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Original Research

Assessment of Co-Infection in Tuberculosis Patients Prior to their Enrollment to the Direct Observed Treatment (Dot) Program in Buea, Limbe, and Douala Tuberculosis Treatment Centers

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ABSTRACT

Introduction: Co-infections during Tuberculosis infection are among the leading causes of morbidity and mortality worldwide. These infections are a major public health problem among patients in most developing countries, including Cameroon. One of the important public problems in co-endemic areas is co-infection with TB in humans. Moreover, there is no adequate research hitherto that provides information on the level of co-infection in Tuberculosis patients. Therefore, this study aimed to assess the prevalence and intensity of co-infections in newly diagnosed Tuberculosis patients prior to their enrollment to the Direct Observed Treatment (DOT) program in Buea, Limbe and Douala Tuberculosis treatment centers. **Methods:** This cross-sectional study was conducted from March to November 2020 using a purposive sampling technique. A fresh sputum sample was collected, processed, and examined through a smear microscopy and culturing technique. Blood samples were collected through finger pricking for the detection of blood parasites using thin and thick blood smears. Stool samples were also collected and processed using a direct wet mount, and Kato-Katz and Modified Ziehl Neelsen staining methods. The data was analyzed using SPSS version 20.0. A Chi-square test was done to assess the prevalence of co-infection among all TB patients recruited for this study. **Results:** A total of 200 newly diagnosed TB-positive patients were recruited from the three study sites. Out of the total participants recruited, 29.0% were diagnosed positive for blood parasites, 2.0% for intestinal parasites and 15.0% for viral infections, bringing the overall prevalence of co-infection to 46.0%. When specific infections were considered, malaria, filaria, intestinal parasites, HIV and HBV constituted 28.0%, 1.0%, 2.0%, 14.5% and 0.5% respectively. When dual-infection was considered, the results were 0.5% malaria/filaria, 1.5% malaria/intestinal parasites and 2.0% malaria/HIV. The mean parasite densities for all samples diagnosed positive for malaria, filarial and intestinal parasites were 107.0 ± 338.8 parasites/uL, 25.7 ± 356.4 mf/mL and 85.0 ± 85.4 EPG, respectively. **Conclusion**: The results indicate that there is an overlap of co-infections in TB patients with their interactions expected to lead to complications and difficulty when managing the TB patients, calling for more research and recommending more assessment for co-infection in TB patients before starting treatment.

Key words: Tuberculosis, Assessment, Co-infections, Treatment Centers

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INTRODUCTION

Tuberculosis (TB) is one of the leading causes of morbidity and mortality worldwide[1,2]. TB is caused by *Mycobacterium tuberculosis* (MTB), which is predominantly airborne. A quarter of the world's population carries MTB in their bodies. Infecting more than 10 million people worldwide and killing up to 2 million people makes TB the deadliest infectious disease globally[3].

Africa has the highest rate of TB cases at 237 per 100,000 population[4]. In Cameroon, TB infection is the most common opportunistic infection associated with AIDS which may account for the high prevalence in the middle age group[5]. Both tuberculosis and parasitic diseases cause significant harm in humans[6,7] and they mostly affect people of a low socioeconomic status living in disadvantaged communities[8]. These diseases are not only related to poverty but, they also overlap in the same geographical region and are found to have significant consequences for public health[9]. Co-infection diseases are the leading cause of disability in low-income countries[10]. In addition, these diseases are not restricted to the elderly as young people are also greatly affected[11]. Intestinal parasitic infections are among the most common infections, affecting approximately 3.5 billion people worldwide[7] and causing more than 450 million ill health problems[12]. Protozoa, helminths, and ectoparasites are the three

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major parasite groups that can cause disease in humans [13]. Acute respiratory infections, HIV/AIDS, diarrhea, malaria and TB, with malnutrition as a common contributor, are the world's five foremost deadly infectious diseases [10].

Co-infection diseases are widely distributed in Cameroon like most developing countries, partly due to the geographical location of the region, the low level of environmental and personal hygiene, and the consumption of undercooked meat. It is therefore of utmost importance to assess the prevalence of co-infection in TB patients in public health activities. To this end, this research was conducted in the TB treatment centers of Buea, Limbe and Douala to assess the rate of infectious diseases (blood and intestinal parasites, HIV and hepatitis) among newly-diagnosed patients with TB.

METHODS

Study sites

A hospital-based cross-sectional study was conducted to recruit newly-diagnosed TB patients from three sites across Cameroon. The sites were two treatment centers in the Southwest region, namely Buea and Limbe. The third study area was in Douala. Located in the Littoral region, there are six health districts (Newbell, Logbaba, Deido, Cité des Palmiers, Cite-sic and Bonassama health districts) and a Catholic Hospital (Barcelone). The treatment centers were selected due to the ease of access to the health centers and patient flow.

Study design, population and sampling technique

Newly diagnosed smear-positive TB patients were recruited prior to their enrollment in the Direct Observed Treatment (DOT) program. Samples from bacteriologically confirmed patients with TB along with the patients' demographic and medical history were obtained using a purposive sampling technique from March 2020 to November 2020. Blood and stool samples were analyzed using microscopy. To calculate the sample size, a single population proportion formula was used. The participants in the study were enrolled consecutively until the target sample size of 200 was achieved. The purpose of the study was explained to the participants before the administration of the questionnaire including the procedure, the duration of the study, and the risks and benefits associated with the study. The participants were also informed of the study's anonymity and that during the research period only codes were to be used regarding them. The participants signed the consent form after they agreed to participate in the study.

A structured questionnaire containing questions on the participants' socio-demographic and disease characteristics was used. Socio-demographic variables including age, gender, marital status, level of education, place of residence and occupation were collected from the participants.

A calibrated weighing scale (Seca 760) and stadiometer (Seca 213) were used to measure the patient's body mass index (BMI). A digital thermometer (Royal Caremodel: MT1027, SOJOY ELECTRONICS, China) was used to measure the axillary temperature of the participants. Furthermore, by looking for the BCG scar on the participant's arms, the Bacillus Calmette Guerin (BCG) vaccination status of the individuals was also collected.

Eligibility criteria

Patients with newly diagnosed smear-positive TB were enrolled in the study. Pregnant women and individuals under the age of 18 years, patients who were critically ill or patients with severe complications such as mental illness and TB patients under treatment were excluded from this study.

Sample collection and processing

Before the start of the TB treatment, the desired quantity of fresh sputum sample was collected using falcon tubes from the recruited patients. The sputum samples were kept in ice-cooler containers for transport from the study sites to the Research Foundation for Tropical Diseases and Environment (REFOTDE) lab for further analysis. Each fresh sputum sample collected from each individual was processed and examined on the same day to identify Acid-Fast Bacilli (AFB) using the Ziel Neelsen staining (ZN) technique for smear microscopy. To confirm the smear microscopy, a sputum culture based on the Lowenstein-Jensen medium was also done.

After the levels of hemoglobin (Hgb) and hematocrit (Hct) were measured, approximately 6 μL of blood was placed on a clean microscope slide for a thick smear and 3 μL for a thin smear for malaria parasite detection and speciation, respectively. Using 75μL capillary tubes, thick blood films for filarial detection were prepared separately using standardized 50 μL finger pricked blood. The thin film for malaria and thick film for filaria blood smears were methanol-fixed for one minute, and both thick and thin blood smears were later stained with a 10% Giemsa stain solution. The malaria parasitemia (MP) (parasites/μL)

was determined by counting the number of parasites present in the thick blood smear to 200 white blood cells and using 8000 white cells per μL of blood [14] as a reference.

The desired quantity of fresh stool specimens were collected from the recruited patients and were analyzed at the end of each day. The samples were processed using a direct wet mount and Kato-Katz and Modified Ziehl Neelsen (mZN) staining methods.

Data analysis and interpretation

All data was entered into Microsoft Excel 2013 and analyzed using the Statistical Package for Social Science (SPSS) version 20.0. The data was summarized into means (x̄) and standard deviation (SD) and percentages were used in the descriptive statistics assessment. The Chi-square test was calculated to assess the prevalence of the possible co-infections among all TB patients by age and sex and according to their socio-demographic and clinical characteristics. The prevalence and outcome were considered to be statistically significant at a confidence interval of 95% and a p-value of less than or equal to 0.05.

Ethical considerations

Ethical approval for this study was obtained from the Faculty of Health Sciences Institutional Review Board (FHSIRB) of the University of Buea (Reference number: 2020/1205-05/UB/SG/IRB/FHS. Written and verbal consent was obtained from the participants before they were enrolled in the study.

RESULTS

Socio-demographic characteristics

A total of 200 newly diagnosed TB positive patients were recruited from the three study sites, namely Douala (148, 74%), Limbe Regional Hospital (28, 14%) and Buea Regional Hospital (24, 12%) TB treatment centers. The socio-demographic characteristics of the study participants have been summarized in **Table 1**. The majority of the participants were male with a mean age of 36.0 (SD $\pm$ 12.6) years. The majority of the study participants were urban residents (89.5%), married (52.4%) and had attained secondary (72.0%) or tertiary (13.5%) education.

Clinical characteristics

The proportions of participants reporting a cough, hemoptysis (blood in sputum), dyspnea (shortness of breath), chest pain, and fever were 98.0%, 17.1%, 47.5%, 41.2% and 41.2%, respectively. Out of the 196 study participants, 39.3% had normal hemoglobin levels, 25.5% moderate, 31.6% mild and 3.6% were severely anemic with a mean hemoglobin concentration of 12.3 g/dL (SD $\pm$ 2.4). Regarding temperature (n = 182), 1.6% were hypothermia, 57.7% normal and 40.7% hyperthermic with a mean body temperature of 37.2^{o}C (SD $\pm$ 0.9). The majority of the study participants (n = 168) 81.0% were assessed as having a scar on their arm from the BCG vaccination. The BMI analysis revealed 24.5% of the participants to be underweight, 9.5% overweight and 64.0% of a normal weight with a mean BMI of 20.9 kg/m^2 (SD $\pm$ 3.5). The mean hematocrit level and Middle Upper Arm Circumference (MUAC) of the participants was 36.3% (SD $\pm$ 7.2) and 25.5cm (SD $\pm$ 3.3), respectively.

Prevalence of co-infections

As shown in **Table 2**, out of the total participants recruited to the study, 58 (29.0%) were diagnosed positive for blood parasites, 4 (2.0%) for intestinal parasites and 30 (15.0%) for viral infections, hence the overall prevalence of co-infection was 92 (46.0%).

Prevalence of malaria

Based on the thin film microscopic examination of blood specimens for the detection of the malaria parasite, only one species was found, hence the overall prevalence of malaria being 56 (28.0%). All participants with malaria in our study had *Plasmodium falciparum* and it was more common in males 39 (19.5%) than females. A higher prevalence of malaria (17.6%) was found in the participants who had a habit of staying out into the night, who stored water in a closed container, who used a mosquito net every day, who did not use standing water, and who had bushes around their houses.

Prevalence of filarial

Using the thick film microscopic examination of blood specimens, 2 (1.0%) male participants recruited from Barcelone Catholic Hospital and Bonassama District Hospital were diagnosed positive with *Loa-loa* microfilaria.

Prevalence of intestinal parasites

Two species of intestinal parasites were identified in 4 participants (2.0%) following the microscopic examination of the stool specimens. Out of the total detected parasites, 3 (1.5%) were identified as *Ascaris lumbricoides* and one (0.5%) as *Entamoeba histolytica/dispar*. Females and males were both infected, totaling 2 (1.0%) patients, with each of the intestinal parasites and the two participants who were diagnosed with intestinal parasites were from Buea regional hospital. Intestinal parasites were common

Table 1: Socio-demographic characteristics of the study participants

Parameters		Gender		
		Male n (%)	Female n (%)	Total n (%)
Sites	Buea	14 (7.0%	10 (5.0%)	24 (12.0%)
	Limbe	14 (7.0%)	14 (7.0%)	28 (14.0%)
	Total	96 (48.0%)	52 (26.0%)	148 (74.0%)
		124 (62.0%)	76 (38.0%)	200 (100%)
		$\chi 2 = 2.364$, P= 0.307		
Age groups (years)	18-19	4 (2.0%)	4 (2.0%)	8 (4.0%)
	20-29	44 (22.0%)	20 (10.0%)	64 (32.0%)
	30-39	34 (17.0%)	30 (15.0%)	64 (32.0%)
	40-49	17 (8.5%)	14 (7.0%)	31 (15.5%)
	50-59	15 (7.5%)	5 (2.5%)	20 (10.0%)
	60-69	9 (4.5%)	2 (1.0%)	11 (5.5%)
	≥70	1 (0.5%)	1 (0.5%)	2 (1.0%)
	Total	124 (62.0%)	76 (38.0%)	200 (100%)
		$\chi 2 = 7.932$, P= 0.243		
Occupation	Business	38 (19.0%)	21 (10.5%)	59 (29.5%)
	Employed	54 (27.0%)	23 (11.5%)	77 (38.5%)
	Farmer	7 (3.5%)	3 (1.5%)	10 (5.0%)
	Housewife	0 (0.0%)	2 (1.0%)	2 (1.0%)
	No job	13 (6.5%)	16 (8.0%)	29 (14.5%)
	Student	12 (6.0%)	11 (5.5%)	23 (11.5%)
	Total	124 (62.0%)	76 (38.0%)	200 (100%)
		$\chi 2 = 10.412$, P= 0.064		
Marital status	Single	59 (34.7%)	30 (17.6%)	89 (52.4%)
	Married	41 (24.1%)	19 (11.2%)	60 (35.3%)
	Divorced	3 (1.8%)	5 (2.9%)	8 (4.7%)
	Widowed	5 (2.9%)	8 (4.7%)	13 (7.6%)
	Total	108 (63.5%)	62 (36.5%)	170 (100%)
		$\chi 2 = 6.756$, P= 0.080		
Education level	Illiterate	10 (5.0%)	3 (1.5%)	13 (6.5%)
	Primary	11 (2.5%)	5 (2.5%)	16 (8.0%)
	Secondary	89 (44.5%)	55 (27.5%)	144 (72.0%)
	Tertiary	14 (7.0%)	13 (6.5%)	27 (13.5%)
	Total	124 (62.0%)	76 (38.0%)	200 (100%)
		$\chi 2 = 2.721$, P= 0.437		
Residence	Rural	11 (5.5%)	10 (5.0%)	21 (10.5%)
	Urban	113 (56.5%)	66 (33.0%)	179 (89.5%)
	Total	124 (62.0%)	76 (38.0%)	200 (100%)
		$\chi 2 = 0.921$, P= 0.337		
Income level	High	21 (13.5%)	12 (7.7%)	33 (21.3%)
	Moderate	40 (25.8%)	12 (7.7%)	52 (33.5%)
	Low	41 (26.5%)	29 (18.7%)	70 (45.2%)
	Total	102 (65.8%)	53 (34.2%)	155 (100%)
		$\chi 2 = 4.553$, P= 0.103		
House type	Plank	31 (18.2%)	12 (7.1%)	43 (25.5%)
	Cement	76 (44.7%)	47 (27.6%)	123 (72.4%)
	Others	1 (0.6%)	3 (1.8%)	4 (2.4%)
	Total	108 (63.5%)	62 (36.5%)	170 (100%)
		$\chi 2 = 4.085$, P= 0.130		

Table 2: Prevalence of co-infections among the study participants

Variables		Gender		
		Male=124 n (%)	Female=76 n (%)	Total=200 n (%)
Co-infections				
Malaria	Negative	85 (42.5)	59 (29.5)	144 (72.0)
	Positive	39 (19.5)	17 (8.5)	56 (28.0)
Filaria	Negative	122 (61.0)	76 (38.0)	198 (99.0)
	Positive	2 (1.0)	0 (0.0)	2 (1.0)
Intestinal parasites	No	99 (49.5)	52 (26.0)	151 (75.5)
	Stool not provided	23 (11.5)	22 (11.0)	45 (22.5)
	Yes	2 (1.0)	2 (1.0)	4 (2.0)
HIV	Negative	113 (56.5)	55 (27.5)	168 (84.0)
	Status unknown	2 (1.0)	1 (0.5)	3 (1.5)
	Positive	9 (4.5)	20 (10.0)	29 (14.5)
Hepatitis B Virus	Negative	123 (61.5)	76 (38.0)	199 (99.5)
	Positive	1 (0.5)	0 (0.0)	1 (0.5)
Malaria/filaria	Negative	123 (61.5)	76 (38.0)	199 (99.5)
	Positive	1 (0.5)	0 (0.0)	1 (0.5)
Malaria/intestinal	Negative	123 (61.5)	74 (37.0)	197 (98.5)
	Positive	1 (0.5)	2 (1.0)	3 (1.5)
Malaria/HIV	Negative	123 (61.5)	73 (36.5)	196 (98.0)
	Positive	1 (0.5)	3 (1.5)	4 (2.0)

among those who did not take worm medicine regularly, who did not use the community stream for bathing, who did not eat improperly cooked meat, and who were urban residents.

Prevalence of HIV

The overall prevalence of HIV was 29 (14.5%) and was more common in females 20 (10.0%) compared to their male counterparts. Moreover, HIV infection was common in participants who were urban residents, not married, had attained a secondary level of education, and were in full-time employment earning a monthly salary of 50,000 FCFA or less. Most infected participants with HIV were from Limbe regional hospital 9 (4.5%) and Deido District Hospital 8 (4.0%).

Prevalence of hepatitis

Only one male participant from Cites des Palmiers District Hospital within the 20-29 age group was diagnosed with hepatitis B Virus (HBV).

Prevalence of malaria/filaria parasites

One (0.5%) malaria/*Loa-loa* co-infection was detected using the thick film microscopic examination of blood specimens (for the detection of microfilaria) and thin and thick blood smears (for the detection of malaria parasites). The co-infection was found in a male from the Bonassama District Hospital who belonged to the 20-29 age group.

Prevalence of malaria/intestinal parasites

Based on the microscopic examination of blood smears for malaria parasites and stool specimens for the detection of intestinal parasites, there were 2 (1.0%) patients with malaria/ascaris from the Buea regional hospital and 1 (0.5%) with malaria/Entamoeba histolytica/dispar co-infection from Deido District Hospital. The co-infections of malaria/ascaris fell into the age groups of 20 - 29 and 50 - 59 years old, while malaria/*Entamoeba histolytica/dispar* co-infection was found in the age group of 40 - 49.

Prevalence of malaria/HIV

The prevalence of malaria/HIV was 4 (2.0%) and was more common in females 3 (1.5%) compared to

males. The dual infection of malaria/HIV among the participants was common among those who had attained a secondary education level, who were urban residents and who were individuals who lived in the cement block house type. The co-infected participants were from Limbe regional hospital (1.0%) and Cites des Palmiers District Hospital (1.0%).

DISCUSSION

Understanding the effect of co-infections in patients with TB has not only reduced mortality due to dual infection and misdiagnosis but also helped to develop effective prevention and control mechanisms to treat the diseases properly. Moreover, there has been no adequate research carried out currently that provides a clear picture of the interactions in co-infections in TB patients. It is therefore to this end that this cross-sectional hospital-based study was done on a total sample of 200 TB positive patients from Cameroon's Southwest and Littoral regions.

The present study found an overall prevalence of co-infection (46.0%) rate among the participants who were TB positive. Regarding the prevalence of specific co-infection conditions in our study, malaria accounts for 28.0%. This finding is higher than that of other studies done in a hospital-based setting in Tanzania at 4.3%[15], Cameroon at 1.5%[9] and Uganda at 2.2%[4] while being lower than a study done in a hospital-based study in Angola[16]. However, the study in Angola was retrospective and included children. *P. falciparum* is most common among children, therefore we expect the rate to not deviate much from our findings.

Furthermore, the high prevalence of malaria reported here corroborates with other studies that have shown that malaria is more prevalent during the rainy season[5] and it appears to be a significant cause of morbidity and mortality in Cameroon. Although substantial progress has been made in the recent past, the disease remains widespread with a high number of confirmed cases[17]. It is still a public health problem in the 10 regions of the country with an estimated prevalence of 29%[18] being exposed to the risk of transmission. The detection of *P. falciparum* in all participants was not unexpected as *P. falciparum* is the predominant species of Plasmodium in Cameroon[17]. The prevalence of filaria as both a mono-infection and dual infection with malaria in our study (TB positive patients) was not reported in the literature and difficult to do a comparison analysis with.

The prevalence of intestinal parasites reported in our study was lower than what is reported in Ethiopia[19,20] and China[21]. More research in the literature has been published from developing countries, and most of these studies report the significant public problem in co-endemic areas, especially in developing countries, of co-infection with TB and intestinal parasites in humans[22]. Because of their overlap in the same geographical areas, co-infections are common with both pathogens[22]. Regular deworming campaigns, run by the Ministry of Public Health of Cameroon, have taken place in the study area which may account for the lower prevalence of IPIs in our study. Additionally, the low prevalence could be attributed to personal hygiene, the differences in the diagnostic techniques used to detect parasites, geographical differences and the majority of the participants in our study being adults as intestinal parasites are very uncommon in this age group[7,23]. *Ascaris lumbricoides* species being the predominant parasite species in our study is in line with the other studies conducted in areas of Cameroon[23]. Furthermore, we were unable to separate *Entamoeba histolytica* from *Entamoeba dispar*. The co-infection of malaria/intestinal parasites was 1.5%. This prevalence is lower than a study conducted among febrile children admitted to the Muyuka district hospital[23] and Mutengene[24] in Cameroon. The low prevalence of malaria and IPI co-infection could be attributed to the same factors responsible for the low prevalence of IPIs in general, as stated above.

The present study found a 14.5% HIV mono-infection and 2.0% co-infection with malaria. The mono-infection of HIV in our study is similar to the results of studies done in Italy and Russia[25], higher than studies conducted in Cameroon[9], Spain and the United Kingdom[25] and much lower than studies done in Angola[16], South Africa[26,27] and the Philippines[28]. The findings on malaria/HIV co-infection are in line with a study done in Cameroon previously[9]. It has been shown that HIV infection increases the risk of TB infection progression[29], latent infection reactivation, and an increased fatality rate[30]. Moreover, HIV has been reported to be the primary factor in HIV endemic settings concerning the failure to reach TB control targets[30].

Cameroon, despite a small decrease in overall incidence from 5.5% to 4.3%, remains one of the world's most affected countries[31]. This is due to HIV overlapping geographically with TB infections. In addition, Cameroon has still been listed as one of the 41 countries with a high TB/HIV co-infection[32]. The prevalence of HIV infection in our study was as common in females compared to males, which can be attributed to a low level of immunity[33]. The prevalence of HBV (0.5%) reported in our study is much lower

than the prevalence of HBV in the rural general populations (8.0%)[34] and among pregnant women (12.1%) in Cameroon[35]. This difference may be due to the different socio-economic statuses and the natural differences between the different geographical zones.

CONCLUSIONS

The results of this study indicate that co-infections have overlapped with newly diagnosed TB-positive patients according to the smear microscopy and culture methods. With co-infections overlapping in patients with TB, there is rising evidence of one disease fueling the other. The high prevalence of these infections indicates that said prevalence will rapidly increase, making it a huge public health problem in the next decade. We believe that assessing the prevalence of co-infections in TB positive subjects, which could be later on compared to their TB negative household contacts, might disclose important information on the occurrence of co-infections. The Ministry of Public Health should strongly assess these infections and the overall health of the patient for proper management to reduce mortality due to dual infection and misdiagnosis.

ABBREVIATIONS

AFB: Acid Fast Bacilli, **AIDS**: Acquired Immune Deficiency Syndrome, **BCG**: Bacille, Calmette Guerin, **BMI**: Body Mass Index, **CI**: Confidence Interval, **DOT**: Direct Observed Treatment, **EPG**: Eggs Per Gram, **HBV**: Hepatitis B Virus, **Hct**: Hematocrit, **HCV**: Hepatitis C Virus, **Hgb**: Haemoglobin, **HIV**: Human Immunodeficiency Virus, **IPIs**: Intestinal Parasitic Infections, **LJ**: Lowenstein Jensen, **M**: Microfilariae, **MTB**: Mycobacterium Tuberculosis, **MUAC**: Middle Upper Arm Circumference, **SD**: Standard Deviation, **TB**: Tuberculosis, **WHO**: World Health Organization

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AUTHOR'S CONTRIBUTIONS

Samuel Wanji participated in study design and coordination. Jerome Fru-Cho, Eyoab Iyasu and Samuel Wanji supervised, reviewed and corrected the manuscript. Michael Weldeslassie, Nkimbeng Akumtoh, Chefor Magha and Gladys Diana collected and processed study specimens. Michael Weldeslassie contributed to the conception of the study, designed the study, participated in the data collection and specimen examination, conducted laboratory data analysis, and prepared the manuscript for publication. All authors read and approved the final manuscript for publication.

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AVAILABILITY OF DATA AND MATERIALS

Data and materials used and/or analyzed during the current study are available from the corresponding author on reasonable request.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was conducted in accordance with the amended Declaration of Faculty of Health Sciences Institutional Review Board (FHSIRB), University of Buea (Reference number: 2020/1205-05/UB/SG/IRB/FHS on 13th of June, 2020. The institutional review board approved the study, and all participants provided written informed consent.

CONSENT FOR PUBLICATION

Not applicable.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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