BMJ Open

Inflammatory bowel disease in sub-Saharan Africa: a protocol of a prospective registry with a nested case–control study

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ABSTRACT

Introduction The epidemiology of inflammatory bowel disease (IBD) in sub-Saharan Africa is poorly documented. We have started a registry to determine the burden, phenotype, risk factors, disease course and outcomes of IBD in Zimbabwe.

Methods and analysis A prospective observational registry with a nested case–control study has been established at a tertiary hospital in Harare, Zimbabwe. The registry is recruiting confirmed IBD cases from the hospital, and other facilities throughout Zimbabwe. Demographic and clinical data are obtained at baseline, 6 months and annually. Two age and sex-matched non-IBD controls per case are recruited—a sibling or second-degree relative, and a randomly selected individual from the same neighbourhood. Cases and controls are interviewed for potential risk factors of IBD, and dietary intake using a food frequency questionnaire. Stool is collected for 16S rRNA-based microbiota profiling, and along with germline DNA from peripheral blood, is being biobanked. The estimated sample size is 86 cases and 172 controls, and the overall registry is anticipated to run for at least 5 years. Descriptive statistics will be used to describe the demographic and phenotypic characteristics of IBD, and incidence and prevalence will be estimated for Harare. Risk factors for IBD will be analysed using conditional logistic regression. For microbial analysis, alpha diversity and beta diversity will be compared between cases and controls, and between IBD phenotypes. Mann-Whitney U tests for alpha diversity and Adonis (Permutational Multivariate Analysis of Variance) for beta diversity will be computed.

Ethics and dissemination Ethical approval has been obtained from the Parirenyatwa Hospital's and University of Zimbabwe's research ethics committee and the Medical Research Council of Zimbabwe. Findings will be discussed with patients, and the Zimbabwean Ministry of Health. Results will be presented at scientific meetings, published in peer reviewed journals, and on social media.

Trial registration number NCT04178408.

Strengths and limitations of this study

► Research in a unique, under-studied population at an early stage of the IBD epidemic.
► The study uses contemporary microbiota analysis techniques to investigate determinants of IBD phenotype in an African population.
► The study includes a control group, addressing a deficiency of most registry-based studies.
► Potential challenge in achieving the desired sample size as IBD is potentially rare, and underdiagnosed in this population.

countries, and is reportedly rare in sub-Saharan Africa.[1] However, many countries in sub-Saharan Africa are experiencing a rapid increase in diseases traditionally associated with westernisation, particularly, diabetes mellitus and obesity.[2 3] Furthermore, the incidence of other large bowel diseases, which were previously considered rare, such as colorectal cancer, is increasing.[4] Thus, it is reasonable to expect that the incidence of IBD could also be on the rise.

However, there is limited data on the burden of IBD in sub-Saharan Africa. Though relatively uncommon, ulcerative colitis is believed to be the predominant phenotype, while Crohn's disease is rarely diagnosed.[5] This is consistent with historical accounts of IBD in industrialised countries, which suggest that ulcerative colitis came to the fore before Crohn's disease.[6] The earlier increase in incidence of ulcerative colitis has also been described in the newly industrialised countries of Asia and South America.[6] However, the situation in sub-Saharan Africa is mostly speculative, and the precise epidemiology, phenotype, course and associated risk factors have not been comprehensively studied.

INTRODUCTION

Inflammatory bowel disease (IBD) is considered mostly a disease of high-income

Therefore, we are establishing a prospective registry in Zimbabwe to describe the phenotype, disease course and outcomes of IBD in a population in sub-Saharan Africa. Furthermore, a nested case–control study will be performed to establish the risk factors of IBD in this population. This registry offers a unique opportunity to study the evolution of a potentially early epidemic of IBD using powerful modern techniques that were not available at a similar stage of the epidemic in Europe and North America.

OBJECTIVES

The aim of this study is to establish a prospective registry of IBD registry at a tertiary care hospital in Harare, Zimbabwe. The database will be used to: (1) describe the demographic and phenotypic characteristics of IBD in the Zimbabwean population, (2) estimate the incidence, and prevalence of IBD in Harare, (3) determine the risk factors of IBD among Zimbabweans, (4) describe the microbial networks associated with IBD, and disease phenotype in this African population and (5) establish a biobank with faecal samples, intestinal biopsies and germline DNA for subsequent research on the aetiology, and evolution of IBD in an African population at an early stage of the potential IBD epidemic.

METHODS AND ANALYSIS

Study design

This is a prospective observational registry, with a nested case–control study. The registry will be based in Harare, Zimbabwe, but recruitment will be open to patients from across the country. The nested case–control study will enable the registry to have a control group.

Study population

The registry will be based at the gastrointestinal clinical at Parirenyatwa Hospital in Harare, Zimbabwe. Parirenyatwa Hospital is one of the two teaching hospitals of the University of Zimbabwe, and functions as a tertiary referral centre for the northern two-thirds of the country, and a secondary level hospital for Harare. The gastrointestinal clinic at Parirenyatwa Hospital was established in January 2018, after a hiatus of more than 20 years. It is the only dedicated gastroenterology clinic in the public sector in Zimbabwe. Although the registry will be based at the gastrointestinal clinic, patients from other hospitals throughout the country, including private institutions and medical practices, will be eligible for recruitment. It is hoped that this will facilitate the provision of specialist gastroenterology services across the country, particularly for suspected cases of IBD. As of March 2020, Zimbabwe, with 14 million people, has only three practising gastroenterologists, all of them in Harare.

A nested case–control study will also be performed with the aim of determining the risk factors of IBD. Two controls will be recruited for each case. These will be either a sibling for each case or second-degree relative of a similar age (±5 years), and an individual from the same neighbourhood, matched for age (±5 years) and for gender. Recruiting relatives should be quite feasible in Zimbabwe, as the extended family structure is intact, and considered as important as the nuclear family.

Eligibility criteria

Any patient, of any age with confirmed IBD in Zimbabwe, with disease duration of at least 3 months will be eligible for enrolment into the registry. The diagnosis will require concurrence of two gastroenterologists, and pathology slides will be independently reviewed at the University of Bern, Switzerland. The case–control study will be restricted to adult cases; thus, all the corresponding controls will be older than 18 years of age. These controls will be a sibling or second-degree relative, and a resident in the same neighbourhood of a similar age (±5 years), and gender. All persons with unexplained gastrointestinal symptoms, including diarrhoea, vomiting, chronic abdominal pain, weight loss, rectal bleeding and recent change in bowel habits, will be excluded as controls. Unexplained major gastrointestinal surgery in the past will be an exclusion criterion, to avoid inadvertently, including a previously undiagnosed case of IBD as a control. All potential controls will have a baseline calprotectin, and those with a sustained level >200 mg/kg will be screened for IBD. If uncertainly remains about whether they actually have IBD, they will be excluded from the study.

Study procedures

Patients with IBD will be approached during routine visits to the gastroenterology clinic, and invited to participate. Clinicians in other hospitals and in private practice will also be informed about the registry and encouraged to refer patients for recruitment. Information about the registry will be disseminated to gastroenterologists, physicians, surgeons, general practitioners and pathologists around the country using various channels, particularly, meetings of various professional associations, and appropriate professional social media groups. Both incident and prevalent cases will be considered for enrolment.

After informed consent, demographic and clinical data will be obtained for all cases using standardised case report forms at baseline, after 6 months and annually thereafter. A stool smear will be evaluated for parasites. Concurrently, two healthy controls per case will be recruited for a nested case–control study. The controls will be the first consenting sibling nearest in age to the case. If there is no appropriate sibling, a second-degree relative meeting the eligibility criteria will be invited to participate. The neighbourhood control will be randomly selected from the same local government ward as the corresponding case, by screening from door to door until an eligible individual is encountered. Care will be taken to ensure confidentiality in this process.

Both cases and controls will be interviewed using a questionnaire for risk factors of IBD, adapted from the

International Organisation for the study of Inflammatory Bowel Disease (online supplemental file A).[7-9] The adapted questionnaire takes into account factors unique to sub-Saharan Africa, such as the high burden of infectious diseases, including HIV and tuberculosis (TB), and cultural factors. Dietary factors will be assessed in both cases and controls using a validated semi-quantitative food frequency questionnaire (online supplemental file B).[10 11] Inflammation will be confirmed in cases, or excluded in controls using calprotectin measurements, and clinical evaluation. Whenever necessary, tissue slides from the various pathology laboratories will be reviewed by a single study pathologist (RM-M), and all slides will undergo a blinded review at the University of Bern (AB). The diagnosis of IBD will require the concurrence of two gastroenterologists in all cases (LK, WFM and IG). Microbiota profiling of cases and controls will be carried out at the University of Bern using the Ion Torrent PGM platform in which V5–V6 region of bacterial 16S rRNA will be sequenced. A subset of samples will be sequenced on the Illumina platform for deeper microbiota resolution. In parallel, the absolute number of bacteria per gram of faecal samples will be assessed using flow cytometry. Serial stool specimens, and germline DNA from peripheral blood in both cases and controls will be biobanked at −80°C in the Department of Medicine, University of Zimbabwe.

Study factors

The following parameters will be collected from the participants in this study:
► Demographic data (age, gender, ethnicity, etc).
► For cases only:
  – clinical data, including age at diagnosis, disease phenotype (Crohn's disease vs ulcerative colitis, location and behaviour) and other clinical, endoscopic, histological and radiographic features.
  – Current and previous treatment, including surgery.
  – Assessment of disease activity (Modified Truelove and Witts Activity Index and Harvey Bradshaw Index) at least yearly. Intermittent flares or IBD-related hospitalisations will be recorded.
  – Laboratory values: we aim for annual calprotectin measurements in all cases.
  – Long-term effects of IBD on health of participants (eg, comorbidities, malignancies, osteoporosis, pain, psychiatric comorbidities, etc).
► HIV status.
► Medical history.
► Current and previous TB status (a WHO TB screening tool will be administered to all participants). We recognise that intestinal TB and Crohn's disease can be virtually indistinguishable at times and it is the policy of our unit to start TB treatment before starting any immunosuppressive medication in such cases.
► Family history of IBD.
► Socioeconomic data—income, education and occupation.

► Data on common environmental risk factors of IBD, including nutrition, body mass index, smoking history, breastfeeding, exposure to mass drug campaigns in childhood, family size and various markers of sanitation and hygiene.
► Migration history (rural to urban, Zimbabwe to other countries).

Biobanking

Annual stool samples, germline DNA and urine specimens will be collected and stored at −80°C in the Department of Medicine, University of Zimbabwe, for future studies. Broad consent will be obtained from all participants, and this has already been approved by relevant ethical review committees.[12]

Statistical plan

The sample size was calculated for the nested case–control study as the registry will mainly generate descriptive data. Exposure to farm animals was taken as a proxy for risk factors for IBD. An inverse relationship between exposure to farm animals and IBD has been previously demonstrated in an umbrella review of meta-analyses of environmental risk factors (OR: 0.44; 95% CI: 0.14–0.74).[13] According to the Zimbabwe Health and Demographic Survey of 2015, 66% of the population have no access to electricity, and we assumed that this should roughly correspond to the proportion of the population living near farm animals.[14] Therefore, the estimated sample size was 86 IBD cases and 172 non-IBD controls, to detect such a difference with a χ^2 test, two matched controls per case, a correlation coefficient of 0.2, power 80% and two-sided alpha of 0.05.[15] The registry is expected to run indefinitely, beginning March 2020, with 5 years as the absolute minimum, which will maximise the sample size.

Descriptive statistics, including percentages (with 95% CIs), mean (with SD) and median (with IQR) will be used to summarise data on demographic and phenotypic characteristics of IBD. This data will also be compared with the Swiss IBD cohort study.[16] The incidence of IBD in Harare will be estimated from the number of new cases identified per year, and the current estimated population of Harare. While cases from outside Harare will be included in the registry, they will be excluded in computing the incidence and prevalence of IBD, so that we have a defined denominator. The numerator for the incidence and prevalence should become more precise as the registry becomes established, and recruitment from the population approaches saturation level. The next census in Zimbabwe is scheduled for 2022, and this will give a contemporary denominator. If this data are not released timeously, then projections from the last census in 2012 will be used.

For the case–control study, we recognise that the sibling control may potentially mask significant environmental factors. We will identify such factors by carrying out the statistical analyses in the following sequence: cases versus neighbourhood controls, cases versus sibling controls

and cases versus neighbourhood and sibling controls combined. In all instances, cases and controls will initially be compared using χ^2 test for categorical and t-test for continuous data. Univariate and multivariate logistic regression will be performed to determine the independent risk factors associated with IBD in this population. ORs and their CIs will be computed for this purpose.

For the microbial analysis, alpha diversity (observed operational taxonomic units and Simpson and Shannon indices) and beta diversity (Bray-Curtis genus-level community dissimilarities) will be calculated. Mann-Whitney U tests for alpha diversity and Adonis (Permutational Multivariate Analysis of Variance) for beta diversity will be used to confirm that the strength and statistical significance of groups in the same distance metrics in the QIIME pipeline and phyloseq.[17 18]

Patient and public involvement

This study was partly borne out of the question the lead author gets from patients about whether this disease is common in Africa. Often, the patients with IBD in Zimbabwe have not met anyone else with the disease. So, we have had discussions at the individual patient level about the need for studies to document the disease in the population. These discussions occurred before the protocol was written and continued throughout the design phase. However, the patients were not formally asked about their opinions regarding various aspects of the study design or the burden the study will place on their time. There are plans to involve the patients in the dissemination of the results as the registry will be a platform to form a patient advocacy groups as this is a neglected disease in Zimbabwe, with very little support for investigations and treatment.

ETHICS AND DISSEMINATION

Ethical approval has been obtained from the Parirenyatwa Hospital's and University of Zimbabwe's joint research ethics committee (JREC) (JREC 159/19), and the Medical Research Council of Zimbabwe (MRCZ) (MRCZ/A/2499).

The proposed study is an observational registry, and poses minimal risk to participants. All data will be anonymised before entry into electronic databases. Broad consent will be obtained from participants for purposes of biobanking,[12] and the consent process has been comprehensively evaluated by the ethical review committees (JREC and MRCZ). Community engagement is critical in this study, and on-going and discussions with IBD patients are underway. The findings will be disseminated to patients, the major hospitals in Zimbabwe and the Zimbabwean Ministry of Health. There are no IBD patient groups in Zimbabwe at the moment, and the findings will be used for advocacy, and to motivate the formation of such groups and other support services. They will be presented at national, and international meetings, and published in peer-reviewed journals, and disseminated using social and broadcast media.

DISCUSSION

This study is expected to provide seminal findings on IBD in sub-Saharan Africa, with a potential for substantial impact on clinical management and resource allocation in this population. Furthermore, the registry represents a unique opportunity for a prospective study in a population where data on the burden, disease phenotype and outcomes of IBD are almost non-existent.

The study team and collaborating institutions have diverse strengths, which will enable comprehensive research on the disease in an under-studied population. It is expected that cutting-edge techniques on the interactions between the microbiome and the host in IBD will provide unique insights on the disease evolution and the factors determining phenotype. Commendably, the study was conceptualised by the local researchers in Zimbabwe, a low-resource country and this will facilitate an equitable north–south collaborative relationship.

Furthermore, we have included a healthy control group from the outset, which will minimise the limitations arising from lack of comparison groups that are intrinsic to most registry-based studies. Our major concern is the possibility of a slow accrual rate and ultimately a small sample size. The available, though limited data, suggests that IBD occurs infrequently in this population.[5] We anticipate that this will be mitigated by the relatively low mortality associated with IBD, and the open-ended study period. With a low mortality, an appreciable number of cases will eventually accrue over time if retention mechanisms are robust (although slowly). Another potential limitation arises from referral bias. Since the registry will be hospital based, it is possible that a disproportionate amount of the severe cases will be recruited, while mild cases in the community may never get referred and reported. This will be acknowledged in discussing the results, and may affect descriptions of the phenotypes, and underestimate the incidence and prevalence of IBD in Zimbabwe. However, we plan to use the study to raise the awareness of IBD among practitioners in Zimbabwe, and the general population, and this may reduce the impact of such referral bias in the long-term.

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