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Symptoms, immunology, and environmental risk factors of visceral leishmaniasis and malaria co-infections in West Pokot County, Kenya

Submission date
17/06/2022

Recruitment status
No longer recruiting

Registration date
11/07/2022

Overall study status
Completed

Last Edited
27/12/2024

Condition category
Infections and Infestations

[X] Prospectively registered

[X] Protocol

[_] Statistical analysis plan

[X] Results

[_] Individual participant data

Plain English summary of protocol

Background and study aims
Malaria and visceral leishmaniasis (VL) are two deadly diseases caused by parasitic infections. Both infections are commonly seen in the Kenyan county of West Pokot. As a result, the local population is at considerable risk of being infected with both VL and malaria at the same time. Nevertheless, few scientific studies have described the clinical presentation of patients with a VL-malaria co-infection. It is also unclear how the human immune system responds in these patients. During a co-infection, it is possible that the immune reaction caused by one infection will have a positive or negative effect on the course of the other, resulting either in milder or more severe symptoms. Because both VL and malaria are transmitted from human to human by blood-feeding insects, the risk of getting infected is largely dependent on exposure to these insects. In turn, insect exposure has been associated with environmental and household factors. So far, it has not been studied which household conditions are important for overlapping risk of VL and malaria in West Pokot. Because of these important knowledge gaps, the aim of this study will be to describe three different aspects of VL-malaria co-infections in West Pokot County, Kenya: 1) the clinical picture, 2) household and environmental risk factors associated this condition, and 3) the immunological profile of co-infected patients. Each of these three aspects of VL-malaria co-infections will be addressed in a separate part of this study.

Who can participate?
In the first part of this study, the clinical picture of VL-malaria co-infections will be studied. Patients coming to the Kacheliba District Hospital with symptoms of VL and/or malaria (any age and gender) can participate.
The second part of this study will look into household and environmental risk factors for getting infected with both VL and malaria. All participants of the first study part will automatically participate in this second part as well. Specifically for this second part, healthy volunteers from local communities are also invited to participate. These healthy controls need to have the same age, gender and village of residence as one of the VL-malaria co-infected participants of the study.
The third part of this study will investigate the immune response of patients with VL and/or malaria. Patients who are already participating in the first study part with a single VL or malaria

infection, or a VL-malaria co-infection, will be invited to participate in this follow-up study as well. Both male and female patients can participate, but these patients have to be between 6 and 30 years. Healthy household members (any gender, aged 6 or older) of these clinically ill participants of the follow-up study will also be asked to participate.

What does the study involve?
In the first part of this study, patients coming to the Kacheliba District Hospital with symptoms of VL and/or malaria will donate a few drops of finger prick blood. This will be used for standard VL and malaria diagnosis. If a patient tests positive for one or both diseases, he/she will be included in the study. Clinical symptoms will be documented and the already collected finger prick blood will be used for quantitative PCR. This technique can quantify the number of VL and malaria parasites in the blood. The clinical and PCR data will be used to describe the symptoms and parasite burden of patients with a VL-malaria co-infection, and to compare them to patients with a single VL or malaria infection.
For the second part of the study, a trained interviewer will administer a household questionnaire to all VL- and/or malaria-infected participants of the first study part. This questionnaire will ask about the participant's living and housing conditions, work and travels. Additionally, healthy volunteers will be recruited in the villages of residence of VL-malaria co-infected study participants. When a healthy volunteer has tested negative for VL and malaria with a rapid diagnostic test on finger prick blood, this person will be interviewed with the household questionnaire. The questionnaire outcomes of patients and healthy controls will be compared. In this way, associations between exposure to certain environmental and household factors and VL-malaria infections will be investigated.
In the third part of this study, small cohorts of patients with VL, malaria and VL-malaria co-infections will be followed during their standard treatment at the Kacheliba Hospital. Clinical symptoms and venous blood samples will be collected on different moments during the treatment of their infection(s): before the start of treatment (day 0), halfway during treatment (day 1 for malaria, day 7 for VL) and at the end of treatment (day 3 for malaria, day 17 for VL). Additionally, a healthy control group will be recruited among household members of participating patients. Symptomless household members will first be tested for VL and malaria using finger prick blood. When both tests are negative, the individual is included as healthy control. In case a person tests positive for VL or malaria but is not feeling ill, he/she will be included as asymptomatically infected participant. After the VL and malaria diagnosis is available, venous blood will be collected once from healthy and asymptomatic participants. When an asymptomatic participant requires treatment for the infection, this participant will also be followed up and sampled during the treatment, similar to the clinically ill participants of the cohort study.
The venous blood samples from the all participants of the cohort study will be analysed in the lab. Here, the blood levels of immunological markers (called cytokines) and VL and malaria parasites will be measured. In this way, the immune response in VL-malaria co-infected patients can be described and compared to the response in symptomatic and asymptomatic single infections.

What are the possible benefits and risks of participating?
As the study does not involve any treatment or dangerous clinical sample collection, the risks of participating are negligible. There is no direct benefit of participating in this study. However, the knowledge gained with this research could be helpful for future patients with VL and/or malaria in West Pokot.

Where is the study run from?
This study is coordinated by the unit of Experimental Parasitology of the Amsterdam University Medical Centres (Amsterdam UMC) in the Netherlands, and Amref Health Africa in Nairobi, Kenya.

When is the study starting and how long is it expected to run for?
November 2021 to February 2023

Who is funding the study?
The costs of this study will be paid by internal funding of the Experimental Parasitology unit of the Amsterdam UMC (Netherlands)

Who is the main contact?
Norbert van Dijk (Principal Investigator), n.j.vandijk@amsterdamumc.nl

Contact information

Type(s)
Principal Investigator

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Additional identifiers

EudraCT/CTIS number
Nil known

IRAS number

ClinicalTrials.gov number
Nil known

Secondary identifying numbers
Nil known

Study information

Scientific Title

Characterisation of clinical features, environmental risk factors and immunological interactions in visceral leishmaniasis and malaria co-infections in West Pokot County, Kenya

Acronym
LEISHMAL

Study objectives
Visceral leishmaniasis (VL) and malaria are two deadly parasitic diseases that co-exist in the Kenyan county of West Pokot. The local population is at considerable risk of being co-infected with VL and malaria. Nevertheless, few studies have described the clinical implications of this co-morbidity. Questions remain regarding potential aggravation or alleviation of VL and malaria symptoms during a co-infection, as well as the immune responses responsible for a possible predisposing or protective effect. Moreover, characterisation of household and environmental risk factors for co-acquiring VL and malaria is warranted, to increase awareness and guide implementation of targeted control strategies.

Ethics approval required
Old ethics approval format

Ethics approval(s)
Approved 26/05/2022, Amref Ethics and Scientific Review Committee (PO Box 30125-00100, Nairobi, Kenya; +254 (0)20 6994000; esrc.kenya@amref.org), ref: ESRC P1196/2022

Study design
Single-centre observational cross-sectional study with an embedded observational longitudinal cohort study and an ancillary observational case-control study

Primary study design
Observational

Secondary study design
Cross-sectional study with an embedded longitudinal cohort study and an ancillary case-control study

Study setting(s)
Hospital

Study type(s)
Other

Participant information sheet
Not available in web format, please use contact details to request a participant information sheet.

Health condition(s) or problem(s) studied
Clinical features, immunological interactions and environmental risk factors in patients with visceral leishmaniasis and malaria co-infections.

Interventions
Patients at the Kacheliba Sub-County Hospital with laboratory confirmation of visceral leishmaniasis (VL) and/or malaria will be included in the study. Their routine VL and malaria

diagnostic test results and clinical features will be documented, and finger prick blood will be collected for haemoglobin measurement and molecular diagnosis of malaria and VL through PCR. Additionally, a structured questionnaire will be administered to all study participants to investigate individual and household determinants associated with VL-malaria co-infections. This questionnaire will also be administered to village-, age- and sex-matched healthy control individuals in local communities.

Moreover, a small cohort of VL and malaria mono- and co-infected patients will be followed up during standard treatment of their infection(s). On multiple time points, clinical features will be registered and venous blood samples will be collected. These samples will be used for haemoglobin measurement, white blood cell counting, parasite density monitoring with PCR and for longitudinal serum cytokine measurements.

Healthy controls and asymptomatic VL and/or malaria cases will also be recruited among household members of the participants of the follow-up cohort. Their healthy or asymptomatic status will be determined through routine diagnostic tests on finger prick blood. In healthy controls, a single venous blood sample will be taken for haemoglobin measurement, white blood cell counting and serum cytokine measurements. The same will apply to subjects with asymptomatic VL and/or malaria, unless the local hospital clinician decides that the infection(s) needs to be treated. In that case, the asymptomatic patient will undergo the same procedures as the clinical patients in the follow-up cohort.

Intervention Type
Other

Primary outcome measure
For the cross-sectional study assessing the clinical features of VL and malaria (co-)infected patients, there is no primary outcome measure.
For the case-control study assessing individual and household determinants of VL-malaria co-infections, the primary outcome measures are the calculated odds ratios between the co-infected cases and healthy controls for the determinants measured with the structured questionnaire at baseline.
For the follow-up cohort study, the primary outcome measure is the cytokine profile measured using a Luminex-based assay at baseline (day 0) prior to treatment initiation

Secondary outcome measures
1. Cytokine profile measured using a Luminex-based assay at day 1 of malaria treatment
2. Cytokine profile measured using a Luminex-based assay at day 7 of VL treatment
3. Cytokine profile measured using a Luminex-based assay at the end of VL and/or malaria treatment

Overall study start date
01/11/2021

Completion date
01/02/2023

Eligibility

Key inclusion criteria

Inclusion criteria for clinically ill patients in the cross-sectional and case-control household questionnaire study:
1. Signed informed consent form of the cross-sectional study. In case of children, informed consent form is signed by parent or legal guardian; additionally, assent is also sought for children aged 12 – 17 years.
2. Showing symptoms suggestive of malaria and/or VL.
3. Having a laboratory-confirmed malaria diagnosis (positive malaria RDT, or thin and thick blood film, any species, parasite count <250,000/µL of blood) and/or VL diagnosis (DAT titre ≥ 1:3200 and/or positive rk39 RDT).
4. Living in the catchment area of the study hospital.

Inclusion criteria for healthy controls in the case-control household questionnaire study:
1. Signed informed consent form of the questionnaire study. In case of children, informed consent form is signed by parent or legal guardian; additionally, assent is also sought for children aged 12 – 17 years.
2. Living in the village of residence of a VL-malaria co-infected participant of the cross-sectional study.
3. Showing no symptoms suggestive of malaria and/or VL.
4. Having no history of clinical malaria in the preceding 2 weeks.
5. Having no history of clinical VL.
6. Having a negative malaria diagnosis with RDT.
7. Having a negative VL diagnosis with rk39 RDT.

Inclusion criteria for clinically ill patients in the follow-up cohort study:
1. Included in the cross-sectional study.
2. Signed informed consent form of the follow-up study. In case of children, informed consent form is signed by parent or legal guardian; assent is sought for children aged 12 – 17 years.
3. Between 6 and 30 years of age.
4. Showing symptoms suggestive of malaria and/or VL.
5. Having a laboratory-confirmed malaria diagnosis with P. falciparum (parasite count between 1000 and 250,000 /µL of blood) and/or VL diagnosis.
6. Being enrolled in the national treatment programme for malaria and/or VL after a laboratory-confirmed diagnosis of either disease.
7. Living in the catchment area of the study hospital.

Inclusion criteria for patients with asymptomatic infections and healthy endemic controls in follow-up cohort study:
1. Signed informed consent form. In case of children, informed consent form is signed by parent or legal guardian; assent is sought for children aged 12 – 17 years.
2. Living in the household of one of the clinically ill participants of the follow-up study.
3. More than 6 years of age.
4. Showing no major symptoms suggestive of malaria and VL.
5. Having positive or negative results of tests for malaria and/or VL
6. Having a body temperature below 37.5°C.
7. Having no history of a clinical VL infection.

Participant type(s)
Mixed

Age group
Mixed

Sex
Both

Target number of participants
Cross-sectional study: 484; case-control study: 10-60 cases, 30-180 controls; cohort study: 6 groups (clinical and asymptomatic VL/malaria mono-infections, VL-malaria co-infections, healthy controls), 20-30 per group

Total final enrolment
168

Key exclusion criteria
The following exclusion criteria apply to all study components (cross-sectional, case-control and cohort):
1. Not giving their informed consent.
2. Showing signs or symptoms as the ones described for active VL or malaria, but with no laboratory confirmation of an ongoing malaria or VL infection (negative rk39 RDT and negative DAT for VL, and negative blood smear for malaria).
3. Having a Plasmodium parasite count >250,000/µL of blood.
4. Being already under drug treatment for malaria and/or VL.
5. Having a positive VL diagnosis in their medical history (in order to exclude false-positive VL diagnoses based on positive serology, and to exclude relapses).
6. Being pregnant.
7. Not living in the selected catchment area.

The following exclusion criteria apply specifically to all participants (clinically ill, asymptomatic and healthy) of the cohort study:
1. Being younger than 6 years.
2. Having a haemoglobin level of ≤7 g/dL.
3. Being diagnosed with malaria caused by a Plasmodium species different than P. falciparum.
4. Suffering from any other infectious disease, acute or chronic, different from malaria and/or VL, of which the patient has knowledge (e.g. tuberculosis, HIV, helminth infections).
5. Suffering from any immune system disorder, acute or chronic, of which the patient has knowledge, such as rheumatoid arthritis, asthma, leukaemia or immunosuppressive disorders.
6. Being under antimicrobial and/or anti-inflammatory treatment.
7. Being under immune-suppressive or immune-stimulatory treatment.

The following exclusion criteria apply to only the clinically ill patients of the follow-up cohort study:
1. Not eligible for first-line treatment for VL and/or malaria infection as stated in the national treatment guidelines (e.g. due to contraindication).
2. Being older than 30 years.

Date of first enrolment
01/11/2022

Date of final enrolment
08/01/2023

Locations

Countries of recruitment
Kenya

Study participating centre
Kacheliba Sub-County Hospital
P.O Box 50
Kacheliba
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Kenya
30601

Sponsor information

Organisation
Academic Medical Center

Sponsor details
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Sponsor type
Hospital/treatment centre

Website
https://www.amsterdamumc.org

ROR
https://ror.org/03t4gr691

Funder(s)

Funder type
Other

Funder Name
Investigator initiated and funded

Results and Publications

Publication and dissemination plan
Current publication and dissemination plan as of 12/07/2023:

The peer-reviewed study protocol has been published in BMJ Open. Once the study has been completed, findings regarding the case-control analysis of the cross-sectional data, the household analyses, and immunological investigations of the follow-up study will all be communicated to the national health authorities of Kenya. Scientists included in the research team will write scientific papers on the results of the study, which will be submitted to peer-reviewed international open-access scientific journals. In addition, the results of the study will be presented at national and international scientific meetings addressing the topic of malaria and VL. Furthermore, the results will also be spread to the local staff and to the involved community.

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Previous publication and dissemination plan:

The study protocol is planned to be submitted for publication prior to completion of data collection. Once the study has been completed, findings regarding the case-control analysis of the cross-sectional data, the household analyses, and immunological investigations of the follow-up study will all be communicated to the national health authorities of Kenya. Scientists included in the research team will write scientific papers on the results of the study, which will be submitted to a peer-reviewed international open-access scientific journals. In addition, the results of the study will be presented at national and international scientific meetings addressing the topic malaria and VL. Furthermore, the results will also be spread to the local staff and to the involved community.

Intention to publish date
01/11/2024

Individual participant data (IPD) sharing plan
The datasets generated during and/or analysed during the current study will be available upon request at the principal investigator, Norbert van Dijk (n.j.vandijk@amsterdamumc.nl). The data that will be available for sharing will include participant clinical data, diagnostic data, household questionnaire data and data generated through laboratory analysis of blood samples (parasite densities, cytokine concentrations, haemoglobin level, white blood cell count). Each dataset will be made available for an indefinite period, simultaneously with the publication of the scientific article following from the analysis of this particular dataset. The access criteria and sharing mechanism will be established at a later time point. All study participants will be asked to give their informed consent upon enrollment for the use of their collected data and samples in future research studies. All stored data will be fully anonymized and linked to a unique patient identification number. Sharing of the datasets with external parties will be preceded by signing of a Data Transfer Agreement, drafted with support of the legal department of the Amsterdam UMC.

IPD sharing plan summary
Available on request

Study outputs

Output type	Details	Date created	Date added	Peer reviewed?	Patient-facing?

Protocol article	17/04/2023	18/04/2023	Yes	No
Results article	04/09/2024	27/12/2024	Yes	No
Results article	23/09/2024	27/12/2024	Yes	No