatinine were measured using an automated biochemical analyser (BIOSMART 240: Bionline-.S.R.L, Chile) according to manufacturer’s instructions. Result output were projected on the screen, verified and printed out.

The haematological parameters were determined using an automated haematology analyser (URIT 3300: URIT Medical Electronic Group Co., LTD, China), which performs full blood count. To determine the differential WBC count, 2 experienced personnel independently counted the blood cells and the average for each cell type was calculated and recorded. After analysis, results of each participant were validated by the laboratory head, placed in sealed envelopes and made available for collection at the reception.

Quality control

Biochemical and haematological analysis were done in accordance with the standard operating protocol (SOP) and manufacturer’s instructions. The test was conducted in a certified laboratory by trained and certified laboratory technologists with experience in the use of the automated analysers. For every automated machine, daily quality assurance checks were performed in accordance with the manufacturer’s recommendations.
Ethical considerations

An ethical approval was obtained from the National Ethics Committee for Health Research on Humans (CNERSH) and the clinical trial is registered under International Standard Randomised Controlled Trial Number (ISRCTN) 11881662. Individual informed consent was obtained from each participant. Patient information sheets, assent and consent forms were printed in French and English, the official languages in Cameroon. Confidentiality was assured by assigning unique codes to all participants and their identification files were kept in a secured locker.

Data analysis

Data was entered by two data clerks using REDCap, compared and any inconsistencies corrected. The validated data was then exported to Excel 2013 and analysed using SPSS version 20. Summary frequency tables were generated and parameters were categorised into normal and abnormal based on reference ranges set by the trial and the reference laboratory of the Bamenda Regional Hospital.

Results

Socio-demographic

In total, 222 podoconiosis individuals were included in the study. Of these, 191 (86 %) and 31 (14 %) of the podoconiosis participants were females and males respectively. One hundred and thirty of the participants were above 40 years old and 113 (50.9 %) of them had as occupation farming. Most of the participants had stages 3 (55.4 %) and 2 (41.4 %) as their highest stages of podoconiosis (Table 1).

Table 1: Socio-demographic characteristics of study population

Vital signs and co-morbidities

Clinically, most of the participants had vital signs within normal range, with 5 of them being hypertensive (Table 2). On physical examination, the most prevalent health issues identified were gastritis (21/222:9.46 %) and poor vision (6/222: 2.7 %). Evidence of surgery was also observed
in 12 (5.41 %) participants (Supplementary table 1). The following reported comorbidities were identified among podoconiosis patients: gastritis (10), hypertension (9), diabetes mellitus (2), leg ulcer (2), asthma (1), cataract (1) and tinea versicolor (1) [Supplementary table 2].

Table 2: Vital sign presentation of study participants

Haematological profile
The haematological parameters were within normal range (Table 3). However, for the following parameters, MPV (47.7 %), PCT (58.1 %), PDW (66.2 %), MCV (67.6 %) and RDWSD (79.3 %), more than 40 % of the study participants had values out of the normal range (supplementary table 3). Similar observation was made with the RBC, WBC and platelet picture in the study participants as no abnormality prevalent within a particular group of study participants. The abnormalities recorded for RBC, WBC and platelets are presented in table 4. For the blood picture, 17.6 % of the study participants had microcytic red blood cells; 10.4 % of the participants had neutropenia, and 22.5 % had thrombocytosis.

Table 3: Haematological profiles of study participants

Table 4: Red blood cell, white blood cell and platelet picture of study subject

Biochemical profile
The biochemical profile of blood serum were within normal range for most of the participants. Less than 3 % of the study participants had abnormal ALT, AST and gamma GT values while 9 % had abnormal creatinine (Table 5). Biochemical measurements of urine of the podoconiosis study participants were also in majority within normal ranges (supplementary table 4).

Table 5: Biochemical parameters of blood serum of podoconiosis study participants

Discussion

The PodoLEDoxy clinical trial has as aim to test the efficacy of a six weeks doxycycline regimen as a treatment option for the non-filarial lymphoedema due to podoconiosis. This is a phase II trial involving the administration of 200 mg/day of doxycycline treatment for six-weeks which has been shown to halt or improve disease severity in filarial lymphoedema (Mand et al., 2012). Building on the successes of the regimen in filarial lymphoedema, the trial seeks to determine if a similar
outcome can be observed with non-filarial lymphoedema. Prior to carrying out clinical trials, it is important to assess the health status of the participants. This study therefore presents the clinical, haematological and biochemical profiles of podoconiosis study participants screened for enrollment into the trial.

To the best of our knowledge, this is the first work attempting to investigate and document the clinical, haematological and biochemical profile of podoconiosis patients. Due to the long duration of the treatment regimen which exerts significant pressure on liver function, profiling these individuals suffering from a health condition with no specific diagnostic and treatment tool is important.

On clinical examination, most of the participants were clinically fit except for their lymphoedema of stages 2 and 3, which was of interest in the PodoLEDoxy clinical trial. This is clearly demonstrated in the fact that 75.7% and 69.0% had no comorbidities and health issues on physical examination respectively. This is important because it ensures that the values measured from our study participants will largely reflect effects due to the podoconiosis condition and not due to any other comorbidity which could have significantly affected the parameters investigated. In addition, it is also important as it ensures that during trial follow ups, it will be much easier to identify any effect of the doxycycline treatment.

With respect to the haematological profile, a significant proportion of the participants had values out of the normal. The most noticeable abnormality was with the size and proportion of platelets (PDW and PCT) and red blood cells (RDW and MCV). The blood picture also revealed a considerable percentage of microcytic RBC in podoconiosis participants. This abnormality is associated to several diseases or clinical state such as thalassemia, anaemia of chronic inflammation, lead poisoning, iron deficiency anaemia and sideroblastic anaemia (Jones, 2009;
Ford, 2013). Since the major morbidity in our study participants is podoconiosis, abnormalities in the parameters mentioned may be as a result of lymphoedema and thus may be worth further investigation. Examining the biochemical profile, the liver and kidney function parameters measured were largely within the normal range indicating that the liver and kidney might not be implicated in podoconiosis disease.

This study was limited to the profiling of the clinical, haematological and biochemical picture of podoconiosis patients who satisfied baseline eligibility criteria for screening into the PodoLEDoxy clinical trial. Thus, it did not include podoconiosis patients with underlying comorbid conditions, which could have affected significantly the parameters measured. In contrast, this made the strength of the study because the results could be related more directly to podoconiosis since the participants largely were not suffering from other comorbid conditions that could significantly influence the results.

With large gaps still remaining in the understanding of the immunology and pathophysiology of podoconiosis, studying the clinical and laboratory profiles of podoconiosis participants screened for the clinical trial was an opportunity to explore potential patterns associated with people suffering mainly of podoconiosis. The information presented might therefore identify and open avenues for further clinical studies or research.

**Conclusion**

Upon physical examination, 68.92 % of the enrolled participants presented with no medical condition, while gastritis (9.46 %), poor vision (4.05 %) and hypertension (2.25 %) were the most recorded co-morbid conditions. The haematological profile of study participants was also within normal range for most of the study participants. High proportions of participants with abnormal haematological values was recorded for the following parameters; MPV (47.7 %), PCT (58.1 %),
PDW (66.2 %), MCV (67.6 %) and RDWSD (79.3 %). For the biochemical parameters, >97% of the study participants had measurements within the normal ranges.
Authors’ contributions;

Conceptualization: SW, AH

Study protocol: UKS, MR, AH, SW

Clinical assessment: INN, WPCN, BLN, GTN

Sample analysis and interpretation of data: RTM, WPCN, TY, GNA, RAA, MEE, JFC BLN, GTN, CAK

Drafted the manuscript: BLN, GTN, CAK, RTM, AJN, SW, FFF

Critically revised the manuscript for intellectual content: KD, AJN, MR and PE

Guarantors of the paper: AH, SW

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Competing interests

None declared.

Ethical approval.

This study received ethical approval for the Cameroon National Ethics Committee and a written informed consent was obtained from each study participant before enrolment in the trial. In addition, the clinical trial is registered under ISRCTN 11881662.
REFERENCES

Davey G (2010). Podoconiosis, non-filarial elephantiasis, and lymphology. Lymphology. 43(4):168–77. PMID: 21446572.

Davey G, Tekola F, Newport MJ (2007). Podoconiosis: non-infectious geochemical elephantiasis. Trans R Soc Trop Med Hyg. 101:1175-80.

De Groot V, Beckerman H, Lankhorst GJ, Bouter LM (2003). How to measure comorbidity. A critical review of available methods. J Clin Epidemiol. 56 (3):221–9. https://doi.org/10.1016/S0895-4356 (02) 00585-1 PMID: 12725876

Deribe K, Wanji S, Shafi O, Edrida MT, Umulisa I, Molyneux DH and Davey G (2015a). The feasibility of eliminating podoconiosis. Bull World Health Organ. 93(10):712–8. https://doi.org/10.2471/BLT.14.150276 PMID: 26600613.

Deribe K, Cano J, Newport MJ, Golding N, Pullan RL, Sime H, Gebretsadik A, Assefa A, Kebede A, Hailu A, Rebollo MP, Shafi O, Bockarie MJ, Aseffa A, Hay SI, Reithinger R, Enquselassie F, Davey G, Brooker SJ (2015b). Mapping and modelling the geographical distribution and environmental limits of podoconiosis in Ethiopia. PLoS Negl Trop Dis. 9 (7):e0003946.

Deribe K, Beng AA, Cano J, Njouendou AJ, Fru-Cho J, Awah AR, et al. (2018a). Mapping the geographical distribution of podoconiosis in Cameroon using parasitological, serological, and clinical evidence to exclude other causes of lymphedema. PLoS Negl Trop Dis 12(1): e0006126. https://doi.org/10.1371/journal.pntd.0006126

Deribe K, Cano J, Trueba ML, Newport MJ, Davey G (2018b) Global epidemiology of podoconiosis: A systematic review. PLoS Negl Trop Dis 12 (3): e0006324.https://doi.org/10.1371/ journal.pntd.0006324
Deribe K, Cano J, Njouendou AJ, Eyong ME, Beng AA, Giogi E, Pigott DM, Pullan RL, Noor AM, Enquaselassie F, Murray CJL, Hay SI, Newport MJ, Davey G, Wanji S (2018c). Predicted distribution and burden of podoconiosis in Cameroon. *BMJ Glob Health* 3:e000730. Doi:10.1136/bmjgh-2018-000730.

Deribe K, Mbituyumuremyi A, Cano J, Bosco MJ, Giorgi E, Ruberanziza E, Bayisenge U, Leonard U, Bikorimana JP, Rucogoza A, Turate I, Rusanganwa A, Pigott DM, Pullan RL, Noor AM, Enquaselassie F, Condo JU, Murray CJ, Brooker SJ, Hay SI, Newport MJ, Davey G (2019). Geographical distribution and prevalence of podoconiosis in Rwanda: a cross-sectional country-wide survey. *Lancet Glob Health*; 7: e671–680. http://dx.doi.org/10.1016/S2214-109X(19)30072-5

Desta K, Ashine M, Davey G (2003). Prevalence of podoconiosis (endemic non-filarial elephantiasis) in Wolaitta, Southern Ethiopia. *Trop Doct.* 32:217-20.

Fuller LC (2005). Podoconiosis: endemic nonfilarial elephantiasis. *Curr Opin Infect Dis.* 18:119-22.

Glynn LG, Valderas JM, Healy P, Burke E, Newell J, Gillespie P (2011). The prevalence of multimorbidity in primary care and its effect on health care utilization and cost. *Fam Pract.* 28 (5):516–23. https://doi.org/10.1093/fampra/cm013 PMID: 21436204

Hofstraat K and Brakel HV (2016). Social stigma towards neglected tropical diseases a systematic review. *Int Health.* 8 (Suppl 1): i53-i70. doi: 10.1093/inthealth/ihv071

Jose M. Valderas, Babara Starfeid, Bonnie Sibbald, Chris Salibary, Martin Rdard (2009). Defining Comorbidity: Implications for understanding Health and Health services. *Am FarmMed* 7: 357:363. doi: 10.1370/afm.983.

Kebede B, Martindale S, Mengistu B, Kebede B, Mengiste A, H/Kiros F, et al. (2018) Integrated morbidity mapping of lymphatic filariasis and podoconiosis cases in 20 co-endemic
districts of Ethiopia. *PLoS Negl Trop Dis* **12**(7): e0006491. https://doi.org/10.1371/journal.pntd.0006491

Korevaar DA and Visser BJ (2012). Podoconiosis a neglected tropical disease. The *journal of tropical medicine*. **70**: 210-214.

Le Blond J, Cuadros J, Molla Y, Berhanu T, Umer M, Baxter PJ, Davey G (2015). Weathering of the Ethiopian volcanic province: A new weathering index to characterize and compare soils. *Am. Mineral.* **100**: 2518–2532. [CrossRef]

Mand, S, Debrah, AY, Klarmann, U, Batsa, L, Marfo-Debrekyei, Y, Kwarteng, A, Specht, S, Belda-Domene, A, Fimmers, R, Taylor, M, Adjei, O and Hoerauf, A (2012). Doxycycline Improves Filarial Lymphedema Independent of Active Filarial Infection: A Randomized Controlled Trial. Clinical Infectious Diseases 2012;55(5):621–30

Molla YB, Wardrop NA, Le Blond JS, Baxter P, Newport MJ, Atkinson PM, Davey G (2014). Modelling environmental factors correlated with podoconiosis: A geospatial study of non-filarial elephantiasis. *Int. J. Health Geogr.* **13**:24. [CrossRef] [PubMed]

Morgan P, Franks PJ, Moffatt C (2005). Health related quality of life with lymphedema: Areview of the literature. *International Wound Journal* **2**(1): 47-62. Doi; 10.1111/j.1742-4801.2005.00066.x

Negussie H, Kassahun MM, Fegan G, Njuguna P, Enquselassie F, Mckay A, Newport M, Lang T, and Davey G (2015). Podoconiosis treatment in northern Ethiopia (GolBet): study protocol for a randomised controlled trial. Trials; **16**: 307.

Palladino R (2016). Associations between multimorbidity, healthcare utilisation and health status: evidence from 16 European countries. *Age Ageing.* **45**(3):431–5. https://doi.org/10.1093/ageing/afw044 PMID: 27013499
Price EW (1976). The association of endemic elephantiasis of the lower legs in East Africa with soil derived from volcanic rocks. *Trans R Soc Trop Med Hyg.* **70:**288-95.

Semrau M, Davey Gail, Amuam Andrew Andrew Beng, Winston Patrick Chounna Ngongmo, Abdel jelil Njouendou, Samuel Wanji and Kebede deribe, (2019). Depressive symptoms amongst people with podoconiosis and lower limbs lymphedema of other cause in Cameroon: A crossectional study. *Trop.Med.Infect.* doi: 10.3390/tropicalmed4030102

Tekola F, Adeyemo A, Finan C, et al. (2012). HLA Class II Locus and Susceptibility to Podoconiosis. *N Engl J Med.* 366; 13:1200-8.

Tekola F, Ayele Z, HaileMariam D, Fuller C, Davey G (2008). Development and testing of a de novo clinical staging system for podoconiosis (endemic non-filarial elephantiasis). *Trop Med Int Health.* **13**(10):1277–83.

Tekola F, HaileMariam D, Davey G. Economic costs of endemic non-filarial elephantiasis in Wolaita Zone, Ethiopia. *Trop Med Int Health* 2006; 11: 1136–44.

Wanji S, Tendongfor N, Esum M, Che JN, Mand S, Tanga Mbi C, Enyong P, And Hoerauf A (2008). Elephantiasis of non-filarial origin (podoconiosis) in the highlands of north-western Cameroon. *Annals of Tropical Medicine & Parasitology.* **102** (6), 529–540.

Wanji S, Kengne-Ouafo JA, Datchoua-Poutcheu FR, Njouendou AJ, Tayong DB, Sofeu-Feugaing DD, Amvongo-Adija N, Fovennso BA, Longang-Tchounkeu YF, Tekola FA, Enyong PA, Newport MJ, and Davey G (2016). Detecting and staging podoconiosis cases in North West Cameroon: positive predictive value of clinical screening of patients by community health workers and researcher. *BMC Public Health.* **16:**997. DOI 10.1186/s12889-016-3669-6
Table 1: Sociodemographic characteristics of study subjects

| Class  | Frequency | Percentage (%) |
|--------|-----------|----------------|
| Age    |           |                |
| 18-30  | 40        | 17.9           |
| 31-40  | 54        | 24.1           |
| 41-50  | 58        | 25.9           |
| 51-60  | 72        | 32.1           |
| Sex    |           |                |
| Female | 192       | 85.7           |
| Male   | 32        | 14.3           |
| Occupation |     |                |
| Farmer | 100       | 44.64          |
| Business | 40    | 17.86          |
| Student | 15      | 6.70           |
| Teacher | 12      | 5.36           |
| Tailor | 11       | 4.91           |
| Others | 32       | 14.29          |
| Farmer + (other) | 14 | 6.25 |
| Podoconiosis stage |     |                |
| Stage 2 | 92     | 41.07          |
| Stage 3 | 123    | 54.91          |
| Stage 4 | 07     | 3.42           |
Table 2. Vital sign presentation of study participants

| Reference ranges | Class                    | Frequency | Percent (%) |
|------------------|--------------------------|-----------|-------------|
| **Temperature**  | Normal                   | 220       | 99.1        |
| 36.5-37.5        | High                     | 2         | 0.9         |
| **Pulse**        | Normal                   | 220       | 99.1        |
| 60-100           | Low                      | 2         | 0.9         |
| **Blood pressure** | Normal                   | 169       | 76.1        |
| <120/80          | High blood pressure      | 48        | 21.6        |
| >130/80-120      | Hypertensive crises      | 5         | 2.3         |
| >180/>120        |                          |           |             |
| **Total**        |                          | 222       | 100.0       |
Table 3: Haematological profiles of study participants

| Parameter          | Classification     | Stage 2 n (%) | Stage 3 n (%) | Stage 4 n (%) | Total n (%) | Reference range |
|--------------------|--------------------|---------------|---------------|---------------|-------------|-----------------|
| **WBC (x10^3/µl)**| Normal             | 69 (75.0)     | 95 (77.20)    | 5 (71.40)     | 169 (76.10) | 4.0-10.0        |
|                    | Abnormal           | 23 (25.0)     | 27 (22.0)     | 5 (28.60)     | 52 (23.40)  |                 |
| **HCT (%)**        | Normal             | 71 (77.20)    | 75 (61.00)    | 5 (71.40)     | 151 (68.00) | 36.0-48.0       |
|                    | Abnormal           | 21 (22.80)    | 47 (38.20)    | 2 (28.60)     | 70 (31.50)  |                 |
| **HBG (g/dL)**     | Normal             | 92 (100.00)   | 122 (99.20)   | 7 (100.00)    | 221 (99.50) | 11.0-15.0       |
| **MCH (g/dL)**     | Normal             | 32 (34.80)    | 37 (30.10)    | 0 (0.00)      | 71 (32.00)  | 80.0-99.0       |
|                    | Abnormal           | 60 (65.20)    | 85 (69.10)    | 5 (71.40)     | 150 (67.60) |                 |
| **MCV**            | Normal             | 63 (68.50)    | 79 (64.20)    | 3 (42.90)     | 145 (65.30) |                 |
|                    | Abnormal           | 29 (31.50)    | 44 (35.80)    | 4 (57.10)     | 77 (34.70)  |                 |
| **Eosinophil (%)** | Normal             | 90 (97.80)    | 119 (96.70)   | 7 (100.00)    | 216 (97.30) | 45-75           |
|                    | Abnormal           | 2 (2.20)      | 3 (2.40)      | 0 (0.00)      | 5 (2.30)    |                 |
| **Monocytes (%)**  | Normal             | 53 (57.60)    | 77 (62.60)    | 4 (57.10)     | 134 (60.40) | 21-45           |
|                    | Abnormal           | 39 (42.40)    | 45 (36.60)    | 3 (42.90)     | 87 (39.20)  |                 |
| **Neutrophil (%)** | Normal             | 33 (35.90)    | 55 (44.70)    | 0 (0.00)      | 92 (41.40)  | 0.10-0.28       |
|                    | Abnormal           | 59 (64.10)    | 67 (55.30)    | 0 (0.00)      | 129 (58.10) |                 |
| **PCT (%)**        | Normal             | 30 (32.60)    | 42 (34.10)    | 2 (28.60)     | 74 (33.30)  | 10.0-14.0       |
|                    | Abnormal           | 62 (67.40)    | 80 (65.00)    | 5 (71.40)     | 147 (66.20) |                 |
| **PDW**            | Normal             | 43 (46.70)    | 66 (53.70)    | 3 (42.90)     | 115 (51.80) | 7.4-10.4        |
|                    | Abnormal           | 49 (53.30)    | 56 (45.50)    | 1 (14.30)     | 106 (47.70) |                 |
| **MCHC (g/dL)**    | Normal             | 76 (82.60)    | 101 (82.10)   | 5 (71.40)     | 182 (82.00) | 32.0-36.0       |
|                    | Abnormal           | 16 (17.40)    | 21 (17.90)    | 2 (28.60)     | 39 (17.60)  |                 |
| **PLT (10^3uL)**   | Normal             | 92 (100.00)   | 122 (99.20)   | 6 (85.70)     | 220 (99.10) | 100-300         |
|                    | Abnormal           | 0 (0.00)      | 0 (0.00)      | 1 (14.30)     | 1 (0.50)    |                 |
| **RDWSD (fL)**     | Normal             | 20 (21.70)    | 24 (19.50)    | 1 (14.30)     | 45 (20.30)  | 39.0-46.0       |
|                    | Abnormal           | 72 (78.30)    | 98 (79.70)    | 6 (85.70)     | 176 (79.30) | 11.5-14.5       |
| **RDWCV (%)**      | Normal             | 66 (71.70)    | 78 (63.40)    | 5 (71.40)     | 149 (67.10) | 26.0-32.0       |
|                    | Abnormal           | 26 (28.30)    | 44 (35.80)    | 2 (28.60)     | 72 (32.40)  |                 |
| **MCH (pg)**       | Normal             | 67 (72.80)    | 88 (71.50)    | 2 (28.60)     | 157 (70.70) | 36.0-48.0       |
|                    | Abnormal           | 25 (27.20)    | 34 (27.60)    | 5 (71.40)     | 64 (28.80)  |                 |

WBC, White blood cell; RBC, red blood cell; HGB, haemoglobin; HCT, haematocrit; MCV, mean corpuscular volume; MCHC, corpuscular haemoglobin concentration; MCH, mean corpuscular haemoglobin; RDW, red cell distribution width; PLT, platelet count; MPV, mean platelet Volume; PCT, Plateletcrit; PDW, platelet distribution width.
Table 4: Red blood cell, white blood cell and platelet picture of study subject

| Cell types         | Frequency | Percent |
|--------------------|-----------|---------|
| **RBC SIZE**       |           |         |
| Macrocytic         | 4         | 1.8     |
| Microcytic         | 39        | 17.6    |
| Normocytic         | 170       | 76.6    |
| Others             | 9         | 4.2     |
| **RBC HB conc**    |           |         |
| Hyperchromic       | 3         | 1.4     |
| Hypochromic        | 58        | 26.1    |
| Normochromic       | 153       | 68.9    |
| Other              | 8         | 3.7     |
| **RBC shape**      |           |         |
| Burr cell          | 8         | 3.6     |
| Rouleux formation  | 9         | 4.1     |
| Ovalocyte          | 16        | 7.2     |
| **WBC**            |           |         |
| Normal             | 123       | 55.4    |
| Scanty             | 48        | 21.6    |
| Left shift of neutrophils | 8 | 3.6 |
| Lymphopenia        | 4         | 1.8     |
| Monocytosis        | 7         | 3.2     |
| Neutropenia        | 23        | 10.4    |
| Basophils          | 3         | 1.4     |
| Eosinophilia       | 11        | 5       |
| Leucopenia         | 2         | 0.9     |
| Lymphocytosis      | 5         | 2.3     |
| **Platelet**       |           |         |
| Normal             | 164       | 73.9    |
| Thrombocytosis     | 50        | 22.5    |
| Giants             | 48        | 21.6    |
| Large forms        | 14        | 6.3     |
Table 5: Biochemical parameters of blood serum in between control and all podoconiosis participants

| Parameter          | Classification | Stage 2        | Stage 3        | Stage 4        | Total n (%) | Reference range |
|-------------------|----------------|----------------|----------------|----------------|--------------|-----------------|
| Creatinine (mg/dl) | Normal         | 88 (95.70%)    | 106 (86.20%)   | 7 (100.00%)    | 201 (90.50)  | 0.7-2.8         |
|                   | Abnormal       | 4 (4.30)       | 16 (13.00%)    | 0 (0.00)       | 20 (9.00)    |                 |
| Gamma (U/L)       | Normal         | 90 (97.80)     | 118 (95.90)    | 7 (100.00)     | 215 (96.80)  | 11-100          |
|                   | Abnormal       | 2 (2.20)       | 4 (3.30)       | 0 (0.00)       | 6 (2.70)     |                 |
| AST/GOT (U/L)     | Normal         | 91 (98.90)     | 119 (96.70)    | 7 (100.00)     | 217 (97.70)  | 0-80            |
|                   | Abnormal       | 1 (1.10)       | 3 (2.40)       | 0 (0.00)       | 4 (1.80)     |                 |
| ALT/GPT (U/L)     | Normal         | 90 (97.80)     | 121 (98.40)    | 7 (100.00)     | 218 (98.20)  | 0-80            |
|                   | Abnormal       | 2 (2.20)       | 1 (0.80)       | 0 (0.00)       | 3 (1.40)     |                 |

ALT: alanine transaminase; AST: aspartate transaminase; GAMMA GT: gamma-glumyltransferase.