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RESEARCH ARTICLE

Mycobacterium ulcerans Infection (Buruli Ulcer) in Southwest Nigeria

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ABSTRACT

Objectives: Buruli Ulcer Disease (BUD) caused by *Mycobacterium ulcerans* is a severe neglected tropical disease of the skin, which has been reported in over 33 countries globally including Nigeria where the disease was first reported in 1967 and later in 1975. Since these reports, there has been no research on BU in the southwest Nigeria. In this study we assessed the presence of BUD in five states of the southwest Nigeria.

Method: This was a community-based, cross-sectional study where BU awareness sessions preceded active search for suspected cases. Questionnaires were administered for participants' demography. Swab and fine needle aspirate specimens from suspected BU lesions were subjected to *IS2404*-based Nested PCR and Real time (qPCR) techniques to confirm BUD.

Results: A total of 256 samples were collected and analyzed between April, 2016 and December, 2018. 157 (61.3%) samples were positive to IS2404 of *M. ulcerans*. Children below 15 years of age and adults constituted 42 cases (26.8%) and 115 cases (73.2%), respectively. Index BU cases were confirmed in Ekiti 4 (2.5%), Lagos 11 (7.3%), Ondo 16 (10.6%), Osun 61 (38.9%) and the remaining 64 (40.4%) were found in Ogun State. Ogun and Osun States accounted for 79.3% of all confirmed BU cases in this study.

Conclusions: Conclusion: BU cases, for the first time, were confirmed in five states of SW Nigeria with two of them (Ogun and Osun States) indicating endemic situation, hence the need for those states to be kept under surveillance as potential BU flash points. On a larger scale, a robust BU awareness program nationwide should be embarked upon by the government and other stakeholders. *J Microbiol Infect Dis 2020; 10(2):82-88.*

Keywords: *Buruli ulcer disease, index cases, endemic areas, Mycobacterium ulcerans, Southwest Nigeria*

INTRODUCTION

Buruli ulcer disease (BUD) is a chronic necrotizing infection of the skin, subcutaneous adipose tissue and occasionally, bones are infected [1]. The disease has been reported in at least 33 tropical and subtropical countries globally especially in Central and South America, Australia, Southeast Asia and Africa [1,2]. The causative agent is a slow-growing environmental pathogen called *Mycobacterium ulcerans* which is closely related to the mycobacteria that cause tuberculosis and leprosy [3,4]. Buruli Ulcer (BU) is now regarded as the third most common mycobacterial disease of humans after tuberculosis and leprosy [4]. While in some communities in West African countries like Benin Republic, Cote d'Ivoire and Ghana, BU has overtaken both tuberculosis and leprosy to become the most common [2]. West Africa is presently the epicenter of the disease in the world [5].

The mode of transmission of this neglected tropical disease is largely unknown and as person-to-person transmission is not proven, it has been hypothesized that *M. ulcerans* is acquired through environmental contact**.** While the *M. ulcerans* DNA has been found in aquatic bugs, plants, fish and in mosquitoes in Australia [4,6] few studies have made efforts beyond detecting DNA. They have actually recorded

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isolation of the pathogen in pure culture from the environment [7-9].

However, proximity to water bodies and contact with an endemic region are regarded as the major risk factors for the disease acquisition [10] as it primarily affects people living in remote and rural wetlands and swampy areas with stagnant lakes or slow-flowing streams [1,11]. *Mycobacterium ulcerans* produces a destructive polyketide endotoxin called mycolactone [12], a virulence factor that is responsible for the progressive necrosis of the skin and cutaneous tissue leading to ulcerative lesions with characteristic undermined edges [5] most especially on the body extremities, resulting in permanent disfigurement, functional impairment and disability with attendant stigmatization in the society, if left untreated, over time [13].

Historically, the first case of BUD in Nigeria was reported in 1967 by Gray et al [14]. Then in 1975, a confirmed case of the disease among a Caucasian family who lived close to a newly constructed dam on the campus of the University of Ibadan in the Southwest (SW) Nigeria was reported. Consequently, further case search yielded 23 more cases within and around Ibadan metropolis [15]. For over two decades thereafter, there was no follow-up search or research on BU in Nigeria. However, between 1998 and 2000 the Institute of Tropical Medicine in Belgium confirmed BU cases from samples sent to it from the Leprosy and Tuberculosis Hospital in Moniaya-Ogoja, Cross River State, Nigeria. Then in 2006, a 5-day BUD case search by a team of World Health Organization (WHO) BU experts in conjunction with the national and the state health authorities in Nigeria had 14 (38%) confirmed cases of BU out of the 37 clinically-suspected specimens harvested from five states in South-South and South-East zones [16].

WHO BU case search was not extended to SW Nigeria where the status of BU was and still largely unknown till date, even though there have been reports of patients crossing over to the Republic of Benin to seek treatment for BU [17,18]. This is a concluded portion of the PhD study of the corresponding author. A pilot study (Presumptive Diagnosis of Buruli Ulcer based on Clinical Presentations) was published in this journal in 2017 [19].

The aim of this study therefore, was to determine the status of BUD in five of the six states of SW Nigeria since index cases of BU had earlier been documented in Ibadan (now in Oyo State) in SW Nigeria [15].

METHODS

Study Sites

The study sites were the designated communities (villages and towns) in Ekiti, Lagos, Ogun, Ondo and Osun States in SW Nigeria (Figure 1).

Study Population

The participants were residents (of the study sites) with skin lesions suggestive of Buruli Ulcer.

Study Design and Methodology

The study was community-based and cross-sectional, involving the sensitization of the target community and active case search for suspected cases of BU in the community [20]. The case search involved on-site screening of participants for BU disease by identifying cases consistent with the WHO clinical definitions. There was also the use of participants and other residents to identify additional cases that were not presented to the researchers on-site.

Advocacy visits to the community authorities was followed with the gathering of the residents at designated places where the sensitization activities (video show, distribution of education and communication materials, question and answer sessions) took place. These interactive sessions were carried out prior to specimen collection at the study sites in all the five states. Sampling was preceded by identification of clinically suspected cases, obtaining written informed consent from the participants and legal guardians (for minors) in addition to administering simple questionnaire to obtain their demographic and related details. Photographs of lesions were taken with permission.

Sampling Method

Swab and Fine needle aspiration (FNA) samples were collected according to WHO guidelines [21].Samples were appropriately labelled, transported in cooling boxes to the laboratory and stored at -20 ^{0}C until processed.

Laboratory Diagnostic Analysis

a) Specimen Preparation: Optimization of bacteria release from swab and FNA specimens using sterile PBS was done as previously described by Yeboah-Manu et al [22].

b) DNA Extraction and Purification: The extraction of DNA from the prepared 0.5ml suspension was done using Bacterial DNA Preparation kit (Jena Bioscience GmbH, Germany) according to the manufacturer's instructions.

c) DNA Amplification: The purified DNA samples were subjected to Nested and Real-time IS2404-PCR techniques for the amplification of the target DNA in a PCR machine as described by Stienstra et al [23] and manufacturer [24,25], respectively.

Figure 1. Map of SW region of Nigeria showing the study sites (colored).

Agarose gel electrophoresis: Agarose gels (1.5%) in Tris-borate-EDTA buffer were prepared with ethidium bromide. Nested PCR products were run on the gels as described by Stienstra et al [23]. The DNA fragments were visualized with UV-trans-illuminator. Figure 2 is the image of a gel electrophoresis documentation of the PCR products as visualized under the illuminator. For the TaqMan Real Time PCR, no default threshold was used for the analysis and CT ≤ 35 was considered positive with the examination of each amplification curve.

The qPCR replaced Nested PCR when the laboratory was upgraded with the installation of a Real TimeBio-Rad CFX96 Thermocycler. Altogether there were 140 samples run with Nested PCR while qPCR was used for the remaining 116 samples. Table 1 shows the list of all primers used in this work.

Statistical Analysis

Data was analyzed using SPSS version 25 as Descriptive Statistics. Statistical significance was set at $p \leq 0.05$. The chi-square test was used to compare categorical proportions and the student's t-test was used in the case of continuous variables.

Ethical Approval

Ethical approval to carry out this study was obtained from the Ethical & Research Review Committee of the Ministry of Health of each state. (LSMH/ 4148/ VOL1; EKSUTH/ A67/ 2016/01/03; OSHREC/ PRS/ 569T/ 118; LTH/ REC/ 2016/12/08/ 283; MR/GEN. AD/1032/1015).

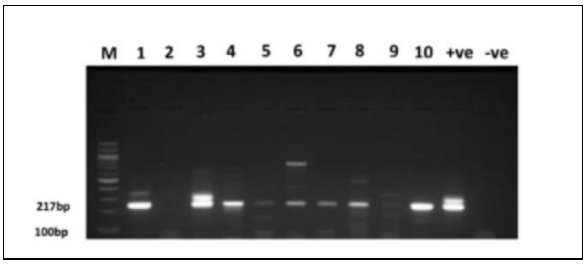

Figure 2. Agarose Gel picture of PCR amplified *IS2404* gene of suspected cases.
Lane M: DNA size marker, Lanes 1 to 10 are the positive and negative results. Lane +ve is the positive control amplified from *Mycobacterium ulcerans Agy99*, Lane –ve is the negative PCR control.

RESULTS

A total of 256 patients (n=256) with suspected BU lesions comprising 138(54%) males and 118(46%) females were recruited into this study which involved over 30 communities in 25 Local Government Areas (LGA) across five states (Ekiti, Lagos, Ogun, Ondo and Osun) of SW Nigeria. Of this number, 157 (61.3 %) were confirmed positive by IS2404 PCR for Buruli ulcer. Figure 3 is a bar chart showing the number and corresponding percentages of all the suspected BU cases in relation to positive cases across all the states.

Among the BU positive participants were 84 (54%) males and 73 (46%) females. The overall mean age was 34 ± 21 years with age range between 4 and 85 years while the most frequent age was 40 years. The mean age of confirmed male and female BU participants were 35 and 29 years respectively. The majority of BU patients, 115 (73.2%) were above 15 years of age while only 42 (26.8%) were children under 15 years. However, 32 (76%) of the 42 children

under 15 years of age seen in this study were from Ogun State (Table 2) and the incidence of disease was observed to decrease with age.

Table 1. List of Primers.

Protocol / Concentration	Primer	Primer Sequence
Nested PCR to amplify 217 bp of Mycobacterium ulcerans		
Primer pair 1 (25 pmol)	pGp1	5' - AGGGCAGCGCGGTGATACGG - 3'
	pGp2	5' - CAGTGGATTGGTGCCGATCGAG - 3'
Primer pair 2 (25 pmol)	pGp3	5' - GGCGCAGATCAACTTCGCGGT - 3'
	pGp4	5' - CTGCGTGGTGCTTTACGCGC - 3'
Real-Time PCR to amplify 479 - 526 bp of *Mycobacterium ulcerans*		
Primer pair (100pmol)	*IS2404*F	5' - GATCAAGCGTTCACGAGTGA - 3'
	*IS2404*R	5' - GGCAGTTACTTCACTGCACA - 3'

X^2= 6.15, p value= ≤0.001.

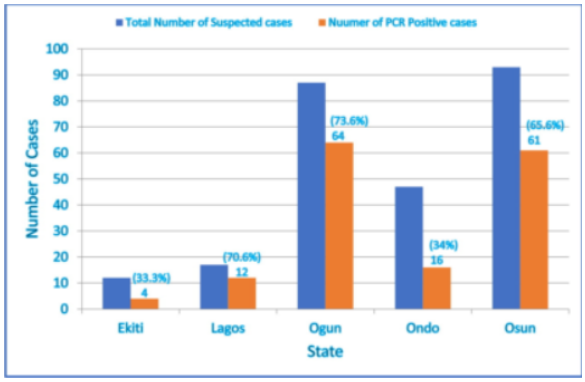

Figure 3. Bar chart showing of suspected cases in the states.

While 112 (71.3%) lesions were located on the lower limbs, 43 (27.4%) occurred on the upper limbs and only 2 (1.3%) were seen on other parts of the body.

By classification of the BU lesions into two major clinical forms(pre-ulcerative and ulcerative), all the nodules, papules, oedema and plaques which were collectively considered as pre-ulcerative accounted for only 26 (17%) while the rest, 131 (83%) presented in the ulcerative form. The distribution of Buruli ulcer patients per state is considered by Local Government Areas. For example, in Ogun State 57(89%) of all the 64 BU cases were found in only two communities (Yewa South and North LGAs). In Osun State 54(88.5%) of 61 BU cases were found in three Local Government Areas. Majority (79.7%) of BU patients seen in this study were from Ogun (40.8%) and Osun (38.9%) States while Lagos and Ekiti States each accounted for less than 10% of BU cases (Table 2). Presented in Plate 1 are some selected BU lesions from some of the study participants.

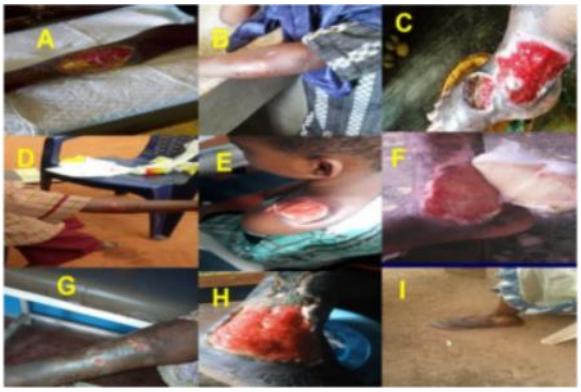

Plate 1. BU lesions of selected patients.

Table 2. Distribution of Buruli ulcer patients by age across the states.

State	≤15 years, n (%)	>15 years, n (%)	Total (%)	P value
Ogun	32 (76.2)	32 (27.8)	64 (40.8)	
Osun	8 (19.0)	53 (46.1)	61 (38.9)	
Ondo	0 (0.0)	16 (13.9)	16 (10.2)	0.000
Lagos	1 (2.4)	11 (9.6)	12 (7.6)	
Ekiti	1 (2.4)	3 (2.6)	4 (2.5)	
Total	42 (100)	115 (100)	157 (100)	

DISCUSSION

Prior to the commencement of this study, two pilot studies were carried out; first, to determine the level of BU awareness among the health personnel in two of the states and secondly, to attempt to presumptively diagnose BU based strictly on clinical presentations. While the results of the latter strongly suggested the possible presence of BU in Ogun State [5], the BU awareness study revealed a very poor knowledge of the scourge even among medical personnel [26]. These findings necessitated the need for a robust BU advocacy among the target communities that was carried out prior to BU case search in this study.

Generally, children of age 15 years and below are more prone to the disease especially in many African countries where BU is endemic [6,10,27]. In this study more cases were recorded among patients above 15 years. However, in specific terms, 32 (76.2%) of the 42 BU cases found in children in this study are actually from Ogun State which is deemed a BU-endemic state.

With near equal distribution results among the gender, BU has no predilection for sex as previously reported [17,20,28,29]. Most of the lesions encountered were located on the lower limbs. The reason for these patterns may not be far-fetched as it has been observed that clothing serves as a kind of protection against acquiring BU infection. The observations that clothed body parts are almost free of lesions suggests that Buruli ulcer occurs on exposed body parts with convincing indication that clothes are sufficient to protect the skin against *M. ulcerans* infection or an injury by inoculating M. ulcerans [9]. The likely explanations for lesions on the head, neck and truck is that these body sites are usually scratched with hands that had been in contact with environmental *M. ulcerans* strains and multiple contacts of anybody wound with sources or reservoirs of M. ulcerans [8].Having the ulcerative form as the majority of the lesions may be largely due to late presentation stemming from the lack of familiarity with the disease, traditional beliefs, stigmatization and absence of health facilities, thus prolonging its diagnosis and subsequent treatment [9,30,31].

It is important to note that about 80% of all cases of Buruli ulcer in SW Nigeria in this study were from two states (Ogun and Osun) which make the states strong candidates for BU endemicity. Considering the distribution of BU cases in the LGAs of each state, particularly in Ogun and Osun States, conclusion could be drawn as to the endemicity of BU in the LGAs identified. It might be safe to conclude that both Yewa North and Yewa South LGAs in Ogun State are BU-endemic. Interestingly both LGAs share borders with Benin Republic where the disease is endemic.

Similarly, Irewole, Iwo and Olorunda LGAs in Osun State; Akoko-South-West, Ile-Oluji/Okeigbo, and Ondo East in Ondo State recorded the highest numbers of BU cases. These areas should be kept under BU surveillance as potential endemic areas.

Currently in Nigeria, there is a gross information deficit about Buruli ulcer among both the healthcare practitioners and the populace as a whole with the consequent inability to correctly identify BU cases. The results from this study therefore necessitate the need to develop BU control mechanisms with specialized BU care facilities at the local government level. Future functional studies will be necessary to identify the epidemiological BU situation at a population level to address the current low surveillance, low monitoring, under-reporting and poor infection prevention and control.

However, we encountered a few limitations in the course of this study which, though could not have significantly affected the results but should be addressed in future studies. For example, we experienced irregular availability of specimen transport medium from the field to the laboratory such that most of the PCR-positive samples produced no growth on culture even after several weeks of incubation. Furthermore, being a self-sponsored project, the financial burden of shuttling between several communities in the five targeted states was huge and overwhelming which led to irregular visits to the study sites. Funding or financial assistance from the stakeholders should be sought for future studies.

Conclusion

The number of confirmed cases of Buruli ulcer disease in this study is considered significant for a region (with predisposing environment) that has not been studied before. The active case

search for Buruli ulcer, which hitherto had not been carried out in five states of SW Nigeria since BU was confirmed in Ibadan in 1975, has been undertaken in this study, providing the research information that has been missing. Furthermore, the identification of BU-endemic states including the most affected communities, as it has happened in some West African countries, will enable the healthcare stakeholders to adopt intervention strategies for the prevention and control of BU in SW Nigeria.

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