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Mycobacterium Ulcerans Infection: Assessment of Risk Factors for Occurrence in Nigeria

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ABSTRACT

Background: Mycobacterium ulcerans infection remains a neglected tropical disease whose control is hampered by the limited knowledge of its definite reservoirs and potential vectors. Modifying the risk factors for its occurrence will therefore enhance the control of the disease.

Objective: To determine the risk factors for the occurrence of Mycobacterium ulcerans infection in Nigeria.

Methods: A cross-sectional descriptive study was carried out among a cohort of 39 Buruli ulcer disease patients referred to a referral hospital in Anambra State between July and December 2021. A semi-structured, interviewer-administered questionnaire was used to collect data on the socio-demographic characteristics the respondents as well as other risk factors for the occurrence of Mycobacterium ulcerans infection. Data were analyzed using SPSS version 22; with alpha set at p-value ≤ 0.05.

Results: Twenty one (53.8%) of the study participants were males while 18 (46.2%) were females. Their ages ranged from 2 to 68 years while their mean age was 28.41±18.668. Thirteen (33.3%) of the respondents were in the age range of ≤ 15 years while 7 (17.9%) were ≥ 50 years. Twenty (51.3%) of the respondents came from Akwaibom State while the 1 (2.6%) each came from Anambra, Delta and Edo States. Thirty eight (97.4%) of the respondents did not wear protective clothing when going to obtain water while 28 (71.8%) had received BCG vaccination. Twenty two (56.4%) of the respondents sought prior care from traditional healers before presenting to the referral centre while 19 (46.1%) sought prior care from hospitals.

Conclusions: M. ulcerans infection is still endemic in Nigeria especially among children aged ≤ 15 years old living in the riverine areas. Public health efforts aimed at patient education and to assist with prevention and early detection of the disease to minimize associated morbidity should be instituted.

Keywords: Mycobacterium ulcerans, infection, risk factors, occurence, Nigeria

1. Introduction

Mycobacterium ulcerans infection is a neglected tropical disease which has been variously called Buruli ulcer, Bairnsdale ulcer, Mossman ulcer, Daintree ulcer, and Searl ulcer depending on the geographic area found. (Zingue, Bouam, Tian, & Drancourt, 2018; Röltgen & Pluschke, 2019; WHO, 2022; Wansbrough-Jones, & Phillips, 2006) This chronic, debilitating, necrotizing disease of the skin and soft tissue is caused by an environmental pathogen called Mycobacterium ulcerans (M. ulcerans). The disease is geographically restricted, being mainly endemic in Western Africa. However, transmission has been reported in endemic areas in more than 30 countries worldwide in Africa, the Americas, Asia and the Western Pacific. (Bratschi, Bolz, & Minyem, et al., 2013; WHO, 2021; Huang, & Johnson, 2014) According to the World Health organization, the annual number of suspected cases reported globally was around 5000 cases until 2010 when it started to decrease, reaching its minimum in 2016 with 1961 cases reported. Since then, the number of cases has started to rise again every year, with up to 2713 cases reported in 2018. In 2020 however, 1258 cases were reported compared with 2271 cases reported in 2019. This reduction in 2020 was said to be linked to the impact of Covid-19 on active detection activities. (WHO, 2015)

In the Americas, Mycobacterium ulcerans infection is a rare disease and little information exists on its prevalence and geographical distribution. There is also limited publications on the occurrence of the infection and only very few cases have been reported to the WHO. (Coloma, Navarrete-Franco, Iribe, & López-Cepeda, 2005; Boleira, Lupi, Lehman, Asiedu, & Kiszewski, 2010; Morris, Gozlan, & Marion, et al., 2014; Reynaud, Millet, Couvin, et al., 2015) In Asia, the severity of the disease seems to be comparable to that caused by M. ulcerans elsewhere (Röltgen, & Pluschke, 2019; Yotsu, Murase,

& Sugawara, 2015; Tsukamura, Kaneda, Imaeda, & Mikoshiba, 1989) while in Australia, endemic foci exist in two geographically and climatically distinct areas. These are the temperate southeastern state of Victoria, where the disease is known as Bairnsdale ulcer; and the tropical Far North Queensland, where the disease is often called Daintree ulcer. (Röltgen, & Pluschke, 2019; Steffen, Smith, & McBride, 2010) Available evidence shows that the major burden of the disease falls on populations living in West and Central Africa, where it typically occurs in rural, isolated foci associated with swampy lowlands and river valleys. (WHO, 2021) For instance, the first cases detected in West Africa were from Nigeria, and they emerged in close proximity to a small stream that was dammed to create an artificial lake. (Oluwasanmi, Solankee, & Olurin, 1976) Subsequently, highly endemic foci were identified in several other West African countries including Benin, Côte d'Ivoire, Ghana, and Cameroon; and more than 40,000 cases have been reported from only these four countries in the last decade. (Debacker, Aguiar, & Steunou, et al., 2004; Noeske, Kuaban, & Rondini, et al., 2004; Marston, Diallo, & Horsburgh, et al., 1995; Kanga, Kacou, & Kouamé, 2006; Amofah, 2002) It is equally believed that the number of cases may be higher in these regions as many cases may go unreported due to limited knowledge of the disease, limited access to reliable diagnostics that is available only in few laboratories, its focal distribution and the fact that it affects mainly poor, rural communities. (Kofie, Attua, & Nabila, 2008)

M. ulcerans has been isolated from biofilms and small aquatic animals of slow-moving or stagnant bodies of water. (Marsollier, Severin, Aubry, et al., 2004*a*; Marsollier, Severin, Aubry, et al., 2004*b*) Although the exact mode of transmission is unknown, it has been postulated that M. ulcerans most likely causes infection through contamination of traumatic wounds. (N'krumah, Koné, & Tiembre, 2016) It has also been postulated that Mycobacterium ulcerans infection is probably acquired through inoculation of the skin either directly from the environment or indirectly via insect bites. (Johnson, Makoutodé, & Sopoh, 2005; Quek, Athan, & Henry, 2007; Benbow, Williamson, & Kimbirauskas, 2008) However, definitive evidence is lacking. Studies show that mosquitoes have tested positive for M. ulcerans by PCR and that epidemiological links exist to suggest that the use of insect repellent on exposed body surfaces and the use of mosquito nets are associated with a reduction in M. ulcerans incidence. (Johnson, Makoutodé, & Sopoh, 2005; Quek, Athan, & Henry, 2007; Landier, Boisier, & Piam, 2011) It has thus been suggested that mosquito bites could be implicated in the mode of transmission of the disease. (Wallace, Mangas, & Porter, 2017) It has also been postulated that mosquitoes carry the organism on their proboscis following contact or feeding with contaminated environmental sources and then directly transmit it through their bite. (Johnson, Makoutodé, & Sopoh, 2005) However, the environmental reservoir and exact route of transmission are not completely understood and it has been proposed that the mode of acquisition may vary in different parts of the world. For instance, in Africa, M. ulcerans has been detected by PCR in aquatic insect species in the order Hemiptera, which are known to bite humans, thereby suggesting that transmission may occur through the bites of these insects as buttressed by findings in laboratory mice. (Marsollier, Robert, & Aubry, 2002) In Australia, it was postulated that aerosols generated by spray irrigation using contaminated water may disseminate M. ulcerans and infect humans through the respiratory tract, or the contamination of skin lesions and minor abrasions. (Veitch, Johnson, & Flood, et al., 1997) Mycobacterium ulcerans infection is not considered a contagious disease. Despite the clustering of cases amongst families, available evidence for now suggests that human to human transmission of M. ulcerans does not occur and that an increased risk of infection is not associated with living with an infected family member. (Jacobsen, & Padgett, 2010; Van der Werf, Stienstra, & Johnson, 2005; Merritt, Walker, & Small, et al., 2010) This assumption has been supported by the finding that the time period between the diagnosis of family clustered cases was shorter than the estimated incubation period of the disease in a study carried out in Australia. In addition, the family cluster isolates were not more genetically related than those of six random isolates from the same geographic region and that isolates derived from two of the three family clusters studied, were not genetically identical. (O'Brien, Wynne, & Buultjens et al., 2017)

M. ulcerans infects individuals at every age especially around the exposed parts of the body (arms or legs). (WHO, 2021) However, the highest incidence has been in children aged between 5 and 15 years. (Debacker, Aguiar, & Steunou, 2004; Vincent, Ardant, & Adeye, et al., 2014) The disease has also been shown to exhibit a bimodal age distribution. For instance, in a case-control study carried out in Benin, an increased risk of the infection in children aged below 15 years and adults aged above 49 years was found. (Debacker, Aguiar, & Steunou, 2004) Similarly, a survey for the disease in Cameroon revealed that the risk is highest in children aged between 4 and 14 years and in the elderly aged above 50 years. (Bratschi, Bolz, & Minyem, 2013; Debacker, Aguiar, & Steunou, 2004; Johnson, Makoutodé, & Sopoh, 2005; Boyd, Athan, & Friedman, et al., 2012) Young adults on the other hand appear to be less often affected by the disease due possibly to the development of a certain degree of immunity to M. ulcerans. Several studies carried out in African and Australian settings show a nearly equal sex distribution among the cases. (Debacker, Aguiar, & Steunou, 2004; Amofah, 2002; Vincent, Ardant, & Adeye, et al., 2014; Boyd, Athan, & Friedman, et al., 2012) However, differences have been observed in the occurrence of cases between groups in some studies with the incidence considerably higher in females than in males.[21,39] M. ulcerans infection foci have been associated with wetlands. (Amofah, 2002; Johnson, Makoutodé, & Sopoh, 2005) Epidemiological studies from several regions reveal that contact with or proximity to water bodies is a risk factor for contracting the infection and that communities living along slow-flowing water bodies such as ponds, swamps and lakes are mostly affected. (Debacker, Aguiar, & Steunou, 2004; Landier, Boisier, & Piam, 2011; Sopoh, Barogui, & Johnson, et al., 2010; Kenu, Nyarko, & Seefeld, et al., 2014; Raghunathan, Whitney, & Asamoa, et al., 2005; Aiga, Amano, & Cairncross, 2004; Nackers, Dramaix, Johnson, et al., 2006; Pouillot, Matias, & Wondje, 2007; Ahoua, Guetta, & Ekaza, 2009) Studies have also suggested that farming close to water bodies and swimming in endemic areas as well as the construction of dams and irrigation systems have also been associated with increased cases and are potential risk factors for the acquisition of the infection. (Aiga, Amano, Cairncross, et al., 2004; Marion, Landier, & Boisier, et al., 2011; Brou, Broutin, Elguero, et al., 2008; Wagner, Benbow, Brenden, et al., 2008; Anagonou, Johnson, Barogui, et al., 2019) Wearing protective clothing and hygiene on the other hand have been associated with a lower risk for the infection. (Quek, Athan, Henry, et al., 2007; Landier, Boisier, & Piam, et al., 2011; Kenu, Nyarko, & Seefeld, et al., 2014; Raghunathan, Whitney, & Asamoa, et al., 2005; Nackers, Dramaix, & Johnson, et al., 2006; Pouillot, Matias, Wondje, et al., 2007)

Mycobacterium ulcerans infection has been associated with poverty, low socio-economic status and ignorance. (Kofie, Attua, & Nabila, 2008) Poor personal hygiene and dirty surroundings have also been recognized as a potential risk factors for the infection. (Debacker, Aguiar, & Steunou, et al., 2004; Johnson, Makoutodé, & Sopoh, et al., 2005; Kenu, Nyarko, & Seefeld, et al., 2014) A vaccine against M. ulcerans would protect individuals at risk

in highly endemic areas as well as shorten the duration of treatment and prevent relapse of infection. The BCG vaccine has been shown to provide some protection against the onset of disease, though this effect is transient and may not last for more than one year. (Jacobsen, & Padgett, 2010) However, previously immunized individuals are less likely to develop ulcers that lead to other complications. Prevention of M. ulcerans infections by modifying its risk factors is crucial to mitigating the debility associated with this disease. This study is therefore aimed to determine the risk factors for the occurrence of M. ulcerans infection in Nigeria.

2. Materials and Methods

2.1. Study Area

The study was carried out in a missionary hospital in Anambra State, Nigeria - St Joseph's Hospital, Adazi Nnukwu. The hospital in addition to providing general health care services to its patients who generally come from Anambra State and its environs also serves as a referral centre for the detection and treatment of Mycobacterium ulcerans infection in Nigeria under the auspices of the Buruli Ulcer Control Programme. This programme is supported and fully funded by the German Leprosy and Tuberculosis Relief Association, Nigeria and the services (including accommodation, drugs, surgery and dressing materials) are rendered at no cost to the patients. The cases are recruited in cohorts form endemic areas and referred to the hospital within treatment periods stipulated by the sponsors. The recruitment is facilitated through active surveillance by a network of community-based focal persons living in the endemic areas or through outreach programmes organized on ad-hoc basis to create awareness on the disease.

2.2. Study Design

This was a cross-sectional descriptive study of a cohort of cases recruited and referred to the hospital between July and December 2021.

2.3. Study Population

Patients with suspected Mycobacterium ulcerans infection recruited and referred to the hospital between July and December 2021.

2.3.1. Inclusion Criteria

Patients clinically diagnosed with Mycobacterium ulcerans infection who gave informed consent to participate in the study.

2.3.2. Exclusion Criteria

Patients clinically diagnosed with Mycobacterium ulcerans infection that were not present on the days of data collection.

2.4. Study Instruments

A semi-structured interviewer-administered questionnaire adapted from the manual for health care providers for the management of Mycobacterium ulcerans disease (Buntine, Crofts, & WHO, 2001) was used for this study. The questionnaires were used to collect information on the socio-demographic characteristics of the respondents as well as other risk factors for the occurrence of Mycobacterium ulcerans infection.

2.5. Data Collection Methods

The semi-structured questionnaires were administered to the eligible respondents presenting at the referral hospital for the disease in Anambra State using face-to-face interviews conducted by the principal researcher and the trained research assistants. Prior to the administration of the questionnaires, each respondent gave verbal informed consent. Each questionnaire took about 20 minutes to administer. Data collection lasted for a period of six months corresponding to the duration for recruiting and referring the patients to the hospital.

2.6. Data Processing and Analysis:

The collected data were inspected for any data collection or coding errors. They were then entered into the International Business Machines-Statistical Package for Social Sciences (IBM-SPSS) version 22 [52] and further cleaned for coding errors. Statistical data analysis was carried out with the aid of IBM-SPSS version 22 (34). Frequency distribution of all relevant variables was developed. Their means and proportions were calculated. The association between the occurrence of the infection and the risk factors were tested using Fishers exact test. Level of statistical significance was set at p-value ≤ 0.05.

2.7. Ethical Considerations

Ethical approval (Ref: COOUTH/CMAC/ETH.C/VOL.1/FN:04/0098) for the study was obtained from the Chukwuemeka Odumegwu Ojukwu University Teaching Hospital Ethics Committee. Permissions to conduct the study were obtained from the German Leprosy and Tuberculosis Control Programme, Enugu; Anambra State ministry of health and the management of St. Joseph's hospital, Adazi Nnukwu. In addition, verbal informed consents were

obtained freely and without coercion from all the respondents and respect for confidentiality of the data obtained from them ensured. The objectives of the study were thoroughly explained to them and they were assured that they were free to opt-out of the study at any time during the study without repercussion.

3. Results

A total of 39 questionnaires were administered to all the clinically diagnosed cases referred to the hospital between July and December 2021. All the questionnaires were retrieved, giving a response rate of 100%.

Table 1: Socio-Demographic Characteristics of the Respondents

Variable	Frequency (n = 39)	Percentage (%)
Age at last birthday (Years)		
<=15	13	33.3
16-32	10	25.6
33-49	9	23.1
>=50	7	17.9
Mean ± SD	**28.41±18.668**	
Minimum, Maximum	**2,68**	
Gender		
Male	21	53.8
Female	18	46.2
Religion		
Christianity	39	100
Islam	0	0
Traditional religion	0	0
Others	0	0
Educational status		
No formal education	2	5.1
Primary	19	48.7
Secondary	15	38.5
Tertiary	3	7.7
Occupation		
Student	14	35.9
Trader	6	15.4
Farmer	4	10.3
Artisan	9	23.1
Unemployed	1	2.6
***Others**	5	12.8
Parent's occupation (n = 19)		
Civil servant	2	10.5
Trader	10	52.6
Farmer	5	26.3
Artisan	2	10.5

***Others = Driver, Pastor, Retired teacher**

Table 1 summarizes the socio-demographic characteristics of the respondents. Their ages ranged from 2 to 68 years while their mean age was 28.41±18.668. Majority of the respondents (33.3%) were in the age range of ≤ 15 years. However, a significant proportion of the respondents were in the age range of 16 – 49 years and when combined, this age range contains the highest proportion (48.7%) of the respondents. All the respondents were Christians and most of them attained some level of formal education. Only 5.1% of them did not attain any level of formal education. Majority of the respondents were students (35.9%) and artisans (23.1%).

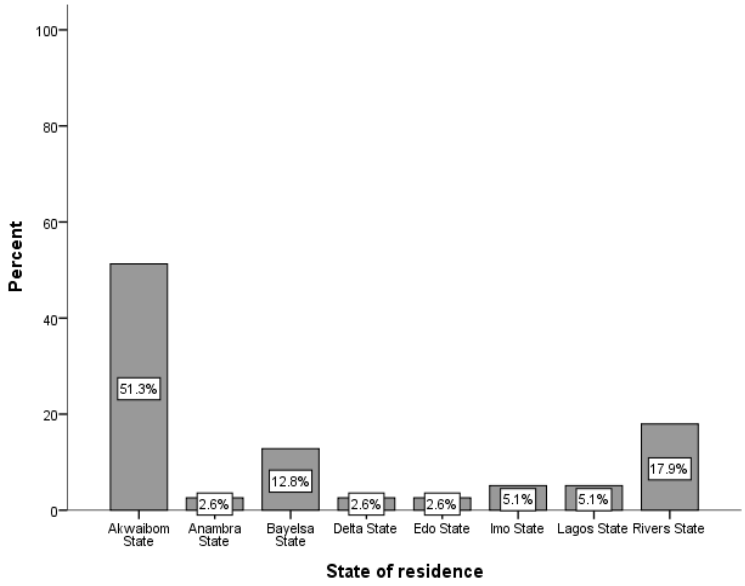

Figure1: State of Residence of the Respondents

Figure1 shows the state of residence of the respondents. More than half of all the respondents (51.3%) came from Akwaibom State while the least proportion of the respondents (2.6% each) came from Anambra, Delta and Edo States.

Table 2: Factors Influencing the Occurrence of Mycobacterium ulcerans Infection among the Respondents

Variable	Frequency (n = 39)	Percentage (%)
Source of water		
Pipe-borne water	3	7.7
Borehole/well	21	53.8
River/stream	14	35.9
Pond/stagnant water	1	2.6
Wears protective clothing while going to obtain water		
Yes	1	2.6
No	38	97.4
Prior care taken before presenting to the referral centre		
Yes	39	100.0
No	0	0.0
Place prior care was sought (multiple answers)		
Chemist shop	7	17.9
Traditional healer	22	56.4
Hospital	19	46.1
Home treatment	1	5.1
Ever used traditional treatment		
Yes	32	82.1
No	7	17.9
Duration of illness (Months)		
3-25	23	59.0
26-49	9	23.0
50-72	4	10.3
>=73	3	7.7
Mean ± SD	**29.33 ± 26.389**	
Minimum, Maximum	**3, 96**	
Family members have the same condition		
Yes	10	25.6
No	29	74.4
Trauma to the site of lesion prior to onset		
Yes	5	12.8

No	34	87.2
Received BCG vaccination		
Yes	28	71.8
No	11	28.2

Table 2 shows the factors influencing the occurrence of mycobacterium ulcerans infection among the respondents. The major sources of water for the respondents were boreholes/wells (53.8%) and rivers/streams (35.9%); and 97.4% of the respondents did not wear protective clothing while going to obtain water. All the respondents had sought prior care before presenting to the referral centre and this was mainly from traditional healers (56.4%) and hospitals (46.1%). Majority of the respondents (82.1%) had ever used traditional treatment and received BCG vaccination (71.8%)

Table 3: Association between the Occurrence of Mycobacterium ulcerans Infection and the Socio-demographic Characteristics of the Respondents

Variable	M. ulcerans infection present (n = 33) (Number, %)	M. ulcerans infection absent (n = 6) (Number, %)	Test statistic	P-Value
Age at last birthday (years)			F = 1.760	0.624
<=15	11 (84.6)	2 (15.4)		
16-32	8 (80.0)	2 (20.0)		
33-49	7 (77.8)	2 (22.2)		
>=50	7 (100)	0 (0.0)		
Gender			F = 0.469	0.493
Male	17 (81.0)	4 (19.0)		
Female	16 (88.9)	2 (11.1)		
Educational status			F = 1.157	0.763
No formal education	2 (100)	0 (0.0)		
Primary	16 (84.2)	3 (15.8)		
Secondary	12 (80.0)	3 (20.0)		
Tertiary	3 (100)	0 (0.0)		
Occupation			F = 2.798	0.731
Student	11 (78.6)	3 (21.4)		
Trader	6 (100)	0 (0.0)		
Farmer	4 (100)	0 (0.0)		
Artisan	7 (77.8)	2 (22.2)		
Unemployed	1 (100)	0 (0.0)		
***Others**	4 (80.0)	1 (20.0)		
Parent's occupation (n = 19)			F = 2.454	0.484
Civil servant	1 (50.0)	1 (50.0)		
Trader	9 (90.0)	1 (100)		
Farmer	4 (80.0)	1 (20.0)		
Artisan	2 (100)	0 (0.0)		
State of residence			F = 13.266	0.066
Akwaibom State	17 (85.0)	3 (15.0)		
Anambra State	0 (0.0)	1 (100)		
Bayelsa State	4 (80.0)	1 (20.0)		
Delta State	1 (100)	0 (0.0)		
Edo State	0 (0.0)	1 (100)		
Imo State	2 (100)	0 (0.0)		
Lagos State	2 (100)	0 (0.0)		
Rivers State	7 (100)	0 (0.0)		

Table 3 shows the association between the occurrence of Mycobacterium ulcerans infection and the socio-demographic characteristics of the respondents at the bivariate level using Fischer's exact test of statistical significance. None of the variables analyzed achieved statistically significant association with the occurrence of Mycobacterium ulcerans infection.

Table 4: Association between the Occurrence of Mycobacterium ulcerans Infection and Some Selected Factors among the Respondents

Variable	M. ulcerans infection present (N = 33) (Number, %)	M. ulcerans infection present (N = 6) (Number, %)	Test statistic	P=Value
Source of water			F = 6.810	0.078
Pipe-borne water	2 (66.7)	1 (33.3)		

Borehole/well	19 (90.5)	2 (9.5)		
River/stream	12 (85.7)	2 (14.3)		
Pond/stagnant water	0 (0.0)	1 (100)		
Wears protective clothing while going to obtain water			F = 0.187	0.666
Yes	1 (100)	0 (0.0)		
No	32 (84.2)	6 (15.8)		
Ever used traditional treatment			F = 0.008	0.929
Yes	27 (84.4)	5 (15.6)		
No	6 (85.7)	1 (14.3)		
Duration of illness (Months)			F = 1.667	0.644
3 – 25	19 (82.6)	4 (17.4)		
26 – 49	7 (77.8)	2 (22.2)		
50 – 72	4 (100)	0 (0.0)		
> = 73	3 (100)	0 (0.0)		
Family members have the same condition			F = 0.220	0.639
Yes	8 (80.0)	2 (20.0)		
No	25 (86.2)	4 (13.8)		
Trauma to the site of lesion prior to onset			F = 0.094	0.759
Yes	4 (80.0)	1 (20.0)		
No	29 (85.3)	5 (14.7)		
Received BCG vaccination			F = 1.663	0.197
Yes	25 (89.3)	3 (10.7)		
No	8 (72.7)	3 (27.3)		

Table 4 shows the association between the occurrence of Mycobacterium ulcerans infection and some selected factors among the respondents. None of the variables analyzed here achieved statistically significant associations with the occurrence of Mycobacterium ulcerans infection.

4. Discussions

This cross-sectional descriptive study was carried out to assess the risk factors for the occurrence of M. ulcerans infection among cases presenting in a referral hospital in Anambra State, Nigeria. The ages of the respondents in this study ranged from 2 to 68 years and had a mean of 28.41 ± 18.668 years. However, majority of the respondents in this study were in the age range of ≤ 15 years. This finding is similar to findings from several studies which report that even though M. ulcerans affects individuals at every age, the highest incidence has been in children under 15 years old. (Bratschi, Bolz, & Minyem, et al., 2013; Yotsu, Murase, & Sugawara, et al., 2015, Debacker, Aguiar, & Steunou, et al., 2004; O'Brien, Wynne, & Buultjens et al., 2017; Vincent, Ardant, & Adeye, et al., 2014) This finding is in keeping with the assumption that the risk of developing the disease should be increased at the age where children have more intense environmental contacts. Individuals in this age range are also mostly school-going children who may be directly exposed to water bodies peripheral to their homes as they walk to school; an assumption that has been corroborated in this study by the majority of the respondents being students. The finding of a large proportion of students in this study may imply that the prolonged morbidity associated with this disease could lead to a serious disruption of schooling or even discontinuation of schooling among these children, thereby resulting in the raising up of a cohort of uneducated individuals with lost opportunities for possible future viable employments. (Yotsu, Murase, & Sugawara, 2015; Debacker, Aguiar, & Steunou, et al., 2004) Moreso, the complications that could arise from this infection may lead to deformities that could prevent the affected individuals from functioning optimally and eventually worsening their socio-economic situations. It has been postulated that young adults appear to be less often affected by M. ulcerans infection due possibly to the development of a certain degree of immunity to the causative organism. (Debacker, Aguiar, & Steunou, et al., 2004) This finding was however not reflected in this study as a significant proportion of the respondents were in the age range of 16 – 49 years. This age group is among the most productive age groups in the society and may therefore have serious socioeconomic implications. For instance, these individuals will likely be incapacitated by this disease and become dependent economically on others for survival throughout the duration of the disease. In addition, the hospitalization of a patient requiring the concomitant hospitalization of a healthy relative to provide indirect care to the patient in most hospitals in developing countries could lead to a considerable loss of productivity and greater poverty to the family.

The distribution of the male and female respondents in this study was almost equal. Several studies carried out in African and Australian settings also show a nearly equal sex distribution among the patients. (Debacker, Aguiar, & Steunou, et al., 2004; Noeske, Kuaban, & Rondini, et al., 2004; Amofah, 2002; Vincent, Ardant, & Adeye, et al., 2014) However, differences have been observed in the occurrence of cases between gender groups in some studies. (Amofah, 2002; Vincent, Ardant, & Adeye, et al., 2014; Hospers, Wiersma, & Dijkstra, et al., 2005) This finding may however be incidental as no explanation was proffered for it. Only a few of the respondents in this study did not acquire any formal education. This finding is similar to the finding by Nwafor et al., in southeastern Nigeria (Nwafor, Meka, & Chukwu, et al., 2019) and is to be expected in view of the consideration of the implementation of compulsory, free universal basic education by the government of Nigeria where it is expected that every government in Nigeria shall provide free, compulsory and universal basic education for every child of primary and junior secondary school age. (ILO, 2004) Acquisition of education has been shown to correlate positively with the ability to process information, acquire skills and model behavior. (NHDS, 2013) This finding in this study that only a few of the respondents did not acquire any formal education is therefore encouraging as it implies that the appropriate health education when instituted will be easily assimilated and result in better health care seeking behaviours.

M. ulcerans infection foci have been associated with wetlands. (Huang, & Johnson, 2014; Marsollier, Severin, Aubry, et al., 2004*a*; Marsollier, Severin, Aubry, et al., 2004*b*) This is in keeping with the findings from this study where the majority of the respondents live in Akwaibom State followed by Rivers State. These states are riverine states with many economic activities carried out along water courses. (About Akwa Ibom, 2022; The Government of Rivers State, 2022) Evidence shows that the major burden of the disease falls on populations living in West and Central Africa, where the disease typically occurs in rural, isolated foci associated with swampy lowlands and river valleys. (Marsollier, Severin, Aubry, et al., 2004*a*; Marsollier, Severin, Aubry, et al., 2004*b*) Epidemiological studies from several regions also reveal that contact with or proximity to water bodies is a risk factor for contracting the infection and that communities living along slow-flowing water bodies such as ponds, swamps and lakes are mostly affected. (Debacker, Aguiar, & Steunou, et al., 2004; Landier, Boisier, & Piam, 2011; Sopoh, Barogui, & Johnson, et al., 2010; Kenu, Nyarko, & Seefeld, et al., 2014; Raghunathan, Whitney, & Asamoa, et al., 2005; Aiga, Amano, & Cairncross, 2004; Nackers, Dramaix, Johnson, et al., 2006; Pouillot, Matias, & Wondje, 2007; Ahoua, Guetta, & Ekaza, 2009) Studies have also suggested that farming close to water bodies and swimming in endemic areas have been associated with increased cases and are potential risk factors for the acquisition of Buruli ulcer disease. (Aiga, Amano, & Cairncross, 2004; Marion, Landier, & Boisier, 2011) These findings have been reflected in this study as an appreciable proportion of the respondents were involved in farming and other activities related to water sources. Moreover, majority of the respondents in this study mentioned that they obtain their water from surface water bodies. M. ulcerans is an environmental pathogen that has been isolated from biofilms and small aquatic animals of slow-moving or stagnant water bodies. (Marsollier, Severin, Aubry, et al., 2004*a*; Marsollier, Severin, Aubry, et al., 2004*b*) Cases of M. ulcerans infection have also been known to occur mostly in areas around bodies of water that are stagnant or slow moving and in areas of endemicity, reservoirs of the mycobacterium appear to be in biofilms in water sources, aquatic insects, mollusks, and fish. (Merritt, Walker, & Small, et al., 2010) Even though statistically significant associations did not exist between the source of water and the occurrence of the disease in this study, possibilities still exist that these individuals could have become exposed to these infective organisms as they make contact with these water bodies. It has been postulated that M. ulcerans most likely causes infection through contamination of traumatic wounds. (Johnson, Makoutodé, & Sopoh, 2005; Quek, Athan, & Henry, 2007; Benbow, Williamson, & Kimbirauskas, 2008) This postulation has been supported by the documentation of the absence of use of protective equipment during agricultural activities as a risk factor for the acquisition of the disease. (N'krumah, Koné, & Tiembre, 2016) This may however not apply to the index study as only 12.8% of the respondents in this study mentioned that they had trauma to the site of the lesion prior to the onset of the disease. The implication of this finding may therefore be that other modes of transmission such as direct inoculation could be implicated just as was found in an epidemiologic research on outbreaks and animal studies in Australia. (Wallace, Mangas, & Porter, 2017) However, the long incubation period of 1-9 months associated with this disease in general could result in the majority of affected patients not recalling an inciting event. (Trubiano, Lavender, & Fyfe, et al., 2013) Therefore, the invasion of the organism into the host through the contamination of traumatic wounds as a mode of infection would not be excluded entirely in this study as almost all the respondents affirmed that they did not wear protective clothing while going to obtain water.

All the respondents in this study had already sought care elsewhere prior to presenting to the referral centre. Majority of them sought care from traditional healers while almost all of them admitted using traditional herbal medicine. This is despite the fact that all the respondents in this study were Christians. The seeking of care from several unorthodox centres prior to presentation could be the explanation for the delay in seeking appropriate treatment observed in this study. Treatment seeking behaviour could be related to the perception of the cause of the disease and the availability of treatment facilities. (Adogu, Igwe, & Chineke, et al., 2018) A study by Adogu et al., on the risk factors for Buruli ulcers in Nigeria showed that majority of the respondents believed that the disease was caused by witchcraft and should therefore be addressed by traditional healers or witchdoctors. Consequently, all the patients interviewed mentioned that they had sought treatment from various traditional and herbal practitioners while majority of them mentioned that they had visited various spiritual homes with no visible improvement or cure before presenting to the referral centre. ((Adogu, Igwe, & Chineke, et al., 2018) Even though the index study did not access perceptions, the possibility of such thoughts would not be excluded entirely as the social characteristics of both study groups could be similar and could connote that these individuals may not been exposed to the appropriate health education and sensitization to appropriate care-seeking, hence the delays. (Anagonou, Johnson, Barogui, et al., 2019; Maman I, Tchacondo T, Kere AB. et al., 2018)

Majority of the respondents in this study mentioned that none of their family members have the disease. This is in keeping with available evidence which suggests that human to human transmission of M. ulcerans does not occur. (Marsollier, Severin, Aubry, et al., 2004*a*; Marsollier, Severin, Aubry, et al., 2004*b*) However, a case–control study in Benin showed that the probability of contracting the disease is five times higher if a family member had previously developed the disease. (Sopoh GE, Barogui YT, Johnson, et al., 2010) This finding was however attributed to genetic predispositions to infection and not really to familial transmission. (Barogui Y, Klis S, Johnson, et al., 2016) Prevention of M. ulcerans infections is crucial as the disease is difficult to be accurately and timely diagnosed in endemic rural areas and could progress to functional limitations. The BCG vaccine has been shown to provide some protection against the onset of disease, though this effect is transient and may not last for more than one year. (Van der Werf, Stienstra, & Johnson, 2005; Nackers, Dramaix, & Johnson, et al., 2006) This may be the case in this study where the majority of the respondents mentioned that they had received BCG vaccination. The development of a vaccine that will prevent M. ulcerans infection would protect individuals at risk in highly endemic areas. It would also be used as a therapeutic vaccine that would shorten the duration of treatment and prevent relapse of infection. (Van der Werf, Stienstra, & Johnson, 2005; Nackers, Dramaix, & Johnson, et al., 2006) This coupled with other public health efforts aimed at prevention, early detection and prompt treatment of the disease will lead to improved clinical outcomes and decreased morbidity from M. ulcerans infection.

5. Conclusions / Recommendations

This study has shown that M. ulcerans infection is endemic in Nigeria especially among children aged ≤ 15 years old living in the riverine areas. Majority of the respondents were exposed to environmental risk factors while all the respondents had sought care elsewhere prior to presenting to the referral centre and this was mainly from traditional healers. Public health efforts aimed at patient education and to assist with prevention and early detection of the disease to minimize associated morbidity should therefore be instituted. Awareness campaigns and intensive appropriate health education on the

prevention and control of M. ulcerans infection should be given to community members by the organizers of the control programmes and sustained by appropriately trained community-based volunteers. This will lead to an increased capacity of community members to adopt positive behaviours, recognize the signs of the disease and refer suspected cases promptly for appropriate medical treatment.

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