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Histopathologic features of Buruli ulcer patients in a referral hospital in South Eastern Nigeria

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Abstract: **Background: Buruli ulcers are characterized by massive subcutaneous tissue destruction which can be treated effectively and completely when detected early. Characteristic histopathologic changes are considered one of the confirmatory laboratory methods for the diagnosis of Buruli ulcers.**

Objective: To assess the histopathologic features of Buruli ulcer cases presenting in a referral hospital in South Eastern Nigeria.

Methods: A cross-sectional prospective study was carried out among a cohort of 39 Buruli ulcer disease patients referred to a referral hospital in South Eastern Nigeria between July and December 2021. A semi-structured, interviewer-administered questionnaire was used to collect data on the socio-demographic characteristics of the respondents while the incisional skin biopsy specimens obtained from the patients in the course of their treatments were sent for histopathologic evaluation. 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 mean age was 28.41±18.668. Epidermal hyperplasia and elastolysis of the dermis were present in 35 (89.7%) of the patients. There was inflammation of the dermis and subcutis in 34 (87.2%) of the patients while necrosis of the subcutis was present in 38 (97.4%) of the patients. AFB was not visualized in any of the lesions of the patients. Overall, the characteristic histopathologic features of Buruli ulcer were demonstrated in 33 (84.6%) of the patients and four (10.3%) of the patients had features of squamous cell carcinoma while 2 (5.1%) had features of varicose veins.

Keywords: **Buruli ulcer, histopathology, hospital, Nigeria, Patient, South East.**

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I. INTRODUCTION

Buruli ulcer is the third most common mycobacterial disease in immuno-competent humans after tuberculosis and leprosy.[1] It occurs in many regions of the world especially in the tropical regions of West and Central Africa, Australia, and Japan.[2,3] Its major endemic foci are Subtropical and swampy terrains. [2,4-7] Mycobacterium ulcerans (*M. ulcerans*), the causative organism for Buruli ulcers, is thought to enter the body either through breaks in the skin or by direct inoculation.[7] Their plasmids contain DNA sequences which encode polyketide synthetases that produce toxins known as mycolactones, which are heat-stable polyketide macrolides.[8-10] Mycolactones are the main virulence factor produced by Mycobacterium ulcerans. They have cytotoxic, analgesic and immunosuppressive properties and are responsible for the massive subcutaneous tissue destructive pathology seen in Buruli ulcers. Mycolactones also aids the micro organism to evade recognition and control by the host immune system.

Several types of mycolactones have been isolated from clinical isolates of *M. ulcerans.*[11,12] However, the major and most potent mycolactones are a mixture of cis-trans isomers - mycolactones A and B. Following release, these toxins diffuse away from where the bacteria are present into the peripheral blood and reach lymphoid organs to accumulate in the fibroblasts and the macrophages.[9,13-15] Mycolactones act through various pathways to exert its effects. They have two main targets - scaffolding proteins such as Wiskott-Aldrich syndrome protein (WASP) and co-translational translocation proteins such as Sec61.[9] WASP is a protein that controls the junctional organization and coordinated migration of epidermal cells while Sec61 translates RNA and translocates proteins from the endoplasmic reticulum to the cytosol. Binding of mycolactones to WASP and other scaffolding proteins leads to their hyper-activation and the attendant disruption of the cell's actin cytoskeleton. The result is a lack of cell adhesion, poor movement of cells, and cell death with the attendant destruction of cutaneous tissues.[16-19] Binding of mycolactones to Sec61 leads to the inhibition of Sec61 translocation resulting in many proteins being degraded. This in turn produces local apoptosis and necrosis of many human cells including adipocytes, fibroblasts, and leukocytes.[9,20-22] Affected patients therefore exhibit global defects in protein metabolism evidenced by decreased levels of total serum proteins and blood urea nitrogen in the absence of malnutrition, liver impairment or kidney impairment.[8] Mycolactone also causes extensive coagulative necrosis through decreasing thrombomodulin expression on the surface of dermal microvascular endothelial cells and impairing the activation of protein C in the process.[23] The fibrin-driven ischemia thus leads to fibrin deposition and tissue necrosis of the affected region. Thrombosis due to mycolactone-associated vascular damage can occur and contribute to tissue necrosis beyond where the bacteria are located. Mycolactones inhibit pain perception. This is through the binding to and the activation of type 2 angiotensin II receptors on neurons leading to potassium-dependent hyper-polarization of neurons, neurite degeneration and cell death.[9,23] Studies carried out with mouse footpad showed that infection with M. ulcerans induces nerve fiber degeneration in advanced ulcers and neurological damages associated with hyposensitivity.[24,25] These studies support the hypothesis that Buruli ulcer associated analgesia may be due to cytopathic effects at the level of the lesion. However, it has not been ascertained that mycolactone diffuses into peripheral nerves to reach the central nervous system. The normal development of inflammatory and cellular immune responses to infections is impaired by mycolactones. Mycolactones exert its immunosuppressive effects by inhibiting the production of interleukin (IL)-12, interferon (IFN)-γ by T helper type 1 (Th1) cells and by suppressing the production of tumour necrosis factor (TNF)-α by monocytes.[26] Mycolactones also alter the immune responses of macrophages and other phagocytic cells, resulting in their difficulty in moving toward their target. The SLC11A1 gene polymorphism is thought to alter the protein function involved in mounting effective immune responses against intracellular pathogens, thereby leading to increased susceptibility to autoimmune and mycobacterial diseases.[27] In addition to mycolactones, it has been postulated that certain human polymorphisms in the SLC11A1 gene results in increased susceptibility to Buruli ulcer.[27]

The hallmark of Buruli ulcer is contiguous coagulation necrosis of the deep dermis and subcutaneous fat tissue with destruction of blood vessels and interstitial edema, epidermal hyperplasia and the presence of fat cell ghosts and extracellular clusters of acid fast bacilli (AFB). It primarily affects the subcutaneous adipose tissue and presents initially as a single, painless, subcutaneous nodule or papule. Eventually, the dermis and epidermis overlying the lesion degenerates, sloughs off, and leaves in its wake ulcers with undermined edges and necrotic sloughs at the base. In early lesions, the necrosis of dermal collagen has a fibrillary appearance, but gradually an amorphous coagulum developes in the center of the lesion with the disappearance of all cellular and structural details, and clusters of extracellular acid-fast bacilli are found primarily in the deep layers of the necrotic adipose tissue.[28] Inflammatory cells are rarely found in the center of active

lesions and cellular responses are defective at both the local and the systemic levels. In advanced lesions the necrotic process may extend through deep fascia and expose deeper structures like muscle or bone. Infection with *M. ulcerans* results in different outcomes. It may be contained by the immune system as evidenced by reports of spontaneous healing[29,30] and of *M. ulcerans*-specific immune responses in exposed but healthy individuals[31-33] or it may lead to a serious dermatologic manifestation and chronic necrotizing disease[34] with the attendant morbidity and socioeconomic burden.[35,36]

Buruli ulcers can be treated effectively and completely when detected early. However, Buruli ulcers can be confused with many diseases in each of its clinical stages and its diagnosis has remained a challenge in resource-poor countries.[20] To establish a definitive diagnosis and ensure the institution of appropriate and adequate treatment, appropriate laboratory tests and procedures are required.[8,37,38] Characteristic histopathologic changes are considered one of the confirmatory laboratory methods for the diagnosis of Buruli ulcers. Histopathologic specimens of Buruli ulcers exhibit extensive coagulation necrosis of the dermal collagen and the subcutaneous fat, together with destruction of cutaneous nerves, blood vessels, and adnexal structures. These necrosis may extend well beyond the edges of the ulceration.[20] Many authors have reported that the most reliable histopathologic feature for the diagnosis of Buruli ulcer disease is necrosis of subcutaneous tissues and dermal collagen accompanied by minimal inflammation and acid fast bacilli (AFB).[20,24,39] However, the histopathologic features of Buruli ulcer disease changes as the lesions evolve from a nodule to an ulcer.[20] For instance, in the early lesions, extracellular clumps of AFB may be seen at the base of the ulcer in the deep subcutaneous tissue. In active lesions, inflammatory infiltrate is usually absent to mild, although a leukocytoclastic vasculitis or thrombosis of small- and medium-sized vessels may be seen. In older lesions, granulomatous reactions occur with fewer organisms present, eventually progressing into cicatrix formation.[20] Epidermal hyperplasia and chronic inflammation with formation of granulomas are also more prominent in the later stages of the disease.[39] Epidermal regeneration occurs in an effort to cover the epidermal tissue defects resulting from the ulcerations.[39] During the pre-ulcerative stages and early in the ulcerative stages, the coagulative necrosis forms a nidus where calcifications and AFB colonies can be easily visualized.[40] However, when the ulcers start healing, and granulation tissue, fibrosis, and granulomatous inflammation become present, AFB becomes difficult to document[24,39] These findings may therefore suggest that new techniques that can be applied to tissues are greatly needed to diagnose Buruli ulcer disease in all stages. However, until these techniques become available, defining the diagnostic histopathologic features of buruli ulcers is essential as it will enable a better understanding of the characteristics of cases, facilitate early definitive diagnosis and make for the institution of early treatment and the prevention of the deforming sequelae of the disease. This study is therefore specifically aimed to assess the histopathologic features of Buruli ulcer cases presenting in a referral hospital in South Eastern Nigeria. The findings from the study are expected to provide the information that will bring to the fore the issue of advocacy for enhancing awareness, diagnosis and early case detection at the community level as well as to guide the policy makers in strengthening and improving the existing Buruli ulcer control programme in the state and elsewhere.

II. BODY OF ARTICLE

2.1. Study Area: This study was carried out at St Joseph's Hospital, Adazi Nnukwu, Anambra State Nigeria. St Joseph's Hospital, Adazi Nnukwu is a missionary hospital run by the Catholic Church Archdiocese in Awka. The hospital provides general health care services to its patients who generally come from Anambra State and its environs. The hospital also serves as a referral centre for the detection and treatment of Buruli ulcers in Anambra State and other parts of 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. In addition, enrolled patients are given weekly feeding allowances for the duration of their admission as well as transport fare to take them back home on discharge. The Buruli ulcer patients 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 Buruli ulcer focal persons living in the endemic areas or through outreach programmes organized on ad-hoc basis to create awareness on Buruli ulcers.

2.2. Study Design: This was a cross-sectional prospective study of a cohort of Buruli ulcer patients recruited and referred to the hospital between July and December 2021.

2.3. Study Population: Patients with suspected cases of Buruli ulcer recruited and referred to the hospital between July and December 2021.

2.3.1. Inclusion Criteria: Patients clinically diagnosed with Buruli ulcer who gave informed consent to participate in the study.

2.3.2. Exclusion Criteria: Patients clinically diagnosed with Buruli ulcer whose incisional skin biopsy specimens lacked subcutaneous adipose tissue.

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[41] and laboratory notes were used for this study. The questionnaires were used to collect information on the socio-demographic characteristics of the respondents while the laboratory notes were used to record the findings from the laboratory.

2.5. Data Collection Methods

2.5.1. Data Collection Method using Questionnaire: The semi-structured questionnaires were administered to the Buruli ulcer patients 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.

2.5.2. Procedure for Collecting and processing the Specimens: Incisional skin biopsy specimens including subcutaneous tissue were obtained from the patients in the course of their treatments. The skin biopsy specimens were collected from the necrotic base of the lesions and the undermined edges of the ulcers in order to increase the yield for the bacilli. The incisional skin biopsy specimens were then fixed in 10% neutral buffered formalin and transported to a standard pathology laboratory in Anambra state. Here, the specimens were converted into formalin-fixed paraffin-embedded tissue blocks which were then cut using a microtome into sections of about 4–6 μm thick and mounted on slides for processing and staining with haematoxylin and eosin stains to visualize details of the cellular and tissue structures; Ziehl–Neelsen stains to identify AFBs; and periodic acid-Schif stains to demonstrate fungal causes of nodules and ulcers and for differential diagnosis of cutaneous ulcerations.

2.6. Data Processing and Analysis: The histopathologic features of Buruli ulcer were evaluated in the three layers of the skin as follows - The presence of hyperplasia in the epidermis; the presence of elastolysis, inflammation and vascular changes in the dermis; and the presence of necrosis and inflammation in the subcutaneous tissue. The presence of AFB in any of the layers of the skin was also evaluated. These features were used to classify the cases as positive or negative for Buruli ulcer. Specimens in which the histopathologic features above with or without the presence of AFB were demonstrated were considered as positive cases while specimens without the histopathologic features above were considered as negative cases unless they have other diagnoses that could account for their presentation. These other diagnoses were listed as the differential diagnoses. Statistical data analysis was carried out with the aid of the International Business Machines-Statistical Package for Social Sciences (IBM-SPSS) version 22.[42] Frequency distribution of all relevant variables was developed. Their means and proportions were calculated. The association between the histopathologic features of Buruli ulcer and the socio-demographic characteristics of the respondents 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 this study was obtained from the Chukwuemeka Odumegwu Ojukwu University Teaching Hospital Ethics Committee. Permission to conduct the study was obtained from the German Leprosy and Tuberculosis Control Programme, Enugu; Anambra State ministry of health, Awka 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.

III. RESULTS

A total of 39 questionnaires were administered to all the clinically diagnosed Buruli ulcer patients referred to the hospital between July and December 2021. Incisional skin biopsy specimens were also obtained from all the patients and sent to the laboratory. All the questionnaires and laboratory results were retrieved, giving a response rate of 100%.

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TABLE I: SOCIO-DEMOGRAPHIC CHARACTERISTICS OF THE PATIENTS

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 I 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%).

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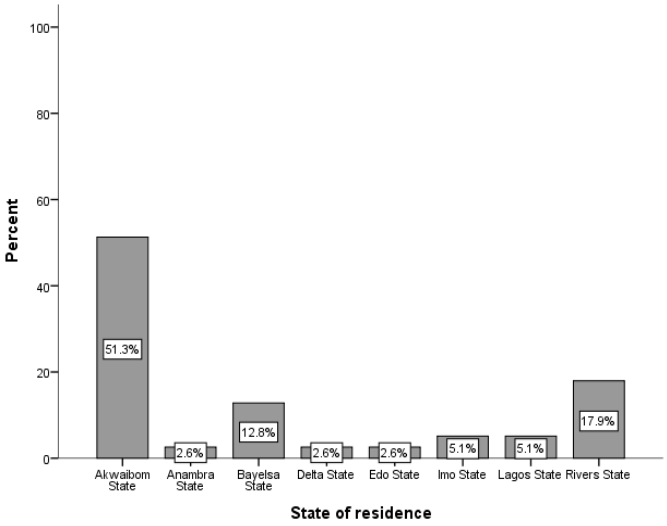

FIGURE1: STATE OF RESIDENCE OF THE PATIENTS

Figure 1 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 II: HISTOPATHOLOGIC FEATURES OF THE LESIONS OF THE PATIENTS

VARIABLE (LOCATION/FEATURES)	FREQUENCY (N = 39)	PERCENTAGE (%)
Epidermis		
Hyperplasia		
Present	35	89.7
Absent	4	10.3
Dermis		
Elastolysis		
Present	35	89.7
Absent	4	10.3
Inflammation		
Present	34	87.2
Absent	5	12.8
Vascular changes		
Present	33	84.6
Absent	6	15.4
Subcutis		
Necrosis		
Present	38	97.4
Absent	1	2.6
Inflammation		
Present	34	87.2
Absent	5	12.8
AFB		
Present	0	0.0
Absent	39	100

Table II details the histopathologic features of the lesions of the patients. Epidermal hyperplasia and elastolysis of the dermis were present in 35 (89.7%) of the patients. There was inflammation of the dermis and subcutis in 34 (87.2%) of the patients while necrosis of the subcutis was present in 38 (97.4%) of the patients. AFB was not visualized in any of the layers of the skin lesions of the patients.

TABLE III: HISTOPATHOLOGIC DIAGNOSIS OF THE PATIENTS

Variable	Frequency (n = 39)	Percentage (%)
Buruli ulcer		
Present	33	84.6
Absent	6	15.4
Squamous cell carcinoma		
Present	4	10.3
Absent	35	89.7
Varicose vein		
Present	2	5.1
Absent	37	94.9
Fungal spores		
Present	0	0.00
Absent	39	100

Table III shows the histopathologic diagnosis of the patients. The characteristic histopathologic features of Buruli ulcer were demonstrated in 33 (84.6%) of the patients. Four (10.3%) of the patients had features of squamous cell carcinoma while 2 (5.1%) had features of varicose veins. Fungal spores were not demonstrated among any of the lesions of the patients.

TABLE IV: ASSOCIATION BETWEEN THE HISTOPATHOLOGIC FEATURES OF BURULI ULCER AND THE SOCIODEMOGRAPHIC CHARACTERISTICS OF THE PATIENTS

VARIABLE	HISTOPATHOLOGIC FEATURES PRESENT (N = 33) (NUMBER, %)	HISTOPATHOLOGIC FEATURES 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)		

F = Fisher's Exact Test; *Statistical significance = P ≤ 0.05

Table IV shows the association between the histopathologic features of Buruli ulcer and the socio-demographic characteristics of the patients at the bivariate level using Fischer's exact test of statistical significance. None of the variables analyzed achieved statistically significant associations with the histopathologic features of Buruli ulcer.

IV. DISCUSSIONS

This cross-sectional prospective study was carried out to assess the histopathologic features of Buruli ulcer cases presenting in a referral hospital in South Eastern Nigeria. Necrosis of subcutaneous tissues and dermal collagen accompanied by minimal inflammation was demonstrated extensively in this study. This is in keeping with the finding in many studies which show that the most reliable histopathologic feature for the diagnosis of Buruli ulcer disease is necrosis of subcutaneous tissues and dermal collagen accompanied by minimal inflammation and AFB.[20,24] The demonstration of minimal inflammation compared to the extensive necrosis observed in this study could be attributed to the toxin, mycolactone, inducing necrosis of the inflammatory infiltrates as they induce the necrosis of subcutaneous tissues and dermal collagen.[32,43] Epidermal hyperplasia is a reflection of the effort of the body to cover epidermal tissue defects due to ulcerations.[36] Epidermal hyperplasia and chronic inflammation with formation of granulomas have been described to be more prominent in the later stages of the Buruli ulcer disease.[24,39] This was also the case in the index study. Here, both the psoriasiform and the pseudoepitheliomatous forms of epidermal hyperplasia were observed and could connote that the ulcers were beginning to heal. Acid Fast Bacillis were not visualized in the histopathologic sections in this study. Many authors have established that during the pre-ulcerative stages and early in the ulcerative stages, the coagulative necrosis forms a nidus where calcifications and AFB colonies can be easily visualized but when the ulcers start healing and granulation tissue, fibrosis, and granulomatous inflammation become present, AFBs become difficult to document[23,39] Perhaps, this explanation could account for the inability to demonstrate AFBs in this study since the cases were all referred and in the chronic stages. However, the findings from a study on the histopathologic features of Mycobacterium ulcerans infection by Guarner et al., that statistically significant differences did not exist in the amount of AFB in the subcutaneous tissues in the preulcerative and ulcerative stages[20] could connote that new techniques that can be applied to tissues are greatly needed to diagnose Buruli ulcer disease in all stages. The WHO recommends that the concentrations of chemicals used in the demonstration of AFBs in Buruli ulcers be slightly different from those often used in other mycobactrial diseases. The fuchsin concentration should be slightly higher (1%) and the methylene blue lower (0.1g in 100ml of distilled water), so that the best possible contrast (that is, strong red bacilli against a light blue background) will be provided.[44] This recommendation was perhaps adopted in the study by Guarner et al.

Histopathology could identify 84.6% of the respondents in this study as having Buruli ulcer. Its sensitivity is therefore very high. This means that accurate diagnosis could be made quickly through histopathology and appropriate treatment initiated promptly thereby leading to the prevention of the deforming sequelae associated with delaying treatment of the disease. Histopathology has also been demonstrated to be used to provide alternative diagnoses of biopsied lesions that are not Buruli

ulcers. This was also the case in this study where histopathology was used to identify 33 cases (84.6%) as Buruli ulcer disease, 4 cases (10.3%) as squamous cell carcinoma and 2 cases (5.1%) as ulcers secondary to arterial-venous insufficiency (varicose veins). Findings from studies have shown that long-standing Buruli ulcers may transform into squamous cell carcinomas.[45,46] Even though it could not be ascertained whether these 3 squamous cell carcinoma cases were Buruli ulcer diseases ab-initio that later transformed into malignancies or not, the precise diagnosis of the cases through histology in this study is still commendable as it connotes that targeted therapy could be initiated promptly and that the outcome of therapy would likely be more favourable. The World Health Organization currently recommends the use of two tests to confirm the diagnosis of Buruli ulcer.[44,47] This is to avoid a misdiagnosis due to false-positive or false-negative results. In areas of endemicity however, the use of only one test may be necessary for the confirmation due to the predictive value of positive results while in areas of non-endemicity where case diagnosis can be difficult, confirmation of the disease requires the use of several diagnostic methods at any of the stages.[20,44] Africa is known to be endemic for Buruli ulcers.[1,48] Histopathology yields results fairly rapidly, has a high sensitivity and is very useful for monitoring response to therapy. Moreover, the procedure does not require strict quality control unlike the other diagnostic methods; and medical assistants can be trained to perform excision procedures and transport the specimens quickly to a district laboratory for processing. Consultations are currently underway to develop rapid diagnostic tests for Buruli ulcers that will lead to the early confirmation of diagnosis and facilitate the timely management of the disease.[49] In the interim, histopathology can be easily adapted for use as a diagnostic tool in Africa.

V. CONCLUSIONS / RECOMMENDATIONS

This study has shown that histopathology could be used to make accurate diagnosis of Buruli ulcers fairly rapidly. It has also shown that histopathology could be used to provide alternative diagnoses of biopsied lesions that are not Buruli ulcers. Histopathologic laboratories and services for the histopathologic screening for the characteristics of Buruli ulcers should therefore be set up in all endemic areas by the government in partnership with the organizers of Buruli ulcer control programmes. This will result in accurate diagnosis being made quickly and appropriate treatment initiated promptly thereby leading to the prevention of the deforming sequelae associated with delaying treatment of the disease.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

REFERENCES

[1] World Health Organization. Buruli ulcer disease (Mycobacterium ulcerans infection). Available from: http://www.who.int/mediacentre/factsheets/fs199/en/. [Accessed April 3, 2021]

[2] G.K.L. Huang, and P.D. Johnson. Epidemiology and management of Buruli ulcer. *Expert Rev Anti Infect Ther*. Vol. 12, pp. 855-865, 2014.

[3] World Health Organization. Buruli ulcer disease factsheet. 2015. Available from: http://www.who.int/mediacentre/factsheets/fs199/en/index.html [Accessed April 3, 2021]

[4] M. Bratschi, M. Bolz, J. Minyem, L. Grize, F. Wantong, and S. Kerber, et al. Geographic distribution, age pattern and sites of lesions in a cohort of buruli ulcer patients from the Mapé Basin of Cameroon. *PLO Negl Trop Dis.* Vol.7, 2013.

[5] World Health Organization. Incidence, prevalence and mapping of Buruli ulcer. Available from: http://www.who.int/buruli/research/priorities/healthmapping/en/ [Accessed April 3, 2021]

[6] P.D. Mavinga, P. Suykerbuyk, P. Saunderson, L.P. Ngwala, M.J.B. Masamba, and D.B. Imposo, et al. Burden of Mycobacterium ulcerans disease (Buruli ulcer) and the underreporting ratio in the territory of Songololo, Democratic Republic of Congo. *PLoS Negl Trop Dis.* Vol. 7, pp. e2563, 2013

[7] R.W. Merritt, E.D. Walker, P.L.C. Small, J.R. Wallace, P.D.R. Johnson, and M.E. Benbow, et al. Ecology and Transmission of Buruli Ulcer Disease: A Systematic Review. *PLoS Negl Trop Dis.* Vol. 4, pp. e911, 2014.

[8] R.O. Phillips, F.S. Sarfo, J. Landier, R. Oldenburg, M. Frimpong, and M. Wansbrough-Jones, et al. Combined inflammatory and metabolic defects reflected by reduced serum protein levels in patients with Buruli ulcer disease. *PLoS Negl Trop Dis*. Vol. 8, No. 4, pp. e2786, 2014.

[9] U. Anand, M. Sinisi, M. Fox, A. MacQuillan, T. Quick, and Y. Korchev, et al. Mycolactone-mediated neurite degeneration and functional effects in cultured human and rat DRG neurons: Mechanisms underlying hypoalgesia in Buruli ulcer. *Mol Pain*. Vol. 12, pp. 1744806916654144, 2016.

[10] N.J. Tobias. Molecular studies of Mycobacterium ulcerans. Thesis. Monash University. 2017. Available from: https://doi.org/10.4225/03/589bc155baf08. [Accessed March 18, 2022]

[11] H. Hong, P.J. Gates, J. Staunton, T. Stinear, S.T. Cole, P.F. Leadlay, and J.B. Spencer. *Chem. Commun.* pp. 2822, 2003

[12] A. Mve-Obiang, R.E. Lee, F. Portaels, and P.L.C. Small. *Infect. Immun.* Vol. 71, pp. 774, 2003

[13] H. Hong, E. Coutanceau, M. Leclerc, L. Caleechurn, P.F. Leadlay, and C. Demangel. Mycolactone diffuses from *Mycobacterium ulcerans*-infected tissues and targets mononuclear cells in peripheral blood and lymphoid organs. *PLoS Negl Trop Dis.* Vol. 2, pp. e325, 2008.

[14] F.S. Sarfo, R.O. Phillips, J. Zhang, M.K. Abass, J. Abotsi, and Y.A. Amoako, et al. Kinetics of mycolactone in human subcutaneous tissue during antibiotic therapy for *Mycobacterium ulcerans* disease. *BMC Infect Dis*. Vol. 14, pp. 202, 2014.

[15] F.S. Sarfo, F. Le Chevalier, N. Aka, R.O. Phillips, Y. Amoako, and I.G.Boneca, et al. Mycolactone diffuses into the peripheral blood of Buruli ulcer patients - implications for diagnosis and disease monitoring. *PLoS Negl Trop Dis.* Vol. 5, pp. e1237, 2011.

[16] D.S. Snyder, and P.L. Small. Uptake and cellular actions of mycolactone, a virulence determinant for Mycobacterium ulcerans. *Microb Pathog*. Vol. 34, pp. 34:91–101, 2003.

[17] L. Guenin-Mace, R. Veyron-Churlet, M.I. Thoulouze, G. Romet-Lemonne, H. Hong, and P.F. Leadlay, et al. Mycolactone activation of Wiskott-Aldrich syndrome proteins underpins Buruli ulcer formation. *J Clin Invest.* Vol. 123, No. 4, pp. 1501–1512, 2013.

[18] J.B. Gama, S. Ohlmeier, T.G. Martins, A.G. Fraga, B. Sampaio-Marques, and M.A. Carvalho, et al. Proteomic analysis of the action of the *Mycobacterium ulcerans* toxin mycolactone: targeting host cells cytoskeleton and collagen. *PLoS Negl Trop Dis*. Vol. 8, No. e3066, 2014.

[19] F.S. Sarfo, R. Phillips, M. Wansbrough-Jones, R.E. Simmonds. Recent advances: role of mycolactone in the pathogenesis and monitoring of Mycobacterium ulcerans infection/Buruli ulcer disease. *Cell Microbiol*. Vol. 18, No. 1, pp. 17-29, 2016.

[20] J. Guarner, J. Bartlett, E.A. Whitney, P.L. Raghunathan, Y. Stienstra, and K. Asamoa K, et al. Histopathologic features of Mycobacterium ulcerans infection. *Emerg Infect Dis.* 2003;9(6):651-656.

[21] K.M. George, L. Pascopella, D.M. Welty, and P.L. Small. A *Mycobacterium ulcerans* toxin, mycolactone, causes apoptosis in guinea pig ulcers and tissue culture cells. *Infect Immun.* Vol. 68, pp. 877–883, 2000

[22] R. Bieri, N. Scherr, M.T. Ruf, J.P. Dangy, P. Gersbach, and M. Gehringer, et al. The macrolide toxin mycolactone promotes bim-dependent apoptosis in Buruli ulcer through inhibition of mTOR. *ACS Chem Biol.* Vol. 12, pp. 1297–1307, 2017.

[23] J. Ogbechi, M.T. Ruf, B.S. Hall, K. Bodman-Smith, M. Vogel, and H.L. Wu, et al. Mycolactone-Dependent Depletion of Endothelial Cell Thrombomodulin Is Strongly Associated with Fibrin Deposition in Buruli Ulcer Lesions. *PLoS Pathog*. Vpl. 11, No. 7, pp. 1005011, 2015.

[24] D.H, Connor, C.M. Washington, and L.H. Fletcher. Buruli ulceration, a clinicopathologic study of 38 Ugandans with *Mycobacterium ulcerans* ulceration. *Arch Pathol*. Vol. 81, pp. 183–99, 1966.

[25] J. En, M. Goto, K. Nakanaga, M. Higashi, N. Ishii, and H. Saito, et al. Mycolactone is responsible for the painlessness of *Mycobacterium ulcerans* infection (Buruli ulcer) in a murine study. *Infect Immun.* Vol. 76, pp. 2002–2007, 2008

[26] A.E. Kiszewski, E. Becerril, L.D. Aguilar, I.T. Kader, W. Myers, and F. Portaels, et al. The local immune response in ulcerative lesions of Buruli disease. Clin Exp Immunol. Vol. 143, No. 3, pp. 445-451, 2006

[27] M. Gazouli, V. Atsaves, G. Mantzaris, M. Economou, G. Nasioulas, and K. Evangelou, et al. Role of functional polymorphisms of NRAMP1 gene for the development of Crohn's disease. *Inflamm Bowel Dis.* Vol. 14, pp. 1323–1330, 2008.

[28] M.T. Ruf, C. Steffen, M. Bolz, P. Schmid, and G. Pluschke. Infiltrating leukocytes surround early Buruli ulcer lesions, but are unable to reach the mycolactone producing mycobacteria. *Virulence.* Vol. 8, pp. 1918–1926, 2017.

[29] D.P. O'Brien, J.W. Wynne, A.H. Buultjens, W.P. Michalski, T.P. Stinear, and N.D. Friedman ND, et al. Exposure risk for infection and lack of human-to-human transmission of *Mycobacterium ulcerans* disease, Australia. *Emerg Infect Dis.* Vol. 23, No. 5, pp. 837–40, 2017

[30] E. Marion, J. Landier, P. Boisier, L. Marsollier, A. Fontanet, and P. Le Gall, et al. Geographic expansion of Buruli ulcer disease, Cameroon. *Emerg Infect Dis.* Vol. 17, No. 3, pp. 551-553, 2011.

[31] D. Yeboah-Manu, S.Y. Aboagye, P. Asare, A. Asante-Poku, K. Ampah, and E. Danso, et al. Laboratory confirmation of Buruli ulcer cases in Ghana, 2008-2016. *PLoS Negl Trop Dis.* Vol. 12, No. 6, pp. e0006560, 2018.

[32] T.M. Gooding, P.D. Johnson, D.E. Campbell, J.A. Hayman, E.L. Hartland, and A.S. Kemp, et al. Immune response to infection with *Mycobacterium ulcerans*. *Infect Immun.* Vol. 69, pp. 1704–1707, 2001.

[33] D. Diaz, H. Döbeli, D. Yeboah-Manu, E. Mensah-Quainoo, A. Friedlein, and N. Soder, et al. Use of the immunodominant 18-kiloDalton small heat shock protein as a serological marker for exposure to *Mycobacterium ulcerans*. *Clin. Vaccine Immunol.* Vol. 13, pp. 1314–1321, 2006

[34] G. Pluschke, and K. Röltgen. (eds). *Buruli Ulcer: Mycobacterium ulcerans Disease.* Cham: Springer International Publishing, 2019.

[35] G. Foxcroft. Witchcraft accusations: A protection concern for UNHCR and the Wider Humanitarian Community? Paper Presented to UNHCR. Lancaster, United Kingdom: Child Rights Information Network. 2009. Available from: www.crin.org/docs/Stepping_stones_witchcraft.pdf. [Accessed January 24, 2022]

[36] K.P. Grietens, A.U. Boock, H. Peeters, S. Hausmann-Muela, E. Toomer, and J.M. Ribera. "It is me who endures but my family that suffers": Social isolation as a consequence of the household cost burden of Buruli ulcer free of charge hospital treatment. *PLoS Negl Trop Dis.* Vol. 2, No. 10, pp. e321, 2008.

[37] K. Asiedu, R. Scherpbier, and M. Raviglione. Buruli ulcer, *Mycobacterium ulcerans* infection. Geneva: World Health Organization; 2000. Available from: https://www.who.int › Publications › i › item [Accessed April 6, 2021]

[38] C.H. King, D.A. Ashford, K.M. Dobos, E.A.S. Whitney, P.L. Raghunathan, and J. Guarner, et al. *Mycobacterium ulcerans* infection and Buruli ulcer disease: emergence of a public health dilemma. In: Scheld WM, Craig WA, Hughes JM, editors. Emerging infections. Washington: American Society of Microbiology Press; Vol. 9, pp. 137–152, 2019

[39] J. Hayman. Out of Africa: Observations on the histopathology of *Mycobacterium ulcerans* infection. *J Clin Pathol*. Vol. 46, pp. 5–9, 1993

[40] J.D. Igo, and D.P. Murthy. Mycobacterium ulcerans infections in Papua New Guinea: correlation of clinical, histological, and microbiologic features. *Am J Trop Med Hyg.* Vol. 38, No. 2, pp. 391-392, 1988

[41] J. Buntine, and K. Crofts. eds. World Health Organization. Global Buruli Ulcer Initiative 2001. Buruli ulcer: management of Mycobacterium ulcerans disease: a manual for health care providers. Available from: https://apps.who.int/iris/handle/10665/67001. [Accessed April 13, 2021]

[42] International Business Machines Corporation. IBM-Statistical Package for the Social Sciences (SPSS) Statistics 20. Somers New York: IBM Corporation, 2011.

[43] A.A. Pahlevan, D.J. Wright, C. Andrews, K.M. George, P.L. Small, and B.M. Foxwell. The inhibitory action of *Mycobacterium ulcerans* soluble factor on monocyte/T cell cytokine production and NF-kappa B function. *J Immunol.* Vol. 163, pp. 3928–3935, 1999

[44] F. Portaels, P. Johnson, and W.M. Meyers. Buruli ulcer: diagnosis of *Mycobacterium ulcerans* disease. In: F. Portaels, P. Johnson, W.M. Meyers, editors. Buruli ulcer: a manual for health care providers. Geneva, Switzerland: WHO/CDS/GBUI, pp. 4–92, 2001

[45] V. Pommelet, Q.B. Vincent, M.F. Ardant, A. Adeye, A. Tanase, and L. Tondeur, et al. Findings in patients from Benin with osteomyelitis and polymerase chain reaction-confirmed Mycobacterium ulcerans infection. *Clin Infect Dis*. Vol. 59, No. 9, pp. 1256-1264, Nov. 2014

[46] E. Minutilli, G. Orefici, M. Pardini, F. Giannoni, L.M. Muscardin, and G. Massi, et al. Squamous cell carcinoma secondary to Buruli ulcer. *Dermatol Surg*. Vol. 33, No. 7, pp. 872-875, 2007

[47] J.K.T. Akoachere, F.S. Nsai, and N.R. Ndip. A Community based study on the mode of transmission, prevention and treatment of Buruli ulcers in Southwest Cameroon: Knowledge, attitude and practices. *PLOS Negl Trop Dis.* Vol. 11, No. 5, Pp. e0156463, 2016.

[48] K. Röltgen, and G. Pluschke. Buruli Ulcer: History and Disease Burden. In: Pluschke G, Röltgen K, editors. Buruli Ulcer: Mycobacterium Ulcerans Disease. 2019. Available from: https://www.ncbi.nlm.nih.gov/books/NBK553836/ [Accessed April 10,.2021]

[49] WHO. Promoting an integrated approach to enhance the detection of neglected tropical diseases of the skin. Available from: https://www.who.int/news/item/15-03- [Accessed April 18, 2022]