Buruli ulcer in Malawi - a first report

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Abstract

One hundred and sixty-one specimens swabbed from as many patients with chronic wounds/ulcers over a period of eight months yielded 3 acid-alcohol fast bacilli (AFB) organisms that slowly grew only at 32⁰C on Lowenstein-Jensen(LJ) medium producing creamy-yellow colonies between 39 and 45 days post-incubation. Mycobacterial organisms harvested from culture were strongly positive when subjected to both catalase spot test and catalase heat stability test indicating the presence of *Mycobacterium ulcerans*, the aetiological agent of Buruli ulcer.

Introduction

Mycobacterium ulcerans infection otherwise called Buruli ulcer is the third most common mycobacterial infection of immunocompetent humans after tuberculosis and leprosy[1]. The disease affects otherwise healthy individuals irrespective of age, sex and race[2] and has rapidly emerged as an important cause of human morbidity around the world[3]. Buruli ulcer has been reported and confirmed in the Americas (Bolivia, French Guyana, Mexico, Peru, Suriname); Asia (China, India, Sri Lanka, Indonesia, Malaysia, Sumatra); and in the Western Pacific (Australia, Karibati, Papua New Guinea). However, most disease occurs in rural equatorial Africa where multiple endemic foci have been reported from Benin, Burkina Faso, Cameroon, Congo, Cote dilvore, Zaire (now DRC), Equatorial Guinea, Gabon, Ghana, Guinea, Liberia, Nigeria, Sierra Leone, Togo, Uganda and Sudan[4]. An index case involving a 6 year old child in Angola has also been reported[5]. Till date, there is no documented evidence of Buruli ulcer in Malawi.

In this communication, the identification of three cases of *M. ulcerans* infection following a bacteriological screening of 161 chronic wounds/ulcers at the Queen Elizabeth Central Hospital (QECH), the largest referral hospital in Malawi, is reported.

Methods

Wounds of more than 4 weeks duration were swabbed from outpatients presenting wounds for dressing at the QECH and the specimens were thereafter transferred to the Microbiology laboratory. However, prior to specimen collection, all presenting ulcers were closely examined while information concerning the patients age, village, occupation, source, duration and the painfulness or otherwise of the wounds were obtained. Previous treatments applied before consulting the hospital were also noted.

All specimens were subjected to Ziehl-Neelsen staining[6] and patients with AFB-positive ulcers were invited to the laboratory where fresh samples were taken and immediately subcultured on Lowenstein-Jensen (LJ) medium with the appropriate controls. All cultures were incubated within minutes of inoculation at 25⁰C, 32⁰C and 37⁰C respectively and observed weekly for 12 weeks. Criteria for positive mycobacterial growth have been previously described by the Centre for Disease Control[7].

Colonies of mycobacterial organisms harvested from culture were subjected to both catalase 'spot' test and test for heat-stable catalase as previously described[6]. Briefly, 2 drops of freshly prepared Tween-peroxide solution were dropped on harvested colonies of the test organisms placed on a microscope slide and the reaction was observed for evolution of bubbles for a few seconds.

For the heat-stable catalase test, colonies of the mycobacterial growth were emulsified with M/15 phosphate buffer/PH 7.0 in a small test tube. The tube was immediately incubated in a water bath at 68⁰C for 20 minutes and after cooling at room temperature, 0.5ml of freshly prepared Tween-peroxide was added. The reaction was observed for bubbles and discarded after about 25 mins.

Results

Three of the 161 (1.9%) specimens analysed were AFB positive with mycobacterial organisms occurring either singly or in clumps. All 3 specimens grew only at 32⁰C on LJ medium and evidence of growth was first observed between 39 and 45 days post incubation gradually producing creamy-yellow colonies. *Mycobacterium tuberculosis* used as controls grew luxuriantly at 37⁰C with traces of growth at 32⁰C. However, both the test and control organisms failed to grow at 25⁰C and neither did the test organisms grow at 37⁰C even after a 12-week incubation period.

The spot test for catalase showed a rapid evolution of bubbles within seconds of adding Tween-peroxide solution to the test organisms and a similar reaction was observed following the test for heat stable catalase. The results of this study are represented in the table. All 3 AFB-positive ulcers had undermined edges with pronounced yellowish-white, cottonwool like necrotic bed (Figure)

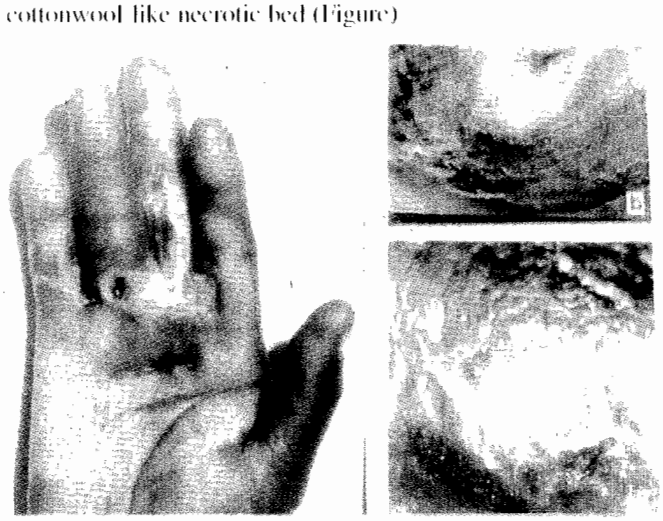

Figure. Three cases of *Mycobacterium ulcerans* infection. (a) Lesion from a 45-year old woman involving the base of all four fingers and half of the middle finger of the right hand. Note the unaffected medial nerve (arrow) of the middle finger. The ulcer had undergone a surgical intervention prior to this study. (b and c) Buruli ulcer on the left leg of two other outpatients. Note the cottonwool-like necrotic bed at the centre of the ulcers.

Discussion

Mycobacterium ulcerans infection or Buruli ulcer has hitherto not been documented in Malawi although certain ulcer presentations tend to support the presence of the disease in the country (Prof Liomba ñ personal communication). Except for an only case reported from Angola[5] a country on the same latitude with Malawi, Buruli ulcer has not been reported from the South African subregion.

In the present study, the identification of 3 painless ulcers with undermined boarders and a pronounced necrotic bed coupled with the isolation of mycobacterial organisms that typically grew slowly (39-45 days) at 32^{0}C[4], and the inability of same to grow at either 37^{0}C (like *M. tuberculosis*) or 25^{0}C (like *M. marinum*) are characteristics compatible with *M. ulcerans*. Being a slow grower, the test organism can be further differenciated from *M. marinum* which is a fast grower (5-14 days) at 30^{0}C[6]. It is also interesting to note that one of the patients, a 45-year old woman (fig 1a) had a history of surgical intervention as a treatment panacea for her ulcer, which is also the treatment of choice for Buruli ulcer[2,4].

Contrary to earlier reports that *M. ulcerans* is fastidious and grows with difficulty in culture,[8,9] organism culture in this study was achieved with relative ease probably because patients presented to the laboratory for samples to be collected and thereafter these samples were rapidly inoculated onto culture media. By so doing, the use of transport medium was excluded, specimen storage was avoided and specimen exposure period to deleterious environmental conditions was drastically reduced, thus maximally preserving the integrity and viability of the test organisms under study.

In the absence of polymerase chain reaction (PCR) technique adjudged most efficient in *M. ulcerans* detection[10] the catalase spot test and catalase heat-stability test described by Koneman et al[6] were used in this study. The positive reaction exhibited by the test organisms further eliminated M. *tuberculosis* and *M. marinum* (which are catalase negative at 68^{0}C)[6] as the casual agents of the infections.

With regards to patient/ulcer demography (Table 1), there were 2 males and 1 female, all adults aged 32, 38 and 45 years respectively. They all agreed to have used local herbs for treatment before presenting to the hospital. All the ulcers were on the extremities and none could remember the source of his/her ulcer.

Presently, the epidemiology and modes of transmission of *M. ulcerans* are not entirely known but there is a strong evidence that, being an environmental organism, *M. ulcerans* probably enters the body through small breaks in the skin from contaminated soil, water or vegetation. This may explain why most infection occurs on the exposed parts of the body especially the extremities with the lower limbs more frequently infected than the upper[4]. The only female patient in this study, a vegetable trader might have been particularly at risk by virtue of her vocation.

In conclusion, the prevalence of *M. ulcerans* infection in this study was 1.86% (3/161) which probably could have been higher if all specimens had been cultured to detect AFB smear-negative otherwise positive specimens or if the PCR technique had been used. On the other hand, QECH being a referral hospital, too much importance should not be attached to the high prevalence of Buruli ulcer observed in this study as it does not represent the national average. On the basis of this result however, there may be need to carry out a larger survey of *Mycobacterium ulcerans* infection in Malawi so as to determine its true prevalence and the possible socio-economic impact on the populace.

Acknowledgements

I wish to gratefully acknowledge the assistance of Mrs Chagomelana (OPD II, QECH) in specimen collection and the technical assistance of N Chilewani and M Ndileke, technicians in the Microbiology laboratory, College of Medicine.

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Table. Patient-ulcer demography

Patient	*Sex*	*Age (yrs)*	*Vocation*	*Village*	*Wound site*	*Source of wound*	*Duration*	*Pain (Yes/No)*	*History of surgical intervention*
A	Female	45	Trading on vegetables	Thyolo	Right hand involving 4 fingers	Unknown	> 7 months	No	Yes
B	Male	32	Retrenched civil servant	Ntcheu	Left leg	Unknown	>5 months	No	No
C	Male	38	Labourer	Nkhatabay	Left leg (mediai side)	Unknown	>4 months	No	No

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