SCHOOL OF PUBLIC HEALTH, COLLEGE OF HEALTH SCIENCES, UNIVERSITY OF GHANA, LEGON

# ANTIBIOTIC TREATMENT OUTCOMES OF BURULI ULCER IN AKWAPEM SOUTH AND SUHUM-KRABOA-COALTAR DISTRICTS

BY

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**DECLARATION**

I hereby declare that with the exception of the references cited to other people's work which has been duly acknowledged, this work is the result of my own research work, done under supervision and has neither in part or whole been presented elsewhere for another degree.

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**DEDICATION**

I dedicate this work to my husband William and my children Charleen, Michelle and Neequaye.

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## ABSTRACT

The World Health Organization (WHO) having recognized Buruli ulcer disease as an important cause of human suffering introduced treatment guidelines of a new protocol of 8-week initial therapy of intramuscular streptomycin and oral rifampicin in 2005. Although there has been some level of success in the treatment of Buruli ulcer with this new antibiotic protocol, some patients do not respond favourably as expected. Also, the response to chemotherapy of BU lesions including large ulcerated forms, which are currently the most common forms in Africa remains insufficiently documented. So this study set out to assess the treatment outcomes of all categories of BU lesions and determine factors that influence the healing of these lesions. This was implemented by employing a non-randomized clinical intervention design by serially recruiting 154 patients over a period of two years. Measurements of lesions were made using tracing sheets to obtain their respective surface areas. Swabs and fine needle aspirates were taken and confirmed by direct smear microscopy for acid-fast bacilli (AFB), polymerase chain reaction or culture.

All patients were given a directly observed treatment (DOT) of a daily combination of intramuscular streptomycin (15mg/kg body weight) and oral rifampicin (10mg/kg body weight) for 8 weeks. There was also daily dressing for all wounds irrespective of size and weekly assessment for all forms of lesions. Patients were counseled regularly on BU management and the necessity for adhering to treatment. All patients were followed up for a minimum of 34 weeks. BU lesions were found among ages 2-84 years. About 37% of the total study participants were less than 15 years and almost equal numbers in the age groups between 15 -49 years and over 50 years old. In those below 15 years of age there was an almost equal gender distribution whilst, in the

older age groups more females than males were affected. It was found that 93.5% (144/154) of the study participants presented with ulcers. All those who reported within two months of noticing their lesions for the first time achieved 100% treatment success irrespective of category of lesion.

There was 97.4% treatment success rate for all BU lesions with no recurrences within 34 weeks of treatment. About 30% of lesions showed an apparent deterioration response to treatment (paradoxical reactions) after an initial improvement at some points during treatment starting from week 2 to week 18 reaching a peak at week 10.

Factors that hastened healing were smaller size of the initial lesion, regular wound dressing, removal of slough, treatment with topical antibiotics and absence of paradoxical reactions. We concluded that the combination of intramuscular streptomycin and oral rifampicin is efficacious in healing all forms of Buruli ulcer disease. Optimal and regular wound dressing regular counseling, consistent wound evaluation and timely interventions play important roles in the healing of BU lesions. In view of these findings we recommended that the National Buruli Ulcer Control Programme in collaboration with the District Assemblies and District Health Management Teams should organize regular health education activities to encourage patients to report early to health institutions and also ensure that drug treatment is combined with wound dressing effectively and efficiently in order to achieve the desired results.

# TABLE OF CONTENTS

## LIST OF TABLES

## LIST OF FIGURES

## LIST OF ABBREVIATIONS

| | |
|---|---|
| AFB | Acid Fast Bacilli |
| AKS | Akwapem South Municipality |
| BU | Buruli Ulcer |
| CBSV | Community-based Surveillance Volunteers |
| cm | Centimeter |
| $cm^2$ | Centimeters squared |
| DNA | Deoxyribonucleic acid |
| FBC | Full blood count |
| FBS | Fasting blood sugar |
| FNA | Fine needle aspiration |
| IUATLD | International Union against Tuberculosis and Lung Disease |
| kPa | KiloPascal |
| LFT | Liver function test |
| mRNA | Messenger ribonucleic acid |
| PCR | Polymerase chain reaction |
| pH | Percentage hydrogen |
| PI | Principal Investigator |
| PRs | Paradoxical reactions |
| R | Rifampicin |
| RBS | Random blood sugar |
| RFT | Renal function test |
| SKC | Suhum-Kraboa-Coaltar District |
| S | Streptomycin |
| S/R | Streptomycin/Rifampicin Combination |
| TNF-β | Tissue Necrosis Factor beta |
| WHO | World Health Organization |
| $^oC$ | Degree Celcius |

# CHAPTER ONE

## 1. INTRODUCTION

### 1.1 Background

Buruli ulcer (BU), also known as Bainsdale ulcer or Searl's ulcer is a chronic, indolent, necrotizing disease of the skin caused by *Mycobacterium ulcerans* (van der Werf, van der Graaf, Tappero, &Asiedu, 1999) Until recently *M. ulcerans* was considered an environmental organism (Durnez *et al.*, 2010; Portaels *et al.*, 2008). It is currently one of the 19 neglected tropical diseases though it is treatable. Globally, it is the third commonest mycobacterial infection in immune-competent people after *M. tuberculosis* and *M. leprae* (which cause tuberculosis and leprosy respectively) (Huygen *et al.*, 2009), but in Ghana it ranks second after tuberculosis (Amofah *et al.*, 2002). Buruli ulcer has been reported in at least 32 countries in Africa, Australia, Western Pacific, Central and Southern America, Southeast Asia and China (Merritt *et al.*, 2010). Since 1980, it has been recognized by the World Health Organization (WHO) as an important cause of human suffering (WHO. Global Buruli Ulcer Initiative., 2004).

The disease is emerging in West African countries (Portaels, Silva, &Meyers, 2009; van der Werf *et al.*, 2005) with a high yearly incidence especially in children less than 15 years (Portaels *et al.*, 2009; Wansbrough-Jones & Phillips, 2006). The highest burden occurs in sub-Saharan Africa (Johnson *et al.*, 2005) as shown in figure 1:1.

The transmission of Buruli ulcer remains widely unknown but it is most common in humid areas that are close to stagnant or slow moving water bodies and in remote rural areas in tropical and sub-tropical climates (Johnson *et al.*, 2007; Wansbrough-

Jones & Phillips, 2006). It is often common in communities associated with rivers, swamps, wetlands, and human-linked changes in the aquatic environment, especially those created as a result of environmental disturbance as mining, sand winning, deforestation, dam construction, and agricultural activities (Merritt *et al.*, 2010).

Though *M. ulcerans* has a wide geographical distribution in the tropical and subtropical belt, the clinical manifestation of Buruli ulcer disease is varied and occurs in focal clusters (Debacker, Aguiar, Steunou, Zinsou, Meyers, Scott*, et al.*, 2004; Amofah *et al.*, 2002). Limited knowledge of the disease, its focal distribution and the fact that it affects mainly the poor in remote rural areas has led to significant under-reporting and under-recognition.

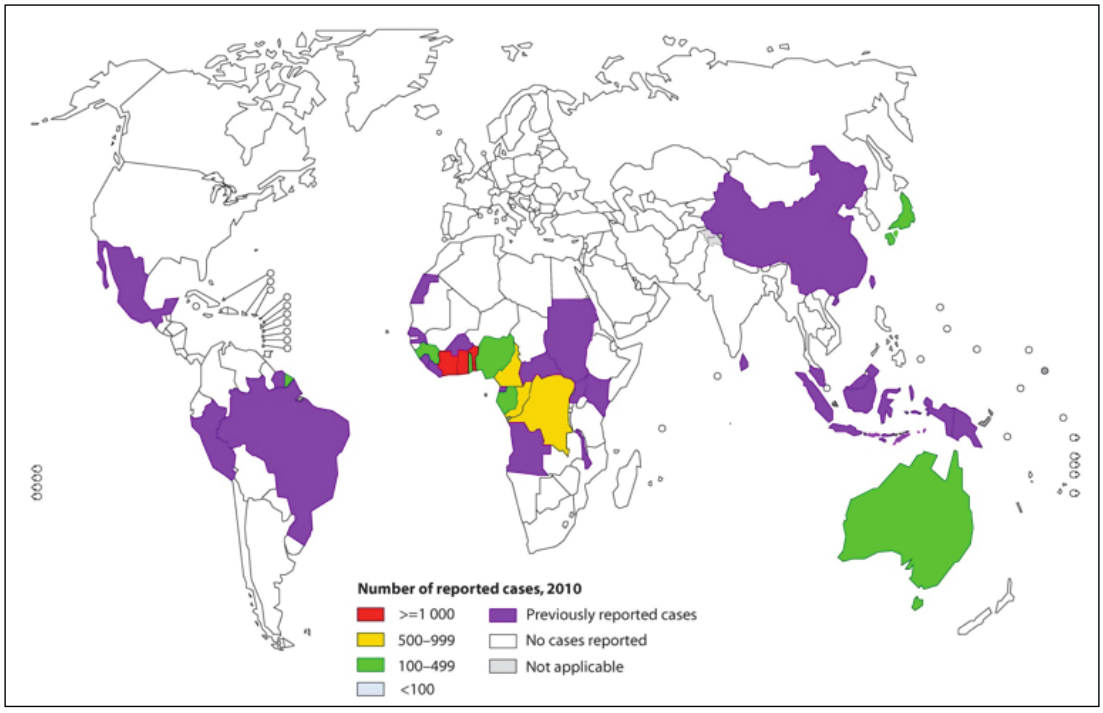



**Figure 1:0:1 Number of reported cases of Buruli ulcer, 2010**

**Source: World Health Organization, Buruli Ulcer 2010**

It is characterized by a painless nodule, papule, plaque or oedema which breaks down centrally into a painless ulcer with undermined edges (Evans *et al.*, 2003). This can progress to a large necrotic lesion especially on the arms and legs leading to extensive destruction of skin and soft tissue and sometimes bone (Portaels *et al.*, 2004; Evans *et al.,* 2003). The infection is sometimes self-limiting, but scar tissue and contractures over joints lead to functional limitations, which can result in stigmatization (Barogui *et al.*, 2009; Stienstra *et al.*, 2005; Evans *et al.,* 2003). Occasionally some cases result in osteomyelitis and this usually leads to amputation (Lagarrigue, Portaels, Meyers, &Aguiar, 2000; Portaels, Chemlal*, et al.*, 2001).

## 1.2 Transmission and Risk Factors of Buruli Ulcer

### 1.2.1 Transmission of Buruli Ulcer

Though the mode of transmission of Buruli ulcer is unknown, several studies have indicated that trauma in the skin contaminated by *M. ulcerans* seems to be essential for its introduction into the skin (Walsh, Portaels, & Meyers, 2008; Meyers, Shelly, Connor, &Meyers, 1974). Others also believe that aerosols from the surface of contaminated water bodies may also be implicated (Portaels, Elsen, Guimaraes-Peres, Fonteyne, & Meyers, 1999; Veitch *et al.*, 1997; Hayman, 1991). Some have proposed that it is rather the exposure of open lesions to infected water that could be a potential source of infection (Williamson *et al.*, 2008).

Human to human transmission of *M. ulcerans* has not been firmly established but there has been evidence of people developing Buruli ulcer at the site of a human bite (Debacker, Zinsou, Aguiar, Meyers, &Portaels, 2003). Some cases have also been reported in same families and this is believed to be due to exposure to a common source or a genetic susceptibility to *Mycobacteria ulcerans* (Stienstra *et al.*, 2001).

### 1.2.2 Risk Factors of Buruli Ulcer

Proximity to marshes and wetlands often created as a result of disturbance of the environment has been shown to be a risk factor for infection (Debacker *et al*., 2006; Asiedu, Raviglione, Scherpbier, &World Health Organization. Global Buruli Ulcer Initiative., 2000). Several cross-sectional surveys and case series have shown that children and adolescents have higher rates of infection by *M. ulcerans* than adults (Hospers *et al.*, 2005; Noeske *et al.*, 2004; Asiedu & Etuaful, 1998).

Several studies have also revealed that poor wound care, failure to wear protective clothing, living or working near water bodies are common risk factors, whereas socioeconomic status, and direct water contact were not significantly associated with the risk of infection (Jacobsen & Padgett, 2010; Johnson *et al.*, 2007).

Failure to immediately wash wounds with soap and water and bandaging them are also associated with increased infection (Nackers *et al.*, 2007; Pouillot *et al.*, 2007; Quek *et al.*, 2007)

Whereas Debacker *et al*. found an increased risk of infection associated with participation in agricultural activities (Debacker *et al.*, 2006), several other studies found no such association (Nackers *et al.*, 2007; Pouillot *et al.*, 2007; Aiga *et al*., 2004).

Some studies have suggested that Bacillus Calmette Guerin (BCG) vaccination provides short-term protection against *M. ulcerans* infection for 6-12 months after vaccination and prevents osteomyelitis when one receives it during the neonatal period (Portaels *et al.*, 2004; Portaels *et al.*, 2002; Smith, Revill, Lukwago, &Rykushin, 1976). However, a case control study by Nackers *et al.* in Benin in 2006

and a systematic review by Jacobson and Padgett in 2010 revealed that BCG vaccination is not significantly associated with the risk of infection (Jacobsen & Padgett, 2010; Nackers *et al.*, 2006).

### 1.3 Clinical Manifestations of Buruli Ulcer

Buruli ulcer disease presents a spectrum of forms but the classical lesion is a necrotic skin ulcer with undermined edges. (See figure 1.2) The disease progresses slowly usually with no pain and no other symptoms (Asiedu *et al.*, 2000). Any part of the body can be affected but mostly on the limbs (Johnson *et al.*, 2005; Amofah *et al.,* 2002). Three clinical stages of the disease have been described with a mean incubation period of 2 to 3 months (Huygen *et al.*, 2009).

The first (pre-ulcerative) stage is characterized by a firm, non-tender subcutaneous nodule, intradermal papule, plaques or oedema (Johnson *et al.*, 2005), which are usually painless. In the second phase, the nodule, plaque or oedema erode the overlying skin and typically break down centrally after a few days to weeks to form an ulcer with undermined edges and a necrotic slough at the base. Thus, the external appearance of the ulcer underestimates the true size of the affected area.

A few patients present with some aggressively progressive oedematous lesion that extends rapidly affecting a whole limb, trunk, or part of the face without any obvious focal lesion. This is usually painful and eventually results in a very large ulcer destroying nerves and other skin appendages, leaving osteomyelitis as a possible complication (En *et al.*, 2008; Johnson *et al.*, 2005).

In the third stage of the untreated disease, a granulomatous healing response takes place followed by fibrosis, scarring, calcification and contractures, with the possibility of permanent disabilities (Barogui *et al.*, 2009; Stienstra *et al.*, 2005).

The WHO clinical case definition for Buruli ulcer divides the disease into two stages: active and inactive stages. The active form is characterized by pre-ulcerative (papules, nodules, plaques, and oedema) and ulcerative disease. The distinctive features of a Buruli ulcer are undermining edges, white or yellow cotton wool-like appearance, and thickening and darkening of the surrounding skin. The ulcers are generally painless and progressive. The inactive form is characterized by a depressed stellate scar with or without sequelae.

### 1.3.1 Classification of Buruli Ulcer Lesions

There are two classes of patients in the presentation of Buruli ulcer lesions. They are classified as New and Recurrent cases. The new patient is a patient with no previous history of Buruli ulcer; and a recurrent patient is a patient who has a past history of Buruli ulcer or previous treatment with streptomycin.

At presentation, Buruli ulcer lesions whether pre-ulcerative or ulcerative are further grouped into 3 different categories according to their size, site and number of lesions per individual. These are:

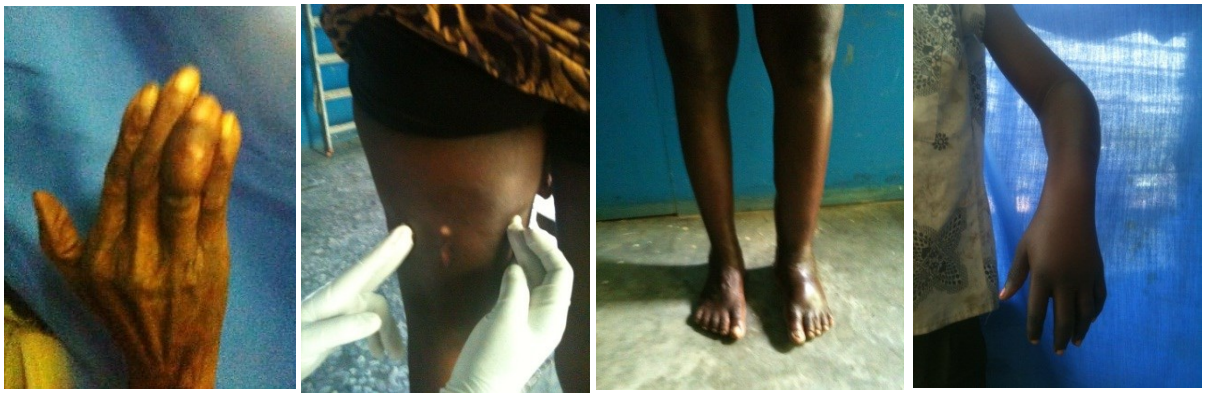
- Category I:- A single lesion less than 5 cm in diameter
- Category II:- A single lesion 5-15 cm in diameter

- Category III: - A single lesion more than 15 cm in diameter, multiple lesions, lesions at critical sites (CS), or osteomyelitis. Critical sites are the head and neck region, breast, genitalia and the perineum.

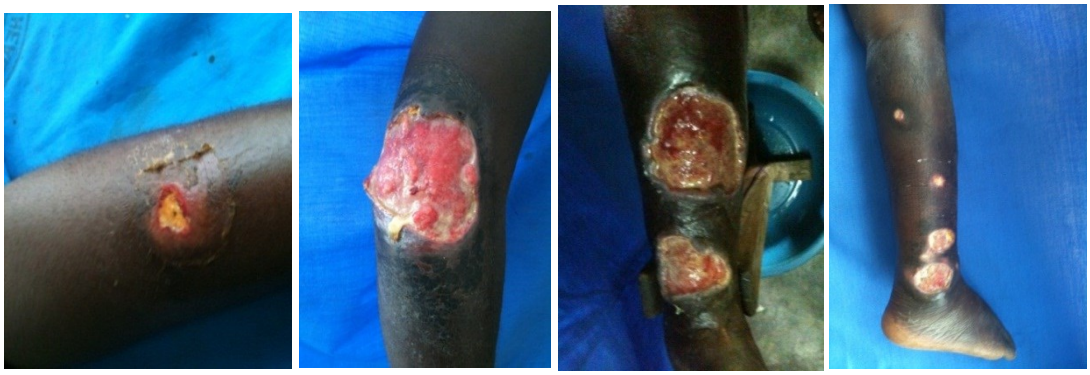
The pre-ulcerative forms of the disease are defined below:

- Papule: a painless, raised skin lesion less than 1 cm in diameter. The surrounding skin is reddened and this form is commonly seen in Australia.
- Nodule: This lesion extends from the skin into the subcutaneous tissue. It is 1–2 cm in diameter and usually painless but may be itchy and the surrounding skin discoloured compared to adjacent areas. This form is commonly seen in Africa.
- Plaque: This is a firm, painless, elevated, well-demarcated lesion more than 2 cm in diameter with irregular edges. The skin over the lesion is often discoloured.
- Oedematous form: This is a diffuse, extensive, usually non-pitting swelling with ill-defined margins. It is usually firm and could be painful or painless involving part or all of a limb or other part of the body. There may be colour changes over the affected area and may be accompanied by fever.

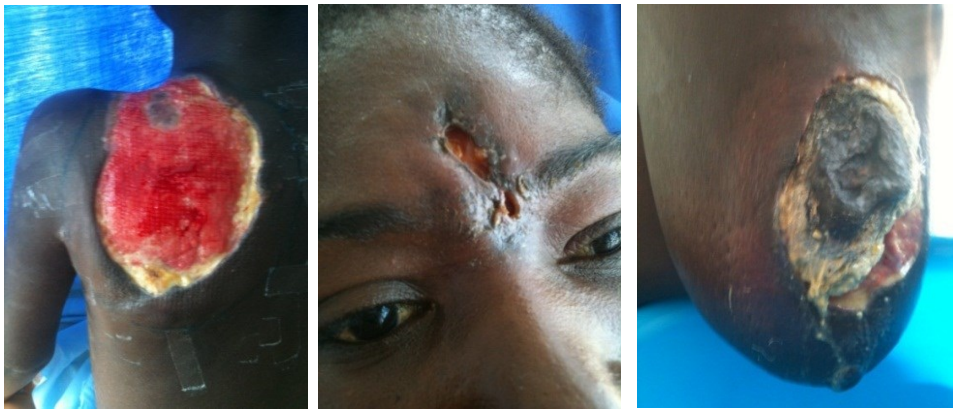
Several conditions can mimic BU disease; some of which are tropical ulcers, leishmaniasis, onchocerca nodules and fungal skin lesions.



Nodule Plaque Oedema Oedema



Category I ulcer Category II ulcer Category III multiple lesions



Category III (single) Category III critical site ulcers

**Figure 1:0:2 Forms and categories of Buruli ulcer lesions**

## 1.4 Diagnosis and Treatment

### 1.4.1 Diagnosis

In a known endemic area the diagnosis of BU can usually be made by an experienced health worker based on clinical grounds. The clinico-epidemiological clues are: patient living in or has travelled to a known endemic area; patient under 15 years of age and most lesions are on the lower limbs. Most diagnosis of the ulcerative forms of Buruli ulcers are made based on the clinical judgment of experienced clinicians, but that of the pre-ulcerative forms is however very difficult and confusing because of the many differential diagnosis.

It is therefore important to confirm the diagnosis with at least one of the currently available four diagnostic laboratory confirmatory tests (Portaels, Johnson, Meyers, &World Health Organization. Global Buruli Ulcer Initiative., 2001). These are detection of acid-fast bacilli (AFB) in a smear stained by the Ziehl-Neelsen technique, positive culture of *M. ulcerans,* positive polymerase chain reaction (PCR) for the detection of *M. ulcerans* DNA and histopathological analysis of excisional biopsies. These methods vary in their sensitivities, specificities, speed of getting results and costs involved.

However these are infrequently used because of logistic and operational difficulties. Laboratory diagnosis can however be used to confirm the clinical diagnosis retrospectively on swab samples, fine needle aspirates (FNA), punch biopsies and surgical biopsies taken before treatment commences. WHO recommends that two confirmatory tests should be obtained for a definitive diagnosis (WHO Advisory Group on Buruli Ulcer. Meeting (7th : 2004 : Geneva Switzerland) & World Health Organization. Global Buruli Ulcer Initiative., 2004).

### 1.4.2 Treatment

The current treatment protocols for Buruli ulcer are antibiotics and complementary treatment (World Health Organization., 2011). The antibiotics kill M. *ulcerans* bacilli, stop further elaboration of mycolactone, arrest the progression of the disease and promote healing. Combinations of antibiotics, which are used for treatment, are rifampicin and streptomycin or amikacin, clarithromycin and moxifloxacin. The complimentary treatment according to WHO are wound care, surgery and interventions to minimize or prevent disabilities (World Health Organization., 2011). Wound care however, is extremely important. Presently, there is an ongoing study in this country using an all-oral antibiotic combination of rifampicin and clarithromycin.

## 1.5 Rationale for the Study

### 1.5.1 Problem statement

Buruli ulcer had traditionally been considered a disease that can only be cured with surgery because of its massively destructive nature. Early reports suggested that wide surgical excision followed by grafting was the only effective treatment (Espey *et al.*, 2002; Aguiar & Stenou, 1997; MacCallum, Tolhurst, &et al., 1948). The marginal benefits demonstrated by early trials with clofazimine (Revill, Morrow, Pike, &Ateng, 1973) and rifampicin/dapsone (Espey *et al.*, 2002) also strengthened this belief.

Many in the endemic regions present late for fear of surgery and their families cannot also afford the time and resources spent in hospital (Aujoulat, Johnson, Zinsou, Guedenon, &Portaels, 2003; Stienstra, van der Graaf, Asamoa, &van der Werf, 2002). Surgical management is often difficult and complicated when the disease is advanced and is presented at a late stage. Outcomes for large ulcers are generally not good simply because of the extensive surgery and subsequent scarring. It has also been

reported that over 50% of those who receive surgical treatment end up with contractures and functional limitations (Stienstra *et al.*, 2005; Teelken *et al.*, 2003).

Meanwhile, significant morbidity results when there is functional limitation from extensive scarring from surgery, contractures at joints and amputation of limbs. Also, recurrence rates after surgery are high varying between 6% and 28% (Schunk *et al.*, 2009; O'Brien *et al.*, 2007; Amofah, Asamoah, & Afram-Gyenin, 1998). This however depended on the extent and type of lesion at hand and the skill and experience of the surgeon as well. Surgery also leads to long periods of hospitalization, serious socio-economic and psychological implications for the patient and family members ( Aujoulat *et al.*, 2003; Stienstra *et al.*, 2002; Asiedu & Etuaful, 1998). The high relapse rates, prohibitive costs and limited access to surgery in most endemic areas (Amofah *et al.*, 2002) led to a renewal of interest in antibiotic therapy again in year 2003.

In previous studies, *in vitro M. ulcerans* had been found to be susceptible to rifampicin (Havel & Pattyn, 1975), clarithromycin (Portaels, Traore, De Ridder, &Meyers, 1998), quinolones (Thangaraj *et al.*, 2000) and aminoglycosides (Dega, Bentoucha, Robert, Jarlier, &Grosset, 2002). Combinations of these drugs have been used successfully in the treatment of *Mycobacterium tuberculosis, mycobacterium leprae* for some years (Stauffer, Dortbudak, &Lahonik, 1991). In previous years, streptomycin and rifampicin had been the mainstay of treatment for tuberculosis. In the same way rifampicin had also been part of the multidrug therapy (MDT) for leprosy.

Based on these, a small pilot study was undertaken in Ghana by Etuaful *et al.* in 2003 under the auspices of the WHO. This study established that an 8-week combination of intramuscular streptomycin and oral rifampicin could sterilize and considerably reduce the size of early (pre-ulcerative) Buruli ulcer lesions. Even four weeks of treatment led to culture negativity of lesions (Etuaful *et al.*, 2005).

Based on these results, the World Health Organization (WHO) introduced new treatment guidelines of an 8-week therapy of intramuscular streptomycin and oral rifampicin with or without surgery (WHO. Global Buruli Ulcer Initiative., 2004). It was anticipated that antibiotics alone might be curative in some patients with early lesions (nodules, papules, plaques, and ulcers of less than 5 cm in diameter), but the primary aims of chemotherapy for more-advanced lesions are to reduce the extent of surgical excision and to prevent recurrence (World Health Organization. Global Buruli Ulcer Initiative., 2005).

Subsequently, Chauty *et al.* reported a case series of 224 patients with pre-ulcerative and ulcerative BU lesions who were treated with this regimen. Of the 215 patients whose lesions healed, chemotherapy alone was successful in achieving cure for 47% of them (Chauty *et al.*, 2007). More recent studies have also demonstrated that a combination of oral rifampicin and oral clarithromycin for 8 weeks is equally effective with no relapses (Chauty *et al.*, 2011; Nienhuis *et al.*, 2010).

This recommended guidelines by WHO has been implemented in Ghana since 2005 and recent evidence has shown some success with this new antibiotic combination. Some patients however, do not respond favourably as expected, fail to respond or relapse after apparent improvement or cure (Schunk *et al.*, 2009; O'Brien *et al.*, 2007).

The level of trust in the formal health system is low in community members because they have not experienced good treatment outcomes where their wounds fail to heal after the stipulated 8-week period of treatment. As a result, some people with Buruli ulcers resign to their fate and remain with their unhealed lesions even after they have completed the 8-week chemotherapy treatment and wound care (Renzaho, Woods, Ackumey, Harvey, &Kotin, 2007). Some affected people also believe that there is no effective medical treatment for the disease and so are reluctant in seeking care (Aujoulat *et al.*, 2003; Stienstra *et al.*, 2002).

According to Renzaho *et al.* 71.8% of BU sufferers in the Ga West district of Ghana first seek treatment from herbalists and only report to the hospital as a last resort at a time when they would have developed very large ulcers. Their main reasons were prolonged hospital stay, cost of transport, loss of earnings of family members associated with attending to patients on admission over extended periods of time, delays in being attended to by medical staff and not knowing the cause of the disease and its required treatment (Renzaho *et al.*, 2007). Again, patients are also poor and so cannot access health services early, increasing the severity of their ulcers (Schunk *et al.*, 2009; Amofah *et al.,* 1998). Also infected people and affected family members end up suffering from social stigmatization and loss of livelihood (Stienstra *et al.*, 2002; Asiedu & Etuaful 1998).

There is the need to break this vicious cycle of poor medical outcomes leading to poor attitude to medical treatment in the community preventing people from seeking care early and subsequently reducing the associated morbidity with its resultant disability. Factors that lead to some patients not responding favourably to treatment could be host and lesion factors, or health system factors. Some of the host and lesion factors

are HIV co-infection, malnutrition, stage of disease presentation and host specific immune responsiveness and size of lesion. Some of the health system factors are poor wound care, incomplete adherence to treatment etc.

There is very little documentation about wound management practices for Buruli ulcer in the Akwapem South and Suhum-Kraboa-Coaltar districts. The role of wound care, and how good wound care practices given at the facility level affect the healing of Buruli ulcers have also not been assessed. The response of BU lesions including large ulcers, which are most common in Africa, also remains insufficiently documented (Kibadi *et al.*, 2010; Phanzu *et al.*, 2006)

### 1.5.2 Justification

The foregoing suggests that most cases of BU occur in resource-limited settings. In addition, the response to chemotherapy of BU lesions including large ulcerated forms, which are currently the most common forms in Africa remains insufficiently documented (Kibadi *et al.*, 2010; Phanzu *et al.*, 2006). Hence, there is the need to assess the treatment outcomes of all categories of BU lesions and identify factors that affect response to treatment. This will help to put in place measures to simplify the care of BU lesions and subsequently improve outcomes, which will invariably motivate people to report early for care.

Chronic wounds cause significant suffering, including profound negative effects on general physical health, socialization, financial status, body image, level of independence, and control to the affected individual and family (Bogie, 2011). They also have negative emotional impact on lives, including fear, social isolation, anger, depression and negative image (Phillips, Stanton, Provan, &Lew, 1994).

Patients suffering from chronic wounds usually find themselves in situations of having to choose between commitments to their work and compliance to treatment and care for their ulcers (Ghanassia *et al.*, 2008; Lanzafame, 2007a). Others who are less fortunate may be permanently impaired from performing their jobs (Lanzafame *et al.*, 2007).

Chronic wounds lead to disability and disability worsens wound outcomes resulting in a vicious cycle (Eberhardt & Raffetto, 2005). Chronic wounds and their care represent a growing problem that results in lost productivity, representing a heavy socioeconomic burden on people and country (Lanzafame 2007a; Eberhardt & Raffetto, 2005). Fast healing of wounds increases efficiency and places less demands on the already thin human resource available in our health institutions and so reduces the overall cost of care (Lanzafame, 2007a).

This study therefore sought to assess the treatment outcomes of all categories of BU lesions and identify factors that influence the healing of Buruli ulcer lesions to this new protocol of 8-week rifampicin plus streptomycin combination.

In a resource-limited country like Ghana, which is one of the highly endemic countries for BU, the documentation of what prevails will help put in place appropriate interventions to control for such factors. The findings in this study will also contribute to bringing care closer to the patient, encourage patients to report early to health facilities and reduce considerably the need for surgery. This will strengthen the local capacity for diagnosis and wound care/management. It will also help in cost reduction in the management of Buruli ulcer lesions and therefore save the nation the much-needed resources for capacity building and other important issues.

## 1.6 Research Questions

1. What are the outcomes of antibiotic treatment for different categories of Buruli ulcer lesions?
2. What factors affect healing of BU when treated with rifampicin plus streptomycin chemotherapy alone?

## 1.7 Objectives

### 1.7.1 General Objective of the Study

The general objective of the study was to determine the factors influencing antibiotic treatment outcomes for Buruli ulcer in the Akwapem South and Suhum-Kraboa-Coaltar districts in the Eastern region of Ghana.

### 1.7.2 Specific Objectives

The specific objectives were to:

1. Describe the demographic characteristics of Buruli ulcer patients
2. Determine the extent to which antibiotic treatment (rifampicin plus streptomycin combination) affect the various categories of BU lesions
3. Determine factors that influence the healing of Buruli ulcer lesions

# CHAPTER TWO

## 2 LITERATURE REVIEW

### 2.1 Historical Background and Epidemiology

#### 2.1.1 The Global Burden of the Disease

Professor Peter MacCallum and his colleagues published the first scientific description of Mycobacterium ulcerans in 1948 when he first identified the pathogen in 1940 (WHO. Global Buruli Ulcer Initiative., 2004). He described the various stages of the disease in two Australian children and four adults in a riverine area near the Bairnsdale district in Victoria, Australia (MacCallum *et al.*, 1948).

Historically however, large ulcers which probably were Buruli ulcers had already been described by Sir Albert Cook, a British physician in 1897 in Africa, when he encountered these patients at Mengo Hospital in Kampala, Uganda (Kwyer & Ampadu, 2006) and later by Klein Schmidt, a missionary physician in north-east Congo in the 1920s (Johnson *et al.*, 2005).

The earliest reports might have come from a country now called Democratic Republic of Congo from an area southwest of Kinshasa, where it is still prevalent (Bafende, Phanzu, &Imposo, 2004; van der Werf *et al.*, 2005). During the 1960s large ulcers were again observed in people living in Kinyara, a refugee camp near the Nile River in the Buruli county in Uganda (now called Nakasongola District), hence the name "Buruli ulcer" (van der Werf *et al.*, 2005).

The occurrence of BU in rural areas, its focal distribution even in endemic regions and poor reporting system make it very difficult to obtain an accurate burden of the

disease (Johnson *et al.*, 2005). Despite these difficulties Buruli ulcer has an annual reported case load of more than 5000 in at least 33 countries worldwide especially in the tropical and subtropical regions of Asia, Latin America, the Western Pacific region, Eastern and Central Africa with West Africa being the region most affected by the disease (Merritt *et al.*, 2010; Portaels *et al.*, 2009). Some of the countries endemic for BU outside Africa are Papua New Guinea, Malaysia, Sri Lanka, French Guyana, Peru and Mexico (Johnson *et al.*, 2005; van der Werf *et al.*, 2005).

BU is uncommon in Australia, but over the years, there have been reports of increases in both the incidence and the number of endemic areas in that country (Johnson *et al.*, 2005). There have also been reports of Buruli ulcers occurring in China (Faber *et al.*, 2000), Japan (Huygen *et al.*, 2009) and Brazil (Menard, Couppie, Sainte-Marie, &Pradinaud, 2003) but its extent is unknown in these countries. Buruli ulcer has occasionally been diagnosed in the United Kingdom, United States of America, Canada and Europe albeit imported (McGann *et al.*, 2009).

Several studies have shown that the disease has been found to be most common in communities associated with rivers, wetlands, swamps with human-linked changes in the aquatic environment especially those created as a result of mining, sand winning, agriculture, deforestation, irrigation and dam construction (Merritt *et al.*, 2010; van der Werf *et al.*, 2005). For instance, there was an outbreak of Buruli ulcer in Phillip Island in Australia from 1992 to 1995 when a dam was constructed for irrigation purposes. Cases declined when the dam was improved (Veitch *et al.*, 1997).

### 2.1.2 Burden of Buruli Ulcer in Africa

Prior to 1980, before Buruli ulcer was declared a major health problem in the world by the World Health Organization (WHO), it had already been reported in several Sub-Saharan countries: Cote d'Ivoire (Martson 1991), Congo (Smith, 1970), Gabon (Burchard & Bierther, 1986), Nigeria (Oluwasanmi *et al.*, 1976), Liberia (Monson, Gibson, Connor, Kappes, &Hienz, 1984), Togo (Meyers, Tignokpa, Priuli, &Portaels, 1996), Uganda Cameroun and Ghana (Bayley, 1971).

Most cases of Buruli ulcer have been reported in Sub-Saharan Africa especially in children younger than 15 years (Ackumey, Kwakye-Maclean, Ampadu, de Savigny, &Weiss, 2011; Debacker *et al.*, 2006). In fact, it has been reported in all countries bordering the Gulf of Guinea (Meyers *et al.*, 1996) with the largest number from riverine rural areas in Benin (Debacker, Aguiar, Steunou, Zinsou, Meyers, Guedenon, *et al.*, 2004), Cote d'Ivoire and Ghana (Johnson *et al.*, 2005; van der Werf *et al.*, 2005; Amofah *et al.,* 2002) where reported cases have alarmingly increased over the years. It is steadily increasing and becoming a serious disease especially in West Africa.

Within the last twenty years increasing numbers have also been reported in Cameroun (Noeske *et al.*, 2004), Cote d'Ivoire (Kanga & Kacou, 2001; Marston *et al.*, 1995), Gabon, Nigeria and Togo (Meyers *et al.*, 1996). Recently, new foci were discovered in Liberia (Johnson *et al.*, 2005), Togo (Meyers *et al.*, 1996) Angola and Democratic Republic of Congo (Kibadi *et al.*, 2008; Kanga & Kacou, 2001; Bar *et al.,* 1998).

Though globally, BU is the third leading cause of mycobacterial infection in immune-competent people after tuberculosis and leprosy, in some countries in East and West

Africa, thousands of cases occur annually and so has displaced leprosy to become the second most prevalent mycobacterial disease of man after tuberculosis (Debacker, Aguiar, Steunou, Zinsou, Meyers, Guedenon, *et al.*, 2004; Amofah *et al.*, 2002; Asiedu *et al.*, 2000).

Point prevalence estimates are varied but as high as 150-280/100,000 populations have been reported in Ghana (Raghunathan *et al.*, 2005; van der Werf *et al.*, 2005; Amofah *et al*., 2002 ). High prevalence levels of 21.5/100,000 have also been reported in Benin (Debacker, Aguiar, Steunou, Zinsou, Meyers, Guedenon, *et al.*, 2004). A national survey in Cote d'Ivoire showed a prevalence as high as 32/100,000 with an annual incidence exceeding 2000 cases (Kanga & Kacou, 2001).

In Africa, approximately 10% of patients develop bone involvement or metastatic osteomyelitis from lympho-hematogenous spread of *M ulcerans* (Walsh, Portaels, &Meyers, 2011). According to Barogui *et al.* almost 50% of BU patients in some African countries have functional limitations (Barogui *et al.*, 2009) and in West Africa about 25% of affected patients are left with permanent disabilities (Johnson *et al.*, 2005).

### 2.1.3 Burden of Buruli Ulcer in Ghana

The first documented probable case of BU in Ghana was identified in 1971 in the Greater Accra region in a child from Nsawam in the Eastern region (Amofah *et al.*, 2002). Later some probable cases were also identified along the tributaries of the Densu River (Bayley, 1971). Later on in 1989 van der Werf *et al.* also described 96 cases in the Asante Akim North district in the Ashanti region (van der Werf, van der Graaf, Groothuis, &Knell, 1989). Then in 1993 Amofah *et al.* described a major

endemic foci in the Amansie West district also in the same region (Amofah, Sagoe-Moses, Adjei-Acquah, &Frimpong, 1993).

In 2002, Amofah *et al.* reported of a national case search conducted in Ghana in 1999 where there were about 5600 people with suspected Buruli ulcer lesions at various stages of development (Amofah *et al.*, 2002). In this same research, Buruli ulcer cases were identified in 90 out of the then 110 administrative districts in Ghana. All regions were affected with Central region having the highest prevalence followed by the Ashanti region. Amansie West district had the highest prevalence with 150.8/100,000. The overall national prevalence then was estimated to be 20.7/100,000 (Amofah *et al.*, 2002) making *M. ulcerans* the second most prevalent Mycobacterial infection in Ghana (Amofah *et al.*, 2002).

According to a WHO report there were more than 11,000 reported cases in Ghana between 1993-2006 (World Health Organization., 2011). Presently Ghana reports an average of 1000 cases annually (Ackumey *et al.*, 2011).

Amofah *et al.* in the national case search to determine the prevalence of BU in Ghana also reported that approximately 48.5% of all suspected BU lesions were at the ulcerative stages whereas 12.5% were at the pre-ulcerative stage, 36.3% had formed scars and 2.7 % had developed deformities (Amofah *et al.*, 2002).

In 2005 the age distribution of BU in Ghana was 53.1% in patients younger than 15 years; 33% in those between 15 years and 49 years, and 12.9% in those 50 years and older (Kwyer & Ampadu, 2006).

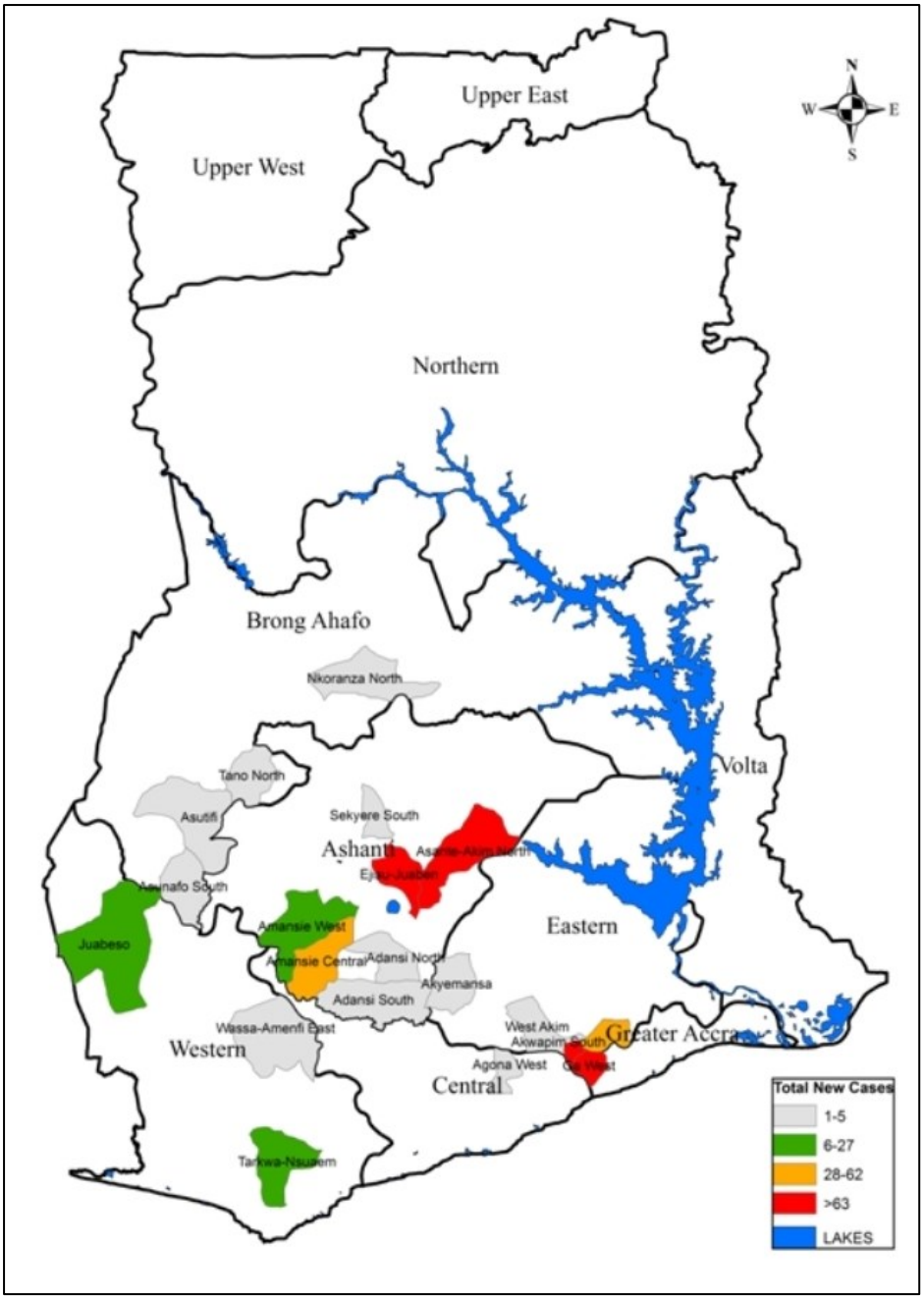



**Figure 2:1 Regional Reported number of Buruli ulcer cases, Ghana 2011**

**Source: National Buruli Ulcer Programme, 2011**

## 2.2 Mycobacterium ulcerans

*Mycobacterium ulcerans* is a slow growing environmental organism with a doubling time of 80 days (Marsollier *et al.*, 2004). It is an acid-fast bacilli that can be cultured from common mycobacterial medium like Lōwenstein-Jensen (L-J) medium. It grows very fast at low temperatures (30-32ºC) and lower pressure than atmospheric oxygen tension below 2.5kPa and within a pH range of 5.4-7.4.

Though *M. ulcerans* is commonly believed to be an environmental organism (van der Werf *et al.*, 2005), it does not freely exist in the environment, but attached to small aquatic animals and biofilms (World Health Organization., 2011). It inhabits water where it can colonize aquatic plants, aquatic insects and herbivorous animals.

However, recent studies and review of data have suggested that *M. ulcerans* is a pathogen (Silva, Portaels, & Pedrosa, 2009) and is genetically very close to the typical intracellular *Mycobacterium marinum* and *Mycobacterium tuberculosis*. According to some studies, it evolved from *M. marinum* by horizontal transfer of a virulent plasmid encoding for mycolactone, a macrolide toxin. *Mycobacterium ulcerans* has the hallmarks of an intracellular parasite and that it produces infections associated with cell-mediated immunity and delayed-type hypersensitivity which are immunologically relevant inflammatory responses (Silva *et al.*, 2009). *M. ulcerans* behaves like the other pathogenic mycobacteria but represents an extreme in the biodiversity of this family of pathogens because of its higher cytotoxicity due to the secretion of mycolactone (Adusumilli *et al.*, 2005).

## 2.3 *M. ulcerans* Toxin and Histopathology of Buruli Ulcer Disease

Once inside the host, *Mycobacterium ulcerans* initially invades the cells in the dermis. It has an incubation period of 2-3 months. The early stages of the infection are governed by two factors: the production of mycolactone and its optimum growth temperature of 30-33$^0$ C.

Mycolactone is the major factor influencing the immunological and pathological manifestations of Buruli ulcer. It is a macrolide consisting of a polyketide side chain attached to a 12-membered core of macrolactone (Kishi, 2011; Tobias *et al.*, 2009). It is encoded by genes found in a large plasmid with a molecular weight of 174-kb called pMUM001 (Stinear *et al.*, 2004). It has cytotoxic, analgaesic and immunosuppressive properties and is responsible for tissue damage, local immunosuppression in BU lesions, fat necrosis and cellular apoptosis in mammalian tissues (Kishi, 2011; Torrado *et al.*, 2007; Kiszewski *et al.,* 2006).

Its immuno-modulatory property is particularly manifested in the inhibition of tumour-necrotic factor production by monocyte and macrophages (Coutanceau *et al.*, 2005). Some studies have suggested that the painlessness and nerve damage associated with Buruli ulcer is also induced by mycolactone (Phillips *et al.*, 2009; En *et al.,* 2008).

The histopathological features of Buruli ulcer disease differ depending on the form and stage of lesion. Buruli ulcer infection unlike other *mycobacterial* diseases is characterized by necrosis of subcutaneous tissue and dermal collagen accompanied by minimal inflammation in the presence of clusters of acid-fast bacilli, and this is considered the most reliable histopathologic feature of BU (Ruf, Sopoh*, et al.*, 2011;

Rondini *et al.*, 2006; Adusumilli *et al.*, 2005; Guarner *et al.*, 2003;). The natural history of BU is such that, with time the immunosuppressive effect of mycolactone wears off in the host, host immunity develops and then healing starts (Johnson *et al.*, 2005).

In the pre-ulcerative forms, the dermis is relatively intact but the subcutaneous tissue which is particularly affected is necrotic and oedematous (Ruf, Sopoh*, et al.*, 2011). Histopathologically, these forms and early ulcers typically show large clumps of extracellular acid fast bacilli surrounded by extensive necrotic tissues with very little inflammatory response (Ruf, Sopoh*, et al.*, 2011; Johnson *et al.,*2005), but in the early stages of the infection there is an initial polymorphonulear cell invasion (Ruf, Sopoh*, et al.*, 2011; Guarner *et al.*, 2003).

In the nodule, there are necrotic foci with fat cell ghosts as a result of minor focal acute infiltration of connective tissue and adipose tissues. Foamy Touton giant cells are found surrounding degrading adipose cells. Abundant bacterial clusters are also located in the necrotic tissue but have no contact with intact leucocytes (Schutte, Umboock, &Pluschke, 2009). In plaques however, after the initial phase of diffuse infiltration and formation of structured leucocyte aggregates, there are also granulomas and a much larger and deeper destruction of subcutaneous tissue (Ruf, Sopoh*, et al.*, 2011).

In the early ulcerative lesion, there is extensive necrosis with decomposing leucocytes and erythrocytes. There is a more substantial acute leucocyte infiltration with haemorrhages found at the borders of the central necrotic area, with some of the leucocytes in close contact with the extracellular mycobacteria showing nucleus

defragmentation (Schutte *et al.*, 2009). There is also a high bacterial load in the necrotic area compared to the untreated nodule. Some studies have however observed an absence of granulomas in relatively early ulcers (Kiszewski *et al.*, 2006).

On the other hand in late ulcers, there are fully developed granulomas towards the margins (Schutte *et al.*, 2009) with epidermal hyperplasia, granulation tissue and fibroblasts which are indications of chronicity or lesions that have started to heal (Kiszewski *et al.*, 2006; Guarner *et al.* 2003).

## 2.4 Demographic characteristics of Patients with Buruli Ulcer

Buruli ulcer affects all ages but the majority is found in those below 15 years of age (Johnson *et al.*, 2005) with an almost equal gender distribution (Asiedu *et al.*, 2000; van der Werf *et al.*, 1999). In 2005 the age distribution of BU in Ghana was 53.1% in patients younger than 15 years; 33% in those between 15 years and 49 years, and 12.9% in those 50 years and older (Kwyer & Ampadu, 2006). According to Amofah *et al*, there is preponderance of lesions on the extremities as their study in 1999 showed that 91% of BU lesions affected the extremities (Amofah *et al.*, 2002). This finding has been corroborated by several other studies (Debacker, Aguiar, Steunou, Zinsou, Meyers, Scott, *et al.*, 2004). Earlier, Marston *et al.* also found similar results of 92% of lesions being on the extremities in Daloa, Cote d'Ivoire (Marston *et al.*, 1995).

Hospers *et al.* in a study of 750 patients in Amansie West in the Ashanti region of Ghana in 2005 observed that there were more lesions on the right side of the body (Hospers *et al.*, 2005). However, van der Werf *et al.* in their study of a case series 15

years earlier found that lesions predominantly affected the right arm in children and the left leg in adults (van der Werf *et al.*, 1989).

Barker *et al.* in their study conducted in 1973 however observed a preponderance of lower limb lesions for boys and equal frequency of lesions on both arms and legs of girls and women (Barker, 1973), whereas Amofah *et al.* observed a preponderance of lower limbs lesions for both gender and all age groups (Amofah *et al.*, 2002). Debacker *et al.* in a study in Benin in 2004 found a preponderance of lower limb lesions in patients older than of 15 years of age (Debacker, Aguiar, Steunou, Zinsou, Meyers, Scott, *et al.*, 2004). In the study by Amofah *et al.* in 2002, lower limb lesions in females were about 240% that of lesions on the upper limbs (Amofah *et al.*, 2002). Children however tend to have lesions on the trunk, head and neck (Debacker, Aguiar, Steunou, Zinsou, Meyers, Scott, *et al.*, 2004).

## 2.5 Antibiotic Chemotherapy

Several anti-mycobacterial agents are being used to treat BU but no one single agent has proven to be consistently effective so they are used in combination with varying success rates (WHO, 2012). These agents include streptomycin, rifampicin, rifabutin, amikacin, clarithromycin, ciprofloxacin, moxifloxacin and azithromycin.

*In vitro, Mycobacterium ulcerans* was susceptible to rifampicin (Havel & Pattyn, 1975), clarithromycin (Portaels *et al.*, 1998), aminoglycosides (Dega *et al.*, 2002) and quinolones (Thangaraj *et al.*, 2000).

Etuaful et al. undertook a small pilot study in Ghana under the auspices of WHO. In this study of 21 pre-ulcerative patients, given 4 weeks of rifampicin and streptomycin led to culture negativity and also reduced the surface area of most lesions by more

than 50%, thus allowing less-extensive surgical excision (Etuaful *et al.*, 2005). Subsequently, Chauty *et al.* reported a case series using this same regimen resulting in a cure for 47% of them (Chauty *et al.*, 2007). In this study, the size of the lesion was the major factor in deciding to treat with antibiotics or by surgery. Seventy-three percent of patients with lesions equal or bigger than 15cm in diameter and 17% of those with lesions with diameter equal or less than 5cm underwent surgery (Chauty *et al.*, 2007).

Considerable progress has been made in the past 7 years with the demonstration of the efficacy of the combination of rifampicin and streptomycin chemotherapy. Its routine implementation has dramatically improved healing whilst reducing relapses. Though this combination was shown to be highly effective for the treatment of Buruli ulcer, ambulatory treatment requiring daily intramuscular injection of streptomycin for 8 weeks is operationally demanding and lack of efficacious oral therapy remains one of the main obstacles to decentralizing care at the local level. To simplify treatment under field conditions, the need to develop effective combined regimens that would be administered orally arose.

In 2007, Ji *et al.* demonstrated that 8 weeks of treatment with orally administered combinations of rifampin-moxifloxacin, rifampin-clarithromycin, or moxifloxacin-clarithromycin were similar to that of rifampin-streptomycin in mice (Ji, Chauffour, Robert, Lefrancois, &Jarlier, 2007). Dossou *et al.* also reported of a case where there was clinical improvement after 8 weeks of oral rifampicin and clarithromycin in a pregnant woman (Dossou *et al.*, 2008). More recent studies have also demonstrated that a combination of oral rifampicin and oral clarithromycin for 8 weeks is equally effective with no relapses in humans (Chauty *et al.*, 2011; Nienhuis *et al.*, 2010).

Presently, there is an ongoing study in Ghana using an all-oral antibiotic combination (rifampicin plus clarithromycin). Clarithromycin being an orally administered drug would most likely be better tolerated than streptomycin (Chauty *et al.*, 2011). If this combination is accepted by the country, patients could be treated at home with supervision by treatment supporters as presently being done for tuberculosis treatment in the country.

### 2.5.1 Histopathological Response to Antibiotic Treatment

Several studies have shown that during antibiotic treatment there is a reversal of the initial immune suppression observed in BU lesions (Ruf, Sopoh*, et al.*, 2011; Schutte *et al.*, 2007). This process starts with diffuse chronic infiltration primarily by macrophages and T-cells. (Ruf, Sopoh*, et al.*, 2011). This initial diffuse infiltration is followed by structured leucocyte aggregates, such as B cell clusters and granulomas, but in the case of plaques this is restricted to the margins of the necrotic areas and that there are large areas showing substantial coagulative necrosis without significant infiltration even after 7-39 days after completion of antibiotic chemotherapy (Ruf, Sopoh*, et al.*, 2011). There is also angiogenesis and this in addition to the leucocyte infiltration promotes tissue debris resorption. In a few pre-ulcerative lesions this resorption is efficient enough to enable healing without ulceration. In plaques however, necrotic areas are too extensive to permit complete resorption without ulceration (Ruf, Sopoh*, et al.*, 2011).

It is generally believed that ulceration results in loss of necrotic tissue, but Ruf *et al.* found that in the case of plaques there was incomplete loss of necrotic tissue (Ruf, Sopoh*, et al.*, 2011). This finding therefore supports the decision to accelerate wound healing by debriding margins of ulcers.

Sarfo *et al.* observed that there is a sustained presence of mycolactone in ulcer exudates during antibiotic treatment, although its serum concentration tended to decrease with time. It persists in cutaneous tissues even after the demise of *M. ulcerans.* This phenomenon could explain why some Buruli ulcers, though culture negative take some time to heal (Sarfo *et al.*, 2011).

### 2.5.2 Outcomes of Buruli ulcer treatment

The outcomes of Buruli ulcer treatment are complete healing, paradoxical reactions, treatment failure, recurrence and functional limitations.

#### 2.5.2.1 Complete healing

Antibiotic treatment of Buruli ulcer is in no doubt very beneficial. It is safe, effective and usually not associated with lesion deterioration requiring surgery. A study conducted by Etuaful *et al.* under the auspices of the WHO to establish whether rifampicin and streptomycin (R/S) treatment of humans with early non-ulcerative BU disease would convert culture positive pre-ulcerative lesions to culture negative and to observe any changes found that most lesions became smaller during treatment and none of them enlarged. They also found that the average reduction in the surface area at the end of treatment was 38% ranging from 29% in those treated for 2 weeks to 52% in those treated for 4 weeks (Etuaful *et al.*, 2005).

Chauty *et al.* in another study carried out in Benin to evaluate the efficacy of the R/S combination on the whole spectrum of BU lesions found that only 47.4% of 215 cases treated were cured with chemotherapy alone. In this study 81% of category I ulcers (less than 5cm), 56% of category II ulcers (5-15cm) and 21% of category III ulcers (larger than 15cm) were cured with chemotherapy alone. For those with pre-ulcerative

lesions (early lesions) 45% of them were cured at 4 weeks of chemotherapy alone (Chauty *et al.*, 2007).

Later on, Nienhuis *et al.* in the first randomized trial using rifampicin, streptomycin and clarithromycin (streptomycin plus rifampicin for 8 weeks in one group; streptomycin plus rifampicin for 4 weeks and rifampicin plus clarithromycin for the next 4 weeks in the other group in lesions less than 6 months' duration and less than 10 in diameter) in the treatment of early limited BU lesions in Ghana found that 96% of all the cases healed by the end of one year after start of treatment (Nienhuis *et al.*, 2010). Here, the median duration of healing was 18 weeks (14-22 weeks) for Category I lesions and 30 weeks for lesions 5-10 cm (Nienhuis *et al.*, 2010). In these studies however, skin grafting was not considered as surgery but an integral part of antibiotic treatment.

In another study conducted in Ghana by Sarfo *et al.* to evaluate the clinical efficacy of this 8-week treatment with R/S combination in 160 PCR-confirmed cases with all forms and categories of Buruli ulcer lesions, they found that 95% of patients healed without undergoing surgery. In this study the median time to complete healing for all ulcers was 12 weeks (4-30 weeks). It was 8 weeks (4-12) for Category I ulcers, 11 weeks (4-20 weeks) for Category II ulcers and 15 weeks (8- 39 weeks) for category III ulcers (Sarfo *et al.*, 2010).

Earlier, in another study by this same group for 26 PCR-confirmed BU lesions, 46% of them healed completely after 8 weeks of antibiotic treatment. Interestingly, the duration of healing for purely oedematous lesions was very wide, ranging from 2 to 48 weeks (Sarfo *et al.*, 2009). In these studies by Sarfo *et al.* they used the diameter

of the lesions instead of the area in assessing the rate of reduction of wound size. They observed that the rate of reduction in the diameter of ulcers depended on the time of treatment (during or after antibiotic treatment). It was 2.6mm/week (1.5-3.6mm/week) during antibiotic treatment and 3.7mm/week (2.9-12.5mm/week) after the end of treatment (Sarfo *et al.*, 2010).

Several studies have reported of complete healing of BU lesions after antibiotic treatment but the conclusions defer depending on the extent of healing; how the healing was measured: diameter, surface area, and duration of healing (median times) etc. The point here is that, complete healing is seen but is variable depending on several factors.

#### 2.5.2.2 Paradoxical reactions

A "paradoxical" reaction describes a deterioration response to treatment of an infection after an initial improvement (Ruf, Chauty*, et al.*, 2011; Lipman & Bren, 2006). Several studies have described these reactions in infections with a number of mycobacterial species, including those causing tuberculosis, mycobacterium avium-intracellulare complex and leprosy (Cheng, Wang, &Yang, 2007; Hawkey *et al.*, 2005). Though they usually occur in severely immuno-suppressed patients with HIV/AIDS who are undergoing antiretroviral therapy (Breton, 2010; Kiertiburanakul, 2010; Muller *et al.*, 2010), they can occur in immune-competent people as well (Cheng *et al.*, 2007; Lipman & Breen, 2006; Carvalho *et al.,* 2006).

In tuberculosis and leprosy, these paradoxical responses are defined as a transient worsening (clinical and radiological) of pre-existing lesions or the development of

new lesions under appropriate therapy not attributable to the normal course of the disease (Cheng *et al.*, 2007).

The pathogenesis relates to an increased exposure to mycobacterial antigens, a decrease in suppressor mechanisms or an enhanced immune response to mycobacterial antigens on treatment that produces deleterious clinical effects (Nienhuis *et al.*, 2012; *Cheng et al.*, 2007; Lipman & Bren, 2006), and is referred to as "immune reconstitution syndrome" in HIV-infected patients with TB. It is unpredictable in its time of initiation and can occur at any time, a few days to months after initiation of anti-mycobacterial treatment

After start of antimicrobial chemotherapy for Buruli ulcer, new or progressive ulceration usually occurs before healing sets in. Schutte *et al.* in a study conducted in Cameroun to elucidate the histopathological processes taking place in Buruli Ulcer lesions in the course of antibiotic chemotherapy found that antibiotic therapy for *M. ulcerans* disease leads to an apparent reversal of the immune-tolerant state of active *M. ulcerans* infection, with phagocytosis of mycobacteria and a rapid onset of local cellular immune responses (Ruf, Chauty*, et al.*, 2011; Ruf, Sopoh*, et al.*, 2011; Schutte *et al.*, 2009).

The formation of epitheloid granuloma with multinucleated giant cells and large clusters of lymphocytes characterize these responses. It has been suggested that antibiotics are likely to facilitate this immune reaction by killing *M. ulcerans* or by liberating mycobacterial antigens from dead organisms and therefore reducing the production of mycolactone leading to a vigorous and intense inflammatory response to the killed organisms (Ruf, Chauty*, et al.*, 2011; Schutte *et al.*, 2009). Though the

time of initiation of paradoxical response is unpredictable and can occur anytime, it is most prominent at the end of 8-weeks of treatment of Buruli ulcer lesions (Nienhuis *et al.*, 2012).

Earlier, in the study by Etuaful *et al.* under the auspices of the WHO to establish whether rifampicin and streptomycin treatment of early pre-ulcerative BU disease would convert culture positive lesions to culture negative and to observe any changes found that most of the lesions became smaller during treatment and none of them enlarged.

Though, recent studies have shown progressive reduction in sizes of most lesions during and after antibiotic chemotherapy, some enlarged before they started to heal (Nienhuis *et al.*, 2012; Sarfo *et al.*, 2010). O'Brien *et al.* in a study in Australia also described "paradoxical reactions" in 2 Australian BU patients during rifampicin and streptomycin treatment where deterioration of lesions occurred after clinical improvement. In the first patient incomplete excision of wound margins showed paradoxical reactions and in the second patient there was an appearance of a distant lesion which ulcerated before the end of treatment (O'Brien, Robson, Callan, & McDonald, 2009).

Meanwhile, Nienhuis *et al.* in their study in Benin observed that peak paradoxical reactions occurred at week 8 in ulcerative lesions (Nienhuis *et al.*, 2012). In that study more than 30% of participants showed an increase in lesion size at week 8 compared to the size at week 6 and as high as 83% of non-ulcerative lesions ulcerated after start of treatment (Nienhuis *et al.*, 2012). Interestingly in the case of a study conducted by Kibadi *et al.* in the Democratic Republic of Congo of large ulcerated lesions (≥10cm),

87.8% (36/41) of all the ZN-positive cases deteriorated at the end of 4 weeks (Kibadi *et al.*, 2010).

Several studies have suggested that limited surgical excision may help to resolve paradoxical reactions by reducing the burden of mycobacterial antigens. In some clinical settings corticosteroids have also been used for down-regulation of immune responses (Troncoso Marino, Campelo Sanchez, Martinez Lopez de Castro, &Inaraja Bobo, 2010). Sarfo *et al.* in their study found that healing rates of BU lesions with paradoxical reactions were not different from those without paradoxical reactions (Sarfo *et al.*, 2010). Several studies have also shown that "paradoxical reactions" do not call for an extension of antibiotic treatment nor spell treatment failure but an adverse consequence of effective treatment (Sarfo *et al.*, 2010; O'Brien *et al.,* 2009).

#### 2.5.2.3 Functional limitations in Buruli ulcer lesions

Localization of lesions at the site of a joint appears to impair movement after healing (Ellen *et al.*, 2003). In West Africa 25% of affected patients are left with permanent disabilities (Johnson *et al.*, 2005). In a study by Stienstra *et al.* to develop a scoring system to assess the nature and severity of functional limitations of Buruli ulcer lesions, they found that 67% of treated patients had functional limitation (Stienstra *et al.*, 2004).

According to Barogui *et al.* in a study in Benin, almost 50% of BU patients had developed some level of functional limitations before treatment (Barogui *et al.*, 2009). In this study, they also found that large lesions (>15 cm cross-sectional diameter) were significantly associated with residual functional limitations. In another study by Sarfo *et al.* in Ghana, of 15 patients who had functional limitation before the start of

antibiotic treatment, 66.7% resolved soon after wound healing and 26.7% resolved one year after start of treatment (Sarfo *et al.*, 2010).

#### 2.5.2.4 Recurrence

Recurrence rates do not differ so much in studies where cases have been treated with the 8-week combination of R/S antibiotic chemotherapy alone. These low rates are attributed to improved quality of clinical management of Buruli ulcer disease with antibiotic chemotherapy (Schunk *et al.*, 2009).

In studies conducted in Ghana by different groups there were no recurrences of BU at the end of one-year follow-up after receiving the 8-week combination of R/S antibiotic chemotherapy (Nienhuis *et al.*, 2010; Sarfo *et al.*, 2010; Etuaful *et al.,* 2005). Chauty *et al.* in a study in Benin found a recurrence rate of 1.9% in those treated with chemotherapy alone within a year following treatment completion (Chauty *et al.*, 2007). Kibadi *et al.* in their study in Democratic Republic of Congo also found the recurrence rate after 2 years follow-up to be 1.1% (Kibadi *et al.*, 2010).

## 2.6 Factors affecting treatment outcomes

Factors that have been found to affect the duration and rate of healing of lesions are many. Some of them are size of initial lesion, age of patient, duration of wound prior to treatment, nutritional status immune response, bacterial burden of the wound, a moist wound environment, quality of wound dressing procedures and the choice of dressing materials used, topical antibiotics, patient education, nursing care, clinical follow-up and several others including co-morbidities (Nicks, Ayello, Woo, Nitzki-George, & Sibbald, 2010; Sarfo *et al.*, 2010; Schultz *et al.*, 2003).

In addition, local factors such as desiccation, necrosis, pressure, maceration, trauma and oedema also affect wound healing (Hess, 2011). In the special case of BU, the presence of mycolactone which is secreted in minute quantities by viable *M. ulcerans* is able to inhibit the production of growth factors necessary for wound healing (Sarfo *et al.*, 2010; Torrado *et al.*, 2007; Coutamceau *et al.*, 2007).

### 2.6.1 Initial size of lesion

In a study to evaluate the efficacy of 8-week rifampicin/Streptomycin combination for BU, it was found that the time to complete healing was dependent on the size of initial lesion (Sarfo *et al.*, 2010). A retrospective observational study undertaken to observe then current wound care practices and to assess the effect of various medical factors on wound healing time on some elderly patients found that wound size was substantively and significantly associated with wound healing time. Larger wounds have longer healing times as shown in several studies (Nienhuis *et al.*, 2010; Sarfo *et al.*, 2010; Chauty *et al.,* 2007).

### 2.6.2 Age and wound healing

Dawes *et al.* in a study to understand the biological basis for age-related differences in posterior capsule opacification in the eye and wound healing rates, found that wound-healing response rate is limited after injury as one ages (Dawes, Duncan, &Wormstone, 2012).

Oriana *et al.* in a study in Italy to document age-related differences in wound healing mechanism after burns in rats also found that wound healing in the aged rats progressed slowly compared to the young ones (Oriana *et al.*, 2012).

In studying the physiologic effect of age on wound healing in human beings Holt *et al.* found that the elderly had a significant delay of 1.9 days than the young in wound epithelialization. Age did not affect collagen synthesis but there was a decreased accumulation of non-collagenous protein in the elderly therefore impairing the mechanical properties of scarring (Holt *et al.*, 1992).

As one ages, skin structure and functions also undergo changes and as a result tissue repair processes are altered (Worley, 2006; Gosain & DiPietro, 2004). However it is critical to note that ageing is largely an individual process and qualitatively, the final result is similar to that in young people (Gosain & DiPietro, 2004).

The current thinking is that after controlling for known associated factors, the effect of age in wound healing is not marked and that wound healing in older people is essentially normal. The age of the patient is not significantly associated with wound healing time and that delay of wound healing in the aged is mainly due to co-morbidities that occur as one ages.

### 2.6.3 Duration of wound prior to treatment

Studies have shown that there is a correlation between longer duration before treatment initiation and poor healing response to compression therapy of wounds. Senescent cells increase as wound ages and according to Harding *et al.* accumulation of greater than 15% senescent fibroblasts is a threshold beyond which wounds become difficult to heal. The ratio of senescent to non-senescent cells is therefore critical to determining response to treatment, and any therapy that modulates this ratio in favour of non-senescent cells is likely to enhance healing (Harding, Moore, & Phillips, 2005). Stanley *et al.* also demonstrated that dermal fibroblasts cultured from

the edges of chronic venous leg ulcers grew slower than fibroblasts from healthy skin and that cells at the margins of chronic wounds are senescent, have lost the capacity to proliferate and thus are less responsive to growth factors (Mendez *et al.*, 1998; Stanley, Park, Phillips, Russakovsky, & Menzoian, 1997).

In addition, wounds of several months' duration will have on average of four to five different microbial pathogens including anaerobic and aerobic gram-negative rods, which are often detected in the course of chronic wound infection (Howell-Jones *et al.*, 2005). According to Landis *et al.*, the longer an ulcer remains unhealed, the more it will acquire significant micro-organism population with more than 50% of these being anaerobes (Landis, 2008) with their presence resulting in failed wound healing (Cutting & White, 2005).

### 2.6.4 Bacteria burden and wound healing

Bacteria in skin ulcers act along a continuum from contamination, through colonization, critical colonization and finally to infection (Frank, Bayoumi, &Westendorp, 2005; Wysocki, 2002). Micro-organisms are acquired from either the indigenous flora of the human host or the environment. It is important to note that the mere presence of bacteria in a chronic wound does not necessarily indicate that infection has occurred or that it will lead to impairment of wound healing (Dow, Browne, &Sibbald, 1999; Kerstein, 1997) because microorganisms are present in all chronic wounds (Landis, 2008; Frank *et al.*, 2005; Schultz *et al.*, 2003).

In most cases wound colonization is poly-microbial (Landis, 2008; Frank *et al.*, 2005; Howell Jones *et al.,* 2005). Wysocki *et al.* in a study to identify the presence of bacteria and then test for their proteolytic activity in ulcers identified 13 different

bacteria species that expressed proteolytic activities against wound matrix substrates, by secreting proteases capable of degrading components of the extracellular matrix important for wound healing. Of the bacteria identified, 6 were Gram-positive and 7 were Gram-negative (Wysocki, Bhalla-Regev, Tierno, Stevens-Riley, &Wiygul, 2012). *Pseudomonas aeruginosa*, beta-haemolytic streptococci and other aerobes have also been identified in ulcers (Ge *et al.*, 2002; Schmidt, Debus, St, Ziegler, &Thiede, 2000).

Critical colonization is not always associated with overt signs of infection but can result in failure to heal, poor granulation tissue, increased wound friability and increased drainage (Cutting & White, 2005; Frank *et al.*, 2005; Schultz *et al.*, 2003). The concept of critical colonization was demonstrated by Sibbald *et al.* in a study where nano-crystalline silver dressing was applied to chronic wounds. Though the wounds did not have clinical signs of infection, the use of the silver dressing resulted in clinical improvement and accelerated healing (Sibbald, Browne, Coutts, &Queen, 2001).

Earlier on, Hansson *et al.* had observed that 86% of ulcers with no clinical signs of infection contained more than one bacterial species (Hansson, Hoborn, Moller, &Swanbeck, 1995). *Staphylococcus aureus* has been found to colonize 43% of infected leg ulcers (Bowler & Davies, 1999) and 88% of seemingly non-infected leg ulcers (Hansson *et al.*, 1995).

Schultz *et al.* observed that though, wound infection may not involve extensive tissue invasion it is sufficient to inhibit wound healing (Schultz, Koenig, Whiteside, &Murray, 2012). This is often accompanied by local pain, warmth, dermal or deeper

erythema, enlarging ulcer, unpleasant or putrid odour and then development of satellite infection (Cutting & White, 2005). As wounds deteriorate and deeper structures become involved, anaerobic flora become part of the local microbial population (Bowler, Duerden, &Armstrong, 2001) representing about 30% of total microbial isolates (Sibbald, Orsted, Schultz, Coutts, &Keast, 2003; Bowler, Davies, &Jones, 1999).

The general observation is that bacterial burden in the wound contributes to a sustained inflammatory state, and inhibits normal progression to the proliferative phase of healing, thereby preventing restoration of tissue integrity (Stojadinovic, Carlson, Schultz, Davis, &Elster, 2008). Matrix degradation delays tissue deposition and repair therefore, treatment of chronic wounds must necessarily include management of colonizing bacteria (Wysocki *et al.*, 2012).

For the normal healing process to resume the barrier to healing must be identified and removed. Therefore, the local management of a chronic wound must necessarily involve an ongoing debridement phase, management of exudate, and resolution of bacterial imbalance (Nicks *et al.*, 2010; Stojadinovic *et al.*, 2008; Schultz *et al.*, 2003).

The most appropriate way of reducing bacterial burden and hence improving wound healing include enhanced host defense mechanisms, debridement, wound cleaning, wound disinfection and topical antibiotics. Most of these can be achieved using the wound bed preparation (WBP) model.

### 2.6.5 Wound Bed Preparation

The wound bed preparation (WBP) model is a systematic way of managing chronic wounds that optimizes achievable patient outcomes. It is an essential element of wound management that removes local barriers to healing and optimizes the tissue environment to accelerate endogenous healing or to facilitate the effectiveness of topical and other therapeutic measures (Stojadinovic *et al.*, 2008; Sibbald, Orsted, Coutts, & Keast, 2007).

The aim of wound bed preparation is to convert the molecular and cellular environment of a chronic wound bed that is failing to heal into that of an acute wound so that healing can proceed through the natural sequential phases. The ultimate aim is to ensure formation of good and quality granulation tissue leading to complete wound closure (Sibbald *et al.*, 2007).

#### 2.6.5.1 Debridement and wound healing

Debridement is the basis of most wound-healing strategies. It may be necessary to remove any devitalized tissue and to facilitate improved wound closure. Necrotic tissue is a toxic contaminant and provides a niche for infection. It prolongs the inflammatory phase of wound healing and mechanically obstructs contraction of wounds and therefore impedes wound re-epithelialization (Frank *et al.*, 2005).

Efficient debridement is an essential step in wound management and should be done regularly to reduce any devitalized and necrotic tissue in order to achieve healthy granulation tissue by leaving a clean surface that will help the wound to heal relatively fast (Lee & Hansen, 2007; Sibbald *et al.*, 2007). It also reduces the bacterial burden of wounds; dead spaces that become niches for bacterial growth are removed,

and therefore assists in reducing tissue destruction. Several studies have also shown that debridement can substantially accelerate wound healing (Cornell, Meyr, Steinberg, &Attinger, 2010; Attinger *et al.*, 2006).

Earlier on, Steed *et al.* in their study to investigate the effectiveness of extensive debridement on diabetic foot ulcers had demonstrated that debridement was a vital adjunct in the care of chronic leg ulcers as the bleeding base left after surgical debridement increases the healing rate of diabetic foot ulcers (Steed, Donohoe, Webster, & Lindsley, 1996).

A well-vascularized wound bed provides nutrients and oxygen to sustain newly formed granulation tissue. It also maintains an active immunological response to microbial invasion.

#### 2.6.5.2 Topical wound treatment

Surface antiseptics or antimicrobial agents can change superficial bacterial burden. Wound disinfection has been an area of controversy because many of these agents have shown toxicity to human fibroblasts *in vitro*. Povidone iodine and chlorhexidine at high concentrations are cytotoxic to tissues and may impede wound healing *in vivo,* but at low concentrations they are considered in certain circumstances to decrease bacterial burden (Nicks *et al.*, 2010; Frank *et al.*, 2005; Sibbald *et al.*, 2003). Iodine, silver-based dressings and topical antibiotics have been shown to be helpful. Cadexomer iodine has been shown to accelerate wound healing in chronic leg ulcers and nano-crystalline silver has also been shown to have broad spectrum antibacterial activity and so accelerates wound healing (Schultz *et al.*, 2003).

Leyden *et al.* also in an open, randomized study observed that topical antibiotic ointment (TAO) significantly increased the rate of wound healing (Leyden & Bartelt, 1987). Berger *et al.* also in another randomized study of TAO versus simple gauze-type dressings in wounds found that TAO was superior to simple gauze-type dressing alone in wound healing and in minimizing scarring of wounds (Berger, Pappert, Van Zile, & Cetnarowski, 2000).

Nicholson *et al.* in another prospective randomized study using topical metronidazole (10%) found that it significantly promotes contraction and epithelialization of wounds (Nicholson & Armstrong, 2004). Recently, Trindade *et al.* in their study in Brazil to assess the efficacy of topical metronidazole (4%) solution in wound healing in rats found that though it facilitated early peripheral epithelialization, it did not affect wound contraction. It rather delayed the appearance of fibroblasts and thereby prolonging healing time. Metronidazole (4%) however almost always eliminated putrid odours emitted by infected wounds. (Trindade *et al.*, 2010).

Despite the controversy surrounding the use of topical antimicrobials, several studies have indicated that they are most appropriate in decreasing the bacterial burden in chronic wounds with active localized infection hence hastening healing ( Lipsky & Hoey, 2009; Frank *et al.*, 2005). Frank *et al.* therefore suggested that there should be a 2-week trial of topical antibiotics with Gram-positive, Gram-negative and anaerobic coverage in cases where wounds are not healing or continue to have exuberant exudate despite optimal management (Frank *et al.*, 2005).

### 2.6.6 Wound dressings

In order to minimize the negative biochemical factors of chronic wound fluid on wound healing, an appropriate wound dressing that can remove copious amounts of wound exudate while retaining a moist environment is desirable. Compression bandaging or highly absorbent dressings are helpful in removing wound fluid. It also allows growth factors to promote angiogenic response leading to wound closure. The choice of wound dressing at one stage of the wound process influences subsequent events in later stages of healing (Kerstein, 1997).

Moist dressings provide a conducive environment for autolytic debridement hence promoting tissue granulation. Wet-to-dry dressings dissolve eschar into a pulp and also induce mechanical separation by physically removing debris as the dressing is removed from the wound bed. A moist wound environment accelerates wound healing by up to 50% compared with exposure to air (Schultz *et al.*, 2003).

Occlusive dressing of wounds favors migration of fibroblasts and therefore matrix formation. It accelerates healing of wounds by promoting autolytic debridement; reduces wound pain and tenderness, reduces fibrosis, decreases wound infection rates and produces a better cosmetic outcome. This is because they are relatively impermeable to exogenous bacteria and also encourage the accumulation of natural substances in wound fluids that inhibit bacterial growth and therefore reduce the burden of necrotic tissue in wounds (Nicks *et al.*, 2010; Fonder, Mamelak, Lazarus, & Chanmugam, 2007).

Several studies have also shown that wounds treated with occlusive dressings are less likely to become infected than wounds treated with conventional dressings (Nicks *et al.*, 2010).

### 2.6.7 Effects of HIV on Buruli ulcer

In a study by Johnson *et al.* in Benin, they found that the overall HIV prevalence was 2.6% among Buruli ulcer patients and 0.3% among controls (Johnson *et al.*, 2008). Several studies have also indicated that HIV prevalence is generally low in patients with Buruli ulcer infection (Debacker *et al.*, 2006; Raghunathan *et al.*, 2005). Though, some studies have shown that HIV infection may increase the risk for Buruli ulcer disease and renders it highly aggressive, its effects have not been fully elucidated (Walsh *et al.*, 2008; Toll *et al.,* 2005).

The disseminated forms of Buruli ulcer with bone involvement are often associated with HIV co-infection (Toll *et al.*, 2005; Pszolla *et al.,* 2003) and in a study in Benin, Johnson *et al.* found that the odds of developing BU was 8.1 (p-value=0.003) in HIV patients compared to controls. Previous studies however, showed that HIV-infected persons are not at increased risk of developing Buruli ulcer (Marston *et al.*, 1995; Darie, Le Guyadec, & Touze, 1993) and that HIV co-infection does not change the treatment outcome of Buruli ulcer disease (Asiedu & Etuaful, 1998).

# CHAPTER THREE

## 3 MATERIALS AND METHODS

### 3.1 Study Area

The study was conducted in two districts in the Eastern region of Ghana, namely the Akwapem South (AKS) district and the Suhum-Kraboa-Coaltar (SKC) district. These districts lie in the Densu River basin and are endemic for Buruli ulcer disease.

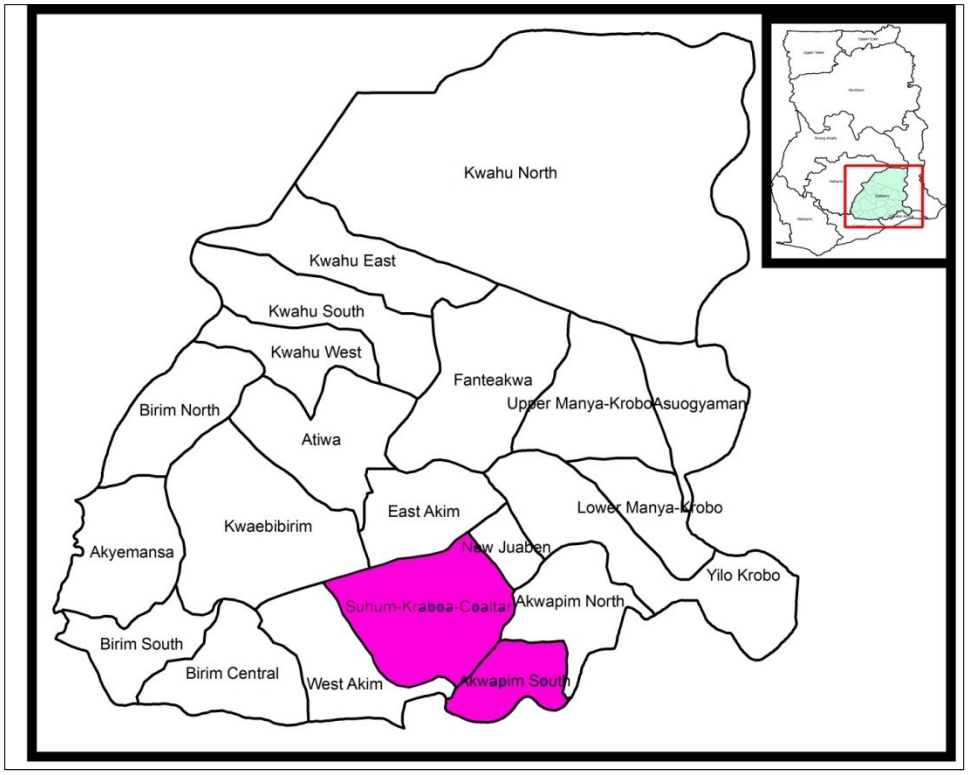



**Figure 3:1 Map of Eastern region showing study area (two districts)**

#### 3.1.1 Akwapem South and Suhum-Kraboa-Coaltar Districts

The Akwapem South District (AKS) is one of the 21 administrative districts in the Eastern region located in the south-eastern part of the region with Nsawam as the municipal capital. It is bounded by the Suhum-Kraboa-Coaltar district in the north-

western part, by West Akim district in the west, the Ga West Municipality in the south-eastern part and the Akwapem North district on the north-eastern part. It covers an area of 440 km² with a population of 135,570 (projected from the 2010 census) and an annual growth rate of 1.4%. It was upgraded from a district to a municipality in January 2008. It consists of 7 sub-municipals namely: Nsawam, Djankrom, Adoagyiri, Pokrom, Pakro/Dego and Aburi sub-municipals.

The Suhum-Kraboa-Coaltar district (SKC) is also located in the southern part of the Eastern Region with Suhum as its capital. The district is essentially rural with Suhum being the only urban town. It covers an area of 1018 km² with a population of 193,981 projected from the 2010 census. It shares boundaries with East Akyem district on the north, Akwapem South on the south, West Akyem on the west and New Juaben and Akwapem North on the east. The main ethnic groups in the districts are Akwapems, Ewes, Akyems and Krobos.

#### 3.1.1.1 Occupation

The main occupations of the inhabitants are farming, trading and bakery. Most of the farmers in both districts are engaged in commercial farming cultivating especially pawpaw, pineapple, cocoa and mango mainly for export. Some of the major crops cultivated are maize, cassava, plantain, sugar cane, oil palm, citrus and cocoyam. Nsawam is well noted for bakery and hawking by petty traders along the main Accra-Kumasi road. The Akwapem South district is particularly endowed with many fruit processing factories and these employ a significant number of the people, especially the youth. There are a few stone quarries and sand-winning activities scattered in both districts.

### 3.1.1.2 Topography Climate and Vegetation

The landscape of the study area is generally undulating with isolated hills and valleys, which form part of the Akwapem range and within the Densu River basin cutting across the northern end to the southern end. There are several streams most of which drain into the Densu River. The climate is typically tropical with the major rainy season from late March to early July and the minor season from early September to early November. The annual rainfall varies between 12.5cm and 200cm. Relative humidity is generally high with maximum and minimum temperatures of 30°C (dry season) and 26°C (rainy season) respectively. The undulating nature of the landscape with several streams traversing has made the low-lying areas swampy with its resultant high humidity. This in addition to the commercial agricultural and sand winning activities have a bearing on the environment hence Buruli ulcer being endemic in these districts.

The vegetation is moist semi-deciduous forest and Guinea savannah grassland that supports the cultivation of cash crops such as cocoa, coffee and non-traditional crops such as pawpaw, pineapple and sugar cane.

### 3.1.1.3 Water and Sanitation

In the Akwapem South district, residents depend on the Densu River as their main source of water supply. Only 30% of the population has access to pipe-borne water and water from boreholes. The remaining 70% depend on unsafe sources of water such as streams, ponds and shallow wells. A community mapping exercise conduced in June 2005 showed that 33% of residents depend on wells as their source of water whilst 17.4% and 13% depend on steams and ponds respectively as their source of water (AKS District Health Directorate Annual Report, 2005).

In the Suhum-Kraboa-Coaltar district the major sources of water are pipe borne water, hand dug wells with pumps and boreholes. A few communities depend on streams, rivers, springs and dug out wells. Only 50% of households have access to potable water. Inadequate water supply in the district has compelled many people in the district to rely on streams and rivers for water, resulting in high incidences of diarrhoea and other water-borne diseases accounting for 6.9% of all out-patient attendance and 7.8% of admissions in health facilities (SKC District Health Directorate, 2010).

The section of the Densu River in the Nsawam municipality is highly polluted and this has largely been attributed to commercial farming, economic development, small-scale mining, encroachment and other human-linked activities very close to it and its tributaries (Akwapem South Municipal Annual Report, 2011) (See figures 3:2 and 3:3). These activities continue to exacerbate the existing pollution and subsequently affecting the lives of the inhabitants

Liquid waste disposal in the Akwapem South district is also saddled with numerous problems with about 60% of residents not having access to decent toilet facilities. Problems that threaten sanitation in the district range from inadequate and unsuitable toilet facilities, overflowing pit latrines to absolute lack of toilet facilities in some communities and also lack of disposal sites for liquid waste.

**Figure 3:2 Sources of water in Akwapem South district**

**Source: AKS DHMT Annual report, 2011**

#### 3.1.1.4 Health Services

In the Akwapem South district, the health sector is divided into 6 sub-municipals namely: Nsawam, Djankrom, Adoagyiri, Pokrom, Pakro/Dego and Aburi for ease of service delivery. The District Health Administration provides technical and administrative support to health service providers in the municipality. These include resource mobilization and distribution, training and research ensuring that services provided are in conformity with national policies. There are 5 hospitals in the municipality which serve as the first referral points. The health facilities are Nsawam Government Hospital, 2 mission and 2 private hospitals, an Orthopaedic Training centre, 6 health centres, 5 functional Community-based Health Planning and Services (CHPS) compounds which provide mainly preventive services, and one optical centre. The municipality is one of the endemic areas for Buruli ulcer with an annual reported case load of about 50 patients. The major health burdens of the district are Buruli ulcer, high maternal mortality, high under-5 mortality, high under-5 malnutrition, environmental sanitation, especially pollution of the Densu River.

The Suhum-Kraboa-Coaltar district in terms of health services is also divided into eight sub-districts, namely Suhum, Asuboi, Anum-Apapam, Kraboa-Coaltar, Akorabo, Nankese Kofi Pare and Dokrokyiwa. The health facilities are one government hospital, 2 private clinics, 2 health centres and 12 functional CHPS compounds.

The study was conducted from two central points: the Pakro Health Centre in the Akwapem South district and the Asuboi Health Centre in the Suhum-Kraboa-Coaltar district.

### 3.1.2 Background of Densu River and Densu River Basin

The Densu River is about 116km long and takes its source from the Atewa-Atwiredu range of mountains near Kyebi in the East Akyem district in the Eastern region of Ghana at altitude 0.64km above mean sea level. As shown in figure 3.3, from the source, the Densu River flows in the south-eastern direction till it reaches Mangoase in the Akwapem North district, then it changes its course and flows generally southwards through the Greater Accra region till it enters the sea at the Gulf of Guinea through the Weija lake and then the Sakumo lagoon at Bortianor, a fishing village which is about 16 km west of Accra.

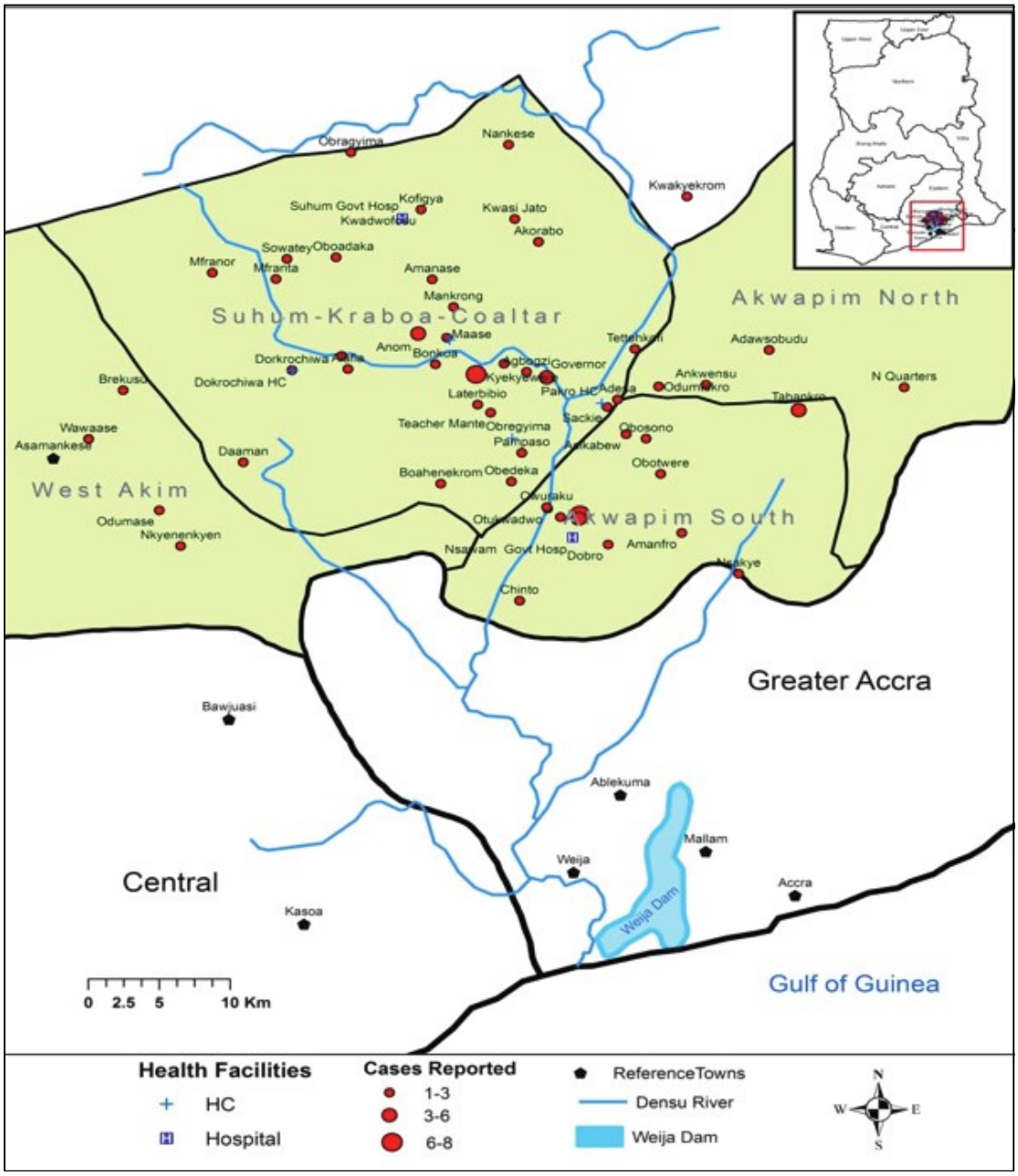



**Figure 3:3 Map of Densu River Basin and Reported Cases of Buruli ulcer, June 2010-May 2012**

**Courtesy: William Opare, NBUCP, Ghana**

The Densu River covers an area of about 2,490km$^2$. Its main tributaries are Kuia, Adaiso, Nsaki and Aprapon rivers. The Densu basin is located in the south-eastern part of Ghana and lies between latitudes 5°30'N to 6°20'N and longitudes 0°10'W to 0°35'W.

The Densu River Basin passes through three regions in Ghana namely Eastern, Greater Accra and Central Regions and falls under ten district administrations. It shares boundaries with the Odaw and Volta Basins to the east and north, the Birim Basin in the northeast and the Ayensu and Okrudu Basins in the west. There are over 200 settlements in the Densu River Basin with a total population of almost 600,000 people. The basin plays a critical role in the socio-economic development of the many towns and satellites villages dotted within it. Most of the urban centers such as Koforidua, Nsawam and Suhum among others get treated water from the Densu River.

Its mean annual runoff is 500x106$m^3$. The Densu Basin is also intensively used for the cultivation of both cash and food crops. Principal food crops cultivated within the basin are cassava, maize, yam, plantain, banana and cocoyam. Cash crops include cocoa, oil palm, papaya, pineapple, sugar cane, vegetables, mangoes and citrus. Other land use activities include housing, sand winning, animal rearing and salt mining.

### 3.2 Study Design

This study was designed as a non-randomized intervention study where the Principal Investigator (PI) was the clinician who treated all enrolled study participants. Randomization of the patients was not done because of the debilitating nature of Buruli ulcer disease. The study was conducted between June 2010 and May 2012. Patients were serially recruited into the study and followed up for a minimum of 8 months and a maximum of 18 months. Study patients were treated on ambulatory basis. Figure 3.4 shows how patients were recruited and enrolled into the study. Decisions to review treatment were based on the responses of the lesions to treatment.

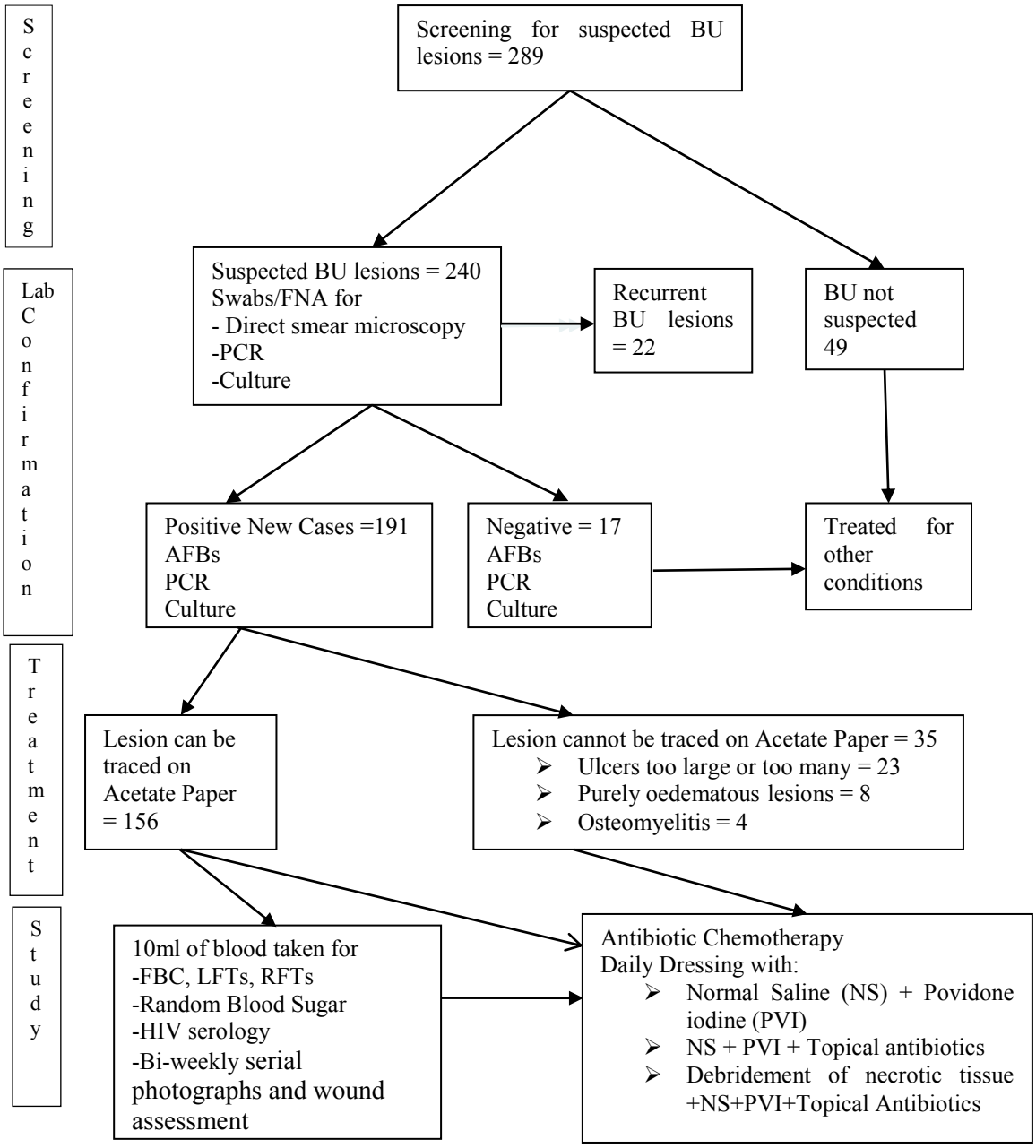



**Figure 3:4 Flowchart of Data Collection in the Clinic**

## 3.3 Study Population and Participants

The study population comprised all residents in the Akwapem South and Suhum-Kraboa-Coaltar districts. All suspected Buruli ulcer patients who met the study's eligibility criteria and consented to enroll in the study were recruited following active case finding activities in endemic communities in both districts (see figure 3:4). All

study participants were counseled regularly and encouraged to comply with drug treatment and wound management procedures till they were discharged from the study.

## 3.4 Case Definition of Buruli ulcer

The case definition of BU used in this study was ulcerative or pre-ulcerative lesion in patients 2 years old and above from the Akwapem South and the Suhum-Kraboa-Coaltar districts diagnosed as Buruli ulcer. Additional criteria established for recruiting the patients are as discussed below.

### 3.4.1 Eligibility Criteria

Any person aged 2 years or more who resided in the Akwapem South and Suhum-Kraboa-Coaltar districts and newly diagnosed as having Buruli ulcer disease was eligible to enter the study.

#### 3.4.1.1 Inclusion Criteria

All newly diagnosed and laboratory confirmed Buruli ulcer patients resident in the Akwapem South and Suhum-Kraboa-Coaltar districts and whose lesions had not been complicated and could be traced on acetate paper.

#### 3.4.1.2 Exclusion Criteria

All suspected Buruli ulcer patients who were:

- Less than 2 years
- Having lesions that had circled or affected the whole arm or leg

- Having cancerous lesions
- Having oedematous lesions
- Lesions complicated by osteomyelitis
- Pregnant: verified by asking females who were 10 years and above about their last menstrual period.
- Past history of treatment with aminoglycosides: history of having received some injections for tuberculosis or receiving some injections for an extended period of time.
- History of leprosy
- Tuberculosis: A history of symptoms and signs of Tuberculosis
- Liver problems: verified by liver function test done
- Kidney problems: verified by renal function test done
- Hearing problems: verified by taking history of difficulty in hearing

**Figure 3:5 Some Ineligible Buruli ulcer lesions for Study**

### 3.5 Definitions of Treatment Outcomes

**Treatment Success**: This was divided into primary treatment success and secondary treatment success

- Primary Treatment Success: Complete wound coverage with healthy granulation tissue of ulcers with antibiotic treatment without surgery.
- Secondary Treatment Success: Complete wound coverage by crust without residual inflammation with antibiotic treatment without surgery.

**Treatment Failure**: Treatment was considered to have failed if in the course of treatment participant died in relation to Buruli ulcer disease or persistence of non-scarring lesions despite appropriate medical and or surgical interventions within 8 months of treatment and wound care.

**Recurrence/Relapse**: a recurrent case was defined as a patient who has had previous treatment with anti-mycobacterial therapy for Buruli ulcer disease and presented with a further Buruli ulcer lesion at the same or a different site within 6 months after the end of the last antibiotic treatment.

**Loss to Follow-Up**: Participants were considered lost to follow-up if they abandoned treatment and could not be retrieved.

### 3.6 Sample Size

From data received from both districts, the facilities receive about 86 patients within a year and out of which 50% achieved complete wound healing. To detect an effect size of 30% in wound healing at 95% confidence level and a power of 80%, a minimum of 90 patients was required. With a sample size of 154 there was a reasonable degree of security against the effects of a decline in success rate.

We recruited a total of 213 confirmed BU patients from June 2010 to May 2012. Fifty-seven patients were excluded due to:

- 35 patients' lesion could not be traced onto acetate paper
    a. 23 - Ulcers too large or too many
    b. 8 - purely oedematous lesions
    c. 4- Osteomyelitis
- 22 were recurrent/non healing BU cases thus had had rifampicin and streptomycin treatment before. (See figure 3:4)

#### 3.6.1 Sampling Technique and Follow-up Procedures

Purposive sampling technique was employed. This was used due to the fact that Buruli ulcer is focally distributed in endemic regions and there is also limited knowledge about it even in affected communities. Anticipating a low reporting of cases in health facilities, case finding activities were performed to augment the reported number of cases. This involved conscious selection of endemic communities for case finding activities. It was done by trained community-based surveillance volunteers, community health workers and the Principal investigator (PI). All consenting patients selected according to the case definition were assessed by the

principal investigator to determine their inclusion into the study based on the study's inclusion criteria.

Eligible patients who presented at the two study-site clinics were screened by the PI and serially recruited into the study until the desired sample size was achieved. Each study participant was given 2 months of antibiotic treatment and wounds were dressed daily till lesions healed completely or till 34 weeks of follow-up. Each participant was followed up for a minimum of 8 months and a maximum of 18 months.

## 3.7 Organization of Field work

### 3.7.1 Community Entry

The study was conducted in close collaboration with the District Health Management Team (DHMT) of the two districts and the National Buruli Ulcer Control Programme staff. A series of meetings were held with the National Buruli Ulcer Control Programme (NBUCP) Manager, the Eastern Regional Director of Health Services, the District Directors of Health Services and the District Chief Executives of both districts to explain the rationale of the study to them and to seek their support. At the community level, meetings were first held with the chiefs, elders and opinion leaders and then followed by mini-durbars. These meetings and durbars sought to enhance community members' knowledge on the clinical manifestations of Buruli ulcer and the treatment options that are available.

The health staff in the sub-districts, with the help of Community-Based Surveillance volunteers (CBSV) who were conversant in Buruli ulcer surveillance activities were recruited to help with the organization of community durbars and case finding activities. They educated community members on Buruli ulcer disease: the signs,

available treatment and its complications. They also encouraged patients to report to the health facilities as soon as they recognized any such symptoms.

### 3.7.2 Field Operations

Data collection was done simultaneously in both Akwapem South (AKS) and Suhum-Kraboa-Coaltar (SKC) districts. There were two central clinics; one in each district: the Pakro Health Centre in AKS district and Asuboi Health Centre in the SKC district. There was a team each for treatment of lesions at the central clinics, which usually comprised the Principal investigator (PI), a Field Technician and a Health Aide. The Field technicians doubled as Research Assistants.

### 3.7.3 Data Collection Techniques, Tools and Procedures

The study employed mainly quantitative data collection techniques and tools in its implementation. The data collection techniques used for this study were semi-structured interviews and participant observation. This involved patient history taking and physical examination. Swabs and fine needle aspirates were taken from ulcerative and pre-ulcerative lesions respectively for laboratory confirmation of Buruli ulcer disease. 10mls of blood was also taken for haematological and blood chemistry examinations.

The data collection tool used was semi-structured questionnaires. Patients were examined by the PI. The semi-structured interview was a combination of both close-ended and open-ended questions. This was designed to elicit both definitive and unexpected kinds of information from the interviewees. Since the data gathering was semi-structured not all respondents were asked the same questions, but the main areas

covered were explored, thus enabling responses to be categorized and coded in order to enable descriptive methods of comparison to take place.

The semi-structured questionnaire was designed by the PI adopting the BU01 form of the NBUCP for research and management of BU. This questionnaire contained questions on participants' background socio-economic, socio-demographic and clinical data as to the form of lesion at presentation, how lesion started, any antecedent trauma, duration of lesion prior to reporting, health seeking behavior, previous treatment received, treatment outcomes and family history of Buruli ulcer, whether ulcers were undermined or not and any associated pain.

Part of the questionnaire was used to assess the form and location of lesion, features and size of the lesion at presentation and to record sequential changes in the features and sizes of lesions during follow up.

### 3.7.4 Training of Field Workers

Health workers and Community-Based Surveillance volunteers who were already involved in BU activities were given a day's training in BU case finding. There was a full day's training session at each of the study sites. These were organized with the support of the District Health Management Teams (DHMTs) and were facilitated by a team from the NBUCP and the PI. Trainees were taken through:

- The rationale and objectives of the study
- Signs of Buruli ulcer disease
- Treatment options available for Buruli ulcer
- Wound dressing

- Prevention of disability (POD) exercises
- Techniques of translating the questions into the local language
- Questionnaire administration and interview techniques

The health workers who were the research assistants to collect data at the clinic were made to practice questionnaire administration and conduction of interviews to be able to collect relevant, consistent and complete information.

### 3.7.5 Pretesting of Instruments

The Principal investigator and Research Assistants carried out the pretesting of the instruments at their respective clinics on patients who had already started treatment and therefore were not going to be included in the study. Some of the questions were reformed and some were entirely dropped. The following were evaluated during the pretesting:

- Whether the questionnaires and acetate paper used for measuring the circumference of lesions were reliable tools for the information needed
- The willingness of the respondents to answer questions and cooperate with researchers
- The time needed to administer the questionnaires
- Whether there was the need to revise the questions
- The sequence of the questions
- Clarity of questions

### 3.7.6 Preparation for Actual Data Collection

Days for conduction of clinics were fixed in consultation with the DHMTs of the study districts. Weekly clinics were run on Wednesdays in Akwapem South district and on Thursdays in Suhum-Kraboa-Coaltar district. The clinics were run for all sorts of ulcers.. Out of the cases that reported at the clinics, suspected cases of BUD were selected and swabs and FNA taken for disease confirmation. Those who turned positive for *M. ulcerans* and met the inclusion criteria were included in the study after informed consent had been sought from adult patients and guardians of minors. Those whose lesions were confirmed as BUD but did not meet the inclusion criteria, those who tested negative and those who were not included for testing were all treated as per the diagnosis given till they were fully healed and discharged from treatment. Those whose lesions could not be managed at the clinics were referred to other centres for further management.

### 3.7.7 Data Collection at the Clinic

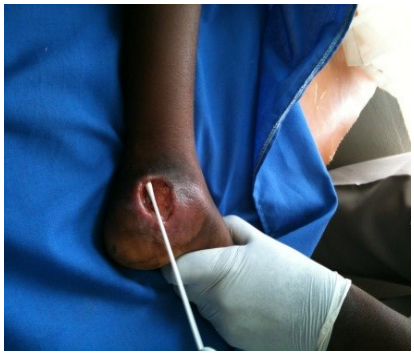
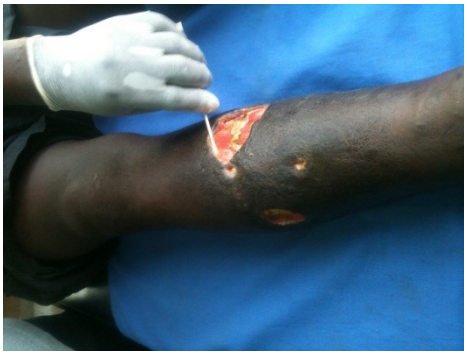
The validated tools and instruments were employed in the actual data collection at the clinic as described below.

#### 3.7.7.1 Pre-treatment Assessment of Patients at the Clinic

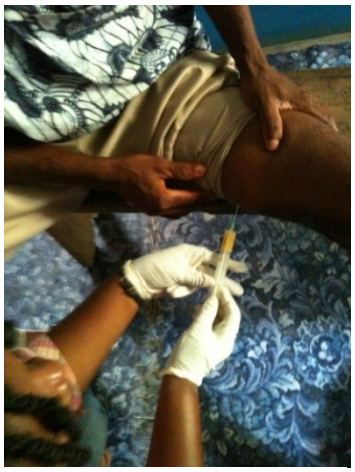
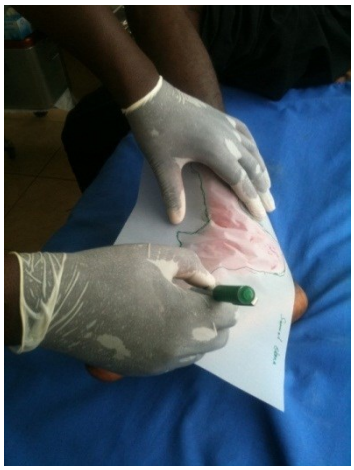
For all cases who were suspected as having Buruli ulcer disease and had consented to enroll in the study, a thorough history was taken to collect information on variables such as age at last birthday, sex, highest educational level attained, ethnicity, marital status, occupation, the form of lesion at initiation and at presentation, any antecedent trauma, duration of lesion prior to reporting, family history of Buruli ulcer.

Thorough physical examinations were performed on patients to assess the number, forms, location of lesions, features and severity of the disease. Swabs were taken from the undermined edges of ulcers and fine needle aspirates (FNAs) from nodules and oedematous lesions on the first day of reporting to the health facilities. These individuals who were clinically examined and diagnosed as probable cases of Buruli ulcer disease had their blood samples taken for blood chemistry to detect any hepatic and renal problems if there be any. Full blood count examination was performed on all suspected cases. All patients were weighed and their heights taken as per the demands of the Ghana Health Service. X-rays were also taken for study participants suspected to be having bone involvement.

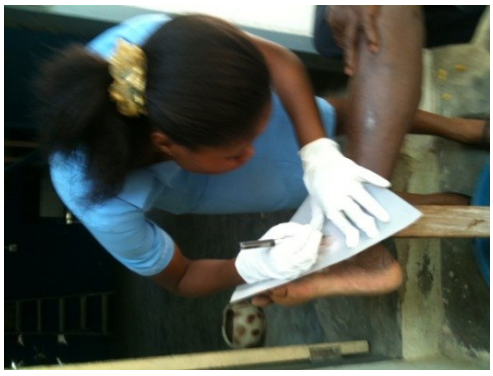
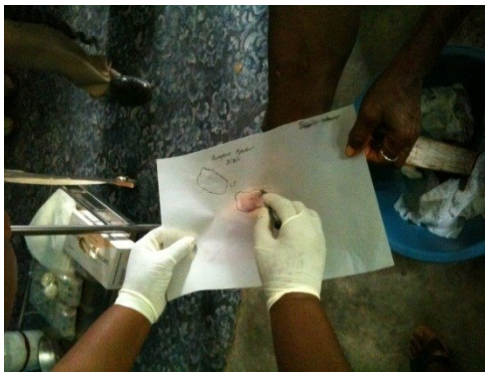
Before treatment initiation photographs of lesions were taken and lesions were traced out using acetate papers as shown in Figures 3:6c and 3:6d. Tracings were serially repeated bi-weekly until lesions were healed or until patients were recommended for skin grafting. Tracing of lesions were done to enable the circumferences of lesions to be measured. (See figure 3:7). The circumferences measured were used to calculate the respective surface areas of lesions.

a: Swabbing of BU ulcers

b: FNA of BU lesion c: Tracing of BU lesion

d: Tracing of BU lesion e: Serial tracing of BU lesion

**Figure 3:6 Swabbing, Fine needle aspiration and Tracing out of Circumferences**


**Figure 3:7 Photographs of serial tracings of BU lesions**

### 3.7.7.2 Specimen Collection and Storage

Swabs from the undermined edges of ulcers and fine needle aspirates (FNAs) from nodules and oedematous lesions, (see figures.3:6a and 3:6b) were taken for direct smear microscopy using the Zeihl-Neelsen staining method. IS*2404*-PCR test was also done to detect *M. ulcerans* DNA (Ross *et al.*, 1997). Two swabs were taken from each suspected case. These cotton swabs were put in plastic containers and sealed. Specimens were labeled with information that included the name of patient, identification number, age, sex and date of collection. Specimens were stored on ice and transported to the laboratory for analysis. 10ml of venous blood was taken from each patient. 3-4ml of this was put in sequesterene bottles for full blood count examination and the rest put in plain bottles for blood chemistry examinations.

#### 3.7.7.3 Detection of *M. ulcerans* in Swab or FNA Specimen

*M. ulcerans* in this study was detected by the use of three confirmatory diagnostic tests. These were direct smear microscopy, polymerase chain reaction and culture.

##### 3.7.7.3.1 Direct Smear Microscopy

Smears prepared from specimens and stained by the Ziehl-Neelsen (ZN) staining method were observed directly under the microscope for acid fast bacilli (AFBs). Briefly, smears were prepared by placing 2 drops of sample specimen on clean glass slides, and gently spread to make thin films. The smears were allowed to air dry and then fixed using Bunsen flame. Next, these fixed smears were flooded with carbolfuchsin stain, and the underside of the slides were heated until steaming (without boiling)

Afterwards the films were rinsed gently with running water until the slides were free of stains. The slides were then decolorized with 20% Sulphuric acid for 2-5minutes, and then again rinsed thoroughly and excess water drained. Counter-staining was then done with 3% methylene-blue chloride solution for a maximum of 120 seconds. This was followed by gentle and indirect stream of water. The slides were then air-dried, and examined under the microscope (oil immersion) for acid-fast bacilli (AFBs). The quantitation used for reporting the results is as shown in Table 3:1.

**Interpretation of Test Results**

**Table 3:1 Quantitation used for Reporting AFBs (IUATLD/WHO Grading scale)***

| No of Index entries found | No. of fields seen | Report |
|---|---|---|
| No AFB / 100 immersion fields | 100 | No AFB observed |
| 1-9 AFB / 100 immersion fields | 100 | Scanty |
| 10-99 AFB / 100 immersion fields | 100 | + |
| 1-10 AFB / 1 immersion field | 50 | ++ |
| >10AFB / 1 immersion field | 20 | +++ |

**+ -Small numbers of AFBs seen in smear, ++ - Moderate numbers of AFBs seen**
**+++ - Numerous numbers of AFBs**
*** International Union against Tuberculosis and Lung Disease (IUATLD)**

### 3.7.7.3.2 Polymerase Chain Reaction (PCR)

This method amplifies minute quantities of DNA to levels that can be detected in the laboratory. The sequence of the DNA that is amplified is determined by the sequence of the PCR "primers" that initiate the PCR amplification.

**Mycobacterial DNA extraction**

Mycobacterial DNA was extracted from 500ul of BU sample using the QIAamp DNA extraction minikit (Qiagen, Hilden, Germany). Briefly 20ul of proteinase K was added to a mixture of 400ul aliquot of BU sample and 400ul of lysis buffer. This was mixed by vortexing and incubated at 56˚ C for 30 minutes. After cooling, 400ul of absolute ethanol was added to facilitate mycobacterial DNA precipitation. A volume of 700ul of the final mixture was transferred to a spin column and centrifuged at 6000g. A collection tube draining the spin column was then discarded with the filtrate. This procedure was repeated for the remaining mixture and the membrane (of spin column)

washed twice with two different washing buffers, firstly with 500ul of washing solution 1 (Buffer AW 1), and then with washing solution 2 (Buffer AW 2). The extracted DNA was then eluted with 100-150ul of the elution buffer provided in the kit as shown in figure 3:8.

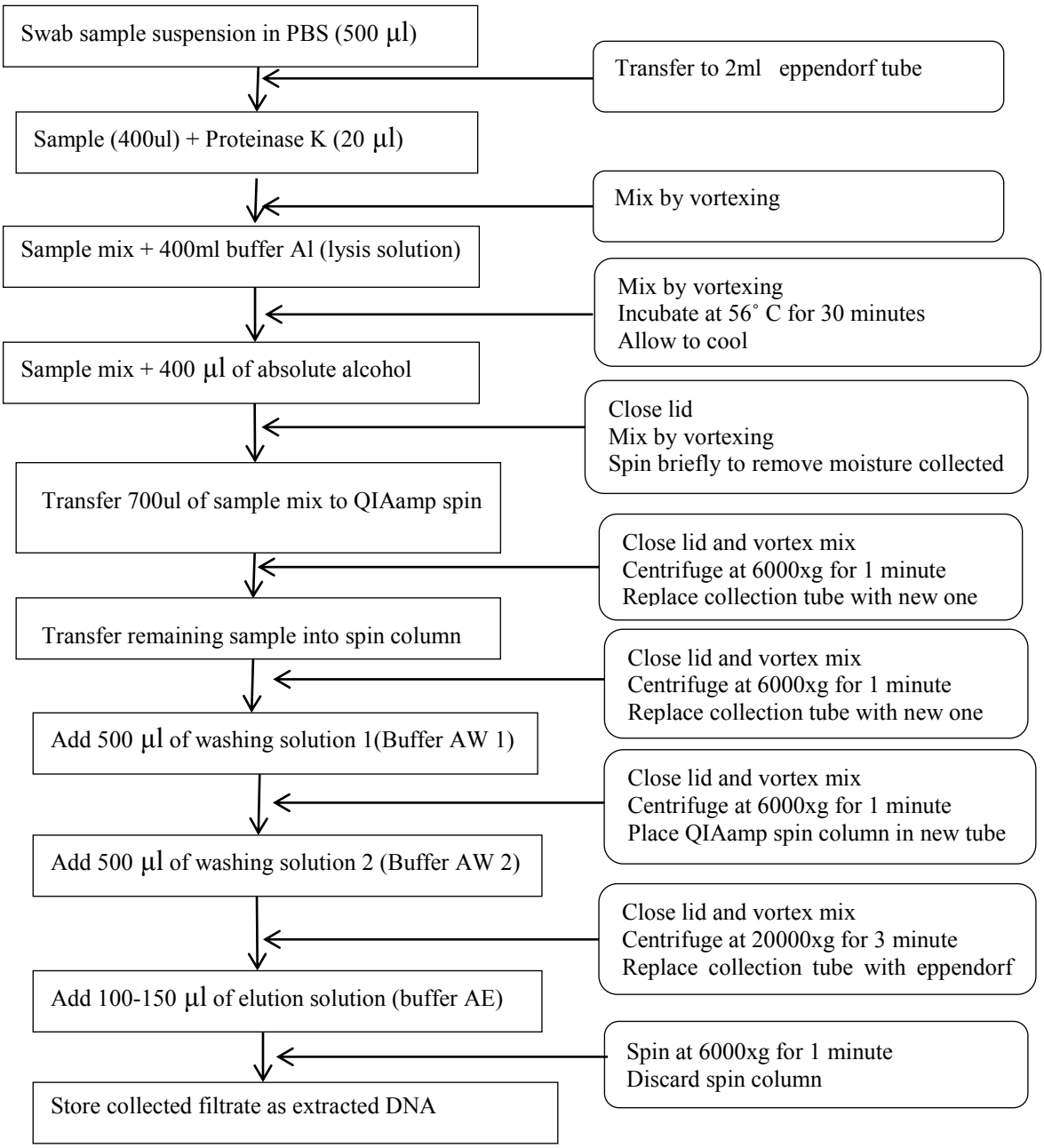



**Figure 3:8 Flow Chart Showing Procedures for Bacterial DNA extraction**

**IS*2404* PCR for the detection of *M. ulcerans***

PCR targeting IS*2404* was performed as described by Ross *et al*. (Ross *et al.*, 1997). The primers pGp1:5'-AGGGCAGCGCGGTGATACGG-3', pGp2: 5'-CAGTGGATTGGTGCCGATCGAG-3', pGp3: 5'-GGCGCAGATCAACTTCGCG GT-3' and pGp4: 5'-CTGCGTGGTGCTTTACGCGC-3', were respectively used for the first and second round PCR amplification.

For the First round, 30 µL reaction volume that contained 3µL DNA, 25pmol/µL of each primer, 3µL of 10x PCR buffer (containing 1.5 mM magnesium chloride), 6.0µL Q-solution, 0.2mM deoxynucleotide triphosphates (dNTPs) and 1.0 U HotStar *Taq* polymerase (Qiagen, Hilden, Germany). For the second run, 1µL of the first run product was added to 24µL reaction volume containing 25pmol/µL of each primer, 2.5 µL of 10x PCR buffer, 5.0 µL Q-solution, 0.2 mM dNTPs and 1.0 U HotStar *Taq* polymerase. Amplification cycles consisted of denaturation at 95°C for 15 min, 94°C for 30 sec, 64°C for 1 min, 72°C for 1 min, 30 sec and a final extension at 72°C for 10 min.

PCR products were analyzed by agarose gel electrophoresis using 2% agarose gel incorporated with ethidium bromide and visualized with a UV trans-illuminator. Amplicon sizes were estimated by comparing the bands to a 100bp ladder (Fermentas Life Sciences, EU). The samples were run concurrently with negative and positive controls. A sample positive for IS*2404* was indicated by the alignment of its band with the band produced by the positive control sample as shown in figure 3:9 below.

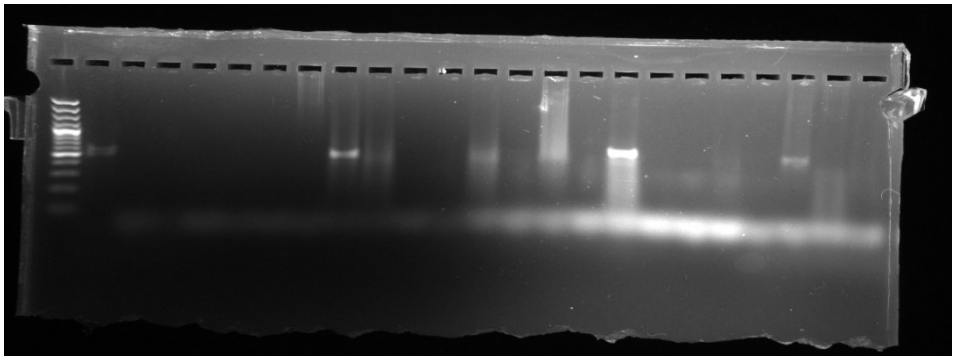


**Figure 3:9 Plate of agarose gel showing electrophoresis of amplified PCR products**

**Lane 1:** 1 kb base pair markers
**Lane 2**: positive control
**Lane 3**: negative control
**Lanes 9 & 17**: Clinical samples strongly positive for IS2404PCR
**Lanes 10, 13, 15, 22**: Clinical samples weakly positive for IS2404PCR

#### 3.7.7.3.3 Culturing of *M. ulcerans*

Samples were decontaminated using the oxalic acid method. Briefly, 2ml of homogenized sample was added to 2ml of 5% oxalic acid. They were mixed by vortexing and then left standing at room temperature for 30mins with intermittent mixing. This mixture was neutralized with 40ml of sterile phosphate buffered saline (PBS) and then centrifuged at 3000xg for 30 minutes. The pellets were re-suspended in 1ml of PBS and 2 drops of this final mixture were inoculated in duplicate Lowenstein-Jenssen (L-J) tubes and incubated at $32^0$C. The tubes were examined daily for contamination for the first week and then subsequently weekly for episodes of growth for 8-12 weeks (Palomino & Portaels, 1998).

**Table 3:2 Results of Confirmatory Diagnostic Tests for *M. ulcerans* in Swab and FNA specimens**

| Test | Number (%) n=154 |
|---|---|
| Direct smear microscopy | 129 (83.7) |
| PCR | 151 (98) |
| Culture | 34 (22.1) |

### 3.7.7.4 Haematological, Blood chemistry and Serology tests

Blood samples were taken from study participants to perform haematological analysis and blood chemistry. The blood chemistry examinations performed were renal function tests (RFTs) and liver function tests (LFTs). Full blood count examination was done for study participants to ensure that none of them were anaemic or had any haematological disorder. Those whose haemoglobin levels were found to be 10mg/dl or lower were treated with haematinics. The liver and kidney function tests were performed to rule out liver and renal problems so that patients on treatment would not unduly suffer from adverse effects of the drugs used for treating Buruli ulcer. Pregnancy was assessed in females who were 10 years and above by enquiring about their last menstrual period.

Finger prick blood was also taken from each patient to estimate the random blood sugar (RBS) levels of patients. In cases where RBS level was above 11mmol/l, fasting blood sugar (FBS) was estimated. The RBS was done using Onetouch Ultra Family Meter and OneTouch Ultra strips.

The blood chemistry, RFTs and LFTs were performed using an automated chemical analyzer (Selectra Junior, Merck Pvt. Ltd). About 1ml of serum was put in a 2ml

cuvette and placed in the analyzer. 30ul of the sample was taken by a probe and mixed together with an appropriate reagent. This was processed in the machine and the results were displayed automatically on the monitor of the chemical analyzer.

Full blood count examinations were also done using a haematology auto-analyzer, KX-2IN (Sysmex, Kobe, Japan). A volume of 2.5ml of blood was put in a sequestrene bottle and mixed thoroughly. Then 50ul of the sample was aspirated by a probe into the analyzer which was processed, and the results come out printed.

Patients were also counseled on the Human immunodeficiency virus (HIV) infection and those who consented to have the test done were tested for HIV antibodies using the OraQuick rapid HIV1/2 antibody test.

**Table 3:3 Haemoglobin, Random Blood Sugar, Renal & Liver Function Tests of study participants from Akwapem South and Suhum-Kraboa-Coaltar districts, June 2010-May 2012**

| Factor | Median | Range (study) | Normal Range |
|---|---|---|---|
| Haemoglobin level | 12.6 g/dl | 9.6-16.7 g/dl | 12.0-18.0g/dl |
| Random Blood Sugar | 6.0 mmol/l | 5.0-8.0 mmol/l | Up to 11 mmol/l |
| Urea | 3.6 mmol/l | 1.1-7.2 mmol/l | 2.0-7.0 mmol/l |
| Creatinine | 89 mmol/l | 58-121 mmol/l | 54-124 mmol/l |
| Bilirubin | 6.9 µmol/l | 2.5-16.8 µmol/l | 3.4-22.2 µmoll/l |
| Total Protein | 84 g/l | 65-88 g/l | 66-87 g/l |
| Albumin | 42 g/l | 28-46 g/l | 35-50 g/l |
| Aspartic Transferase | 30 U/l | 18-38 U/l | 9-44 U/l |
| Alanine Transferase | 20 U/l | 11-35.3U/l | 10-40 U/l |
| Alkaline Phosphatase | 51U/l | 34.7-99U/l | 30-90 U/l |

### 3.7.8 Treatment and Follow-Up

#### 3.7.8.1 Drug treatment

Those diagnosed as having Buruli ulcer and meeting the inclusion criteria, regardless of the clinical form of the lesions were given a directly observed treatment (DOT) of a daily combination of intramuscular streptomycin (15mg/kg body weight) and oral rifampicin (10mg/kg body weight) for 8 weeks (World Health Organization., 2011). All patients were treated on ambulatory basis. Upon diagnosis, participants were given one-week supply of drugs to take to the nearest health facility to receive DOT and daily dressings by health workers. Patients then reported to either the Pakro Health Centre in the Akwapem South district on Wednesdays or the Asuboi Health Centre in the Suhum-Kraboa-Coaltar district on Thursdays every week for review of treatment and drugs and logistics refill. They carried their BU01 forms filled by the health worker who ensured their DOT for the PI to check for treatment compliance.

#### 3.7.8.2 Wound management

There was daily dressing for all wounds irrespective of size and weekly assessment of lesions to review any changes that have occurred as to clinical improvement or deterioration based on changes in size, degree of oedema and appearance of the ulcer base.

During the initial 6 months of the study ulcerative lesions irrespective of their features were given daily dressings with just saline povidone iodine and gauze. Afterwards ulcers were selectively treated based on their features: whether clean or had necrotic tissues (slough). Necrotic tissues covering ulcers were removed (see Figure 3:10). Devitalized skin around ulcers was also removed. All ulcers received daily dressings with povidone iodine and or topical antibiotic ointment (a combination of bacitracin,

neomycin and polymixin-B). Those emitting putrid odour were treated with topical metronidazole for a minimum of 72 hours. Vaseline gauze was then applied, covered with plain gauze and then occluded with crepe bandage. Wound eschars around the edges of ulcers were also removed on weekly basis.

For the first 8 weeks participants were followed up at weekly intervals for clinical assessment of their lesions. At bi-weekly intervals, the outlines of lesions were traced out on tracing sheets to obtain their circumferences and photographs were also taken. At these visits participants were questioned about any symptoms of pruritus, rashes, dizziness and vertigo, abnormal hearing and scleral icterus. At the end of each clinic day all forms were checked for completeness and consistency by the principal investigator.

Removal of necrotic tissues but not skin grafting was considered part of normal wound care in this study. It is important to note that skin grafting speeds up healing but does not affect mycobacterial load of ulcers (Nienhuis *et al.*, 2010). However, removal of necrotic tissue and debridement reduce the bacterial load of ulcers as well as speeding up the healing of wounds (Cornell *et al.*, 2010; Sibbald *et al.*, 2007). All patients received antibiotics and dressings free of charge as per the requirements of the National Buruli Ulcer Control Programme. Safety outcomes were measured by occurrence of adverse events.

#### 3.7.8.3 Counseling and Health information

All patients were counseled on BU infections and management. Information given included

- Drug treatment regimen was for 8 weeks and that
- Complete wound healing may outlive the duration of drug treatment. This depended on the form or size of lesions and several other factors.
- Patients were encouraged not to interfere with wound management by using other drugs or concoctions
- Patients were persuaded to have patience for at least 4 weeks to see if there would be any improvement in their lesions

If there was any improvement then they were motivated to continue with the treatment given. This strategy usually worked. Counseling sessions were held regularly till lesions healed completely. Patients were also encouraged to wear socks or made to occlude scars with crepe bandages until the scars were fully remodeled. All study participants were told to report of any adverse events to the health facility as soon as possible. Some patients were given money for transportation and the children were given biscuits, chocolates and sometimes candies on each clinic day. This motivated them to report to the clinic even if adults did not accompany them.

**Figure 3:10 PI removing necrotic tissue fron ulcers and wound dressing**

Participants were released from treatment as soon as they had finished antibiotic treatment and their lesions healed without any residual inflammation. Participants whose lesions had improved but had not healed completely after the completion of the antibiotic treatment were continued on daily dressings till complete crust coverage without any residual inflammation.

Those whose lesions remained stable with no improvement for 4 weeks continuously were recommended for surgical excision and skin grafting. After completion of treatment all participants were asked to report to the facilities of any lesion recurrence or abnormal events. All participants were followed-up for at least 8 months that is, 6 months after completion of antibiotic treatment. Participants whose lesions turned dry before final healing occurred were asked to cover them with bandages for protection and to visit the facility bi-weekly until final healing.

### 3.8 Quality Control Checks of Data Collection

All field assistants and health workers involved in the recruitment of subjects for the study were given a day's training to ensure uniformity in selecting suspected BU cases. The principal investigator (PI) designed all the data collection tools and questionnaires were with consistency checks to prevent errors at the stage of data collection and at the point of data entry.

All forms were assigned unique identity numbers before they were sent to the field. Questionnaires were administered by field assistants and supervised by the PI. At the end of each clinic day, forms were checked for completeness and any inconsistencies corrected. Data entry screen was also designed by the PI using Microsoft EpiData software with checks. All data entry was done by the principal investigator.

## 3.9 Analysis of Data

The specific objectives of the study were as follows:

1. To describe the demographic characteristics of Buruli ulcer patients;

2. To determine the extent to which antibiotic treatment (rifampicin plus streptomycin combination) affect the various categories of BU lesions and

3. To determine factors that influence the healing of Buruli ulcer lesions

In order to analyze these issues specific analytical methods and models were used.

### 3.9.1 Describing demographic characteristics of Buruli ulcer patients

Data was captured using Microsoft Epi Data version 3.5 and analyzed using Stata 11. The age and sex distribution of respondents were described.

### 3.9.2 Determining the extent to which antibiotic treatment affects the various categories of BU lesions

The duration of healing of the three categories of BU lesions were determined. Appropriate measures of centrality (mean, median) and of dispersion (quartiles, standard deviation, minimum, maximum and standard error of the mean) as well as frequencies (absolute and relative) were computed and summarized in tables. The response analysis of the various categories was done using Kaplan-Meir method of survival estimate.

The Kaplan-Meier method of survival was used in studying the time between entry into the study and development of healthy granulation tissue and achievement of complete healing of BU lesions. It was also used to estimate the proportion of the categories of lesions that would not heal completely in a given length of time under

the same circumstances. Tables and survival curves were also drawn (see table 4.7 and figures 4:3-4:7). The survival analysis was used to produce a curve of wounds not yet healed for each category of lesion; the logrank test was used to compare the survival curves for the three categories of lesions. Exploratory analysis revealed that the relationship between category (size) of lesion and rate of healing varied across time. For the first 4 weeks after initiation of treatment the rate of wound healing was higher for all categories of lesions. (See table 4:7).

### 3.9.3 Determining factors that influence the healing of Buruli ulcer lesions

The association between complete healing of BU lesions and various independent variables of category (size) of lesion, age, nature of the edge of lesion, duration of illness prior to care seeking, use of traditional treatment, pain, family history of Buruli ulcer, paradoxical reaction, presence of necrotic slough, wound dressing with topical antibiotics with or without removal of necrotic tissue (slough) and devitalized tissues was assessed using the Cox Proportional hazard model. Univariate and multivariate analyses were also performed with significance level set at 95% confidence level. Covariates which were found to be significant in the univariate analysis or were known to be associated with outcomes based on previous studies were included in the multivariate analysis

The Cox proportional hazard model which is a semi-parametric hazards model and also a continuous-time hazard model was used to simultaneously explore the effects of several variables on healing of lesions. It is known that there are other variables besides treatment that influence the healing of Buruli ulcer lesions and that these variables cannot be easily controlled in a clinical trial so the Cox proportional hazard model allowed the estimation of the effects of category of lesion from the effects of

other variables. Using the model improves the estimate of the effect of category of lesion by narrowing the confidence interval. Investigating several variables at a time, this procedure models or regresses the healing times (or hazard function) on the explanatory variables.

The hazard function in this study was interpreted as the likelihood of healing at time t provided the probability that an individual's wound would heal within a small time interval, given that the wound had existed up to the beginning of the interval. In this study, the hazard function denoted by h($t$) was estimated using the following equation:

h(t) = $\frac{a}{b}$

$$h(t) = h_0 \exp(b_1 x_1 + b_2 x_2 \ + b_3 x_3 \ldots b_x x_x$$

where

a= number of individuals experiencing complete wound healing in interval beginning at time $t$

$b$ = number of individuals with wounds not yet healed at time, $t$

The hazard function is the dependent variable or the likelihood of healing at time $t$ and $h_0(t)$ is the baseline or underlying hazard function that corresponds to the probability of a wound healing when all the explanatory variables are zero.

The Cox model therefore yields an equation for the hazard as a function of several explanatory variables after adjustment for the other explanatory variables in the model, suggesting no difference in wound healing after adjustment for other factors.

The Cox model has considerable flexibility and its estimates of the hazard ratios have smaller standard errors and hence narrower confidence limits. In this study it was used to analyze the time to occurrence of healing of lesions. The dependent variables were complete healing of lesions and the duration it took the lesions to heal completely.

The independent variables were category (size) of lesion, age marital status, occupation, nature of the edge of lesion, duration of illness prior to care seeking, presence or absence of paradoxical reaction, presence or absence of necrotic slough, use of traditional treatment, wound dressing with povidone iodne alone, topical antibiotics with or without removal of necrotic tissue (slough) and devitalized skin, pain and family history of Buruli ulcer.

Two cases were left censored in this study because their lesions failed to reduce in size for four consecutive weeks. Hazard rates were used to measure the likelihood of healing of lesions within 34 weeks and hazard ratios were used to assess how fast complete healing was achieved. The category of lesion was fitted into the Cox proportional hazard model as Category I, Category II and Category III. Periods during which it was likely for healing to occur were defined in weeks for each person. Each spell of complete healing and time between start of treatment and complete healing constituted an observation. All patients contributed one spell to the analysis. The time-varying independent variable was the size of the lesion.

### 3.10 Ethical Considerations

Approval for this study was sought from the Institutional Review Board of the Noguchi Memorial Institute for Medical Research and the Ethical Review Board of the Ghana Health Service. (See copies in Appendices D and E).

Informed consent was sought from subjects, parents or guardians of minors who agreed to take part in the study. The objectives and the procedures for the study were fully explained to them. (see copy Appendix B). Informed consent forms were administered to patients, parents or guardians on behalf of their wards. Those who agreed to participate gave their consent to participate in the study by signing or thumb printing. (See copy in Appendix C).

Participation in the study was voluntary and subjects could withdraw at any time they so wished. The privacy and confidentiality of subjects were kept at all times. Some participants benefited directly by way of transport remuneration so that they could report to the health facility at all scheduled times for review of treatment. All those who had surgeries whether study participants or not were supported by the study. The results of the study have helped in describing the outcomes of Buruli ulcer treatment in this country and in determining some factors that affect the healing of BU lesions.

Data was kept confidential and was used exclusively for the purpose of this study. This study was funded by the Principal Investigator, DAAD (Germany) and School of Public Health (University of Ghana, Legon).

# CHAPTER FOUR

## 4 RESULTS

### 4.1 Demographic Characteristics

A total of 213 cases of Buruli ulcer were screened and treated in a period of 2 years but 156 cases who met the inclusion criteria were enrolled into the study. 2 study participants left the study 2 weeks into the study because they moved out of their respective communities. Suspected cases of Buruli ulcer that reported at the project clinics for treatment came from all over the study sites and adjoining districts but most of them were from the southern part of the Suhum-Kraboa-Coaltar district and the middle and northern parts of Akwapem South districts. (See figure 4.1 below).

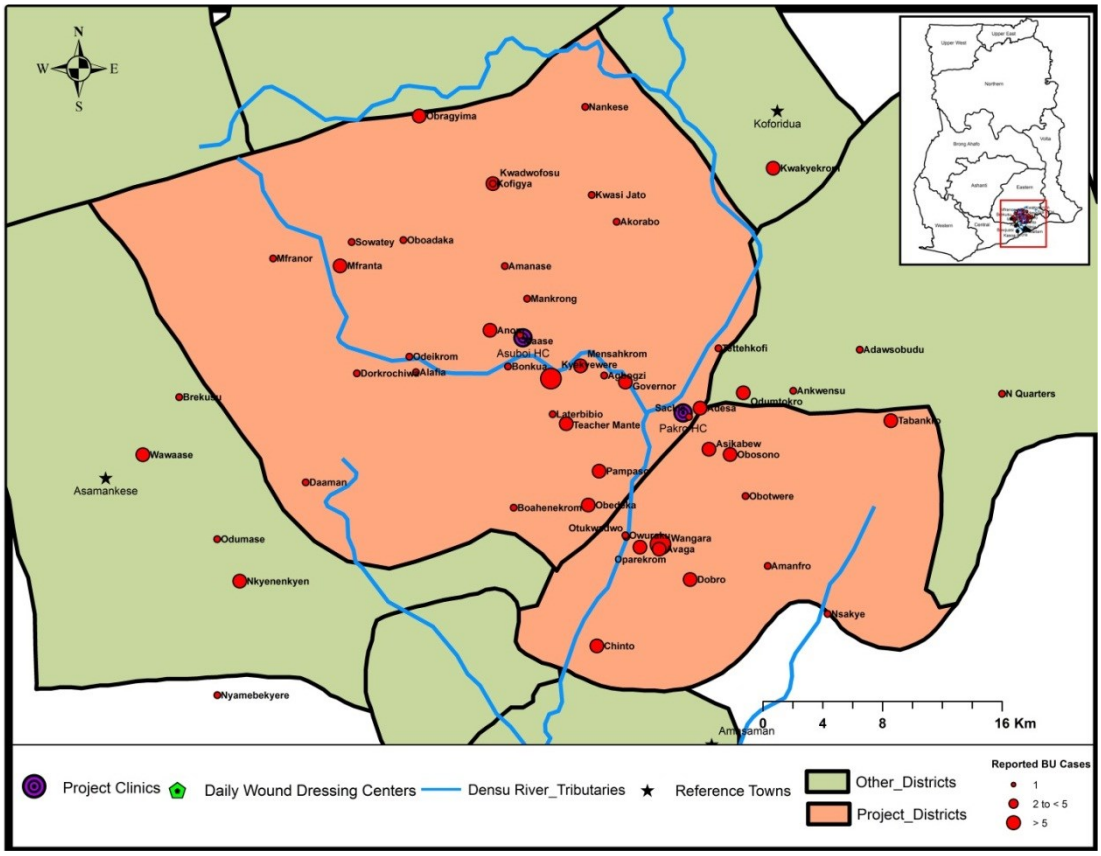
**Table 4:1 The demographic characteristics of study participants from Akwapem South and Suhum-Kraboa-Coaltar districts, June 2010-May 2012**

| Variable | Number (%) of Cases | | |
|---|---|---|---|
| | Male (%) | Female (%) | Total |
| Age (years) | | | |
| <15 | 28 (49.1) | 29 (50.9) | 57 |
| 16-49 | 27 (56.2) | 21 (43.8) | 48 |
| >50 | 16 (32.7) | 33 (67.3) | 49 |
| Total | 71 (46.1) | 83 (53.9) | 154 |

Table 4:1 shows the demographic characteristics of the study participants. There were more females than males with almost 54% of the enrolled participants being females. About 37% of the total study participants were less than 15 years and almost equal numbers in the age groups between 15 -49 years and over 50 years old. In those below

15 years of age there was an almost equal gender distribution, whereas there were slightly more males than females in those between 15-49 years. However, in those above 50 years females were twice as much as males. Apart from children (36.5%), students (15.4%) and the unemployed (5.2%) forming 57.1%, subsistent farmers formed the largest (29.2%) occupational group of the study participants. Akans (47.7%) and Ewes (37.2) dominated the ethnic groups in the area, together forming about 85%.

**Figure 4:1 Map of Project clinics and Reported Cases of Buruli ulcer in the Study area, June 2010-May 2012**




**Courtesy: William Opare, NBUCP**

### 4.1.1 Socioeconomic Characteristics

**Table 4:2 The Socioeconomic characteristics of study participants from Akwapem South and Suhum-Kraboa-Coaltar districts, June 2010-May 2012**

| Factor | Male % | Female % |
|---|---|---|
| **Education** | | |
| None | 19 (26.8) | 30 (36.1) |
| Primary & JHS | 44 (62.0) | 49 (59.0) |
| SHS & Tertiary | 8 (11.3) | 4 (4.8) |
| **Marital Status** | | |
| Single | 46 (64.8) | 35 (42.2) |
| Married | 24 (33.8) | 32 (38.5) |
| Divorced/Separated | 1 (1.4) | 16 (19.3) |
| **Occupation** | | |
| Farmer | 21 (29.6) | 24(28.9) |
| Traders & Artisans | 4 (5.6) | 17 (20.5) |
| Children/Students/Unem* | 46 (64.8) | 42 (50.6) |
| **Ethnicity** | | |
| Akan | 36 (50.7) | 34 (41.0) |
| Ewe | 23 (32.4) | 32 (38.5) |
| Ga/Adangme | 11 (15.5) | 15 (18.1) |
| Others | 1 (1.4) | 2 (2.4) |

**Unem*-Unemployed**

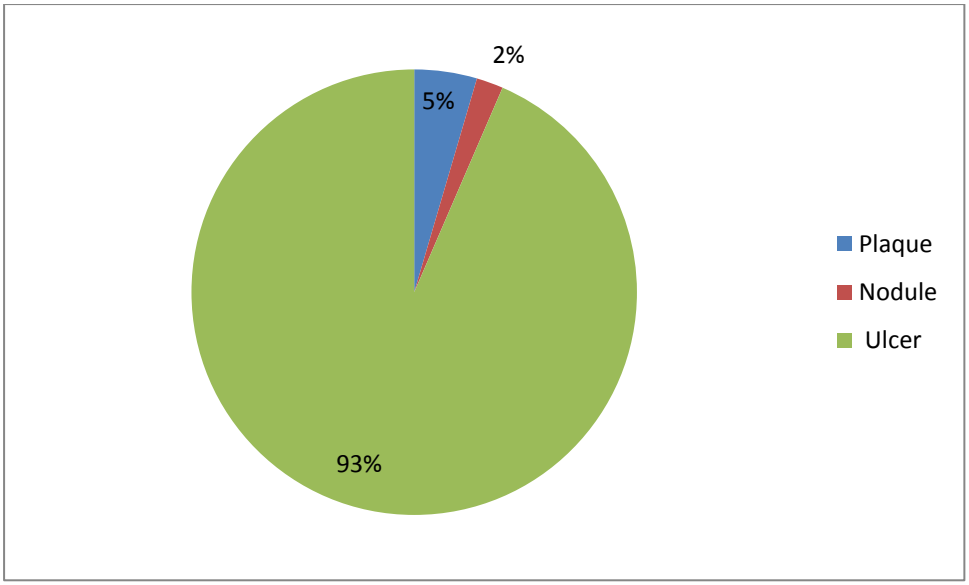



**Figure 4:2 Forms of lesions at presentation of study participants from Akwapem South (AKS) and Suhum-Kraboa-Coaltar (SKC) districts, June 2010-May 2012**

Of the 154 study participants enrolled, majority of them 144 (93.5%) presented with ulcers whilst only 6.5% presented with pre-ulcerative lesions (See figure 4:2). These lesions were classified into various categories based on their sizes and locations in on the body. (See table 4:4).

**Table 4:3 Distribution of lesions by Age group and category of lesions of study participants, AKS & SKC districts, June 2010-May 2012**

| | Age group (years) | | |
|---|---|---|---|
| **Characteristic** | **<15 years (n=57)** | **15-49 (n= 48)** | **≥50 years (n= 49)** |
| Category I | 23 (40.3) | 12 (25) | 11 (22.4) |
| Category II | 21 (36.8) | 17 (35.4) | 23 (46.9) |
| Category III | 13 (22.8) | 19(39.6) | 15 (30.6) |
| Single Lesions | 51 (89.5) | 37 (77.1) | 37 (75.5) |
| Multiple Lesions | 6 (10.5) | 11 (22.9) | 12 (24.5) |

Most (89.5%) of the children under 15 years of age had single lesions. Multiple lesions were more common in those who were 15 years and above. From table 4:5 above as much as 50 % (23/46) of Category I (small) lesions were found in children less than 15 years of age.

**Table 4:4 Distribution of location & size (diameter) of lesions in study participants from Akwapem South and Suhum-Kraboa-Coaltar districts, June 2010-May 2012**

| Characteristic | Number of Cases (%) | | |
|---|---|---|---|
| | Male (n=71) | Female (n=83) | Total (n=154) |
| Location of Lesion | | | |
| Upper Limbs | 13 (18.3) | 21(25.3) | 34 (22.1) |
| Lower Limbs | 53 (74.7) | 52 (62.7) | 105 (68.2) |
| Trunk | 1 (1.4) | 2 (2.4) | 3 (1.9) |
| Critical sites (BP, HN & breast) | 4 (5.6) | 8 (9.6) | 12 (7.8) |
| Category of lesion (d*) | | | |
| I (< 5 cm) | 19 (26.8) | 27 (32.5) | 46 (29.8) |
| II (5-15 cm) | 30 (42.2) | 31 (37.4) | 61 (39.6) |
| III (>15cm+CS+ML) | 22 (31.0) | 25 (30.1) | 47 (30.5) |

**CS – Critical Sites lesions; d* – longest diameter of initial lesion; BP - Buttocks & Perineum; HN - Head & Neck**
**ML – Multiple lesions**

Out of the 154 patients who took part in the study, 139 (90.2%) of them presented with lesions on the extremities. Though upper limb lesions in females were about twice as much as that of males, it was not significant, (OR=1.5; 95% CI = 0.65-3.60). There was an almost equal distribution of lesions on the lower limbs among males and

females, but among females, lower limb lesions were 2.5 times that of upper limb lesions. (See table 4:4). The distribution of lesions on the lower limbs is significantly associated with the age of patient. Patients older than 20 years of age had more lesions on the lower limbs than younger ones (OR=3.04; 95% CI = 1.42-6.55).

People with lesions at critical sites and those with multiple lesions formed 7.8% (12/154) and 18.8% (29/154) of the total number of study participants respectively. About 96% of Category I lesions, 100% of Category II lesions and 72.1% of Category III lesions were all confined to the extremities. 25.5% of all Category III lesions were found on critical sites (CS) and apart from these, 77.1% of the rest of category III lesions were also found on the lower limbs. Only 12.8% (6/47) of the category III lesions were single lesions with longest diameter greater than 15 cm.

## 4.2 Extent to which Rifampicin and Streptomycin combination affected Buruli ulcer lesions

The response to treatment of the various categories of lesions was assessed in terms of the proportion that healed within a period of eight months, the proportion that recurred and the proportion that deteriorated before they finally healed. The proportion that healed within the 8 weeks of antibiotic treatment, the time (duration) it took for the various categories to heal completely and the rate at which they reduced in size until complete healing was achieved were also assessed.

**Table 4:5 Treatment Outcomes of 154 Buruli ulcer patients treated in AKS and SKC districts, June 2010-May 2012**

| Treatment Outcomes | Number (%) |
|---|---|
| Completely Healed | 150/154 (97.4) |
| Category of Lesion | |
| I (n =46) | 46 (100) |
| II (n =61) | 58 (95.08) |
| III (n =47) | 46 (97.87) |
| Paradoxical Reactions | 43/154 (27.9) |
| Recurrence | 0/154 (0) |

Almost all (97.4%) of the 154 participants who received the recommended 8-weeks of rifampicin and streptomycin combination treatment achieved complete healing (secondary treatment success) without any recurrence within the 8 months of follow-up (see figure 4:3 and table 4:5). During the course of treatment almost a third (27.9%) of the study participants experienced immune reconstitution reactions (paradoxical reactions), that is, enlargement of lesion after an initial improvement. (See table 4:5, figures 4:9, 4:11a and 4:11b). All (100%) of category I lesions healed completely, 95.08% and 97.87% complete healing for category II and category III lesions respectively. About 28% of ulcers treated enlarged after initial improvement before they finally healed completely

At the end of the study, the lesions of only 4 (2.6%) patients failed to achieve secondary treatment success after having developed healthy granulation tissue (primary treatment success). (See figures 4.5 & 4.6). These patients were referred for

surgery and subsequent skin grafting. Two (2) out of the four had completely healed by the end of one year after starting treatment: one healed on topical antibiotics only and the other underwent a minor surgery and subsequent skin grafting. Treatment failure rate was therefore 2.6% (4/154) within 8 months of follow-up and 1.3% within one year follow-up.

The duration of healing and the rate of healing for the three categories of lesions were analyzed using a logistic regression model. There was significant difference in the duration of healing of BU lesions between the various categories (p-value <0.0001). The rate of healing was also significantly different between category I and category II (p-value <0.0001) and between category I and category III (p-value<0.0001) but not significantly different between category II and category III lesions, p-value > 0.05 (see figures 4:3, 4:8d and 4:8e). Using the Bonferroni multiple range tests, there was significant difference between category I and category II, p –value < 0.0001 category I and category III, p –value < 0.0001 but no significant difference between category II and category III lesions, p-value >0.05. There were only 4 (2.6%) patients who were HIV–positive and the clinical presentations and achievement of treatment success of their lesions were not particularly distinct from those who were HIV-negative.

**Table 4:6 Duration of Complete Healing for Categories of Lesions**

| Category of Lesion | Duration of Secondary Healing (weeks) | | |
|---|---|---|---|
| | Mean | Median | Range |
| Category I | 6.0 | 6 | 2-16 |
| Category II | 12.4 | 10 | 4-30 |
| Category III (single) | 16.4 | 18 | 8-28 |
| Category III (critical & multiple) | 11.7 | 10 | 4-32 |

**Table 4:7 Rate of Healing (Reduction in wound size (area)) by categories of lesions of study participants from Akwapem South and Suhum-Kraboa-Coaltar districts, June 2010-May 2012**

| | Reduction in Wound surface area (cm²) (95%CI) | | |
|---|---|---|---|
| Category of Lesion | First 4 weeks | Subsequent weeks | Total Period |
| I (< 5cm) | 1.35 (0.92 - 1.78) | 0.02(0.02–0.44) | 0.51(0.33–0.69) |
| II (5-15cm) | 5.6 (3.07 – 8.16) | 0.51 (0.01-1.03) | 1.84 (1.45-2.24) |
| III (>15cm) | 8.46 (0.22-17.1) | 1.34 (0.62-2.06) | 2.61(1.78–3.44) |

**Table 4:8 Distribution of the Proportion of Healed Lesions by Duration of lesion prior to Care Seeking of study participants from Akwapem South and Suhum-Kraboa-Coaltar districts, June 2010-May 2012**

| | Duration (months) Number (%) | | | | | |
|---|---|---|---|---|---|---|
| Characteristic | 1 (n=57) | 2 (n=28) | 3-6 (n=37) | 7-12 (n=14) | >12 (n=18) | Total (154) |
| Healed | 57 (100) | 28 (100) | 36 (97.3) | 14 (100) | 15 (83.3) | 150 (97.4) |
| ≤ 8 weeks | 35 (61.4) | 14 (50.0) | 11 (29.73) | 5 (35.7) | 7 (38.9) | 72 (46.75) |
| > 8 weeks | 22 (38.6) | 14 (50.0) | 26 (70.3) | 9 (64.3) | 11 (61.1) | 82 (53.25) |

Considering the duration of the lesion prior to seeking care at health facilities, most (80%) of the study participants reported within 6 months of noticing their lesions for the first time. (See table 4.8). Out of the 72 (46.7%) patients that achieved secondary treatment success within 8 weeks of treatment, 68% (49/72) of them reported within two months of noticing their lesions for the first time (see table 4:8). The likelihood of achieving secondary treatment success within eight weeks of treatment and wound care among those who reported within two months of noticing the lesions was higher

than those who reported later (RR= 1.55, 95% CI= 1.16-2.07). However, all of those who reported to the health facility within two months of noticing the lesion for the first time achieved 100% treatment success irrespective of the category of lesion. (see table 4:8)

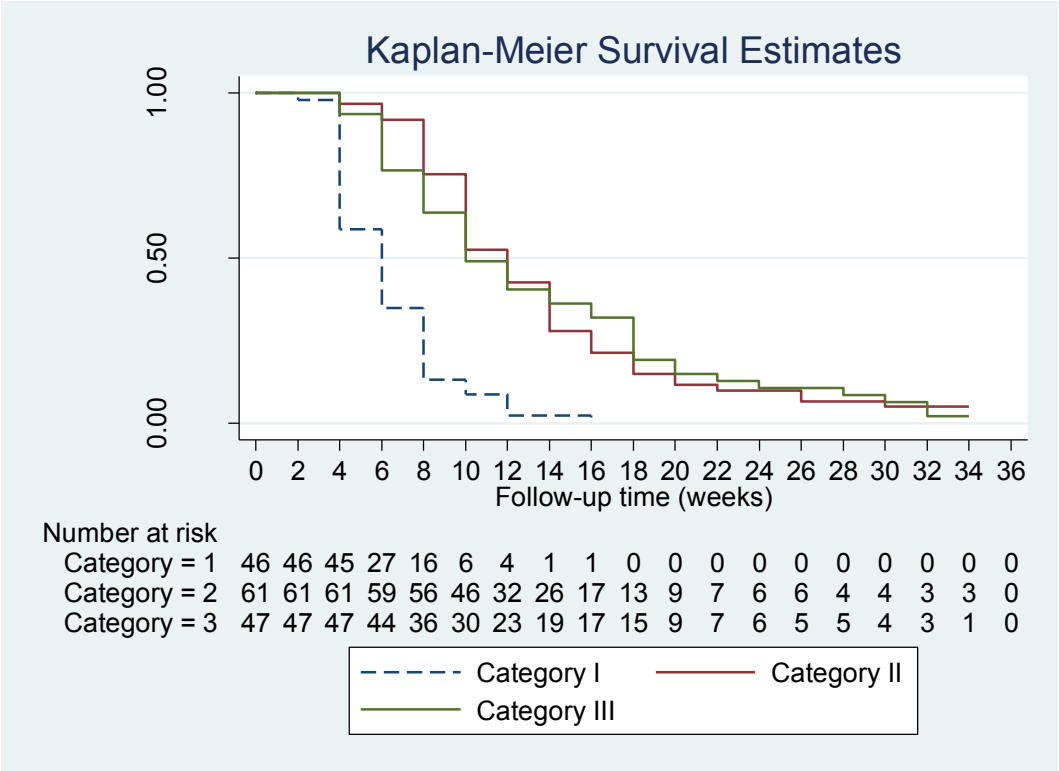



**Figure 4:3 Kaplan-Meier Healing Estimates for Response to Treatment showing Proportion of patients likely to achieve secondary treatment success by category of lesions of study participants from Akwapem South and Suhum-Kraboa-Coaltar districts, June 2010-May 2012**

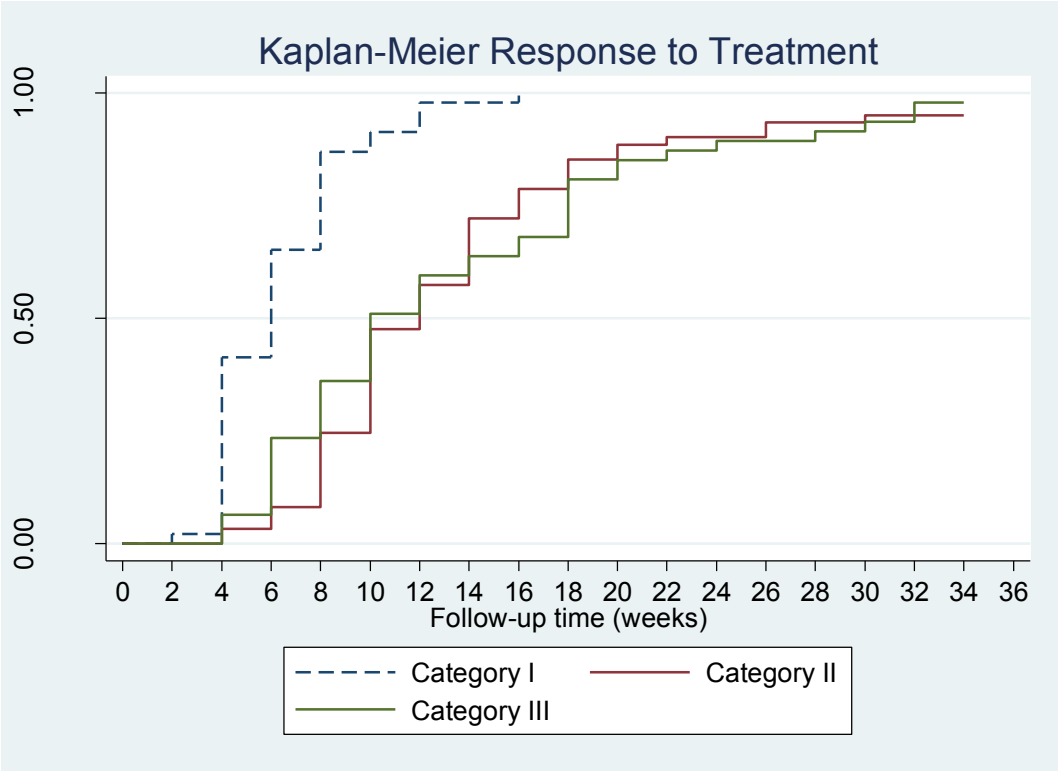



**Figure 4:4 Kaplan-Meier Healing Estimates for Response to Treatment showing Proportion of patients achieving secondary treatment success by category of lesions of study participants from Akwapem South and Suhum-Kraboa-Coaltar districts, June 2010-May 2012**

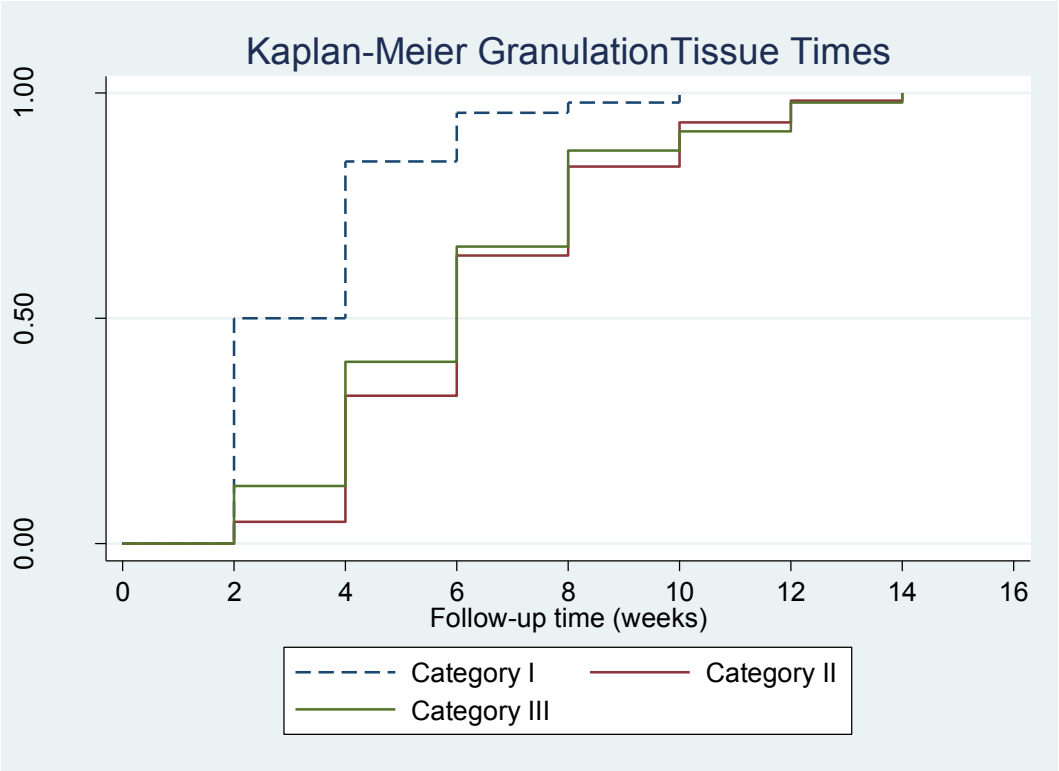



**Figure 4:5 Kaplan-Meier Healing Estimates for Response to Treatment showing Proportion of patients developing healthy granulation tissue by category of lesions of study participants from Akwapem South and Suhum-Kraboa-Coaltar districts, June 2010-May 2012**

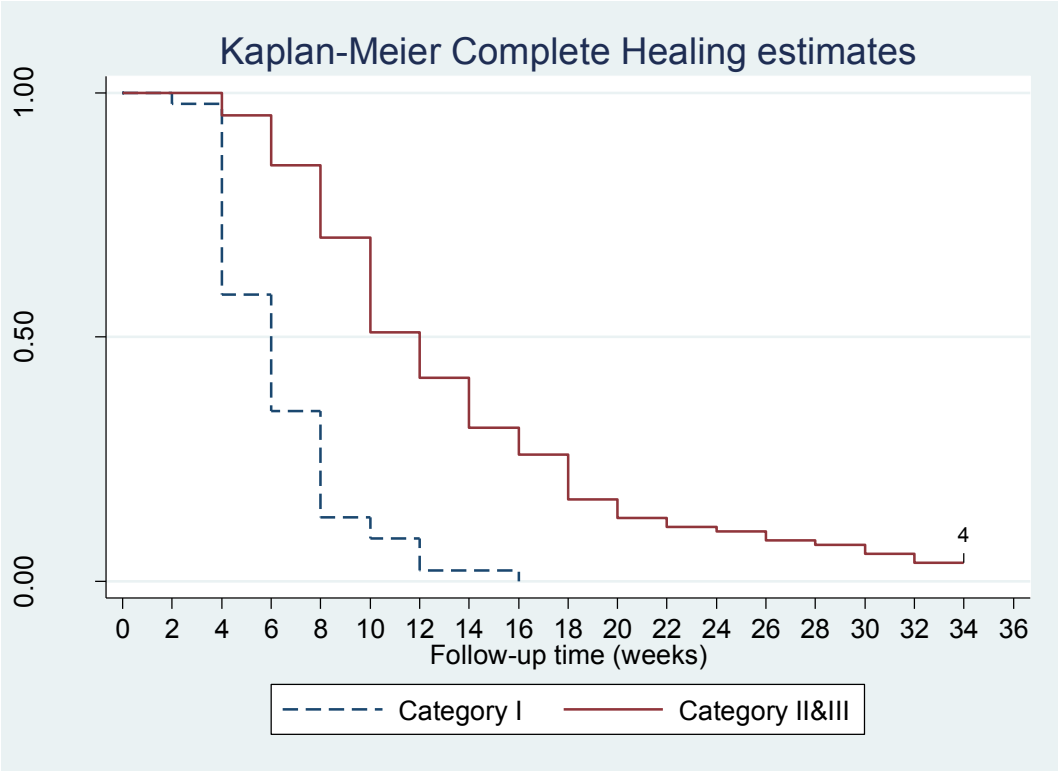



**Figure 4:6 Kaplan-Meier Healing Estimates for Response to Treatment showing proportion of patients achieving complete Healing and those censored by category of lesions of study participants from Akwapem South and Suhum-Kraboa-Coaltar districts, June 2010-May 2012**

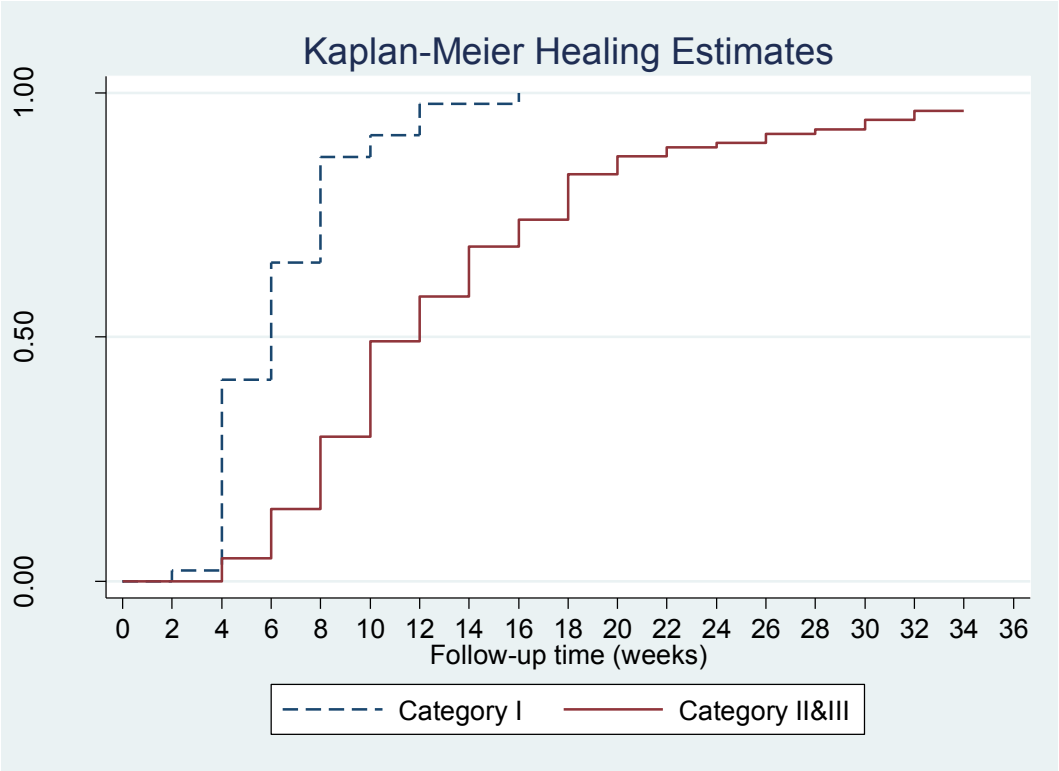



**Figure 4:7 Kaplan-Meier Healing Estimates for Response to Treatment showing proportion of patients achieving secondary treatment success by category of lesions of study participants from Akwapem South and Suhum-Kraboa-Coaltar districts, June 2010-May 2012**

## Category I Lesions

All of category I lesions achieved secondary treatment success by the end of 16 weeks. The median duration of secondary treatment success for category I lesions was 6 weeks (2-16 weeks) while that of their primary treatment success was 3 weeks (2-10 weeks). Eighty-seven percent (40/46) of them achieved secondary treatment success by the end of the 8-week antibiotic treatment with a rate of reduction in wound size (area) of 0.51 (0.33-0.69) $cm^2$ per week. Category I lesions healed at the fastest rate with almost 50% of them healing completely by the end of 6 weeks regardless of the occurrence of paradoxical reactions. (See Kaplan-Meier curves and figures 4:8d and 4:8e). The mean percentage reduction in surface area was 37% at week 2, 65% at week 4 and 70% at week 8 of treatment. However, the rate of wound reduction was

faster during the first 4 weeks of treatment than the subsequent weeks (p-value < 0.05). (See table 4:7).

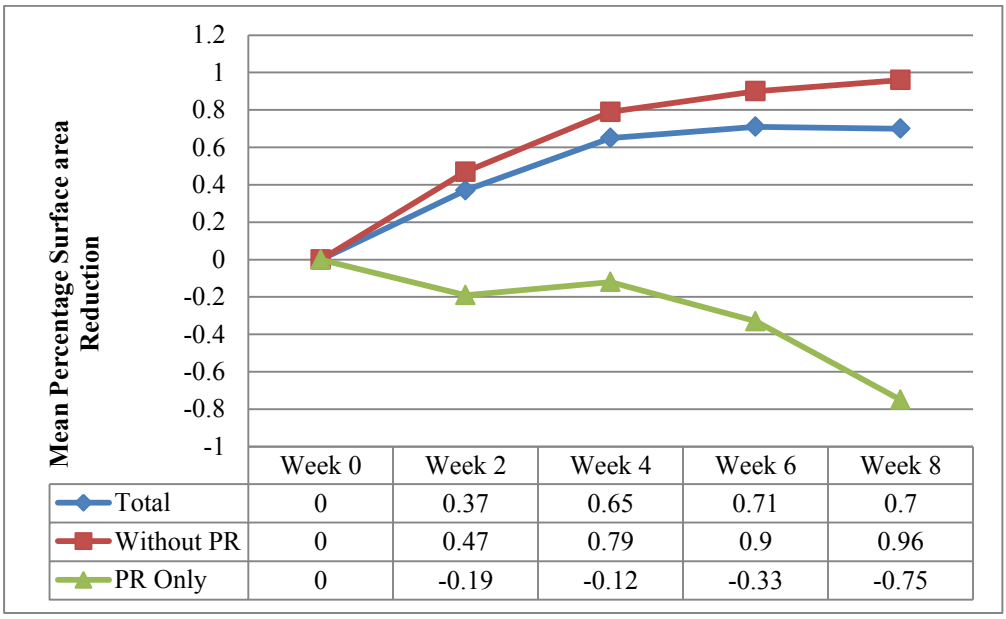
**Category II Lesions**

For category II lesions, 83.6% (51/61) of them developed healthy granulation tissue by the end of the 8-week antibiotic treatment. However, only 24.6 % (15/61) of them achieved secondary treatment success during the same period. The minimum period of time for category II lesions to achieve secondary treatment success was 4 weeks, with a maximum of 30 weeks. The median duration of healing was 10 weeks. The mean reduction in surface area of category II lesions was 1.84 cm² per week (p-value <0.001) over the total period of 34 weeks, though it was fastest during the initial four weeks. (See table 4:7). The mean percentage reduction in surface area was 23% at week 2, 48% at week 4 and 74% at week 8 of treatment for all category II lesions regardless of paradoxical reactions. (See figure 4:8b).

**Category III Lesions**

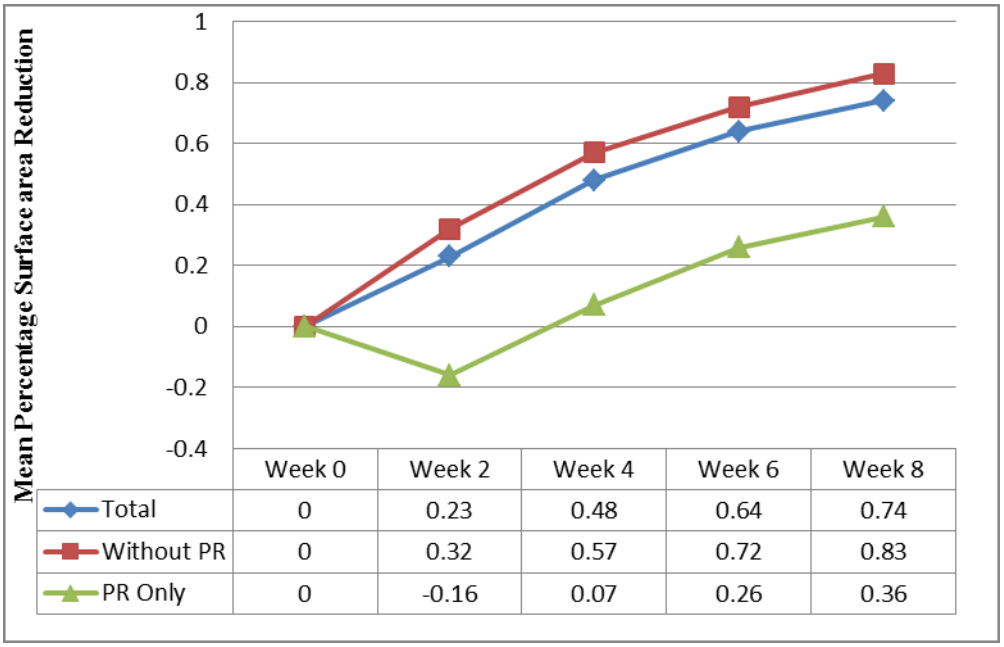
For category III lesions, 87.2% (41/47) of them developed healthy granulation tissue by the end of the 8-week antibiotic treatment and 36.2 % (17/47) of them achieved secondary treatment success by the end of the same period with a mean reduction of 2.6 $cm^2$ per week (p-value<0.001). The median duration of healing for all them was 10 weeks but when they were separated out into single lesions, critical and multiple lesions, the median duration of secondary healing for Category III single, critical and multiple lesions became 18 weeks (8-28 weeks), 7 weeks (4-22) weeks and 10 weeks (4-32) weeks respectively. The mean percentage reduction in surface area was 26% at 2 weeks, 47% at 4 weeks and 63% at week 8 of antibiotic treatment.

For category II and category III lesions bigger than 5cm in diameter (Category III large lesions) their responses to treatment were similar. (See figure 4:10). The response to treatment of category I and category III lesions smaller than 5cm in diameter (small lesions) were also similar (see figure 4:10). The mean percentage reduction in surface area was greater in category I lesions but there was no observable differences between category II and category III lesions (see figures 4:8d and 4:8e).




| | Week 0 | Week 2 | Week 4 | Week 6 | Week 8 |
|---|---|---|---|---|---|
| Total | 0 | 0.37 | 0.65 | 0.71 | 0.7 |
| Without PR | 0 | 0.47 | 0.79 | 0.9 | 0.96 |
| PR Only | 0 | -0.19 | -0.12 | -0.33 | -0.75 |

**Figure 4:8a Mean Percentage Reduction of Surface Area of Category I Lesions of study participants from AKS and SKC districts, June 2010-May 2012**




| | Week 0 | Week 2 | Week 4 | Week 6 | Week 8 |
|---|---|---|---|---|---|
| Total | 0 | 0.23 | 0.48 | 0.64 | 0.74 |
| Without PR | 0 | 0.32 | 0.57 | 0.72 | 0.83 |
| PR Only | 0 | -0.16 | 0.07 | 0.26 | 0.36 |

**Figure 4:8b Mean Percentage Reduction of Surface Area of Category II Lesions of study participants from AKS and SKC districts, June 2010-May 2012**

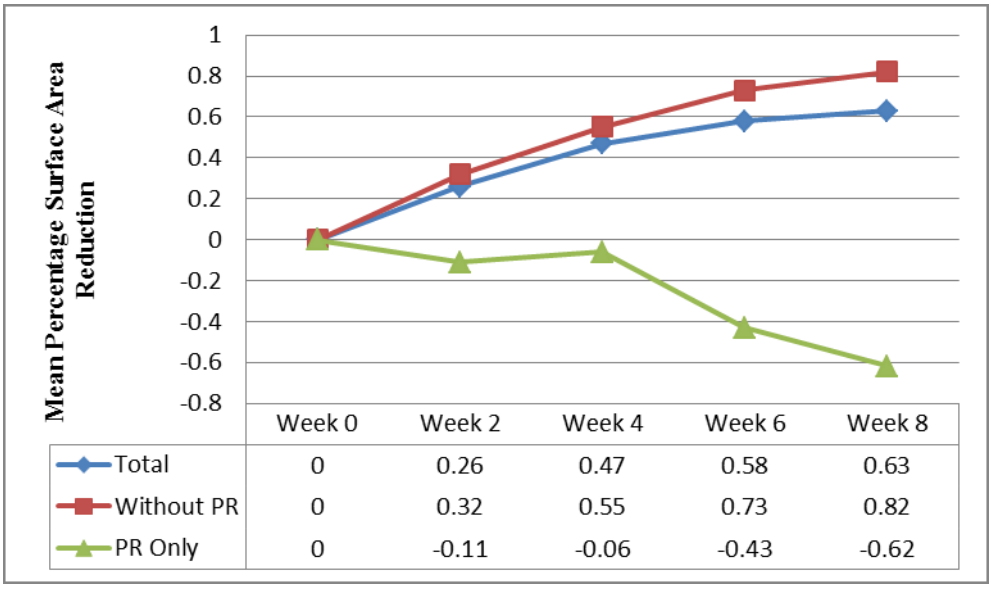



**Figure 4.8c: Mean Percentage Reduction of Surface Area of Category III Lesions of study participants from AKS and SKC districts, June 2010-May 2012**

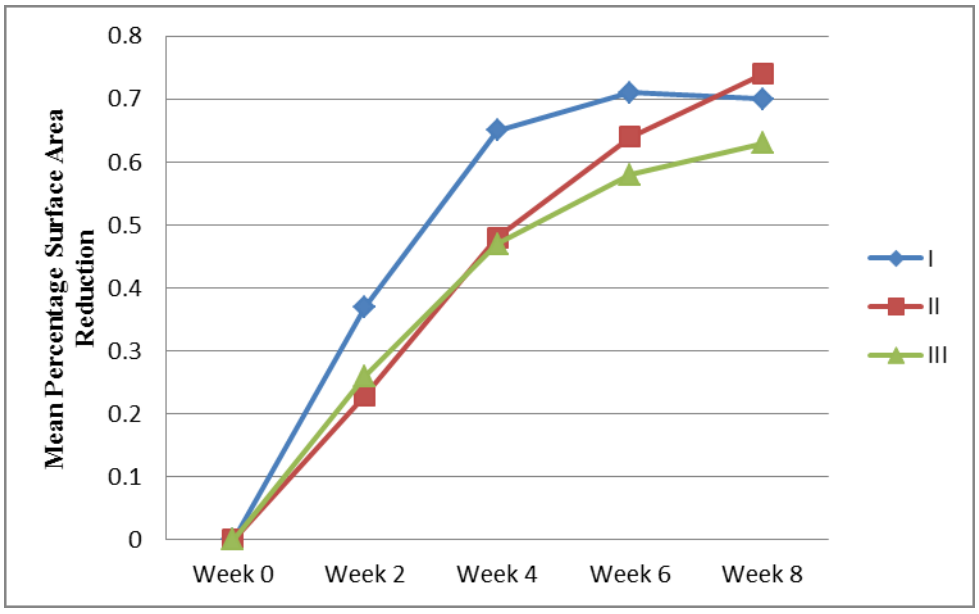



**Figure 4:8d Overall Mean Percentage Reduction of Surface Area of all categories of lesions of study participants from AKS and SKC districts, June 2010-May 2012**

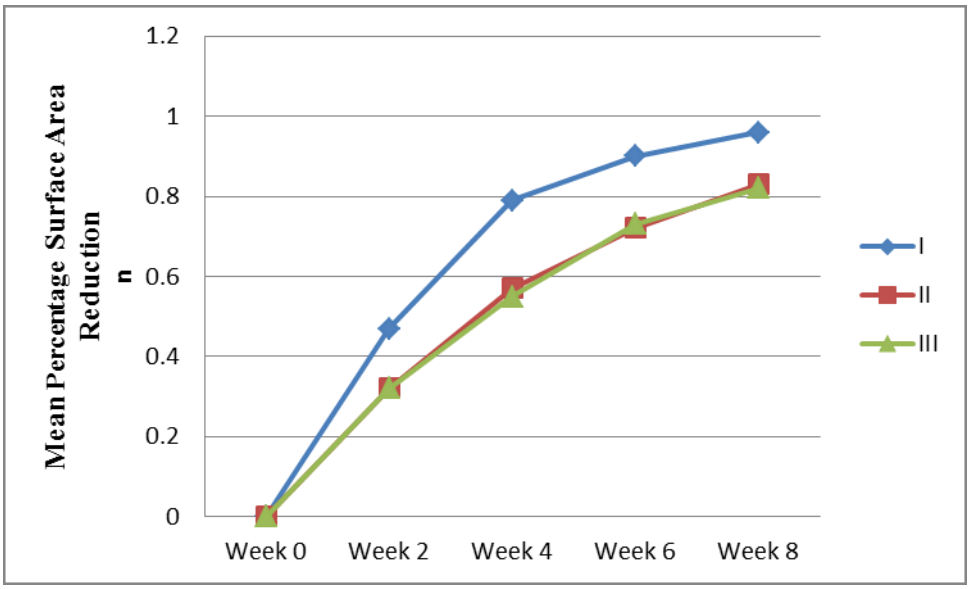



**Figure 4:8e Mean Percentage Reduction of all Categories of lesions with no Paradoxical reactions of study participants from AKS and SKC districts, June 2010-May 2012**

### 4.3 Characteristics and Treatment Outcomes of Buruli ulcer lesions

Majority (84.4%) of the study participants reported with pain of varying degrees. Out of the 144 ulcers that were seen, most (81.9%) of them had undermined edges with 61.8% of them having the ulcer floor covered with necrotic slough. Most of category II (77.2%) and category III (79.5%) ulcers were visibly infected. (See table 4:9). About 78.6% of all lesions were warmer than the surrounding normal skin (See table 4:9). A significant proportion of Category II and Category III lesions were healed on topical antibiotics with or without debridement of necrotic tissues. (see table 4:10)

**Table 4:9 Features of Buruli ulcers of study participants from Akwapem South and Suhum-Kraboa-Coaltar districts, June 2010-May 2012**

| Characteristic | Yes/No |
|---|---|
| Pain | 130/24 |
| Undermined | 118/26 |
| Warm to touch | 121/33 |
| Ulcers covered with necrotic slough | 89/55 |
| Category I | 10/35 |
| Category II | 44/13 |
| Category III | 35/9 |

**Table 4:10 Treatment Outcomes of Category of Lesion by Treatment Options of study participants from Akwapem South and Suhum-Kraboa-Coaltar districts, June 2010-May 2012**

| | Number of healed patients (%) | | |
|---|---|---|---|
| **Treatment Options** | **Category I** | **Category II** | **Category III** |
| Povidone Iodine only | 37 (80.4) | 17 (27.9) | 13 (27.7) |
| Topical Antibiotics | 8 (17.4) | 26 (42.6) | 22 (46.8) |
| Topical Antibiotics + Debridement | 0 (0) | 11(18.0) | 8 (17.0) |
| Recommended Surgery | 0 (0) | 3 (4.9) | 1 (2.1) |
| Total | 46 (100) | 61(100) | 47 (100) |

All wounds were cleaned with saline

**Paradoxical Reactions**

Concerning paradoxical reactions (transient aggravation of lesions) during treatment, that is lesions increasing in surface area after an initial improvement, it was observed that less than 50% of patients experienced this. Of the 144 out of the 154 participants that initially presented with ulcers, 43 (29.8%) experienced paradoxical reactions (PRs). Their respective surface areas increased compared to the previous assessment. Out of the 10 participants who initially presented with pre-ulcerative lesions two (2) of them erupted into ulcers after two weeks of treatment. These ulcers however were smaller than the pre-ulcerative lesions. After two weeks of treatment, about 13% (20/154) of lesions showed an increase in surface area compared to the first assessment. The percentage of those who experienced paradoxical reactions was highest (22.1%) at week 10 and this reduced steadily to week 18 after which there were no more PRs. (see figure 4.9). The occurrence of PRs was more pronounced in category I and category III lesions less than 5cm in diameter. (See figures 4:10 4:11a and 4:11b). The overall percentage reduction in surface area was less for lesions that showed PRs for all the categories of lesions. (See figures 4:8a, 4:8b and 4:8c).

Though there was a negative correlation between the duration of healing of lesions and paradoxical reactions ($r = 0.34$, p-value $< 0.0001$), only one (2.3%) out of the 43 ulcers that experienced PRs failed to heal. Overall, healing was steady but slow for those lesions that showed PRs.

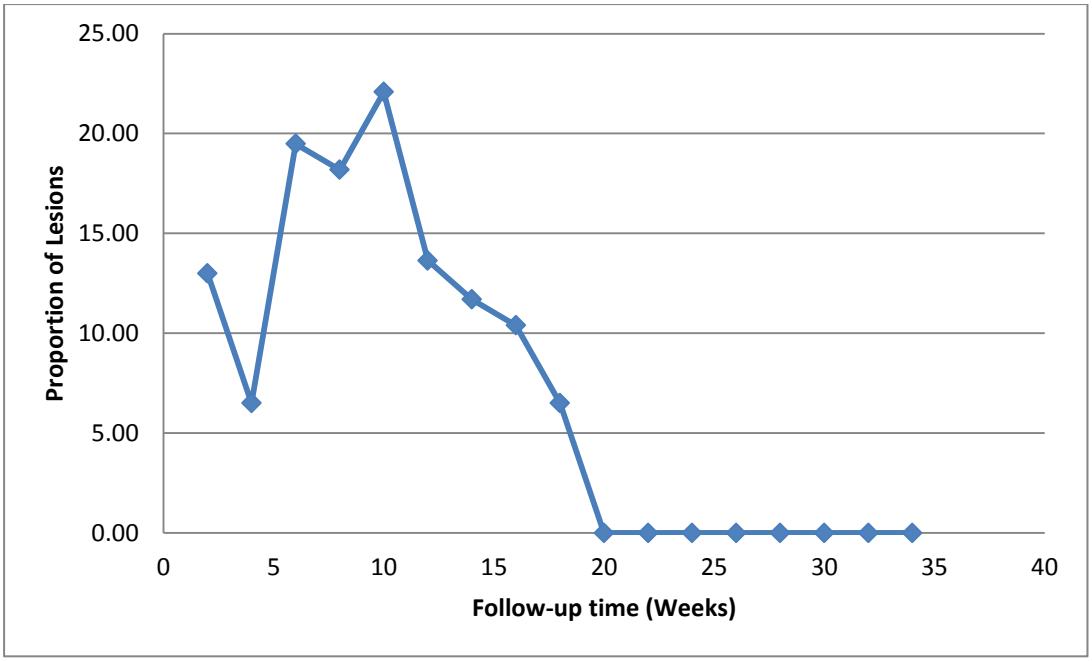



**Figure 4:9 Time points with Paradoxical reactions of lesions of study participants from Akwapem South and Suhum-Kraboa-Coaltar districts, June 2010-May 2012**

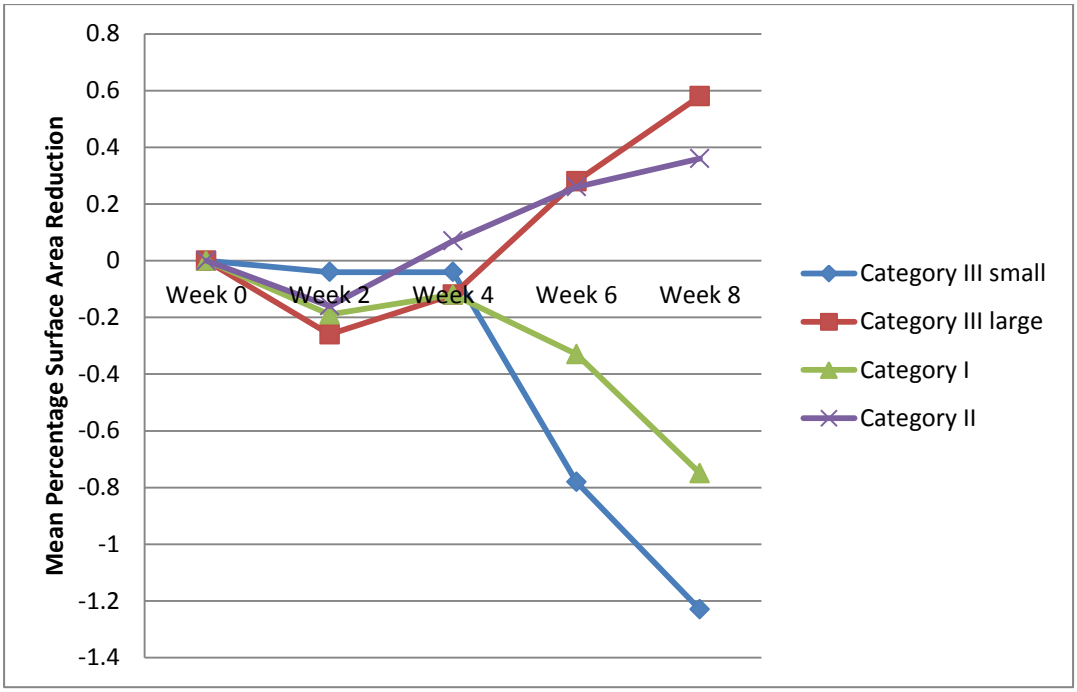



**Figure 4:10 Time points with Paradoxical reactions of all categories of lesions of study participants from Akwapem South and Suhum-Kraboa-Coaltar districts, June 2010-May 2012**

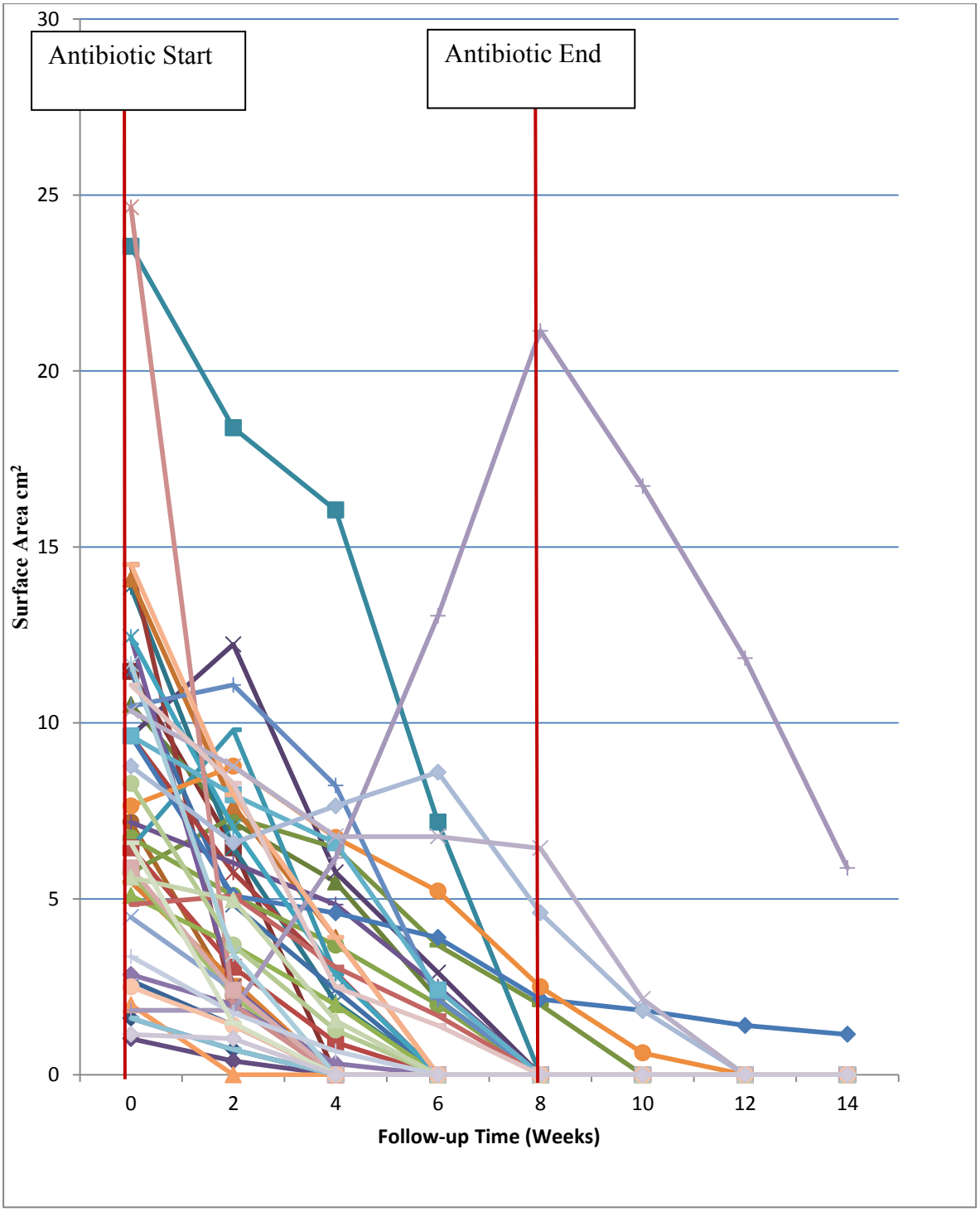



**Figure 4:11a Surface area measurements of category I Buruli ulcer lesions plotted for every individual during and after 8 weeks of antibiotic treatment till complete healing was achieved (n=46, lesions <5cm) of study participants from AKS and SKC districts, June 22010-May 2012**

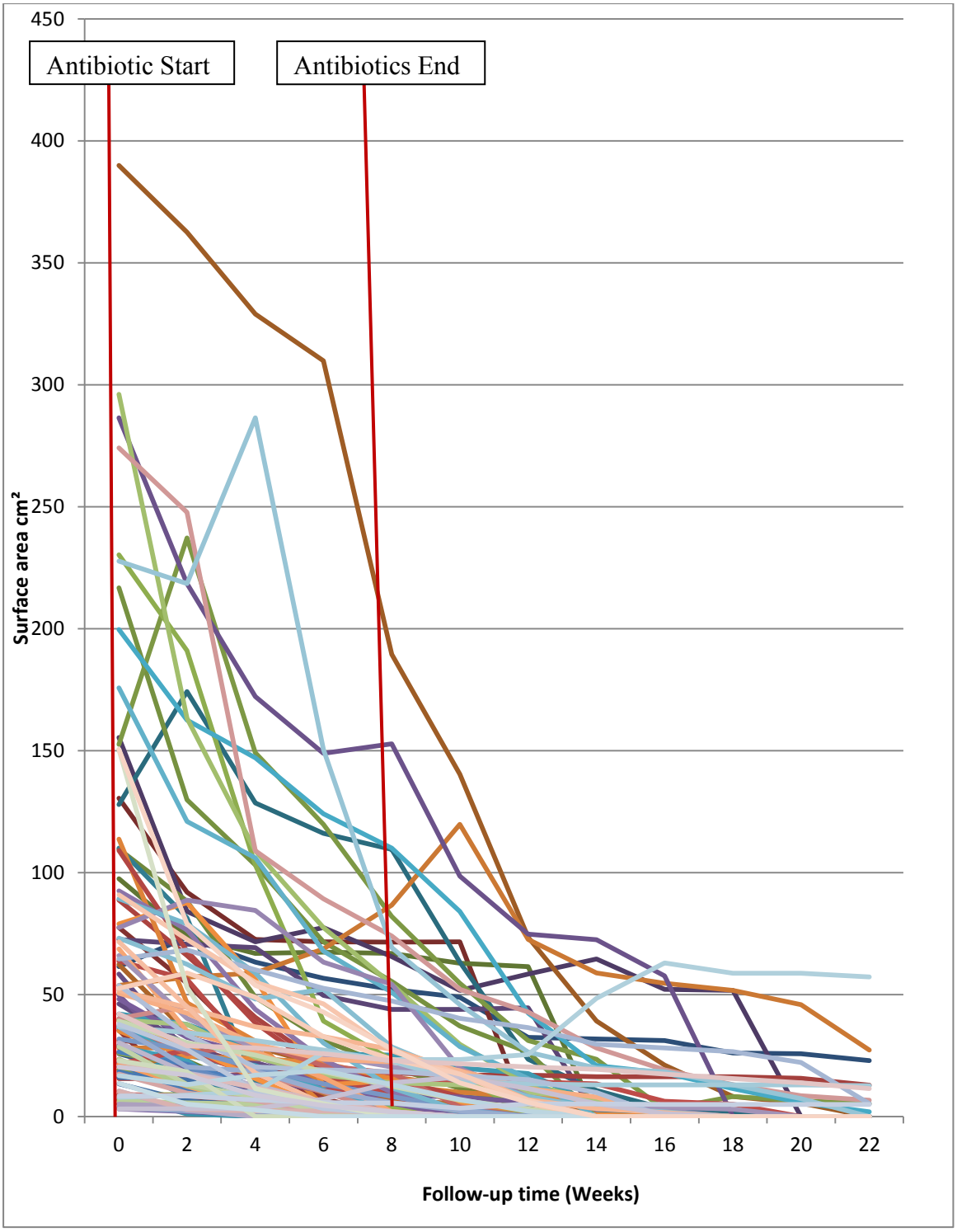



**Figure 4:11b Surface area measurements of category II and category III Buruli ulcer lesions plotted for every individual during and after 8 weeks of antibiotic treatment till complete healing (n=128, Category II lesions 5-15cm and category III are lesions >15cm, multiple lesions and lesions at critical sites) of study participants from AKS and SKC districts, June 2010-May 2012**

## 4.4 Factors that influence the outcomes of antibiotic treatment of Buruli ulcer lesions

**Table 4:11 Relative Hazard (Unadjusted) of selected variables for Healing among Buruli Ulcer Patients according to Categories of Lesions, 150 patients from AKS and SKC districts, June 2010-May 2012**

| Characteristic | Hazard Ratio | Standard Error | (95% CI) | P-value |
|---|---|---|---|---|
| **Demographic Factors** | | | | |
| Age Group | | | | |
| > 50 years | 1 | | ref. | |
| <15 years | 1.3 | 0.27 | 0.90 - 1.97 | 0.15 |
| 15-49 years | 1.0 | 0.22 | 0.69 - 1.57 | 0.84 |
| | | | | |
| Male | 1 | | ref | |
| Female | 1.3 | 0.21 | 0.94 - 1.80 | 0.112 |
| | | | | |
| Marital status | | | | |
| Married | 1 | | ref. | |
| Single | 1.5 | 0.27 | 1.05 - 2.15 | 0.025* |
| Divorced/Separated | 1.4 | 0.42 | 0.82 - 2.54 | 0.202 |
| | | | | |
| Occupation | | | | |
| Farmer | 1 | | ref. | |
| Trader | 2.1 | 0.66 | 1.10 - 3.88 | 0.024* |
| Artisan | 1.0 | 0.39 | 0.48 - 2.17 | 0.96 |
| Others | 1.4 | 0.28 | 0.99 - 2.10 | 0.06 |
| | | | | |
| Educational Level | | | | |
| Tertiary | 1 | | | |
| No education | 1.7 | 0.98 | 0.51 - 5.32 | 0.40 |
| Primary/JHS education | 1.8 | 1.04 | 0.58 - 5.99 | 0.35 |
| SHS | 1.5 | 1.02 | 0.39 - 5.68 | 0.55 |
| | | | | |
| No Family History | 1 | | ref. | |
| Family History | 1.5 | 0.34 | 0.96 - 2.32 | 0.073 |
| | | | | |
| Over 1year | 1 | | Ref | |
| 1 month | 2.9 | 0.89 | 1.59 - 5.31 | 0.001* |
| 2 months | 2.6 | 0.86 | 1.33 - 4.93 | 0.005* |
| 3-6 months | 1.7 | 0.53 | 0.92 - 3.14 | 0.09 |
| 7-12 months | 1.7 | 0.66 | 0.82 - 3.64 | 0.15 |

Cont.

| Characteristic | Hazard Ratio | Standard Error | (95% CI) | P-value |
|---|---|---|---|---|
| **Lesion Factors** | | | | |
| Over 1year | 1 | | Ref | |
| 1 month | 2.9 | 0.89 | 1.59 - 5.31 | 0.001* |
| 2 months | 2.6 | 0.86 | 1.33 - 4.93 | 0.005* |
| 3-6 months | 1.7 | 0.53 | 0.92 - 3.14 | 0.09 |
| 7-12 months | 1.7 | 0.66 | 0,82-3.64 | 0.15 |
| | | | | |
| Category II&III | 1 | | Ref | |
| Category I | 3.8 | 0.77 | 2.6 - 5.7 | 0.000* |
| | | | | |
| Duration of Trad. Treat. | | | | |
| Over 1 month | 1 | | ref. | |
| 1 Week | 2.9 | 0.99 | 1.47 - 5.66 | 0.002* |
| 2 Weeks | 2.1 | 0.60 | 1.17 - 3.64 | 0.012* |
| 3 Weeks | 1.3 | 0.53 | 0.59 - 2.91 | 0.509 |
| 4 Weeks | 1.5 | 0.49 | 0.83 - 2.86 | 0.173 |
| | | | | |
| Pain | 1 | | ref. | |
| No Pain | 1.7 | 0.40 | 1.12 - 2.73 | 0.014* |
| | | | | |
| **Health System Factors** | | | | |
| Povidone iodine (PVI) only | 1 | | | |
| PVI+Topical Antibiotics | 1.2 | 0.23 | 0.87 -1.79 | 0.20† |
| PVI+TAB & Debridement | 1.5 | 0.41 | 0.90 - 2.58 | 0.12† |
| | | | | |
| Necrotic Tissue | 1 | | Ref | |
| No necrotic Tissue | 1.6 | 0.28 | 1.14 - 2.25 | 0.007* |
| | | | | |
| Paradoxical reaction | 1 | | Ref | |
| No Paradoxical react | 2.3 | 0.71 | 1.28 - 4.23 | 0.006* |
| | | | | |
| Lesion not undermined | 1 | | ref. | |
| Undermined lesion | 1.7 | 0.36 | 1.09 - 2.54 | 0.018* |

***significant association between variable and complete healing of Buruli ulcer lesion.**
**†though no significant association, believed to have an effect on healing.**
**TAB- Topical Antibiotics**
**Trad. Treat.- Traditional treatment**

**Demographic factors**

The association between age and healing of BU lesions was assessed and found that the complete healing of BU lesions was not statistically significant. (HR =1.5, 95% CI=0.90-1.97). BU lesions seemed to heal faster in females than males (HR=1.3, 95% CI= 0.69 - 1.57).

Considering the marital status of study patients, the lesions of those who had not married before (singles) healed faster than those who were married (HR=1.5, 95% CI 1.05-2.15).

Traders had a higher rate of achieving complete healing compared to the other occupations (HR=2.1, 95% CI=1.1- 3.88). The level of education did not seem to affect the achievement of complete healing. The healing rate was least in those with tertiary education, although not statistically significant.

The rate of achieving complete healing reduced as the time spent at home prior to seeking care increased and significantly so in the first two months of seeing the lesion, p-value $\leq 0.005$. Those who sought medical treatment within 2 months of noticing the lesion were the ones who responded well to treatment. However those who sought treatment within a month of noticing the lesion healed fastest (HR = 2.9, 95% CI= 1.59- 5.31) followed by those who reported after one month but before the end of the second month (HR=2.6, 95% CI=1.33-4.93). They were about three times likely to achieve complete healing than those who reported a year after having the lesion.

Taking into account education alone, a J-shaped relationship between healing and education was observed. The lowest healing rate was observed in those at the tertiary level of education. Trend analysis showed decreasing healing rate as the level of education increased though this was not statistically significant, p-value $\geq 0.30$.

**Lesion Factors**

Trend analysis of duration of treatment of lesions with traditional medication showed increasing healing rate with shorter duration of use of traditional medication. Healing was better when traditional treatment was used for only two weeks compared to its use over one month. They had the most effect when used for only one week (HR= 2.9, 95% CI= 1.47- 5.66): two weeks (HR=2.1, 95% CI= 1.17- 3.64).

Pain reduced the rate of achievement of complete healing. The absence of pain in lesions significantly improved healing to almost as twice as when there was pain (HR= 1.7, 95% CI= 1.12 – 2.73).

Ulcers without necrotic slough healed faster than those, which were covered with necrotic slough at presentation (HR=1.6, 95% CI=1.14-2.25).

Taking into consideration the edges of lesions, ulcers that were undermined healed faster than those that did not have undermined edges at presentation, (HR=1.7, 95% CI=1.01-2.54). Lesions that did not show immune reconstitution reactions (paradoxical reactions) during the course of treatment healed faster than those, which showed them.

**Health System Factors**

Wound care was given using topical antibiotics and debridement of necrotic slough. The effect of topical antibiotics alone (HR=1.2, 95% CI= 0.89-1.79) or a combination of topical antibiotics and debridement of necrotic slough (HR=1.5, 95% CI= 0.90-2.58) from lesions seemed to increase healing rate slightly more than povidone iodine alone in the univariate analysis. However, they were not statistically significant, p-value = 0.12. However the Odds of wounds healing among wounds in patients who

had wound infection and used topical antibiotics was 15 times that of those who used povidone iodine only (OR =15.2, 95% CI = 5.59-42.57).

**Multivariate (Adjusted) Analysis**

Variables which were found to be significant in the univariate analysis or were known to be associated with healing time based on previous studies were included in the multivariate analysis and the backward elimination procedure was used.

**Table 4:12 Relative Hazard (Adjusted) of selected variables for Healing among Buruli Ulcer Patients according to Categories of Lesions, 150 patients from AKS and SKC districts, June 2010-May 2012.**

| Characteristic | Hazard Ratio | Standard Error | (95% CI) | P-value |
|---|---|---|---|---|
| Category II & III | 1 | | ref. | |
| Category I | 5.9 | 1.52 | 3.59 -9.81 | 0.000 |
| Paradoxical reaction | 1 | | ref. | |
| No Paradoxical reaction | 1.9 | 0.61 | 1.05 - 3.60 | 0.034 |
| Povidone iodine only | 1 | | ref. | |
| PVI+Topical antibiotics | 3.1 | 0.80 | 1.83 - 5.10 | 0.000 |
| PVI+TAB + Debridement | 5.1 | 1.82 | 2.50 - 10.23 | 0.000 |
| Necrotic Tissue | 1 | | ref. | |
| No Necrotic Tissue | 1.9 | 0.5 | 1.13 - 3.16 | 0.016 |

Smaller lesions (HR=5.9, 95% CI = 3.59 -9.81), topical antibiotics alone (HR=3.1, 95% CI = 1.83 - 5.10), removal of slough plus topical antibiotics (HR = 5.1, 95% CI = 2.50 - 10.23), absence of slough (HR = 1.9, 95% CI = 1.13 - 3.16) and absence of paradoxical reactions (HR = 1.9, 95% CI = 1.05 - 3.60) all hastened wound healing in the multivariate model.

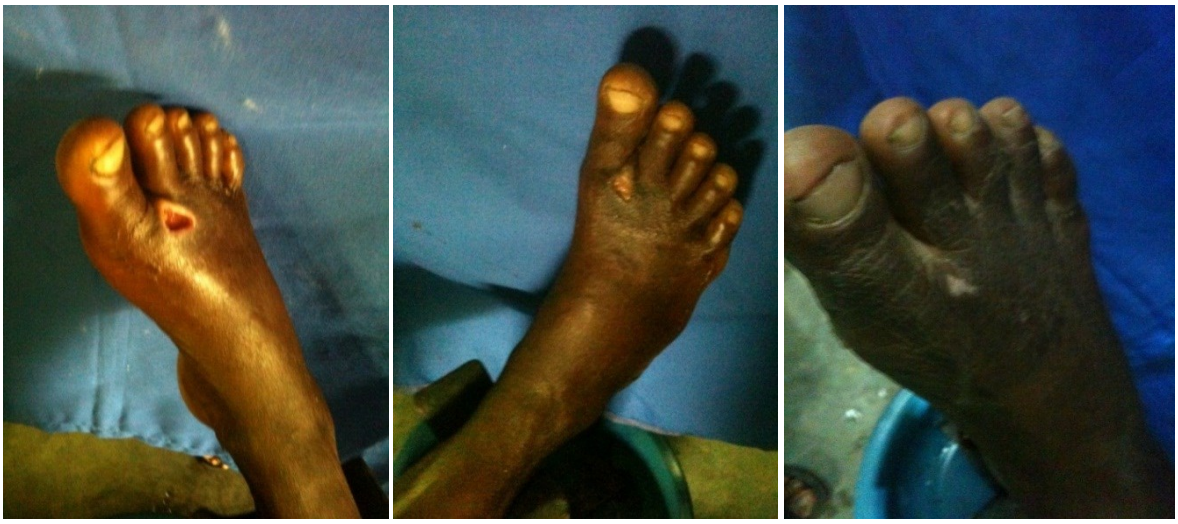
### 4.5 Surgical Interventions

Four patients did not experience secondary healing during the 8 months period. They were recommended for surgery and skin grafting. Only one of them could be admitted for surgery and skin grafting. The rest could not have the surgery and skin grafting done for lack of resources. One of them achieved secondary healing with the application of topical antibiotics alone within one year.

### 4.6 Adverse Events

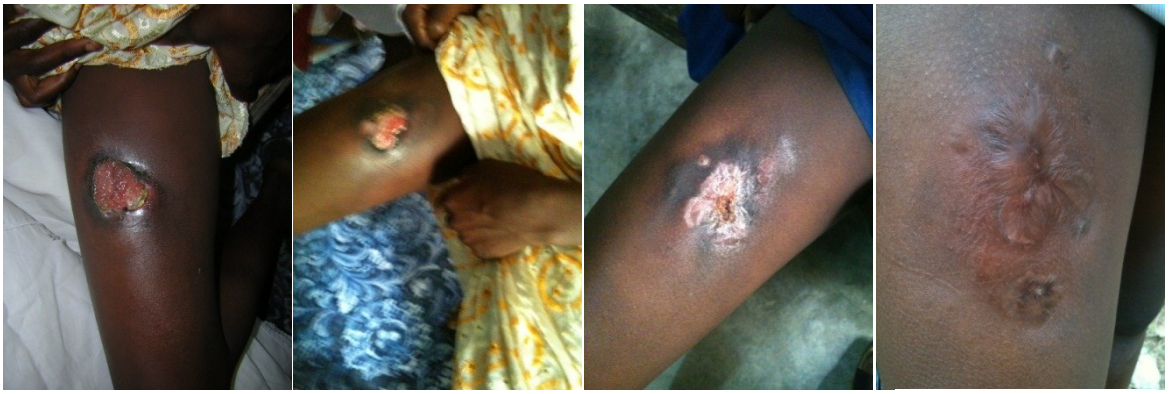
The combination of streptomycin and rifampicin treatment was well tolerated and acceptable to patients. Only one elderly woman experienced dizziness and this was attributed to streptomycin but it happened in the last week of treatment and this was rather mild so she was counseled and made to finish the treatment as scheduled.

**Stages of Healing of different categories of Buruli ulcers**



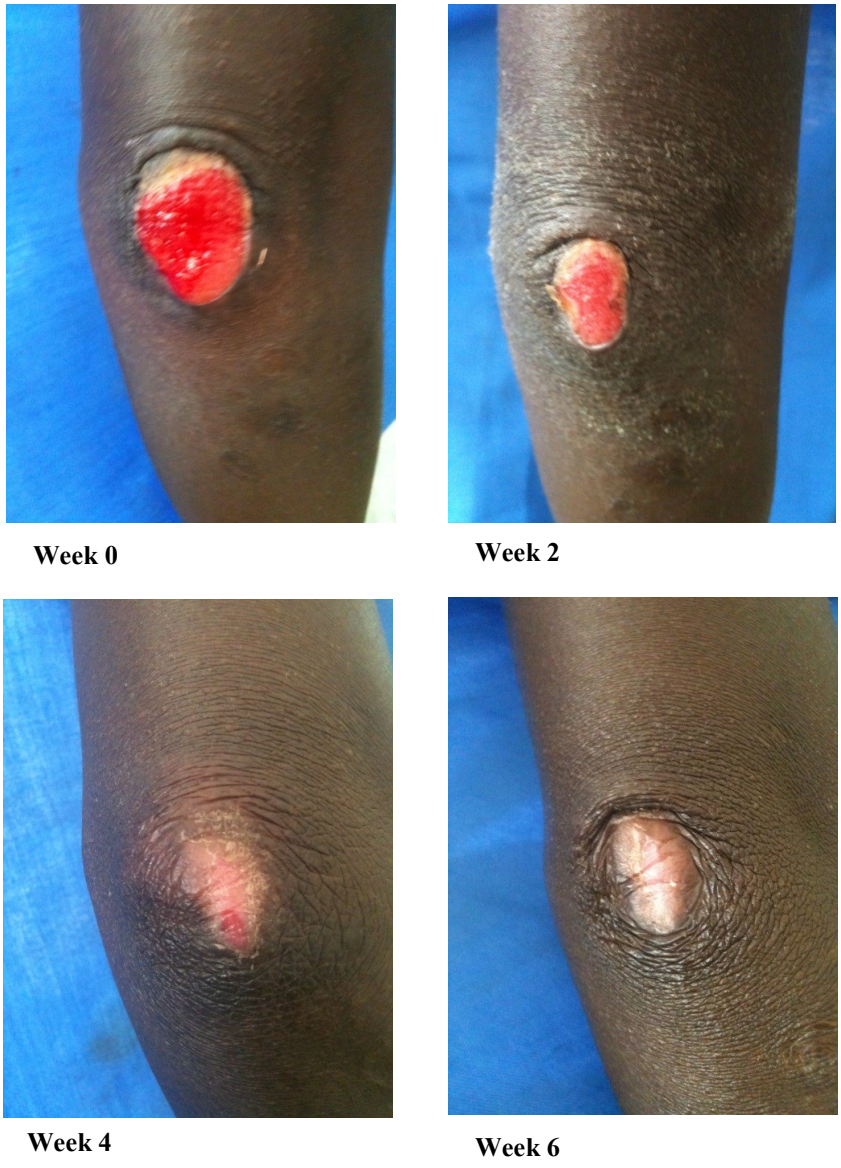
**Week 0** **Week 2** **Week 4**

**Figure 4:12 Category I lesion on the right foot**



**Week 0** **Week 2** **Week 4** **Week 6**

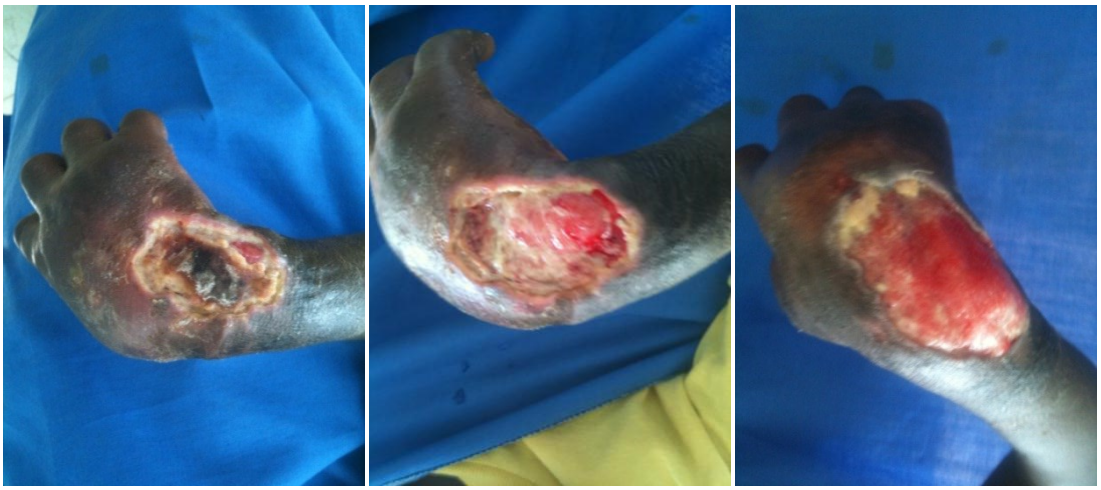
**Figure 4:13 Stages of healing of a category II lesion on the right thigh of a nine-year old girl**



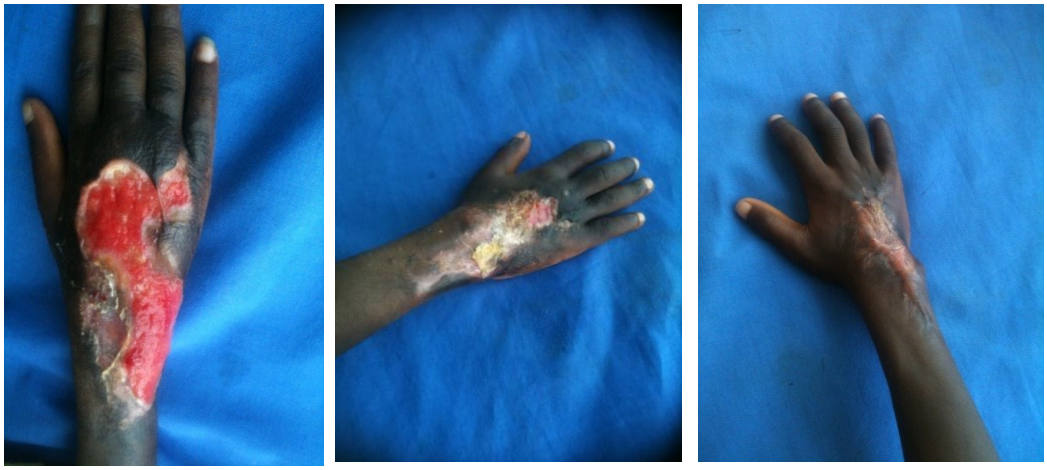
**Week 0** **Week 2**

**Week 4** **Week 6**

**Figure 4:14 Category I ulcer on the elbow**

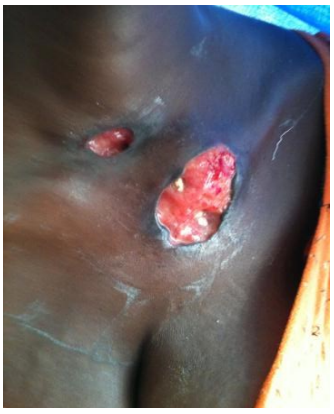


Week 0 Week 0 after De- sloughing Week 2

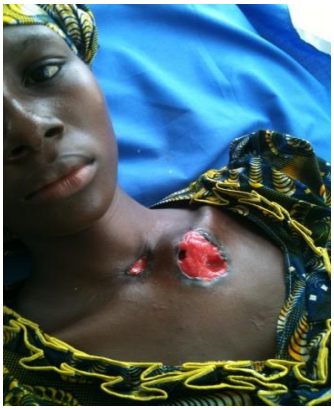


Week 12 Week 20 Week 26

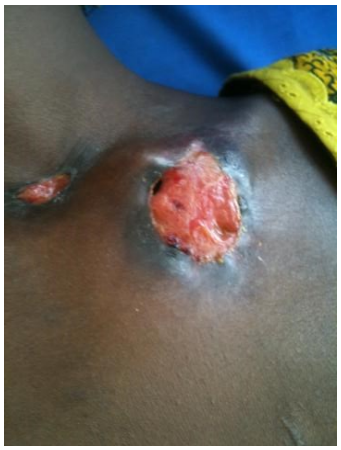
**Figure 4:15 Category II lesion of the right hand of a 7 year old boy**



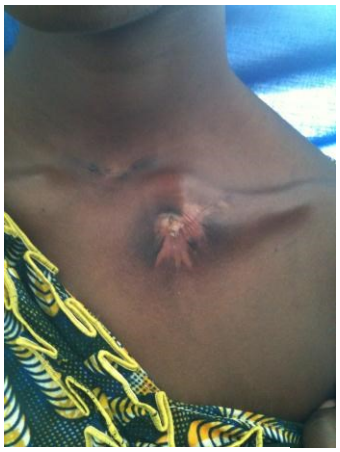
Week 0



Week 2

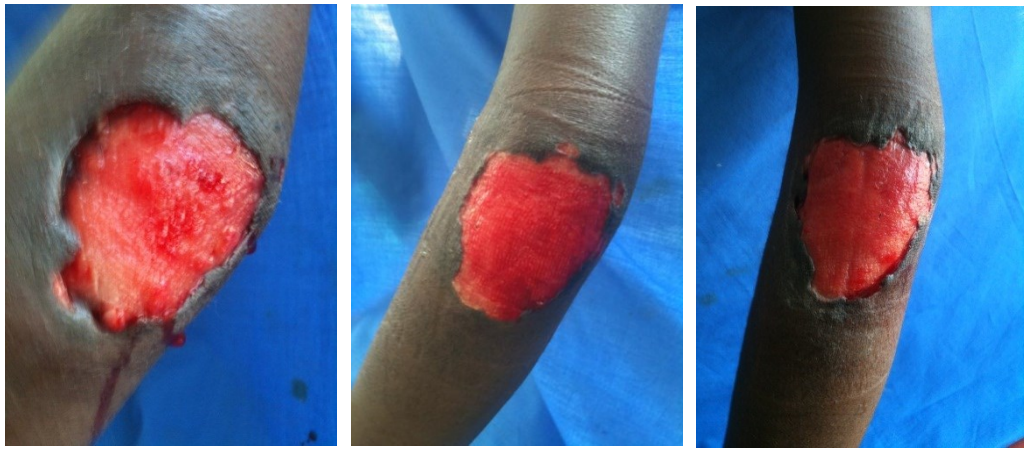


Week 4

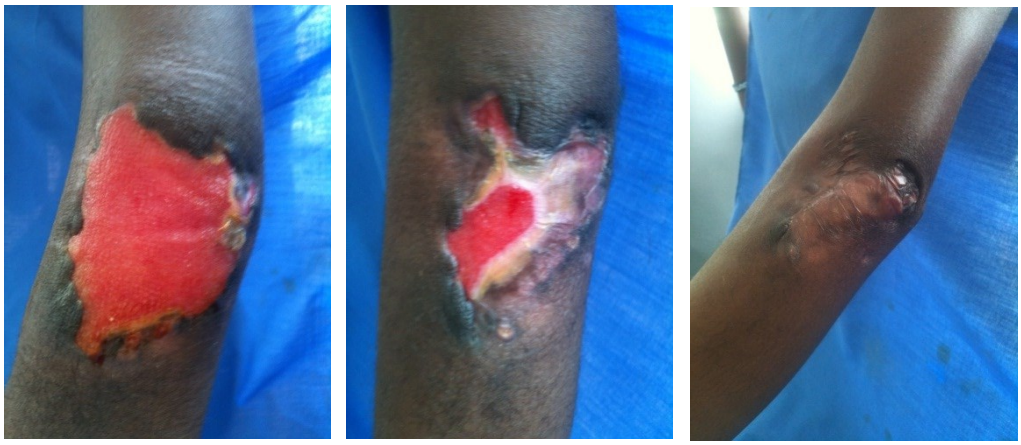


Week 8

**Figure 4:16 Stages of healing of a category III lesion around the clavicle**

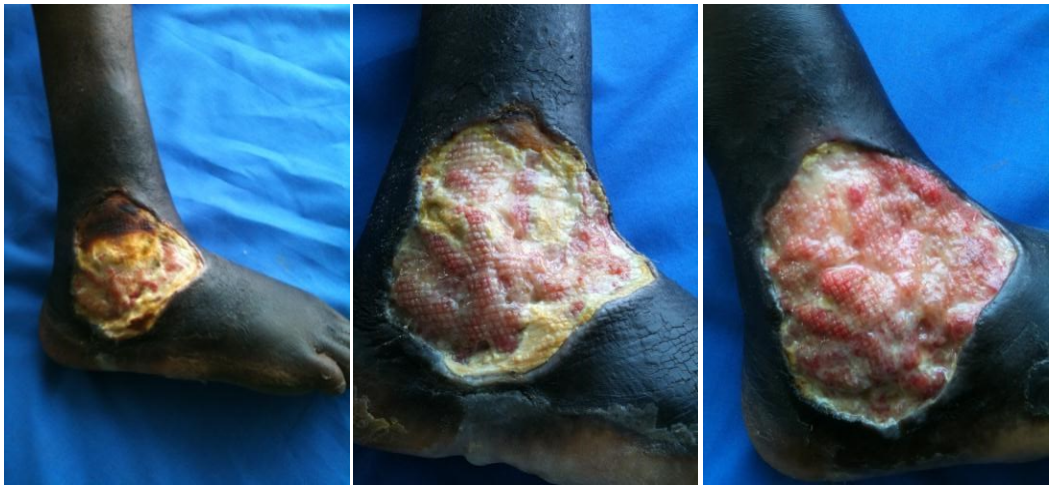


Week 0 Week 2 Week 4

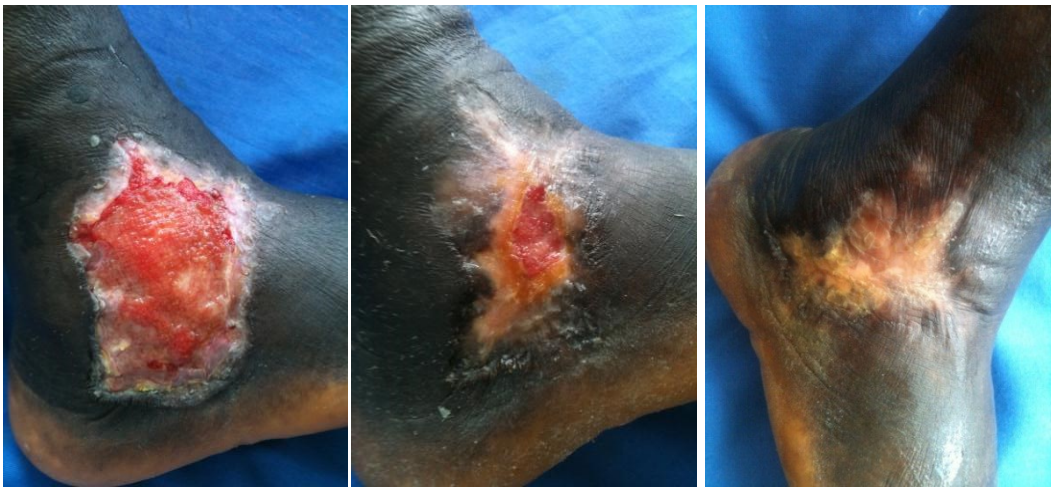


Week 8 Week 14 Week 26

**Figure 4:17 Stages of healing of a category II ulcer around the left elbow of a 17-year SSS student**



Week 0 Week 2 Week 6



Week 16 Week 26 Week 30

**Figure 4:18 Stages of healing of Category II ulcer around the right ankle**

# CHAPTER FIVE

## 5 DISCUSSIONS

The recommendation by WHO to use IM streptomycin and oral rifampicin to treat BU lesions based on one single study by Etuaful *et al.* was most probably the result of frustration and helplessness because before then, the recommended treatment for BU was wide surgical excision and this often caused significant morbidity. Surgery was technically difficult, resource intensive, expensive, and often not easily accessible in endemic areas. (Asiedu K, Etuaful, WHO, 2004). Furthermore, BU recurred after treatment in 16% – 47% of cases if antibiotics or surgery alone was used. The study was based on only 21 pre-ulcerative lesions that were less than 5 cm in diameter (Etuaful *et al.*, 2005). According to the WHO guidelines, the primary aims of chemotherapy for more-advanced lesions are to reduce the extent of surgical excision and to prevent recurrence (World Health Organization. Global Buruli Ulcer Initiative., 2005).

This study set out to determine the factors influencing treatment outcomes for Buruli ulcer disease using antibiotics alone. It was difficult to implement with the limited resources available because studies like, this are usually supported by a team of doctors, nurses and other health workers, but this study was implemented by a team made up of the PI, two field technicians and one health Aide. Despite all the challenges in getting eligible cases for the study, there was no selection bias.

It was implemented by employing a non-randomized clinical intervention design by serially recruiting patients into it over a period of two years until the desired sample size was achieved. In this study, the main finding was that antibiotic treatment of

Buruli ulcer in combination with good wound care practices is effective even for large ulcers with 97.4% (150/154) of patients healing completely without recourse to skin grafting or other surgical interventions and that there were no recurrences. The study also found significant associations between size of BU lesions and time to healing. Other variables, which were associated to time of healing were absence of slough and paradoxical reactions, use of topical antibiotics with or without debridement.

The strengths of this study are that all participants had laboratory confirmation. The health team ensured that all patients received treatment as per the national protocol and follow-up was done for every participant for at least 6 months. Patients with suspected Buruli ulcer lesions living in adjoining districts all reported at the project clinics for treatment because of the results being achieved there.

The findings from this study confirm the efficacy of the combination of intramuscular streptomycin and oral rifampicin in healing all forms of Buruli ulcer disease as achieved by (Nienhuis *et al.*, 2010; Sarfo *et al.*, 2010; Chauty *et al.,* 2007; Etuaful *et al* 2005). This study also showed that wound management also plays an important role in this process. These findings are important for patients with Buruli ulcer disease who mostly live in remote, rural areas in this country where health services are not easily accessible.

In this study with 37% (57/154) of the study participants less than 15 years of age with an equal gender distribution; this is somewhat different from the findings of Kwyer and Ampadu in 2005 when the age distribution in Ghana was 53.1% in patients younger than 15 years (Kwyer & Ampadu, 2006).

As found by Amofah *et al.* in 2002, though, there was a preponderance of lower limb lesions for both gender and all age groups with 90.3% (139/154) having lesions on the extremities, but females had about 2.4 times upper limb lesions as that of males (Amofah *et al.*, 2002). More patients having lesions on the lower limbs and this, being significantly associated with age as patients older than 20 years had more lower limb lesions is most probably as a result of most adult patients being farmers; and so were more likely to traumatize their lower limbs when weeding.

Similarly, Debacker *et al.*in Benin also in 2004 found a preponderance of lower limbs lesions in patients older than 15 years of age (Debacker, Aguiar, Steunou, Zinsou, Meyers, Scott, *et al.*, 2004). On the contrary, Barker *et al.* in their study in Cote d'ivoire in 1973 observed a preponderance of lower limb lesions for boys and that, there was equal frequency of lesions on both arms and legs of girls and women (Barker, 1973). Females having more lesions on the upper limbs could be attributed to sustaining small cuts and bruises in performing their usual household chores.

In this study the aims of treatment were to determine the duration of complete healing of lesions, the rate of healing and the factors that affect complete healing. The median times to complete healing were similar to the 8 weeks, 10 weeks, and 20 weeks for category I, II, and III Buruli ulcer lesions respectively in the study by Sarfo *et al*. (Sarfo *et al.*, 2010). This indicates that bigger lesions take longer times to heal.

All category I lesions healed completely by the end of 16 weeks with 50% (23/46) of them healing by 6 weeks and 87% (40/46) of them by the end of the 8-weeks of treatment but in the study by Chauty *et al.* conducted in Benin, it was 81% of category I ulcers that healed completely (Chauty *et al.*, 2007).

In this study with more than 95% of lesions larger than 5cm healing completely: a feat far higher than the 21%-56% achieved by Chauty *et al.*in 2007, could be a result of application of better wound dressing practices.

The rate of healing was more difficult to measure than the time to complete healing, because it is dependent on the size of the lesion and also had to be done on a regular basis. Whilst Sarfo *et al.* in 2010 and other studies used the diameter to calculate the rate of healing, Etuaful *et al*. in 2005 used the area of the lesion to calculate the rate. In this study, the rate of reduction in area was used as a measure of healing, because whilst the area changed over time the diameter could remain unchanged. There was considerable variation in the healing rates between the categories with the reduction highest in Category III.

There were no apparent differences in the duration of healing between ulcers that evolved from oedematous lesions and ulcers that evolved from other pre-ulcerative lesions apparently because all lesions were occluded with crepe bandage till they finally healed completely.

After start of antimicrobial chemotherapy for Buruli ulcer, new or progressive ulceration (paradoxical reactions) usually occurs before healing sets in (Nienhuis *et al.*, 2010; O'Brien *et al.*, 2009; Schutte *et al.*, 2009). In this study, paradoxical reactions occurred in about a third of the study patients from week 2 to week 18 and reaching a peak at week 10. This is not particularly different from what was observed by Sarfo *et al.* and Nienhuis *et al.* in their studies (Nienhuis *et al.*, 2012; Sarfo *et al.*, 2010).

The extent of PRs observed in category I and category III small (less than 5cm) lesions were similar in magnitude and this was bigger in smaller lesions.

According to some studies the histopathological features of pre-ulcerative lesions and early ulcers typically show very little inflammatory response (Ruf, Sopoh, *et al.*, 2011; Johnson *et al.*, 2005). According to Schutte *et al.* and others when antibiotic therapy is initiated there is an apparent reversal of the immune-tolerant state of active *M. ulcerans* infection as these antibiotics are likely to facilitate this immune reaction by killing *M. ulcerans* or by liberating mycobacterial antigens from dead organisms and therefore reducing the production of mycolactone leading to a vigorous and intense inflammatory response to the killed organisms (Ruf, Chauty, *et al.*, 2011; Ruf, Sopoh, *et al.*, 2011; Schutte *et al.*, 2009). Therefore, the similarities in the pattern of PRs in category I and category III small lesions are not surprising as both are early lesions.

The pathogenesis of paradoxical reactions, which describe a deteriorating response to treatment of an infection after initial improvement, relates to an enhanced immune response to mycobacterial antigens (Schutte *et al.*, 2009; Lipman & Bren, 2006). From this study, though paradoxical reactions decreased the rate of healing, all those who experienced it except one healed eventually. This supports the proposition by O'Brien *et al.* and Nienhuis *et al.* (Nienhuis *et al.*, 2012; O'Brien *et al.*, 2009) that paradoxical reactions in Buruli ulcer lesions are a consequence of effective antibiotic treatment and therefore there is no need to extend antibiotic treatment in patients who seem to have deterioration in their lesions after an initial improvement..

In this study though 36% of patients was seen to have limitation of movements at presentation, apart from one who had already developed a contracture at the elbow joint at the time of presentation only 3.5% (2/56) of them had a persistent functional limitation at the end of follow-up. When limitation of movement was detected at adjacent joints at the time of presentation, patients were taught simple techniques for mobilizing the joints; which they continued until the lesions healed completely and then discharged. This finding supports the WHO strategy for the management of functional limitations associated with Buruli ulcer during treatment (World Health Organization., 2011).

This recurrence rate of 0% in this study is similar to what was found by (Nienhuis *et al.*, 2010) and (Sarfo *et al.*, 2010), though their follow-up period was one year. This is lower than the rates of 2% and 1.1% achieved by (Chauty *et al.*, 2007) and (Kibadi *et al.*, 2010) respectively. This result is far better than the recurrence rate after surgery that has been reported to be 6-28% in various studies (Schunk *et al.*, 2009; O'Brien *et al.,* 2007; Debacker *et al.,* 2005; Amofah *et al.,* 1998) and thus supports the recommended 8-week chemotherapy treatment as has also been supported by studies conducted by Nienhuis *et al.* and Sarfo *et al.* in 2010 (Nienhuis *et al.*, 2010; Sarfo *et al.*, 2010).

Generally factors that affect the healing of wounds include the immune response, nutritional status of the patient, quality of wound dressing procedures (Sarfo *et al.*, 2010; Landis, 2008), wound size, duration of wound prior to treatment, bacterial burden of the wound, a moist wound environment, the choice of dressing material, topical antibiotics, patient education, appropriate environment, nursing care and clinical follow-up (Nicks *et al.*, 2010; Schultz *et al.*, 2003).

Immune response, nutritional status of the patient and bacterial burden of the wound were not quantified as they were not part of the objectives of the study. The quality of wound dressing procedures, choice of dressing material and nursing care were uniform in this study. The full blood counts, renal function tests, liver function tests, random blood sugar levels done in these patients did not show any apparent abnormalities and there were no overt signs of malnutrition or any systemic disease although minor deficiencies could not be ruled out.

The factors that hastened healing were size of the initial lesion, removal of slough, treatment with topical antibiotics and regular wound dressing. Smaller lesions healed faster than larger ones whilst lesions that reported within two months of their initiation healed completely irrespective of their sizes.

Necrotic slough significantly reduced the rate of healing. This was not unexpected because it served as a niche for secondary bacterial growth and acted as a barrier to the effects of topical antiseptics and antibiotics.

Topical antibiotics, minor debridement and removal of slough and eschars all significantly increased the rate of healing. It was important to use topical antibiotics as almost 62% (89/144) of ulcers in this study had necrotic slough. With topical antibiotic ointments these ulcers healed rapidly though lesions with slough healed slower than those without slough. This was not surprising since several studies have found that the presence of bacteria slows down the healing of ulcers as they express proteolytic activities against components of the extracellular matrix important for wound healing, hence contributing to a sustained inflammatory state inhibiting normal

progression to the proliferative phase of healing and thereby preventing restoration of tissue integrity (Schultz *et al.*, 2012; Wysocki *et al.*, 2012; Stojadinovic *et al.*, 2008).

In view of the fact that people with Buruli ulcers report late with 93.5% of them reporting with ulcers and about 62% of these ulcers having necrotic slough and emitting putrid odours, it was necessary if not essential to treat them with topical antibiotics and antiseptics in order to immediately reduce the bacterial burden to speed up healing. The topical antibiotic ointment applied provided immediate relief for those whose lesions were emitting pungent odours. This finding is very important as facilities generally use only saline and plain gauze dressings for managing Buruli ulcers and this is likely to be one of the reasons why Buruli ulcers failed to heal, giving the impression that Buruli ulcers do not heal.

It is also important to note that Hansson *et al.*in 1995 observed that 86% of ulcers with no clinical signs of infection were actually infected and several other studies have also indicated that micro-organisms are present in all chronic wounds (Landis, 2008; Frank *et al.,* 2005; Schultz *et al.*, 2003) with most of them being polymicrobial (Landis, 2008; Howell-Jones *et al.,* 2005). It has also been observed that the longer an ulcer remained unhealed, the more it acquired significant polymicrobial population with more than 50% of them being anaerobes (Landis, 2008). This tends to be consistent with the fact that older lesions heal slowly as was observed in this study.

The findings in this study supports the observations by several studies that topical antimicrobials hasten healing in that they decrease the bacterial burden in chronic wounds with active localized infection (Berger *et al.*, 2000; Frank *et al.*, 2005; Lipsky & Hoey, 2009; Frank *et al.,* 2005; Berger *et al.,* 2000). This finding was also observed

several years ago by Leyden *et al.* also in an open, randomized study that found that topical antibiotic ointments significantly increased the rate of wound healing (Leyden & Bartelt, 1987).

Frank *et al.* suggested that a 2-week trial of topical antibiotics with Gram-positive, Gram-negative and anaerobic coverage should be used in cases where wounds are not healing or continue to have exuberant exudate despite optimal management (Frank *et al.*, 2005). This is because, lesions may be seemingly uninfected but are actually infected as found by some studies that most ulcers with no clinical signs of infection contained more than one bacterial species (Wysocki *et al.*, 2012; Hansson *et al.*, 1995).

The adherence to treatment of the combination of streptomycin and rifampicin and regular wound management with appropriate dressings was accomplished in this study through a few dedicated health workers. We achieved a high rate of compliance through the system of regular counseling, directly observed therapy, optimal and regular wound dressing and documentation. Patients who were very old and those who could not commute daily to the health facilities were managed in their homes by the team each day until their wounds healed or could commute to the health facility. This involved a lot of resources as the team had to rise up early each dawn to treat these patients before the start of their normal work. Treatment was however well tolerated and acceptable to patients.

Although the results of this study are very promising for the management of Buruli ulcer disease in this country, there are several issues that need to be addressed to be able to achieve similar results in our health facilities in future.

First, patients with Buruli ulcer disease are stigmatized by health workers in some of the health facilities in Buruli ulcer endemic areas. This may be as a result of these ulcers being very large, unsightly and emitting putrid odours. Some of the nurses in the health facilities where this study was carried out refrained from caring for BU patients. This is problematic because it has been observed by Shultz *et al.* that nurses play important roles in wound care and monitoring and their intervention can enhance or delay the normal healing process (Schultz *et al.*, 2003). In a situation like this where nurses are not willing to care for Buruli ulcers; who do they expect to do it and how do we expect these lesions to heal completely without their involvement.

Secondly, dressing materials and drugs were sometimes not readily available and these had to be procured by the PI from private pharmacy shops at considerable costs. Patients and their families are generally poor and so cannot afford to buy these drugs and dressing materials from the open market if they had to do it. In that situation they would end up not having optimal care and wounds will not heal as expected and so would resign from treatment despite their unhealed wounds.

This study and several other studies have shown that patients present late with large ulcers with the reason being that, most of these patients are poor and therefore cannot access health services early, which in turn intensifies the severity of these ulcers (Schunk *et al.*, 2009; Amofah *et al.*, 1998; Asiedu & Etuaful, 1998;). If we encourage patients to present early to health facilities to seek help and the health sector does not ensure regular supply of drugs and dressings to give optimal care and as a result wounds fail to heal, all our efforts will be in futility. This will further strengthen the already held belief by many that there is no effective medical treatment for the disease (Aujoulat *et al.*, 2003; Stienstra *et al.*, 2002) and will discourage them further from

seeking care. It will then compound an already existing problem and this will cost the nation a lot of resources, both human and financial.

The number of patients seen would have been smaller but due to the efficacy of the management of lesions at our centers, it encouraged a lot of people to report earlier than usual who also accepted our management protocol even when they had to travel long distances. The high treatment success rate, low treatment failure rate and no recurrence of lesions of this study supports the efficacy of streptomycin/Rifampicin chemotherapy in the treatment of Buruli ulcer disease as was observed by (Nienhuis *et al.*, 2010; Sarfo *et al.*, 2010; Chauty *et al.,* 2007; Etuaful *et al.*, 2005 ). In this study this was achieved in the context of optimal wound care, consistent wound evaluation and timely interventions.

Tarnuzzer and Schultz and Stojadinovic *et al.* observed that appropriate wound bed preparation removes local barriers to healing and optimizes tissue environment to achieve wound healing (Stojadinovic *et al.*, 2008; Tarnuzzer & Schultz, 1996). Also Lanzafame *et al.* and Fivenson and Scherschun also asserted that accurate assessment, prompt treatment and suitable follow-up are essential components in minimizing the potential long-term disability caused by chronic wounds (Lanzafame, 2007a; Fivenson & Scherschun, 2003).

Chronic wounds cause significant suffering, including profound negative effects on general physical health, socialization, financial status, body image, level of independence, and control (Bogie, 2011). They also have negative emotional impact on lives, including fear, social isolation, anger, depression and negative image (Phillips *et al.*, 1994).

Patients suffering from chronic wounds frequently find themselves in situations of having to choose between commitments to their work and compliance to treatment for their ulcers (Ghanassia *et al.*, 2008; Lanzafame, 2007a; Phillips *et al.*, 1994). Others that are less fortunate may be permanently impaired from performing their jobs (Lanzafame, 2007b; O'Donnell, Browse, Burnand, &Thomas, 1977). This situation has been found in a lot of patients in this study with some living with their lesions for well over 10 years or had been incapacitated for the rest of their lives.

Chronic wounds lead to disability and disability worsens wound outcomes resulting in a vicious cycle (Eberhardt & Raffetto, 2005). Chronic wounds and their care represent a growing problem that results in significant disability and lost productivity (Lanzafame, 2007a) and so represents a heavy socioeconomic burden (Eberhardt & Raffetto, 2005). Faster healing of wounds increases efficiency and places less demands on the already thin manpower pools in our health institutions and therefore reduce the overall cost of care.

In view of the above there is the need to intensify efforts to diagnose Buruli ulcer disease early and to manage them promptly to alleviate the suffering of patients, their relatives and to minimize the time and resources used to care for them. Also the immense economic impact of large wounds and for that matter Buruli ulcer disease in our society calls for a higher allocation of resources for research into why patients present late. More work needs to be done to investigate factors that affect the rate of healing of Buruli ulcers and to define the optimal time for surgery and skin grafting as some patients would definitely need surgery and subsequent skin grafting.

# CHAPTER SIX

## 6 CONCLUSIONS AND RECOMMENDATIONS

### 6.1 Conclusions

This study has shown that the combination of intramuscular streptomycin and oral rifampicin is efficacious in healing all forms of Buruli ulcer disease. The treatment success rate was 97.4% on antibiotic chemotherapy and daily wound dressing without any extensive surgery. There were no recurrences of lesions. It has also shown that optimal wound dressing, regular counseling, consistent wound evaluation and timely interventions need to be undertaken to achieve optimal results.

About 37% of the total study participants were less than 15 years with an almost equal gender distribution with exception of those above 50 years where females were twice as much as males. About 90% of lesions were found on extremities with lower limb lesions being significantly associated with older patients. Patients older than 20 years of age had more lesions on the lower limbs than younger ones (OR=3.04; 95%CI = 1.42-6.55).

Almost a third (27.9%) of the patients experienced PRs which peaked (22.1%) at week 10 and were more pronounced in category I and category III small lesions. There was no need to extend antibiotic treatment in those who experienced PRs.

Late presentation to health facilities was common with 93.5% of them having ulcerative lesions and 62% being secondarily infected. However, all lesions that reported to the health facility within two months of appearance achieved 100% treatment success irrespective of category of lesion.

Secondarily infected wounds take longer times to heal completely. Topical antibiotics (HR=3.1, 95% CI=1.83-5.10) or combined with removal of necrotic slough (HR=5.1, 95% CI=2.50-10.23) speed up healing faster than povidone iodine alone.

Smaller sizes of the lesion, debridement and topical antibiotic ointments rapidly and significantly increase the rate of healing of Buruli ulcers especially those ulcers with slough.

Limitation of movements that occurs in Buruli ulcer disease were reduced considerably by teaching patients to practice simple techniques for mobilizing the joints during the period of treatment

## 6.2 Recommendations

In view of the findings in this study it is recommended that all stakeholders involved in Buruli ulcer prevention and control would need to do the following:

The Ministry of Health and Ghana Health Service

- The National Buruli Ulcer Control Programme should ensure the regular supply of drugs and non-drug consumables including topical antiseptics and antibiotic ointments for the management of Buruli ulcer lesions to health facilities in Buruli ulcer endemic areas. This will help in ensuring that Buruli ulcer lesions are diagnosed and managed promptly and effectively leading to complete healing of wounds, and to also alleviate the suffering of patients, their relatives and to minimize the time and resources used to care for them.
- The National Buruli Ulcer Control Programme in collaboration with the District Assemblies and District Health Management Teams of endemic districts should organize regular health education activities to encourage patients to report early to health institutions.
- The National Buruli Ulcer Control Programme should ensure that nurses are trained and re-trained in wound dressing periodically to ensure effective healing of wounds.
- The National Buruli Ulcer Control Programme should ensure that regular counseling sessions are organized regularly to all patients to ensure compliance to treatment.

Health Facilities

- The District Health Management Teams of endemic districts should organize regular training in wound care and infection prevention to health workers involved in Buruli ulcer management to enable them offer optimal wound care to patients.
- The District Assemblies in Buruli ulcer endemic areas should set aside funds to assist DHMTs in defaulter prevention and defaulter tracing activities since these are essential components in ensuring complete healing of lesions hence minimizing the potential long-term disability caused by chronic Buruli ulcers.

Research Institutions

- Research Institutions should work in close collaboration with health facilities to undertake further research into "paradoxical reactions" and its association with wound healing and to define the optimal time for surgery and skin grafting for Buruli ulcers.

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## APPENDIX A

## QUESTIONNAIRE FOR SUBJECTS UNDER BURULI ULCER STUDY IN THE AKWAPEM SOUTH MUNICIPALITY AND SUHUM-KRABOA-COALTAR DISTRICT

**Title: Antibiotic Treatment Outcomes of Buruli Ulcer in Akwapem South and Suhum-Kraboa-Coaltar Districts of Eastern Region, Ghana**

1. ID #_____
2. District_____ Region:_____
3. Name of patient _____
4. Age (years) ☐☐
5. Sex: ☐ Male ☐ Female
6. Ethnicity: ☐ 1.Ewe ☐ 2. Ga/Adangme ☐ 3. Akan ☐ 4. Others
7. Educational Level ☐ 1.No Education ☐ 2.Primary
   ☐ 3. JHS ☐ 4. SHS ☐ 5.Tertiary
8. Marital Status ☐ 1. Single ☐ 2.Married ☐ 3.Divorced/Separated
9. Occupation ☐ 1. Farmer ☐ 2. Trader ☐ 3. Artisan ☐ 4. Others
10. BCG Scar (look on left shoulder) ☐ 1.Yes ☐ 2.No
11. Weight (Kg) ☐☐ . ☐
12. Height (cm) ☐☐ ☐
13. Date of clinical diagnosis (dd/mm/yy) ____/____/____
14. Date of healing (Epithelialization) (dd/mm/yy) ____/____/____
15. Date of healing (complete crust coverage) (dd/mm/yy) ____/____/____
16. Address (village or town)_____

    Landmark:_____

## CLINICAL HISTORY AT DIAGNOSIS

17. Duration of illness before seeking care (weeks) ☐☐

18. Any antecedent event? ☐ 1. Yes ☐ 2. No

19. If yes nature of incident? ☐ 1. Trauma ☐ 2. Pruritus ☐ 3. Other

20. What form did lesion start? ☐ 1. Papule ☐ 2. Plaque
☐ 3. Nodule ☐ 4. Oedema

21. Form of lesion at presentation? ☐ 1. Papule ☐ 2. Plaque ☐ 3.Nodule
☐ 4. Oedema ☐ 5. Ulcer

22. Duration between initial form and form at presentation (weeks) ☐☐

23. No. of lesions ☐☐

24. Pain associated with lesion? ☐ 1. Yes ☐ 2. No

25. Use of traditional treatment? ☐ 1. Yes ☐ 2. No

26. If yes how long? (weeks) …….

27. Type of treatment …….

28. Limitation of movement at any joint ☐ 1. Yes ☐ 2. No

29. Previous history of Buruli ulcer? ☐ 1. Yes ☐ 2. No

30. If yes, how long? (Weeks) .....

31. If yes treatment given? ☐ 1. Antibiotics ☐ 2. Traditional

32. Result of treatment? ☐ 1. Healed ☐ 2. Not healed

33. Any History of TB? 1. Yes ☐ 2. No

34. Any treatment with streptomycin? ☐ 1. Yes ☐ 2. No

35. History of cough? ☐ 1. Yes ☐ 2. No

36. Family history of BU? ☐ 1. Yes ☐ 2. No

37. LOCATION OF LESION(S)

☐ 1. Upper Limb (UL) ☐ 2. Lower limb (LL) ☐ 3.Trunnk (TK)

☐ 4. Buttocks and Perineum (BP) ☐ 5. Eye ☐ 6. Breast

☐ 7. Genitalia ☐ 8. Head ☐ 9. Neck

38. CLINICAL FORMS

☐ Nodule (N) ☐ Plaque (Q) ☐ Oedema (E) ☐ Ulcer (U)

☐ Osteomyelitis (O) ☐ Papule (P)

If there are multiple lesions indicate forms of different lesions below

Form: 1. ---------- 2. ---------- 3. ---------- 4. ----------

39. Is lesion undermined? ☐ 1. Yes ☐ 2. No

40. Is lesion warm to touch ☐ 1. Yes ☐ 2. No

41. Presence of necrotic tissue ☐ 1. Yes ☐ 2. No

42. Is ulcer infected? ☐ 1. Yes ☐ 2. No

43. CATEGORIES OF LESIONS

☐ Category I: A single lesion ≤ 5 cm in diameter

☐ Category II: A single lesion 5 - 15 cm in diameter

☐ Category III: A single lesion >15 cm in diameter, multiple lesions critical sites, osteomyelitis

**LABORATORY CONFIRMATION**

44. Date 1st specimen taken: ____/____/___

45. Date results received: ____/____/___

46. Specimen type: ☐ 1. Swab ☐ 2. Fine Needle Aspiration

**RESULTS**

47. ZN ☐ 1. Positive ☐ 2. Negative

48. PCR ☐ 1. Positive ☐ 2. Negative

49. Culture ☐ 1.Positive ☐ 2. Negative

**TREATMENT TYPE**

50. Wound care given? ☐ 1.Saline/Povidone oidine

☐ 2. Topical antibiotics ☐ 3. Debridement done

51. Treatment given? ☐ 1. Antibiotics alone ☐ 2. Antibiotics + surgery

52. Prevention of Disability done? ☐ 1. Yes ☐ 2. No

53. Dosage of drugs? Rifampicin: _____(mg) Streptomycin: _____(mg)

54. Total Doses received _____

55. Duration of treatment ----------

56. Sickling Status: ☐ 1. Positive ☐ 2. Negative

57. If sickling positive ☐ 1. AS ☐ 2. SS/SD/SβBthal

58. Full Blood Count ☐ 1. Normal ☐ 2. Abnormal

59. Blood urea & electrolytes ☐ 1. Normal ☐ 2. Abnormal

60. Liver Function Test ☐ 1. Normal ☐ 2. Abnormal

61. Random blood sugar (RBS) ☐☐.☐

62. If RBS abnormal, then FBS ☐☐.☐

63. X-ray ☐ 1. Yes ☐ 2. No

64. If yes ☐ 1. Normal ☐ 2. Abnormal

65. Sero-status ☐ 1. Reactive ☐ 2. Non-reactive

66. AFB (TB) ☐ 1. Positive ☐ 2. Negative

67. Paradoxical reaction ☐ 1.Yes ☐ 2. No

68. Wound size circumference (cm) (serial wound measurements)

| Week 0 | Week 2 | Week 4 | Week 6 | Week 6 |
|---|---|---|---|---|
| | | | | |
| Week 8 | Week 10 | Week 12 | Week 14 | Week 16 |
| | | | | |
| Week 18 | Week 20 | Week 22 | Week 24 | Week 26 |
| | | | | |
| Week 28 | Week 30 | Week 32 | Week 34 | Week 36 |
| | | | | |

Wound size Area ($cm^2$) (serial wound measurements)

| Week 0 | Week 2 | Week 4 | Week 6 | Week 6 |
|---|---|---|---|---|
| | | | | |
| Week 8 | Week 10 | Week 12 | Week 14 | Week 16 |
| | | | | |
| Week 18 | Week 20 | Week 22 | Week 24 | Week 26 |
| | | | | |
| Week 28 | Week 30 | Week 32 | Week 34 | Week 36 |
| | | | | |

72. TREATMENT OUTCOMES

☐ 1. Healed ☐ 2. Not healed ☐ Lost to follow up /Died

# APPENDIX B

## CONSENT FORM

## ANTIBIOTIC TREATMENT OUTCOMES OF BURULI ULCER IN AKWAPIM SOUTH AND SUHUM-KRABOA-COALTAR DISTRICTS OF EASTERN REGION, GHANA

Principal Investigator: Nana Konama Kotey

Address: DEPARTMENT OF EPIDEMIOLOGY AND DISEASE CONTROL

SCHOOL OF PUBLIC HEALTH,
UNIVERSITY OF GHANA
P.O.BOX LG13
LEGON-ACCRA

**Introduction**

This Consent Form contains information about the research named above. In order to be sure that you are informed about being in this research, we are asking you to read (or have read to you) this Consent Form. You will also be asked to sign it (or make your mark in front of a witness). We will give you a copy of this form. This consent form might contain some words that are unfamiliar to you. Please ask us to explain anything you may not understand.

**Reason for the Research**

The reason for the research is to find out the factors that determine how people respond to the standard antibiotics treatment for Buruli ulcer.

**General Information about Research**

Buruli ulcer disease (BU) is a chronic debilitating skin disease caused by *Mycobacterium ulcerans*. Buruli ulcer presents in various form including, nodules and ulcers. The ulcer can be so extensive that it affects daily activities of the person affected and for some individuals that the ulcer has healed it leads to disfiguring of the part of the body involved. Ghana is one of the countries in the West Africa that Buruli

ulcer affects. Amongst the districts, Suhum-Kraboa-Coaltar and Akwapem South are also affected. Unfortunately the exact way of getting the disease is not known.

**Your Part in the Research**

You/ward involvement will require that you/ward answer certain questions and, you will be examined, take samples of your lesion and blood samples as well. The number of times blood will be taken from you will depend on how you respond to the treatment; a minimum of one and a maximum of 7 samples will be taken for the whole period. The taking of blood and specimens from lesions will cause some slight pain but this will wear off after a few minutes. You will be treated after the test results are received. This will help us identify the factors responsible for the antibiotic treatment outcome. About 100 people will participate in the research at this site.

**Possible Risks**

There is minimal risk in participating in this research.

**Possible Benefits**

This research will bring benefit to the community by helping us to know how long to continue antibiotics treatment and when to consider other treatment options.

**If You Decide Not to Be in the Research**

You are free to decide if you want to be in this research. Your decision not to participate will not affect your care in any way.

**Confidentiality**

We will protect information about you and your taking part in this research to the best of our ability. You will not be named in any reports. However, the members of our research team may sometimes look at your research records. Someone from the IRB might want to ask you questions about being in the research, but you do not have to answer them. A court of law could order medical records shown to other people, but that is unlikely.

**Compensation**

You will not be paid, since you do not have to take part in this research.

**Alternatives to Participation**

You do not have to participate in the research in order to receive care.

**Leaving the Research**

You may leave the research at any time. If you choose to take part, you can change your mind at any time and withdraw.

When you are no longer in the research, you will still be able to use this clinic.

Please call Nana Konama Kotey (phone 0247910894) if you have questions about the research.

**Your rights as a participant**

This research has been reviewed and approved by the IRB of Noguchi Memorial Institute for Medical Research. An IRB is a committee that reviews research studies in order to help protect participants. If you have any questions about your rights as a research participant you may contact Rev. Dr. Ayete-Nyampong, Chairperson, NMIMR-IRB, mobile 0208152360

## APPENDIX C

## VOLUNTEER AGREEMENT

The above document describing the benefits, risks and procedures for the research on "ANTIBIOTIC TREATMENT OUTCOMES OF BURULI ULCER IN AKWAPIM SOUTH AND SUHUM-KRABOA-COALTAR DISTRICTS OF EASTERN REGION, GHANA" has been read and explained to me. I have been given an opportunity to have any questions about the research answered to my satisfaction. I agree to participate as a volunteer.

_____ _____

Date Signature or mark of volunteer

**If volunteers cannot read the form themselves, a witness must sign here:**

I was present while the benefits, risks and procedures were read to the volunteer. All questions were answered and the volunteer has agreed to take part in the research.

_____ _____

Date Signature of Witness

I certify that the nature and purpose, the potential benefits, and possible risks associated with participating in this research have been explained to the above individual.

_____ _____

Date Signature of Person Who Obtained Consent

# APPENDIX D

NOGUCHI MEMORIAL INSTITUTE FOR MEDICAL RESEARCH
INSTITUTIONAL REVIEW BOARD
(UNIVERSITY OF GHANA)

Phone: +(233) 21 500374 /501178
Fax: +(233) 21 502182
Email: Director@noguchi.mimcom.org
Telex No: 2556 UGL GH

P.O. Box LG581
Legon
Ghana

My Ref. No: DF.22

3rd March, 2010

Your Ref. No:

## ETHICAL CLEARANCE

**FEDERALWIDE ASSURANCE FWA 00001824** **IRB 0001276**

**NMIMR-IRB CPN 043/09-10** **IORG 0000908**

On 3rd March, 2010, the Noguchi Memorial Institute for Medical Research (NMIMR) Institutional Review Board (IRB), at a full board meeting reviewed and approved your protocol titled:

**TITLE OF PROTOCOL** : **Antibiotic treatment outcomes of Buruli Ulcer in Suhum-Kraboa-Coaltar and Akuapim South Districts of Eastern Region, Ghana**

**PRINCIPAL INVESTIGATOR** : **Nana Konama Kotey (PhD Student)**

Please note that a final review report must be submitted to the Board at the completion of the study. Your research records may be audited at any time during or after the implementation.

Any modification of this research project must be submitted to the IRB for review and approval prior to implementation.

Please report all serious adverse events related to this study to NMIMR-IRB within seven days verbally and fourteen days in writing.

This certificate is valid till 2nd March, 2011. You are to submit annual reports for continuing review.

Signature of Chairman: .....
Rev. Dr. Samuel Ayete-Nyampong
**(NMIMR – IRB, Chairman)**

cc: Professor Alexander K. Nyarko
Director, Noguchi Memorial Institute
for Medical Research, University of Ghana, Legon

# APPENDIX E

**GHANA HEALTH SERVICE ETHICAL REVIEW COMMITTEE**

*In case of reply the number and date of this Letter should be quoted.*

*My Ref. :GHS-ERC: 3*
*Your Ref. No.*


Research and Development Divison
Ghana Health Service
P. O. Box MB 190
Accra

*27th May 2010*

*Tel: +233- 0302-681109*
*Fax + 233-0302 685424*
*Email: Hannah.Frimpong@ghsmail.org*

**NANA KONAMA KOTEY - Principal Investigator**

**ETHICAL CLEARANCE - ID NO: GHS-ERC: 07/4/10**

The Ghana Health Service Ethical Review Committee has reviewed and given approval for the implementation of your Study Protocol titled:

**"Antibiotic Treatment Outcomes of Buruli Ulcer in Akwapem South and Suhum-Kraboa-Coaltar District of Eastern Region, Ghana"**

This approval requires that you submit periodic review of the protocol to the Committee and a final full review to the Ethical Review Committee (ERC) on completion of the study. The ERC may observe or cause to be observed procedures and records of the study during and after implementation.

Please note that any modification of the project must be submitted to the ERC for review and approval before its implementation.

You are also required to report all serious adverse events related to this study to the ERC within seven days verbally and fourteen days in writing.

You are requested to submit a final report on the study to assure the ERC that the project was implemented as per approved protocol. You are also to inform the ERC and your mother organization before any publication of the research findings.

Please always quote the protocol identification number in all future correspondence in relation to this protocol

SIGNED.....

MR. ANNOR NIMAKO
(GHS-ERC VICE CHAIRMAN)

Cc: The Director, Research and Development Division, GHS, Accra

# APPENDIX F

# APPENDIX G

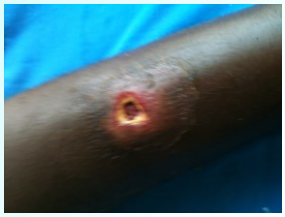
## Antibiotic Treatment Outcomes of Buruli Ulcer in Akwapem South and Suhum-Kraboa-Coalter Districts in Eastern Region, Ghana, 2011

Kotey Nana K. (Mrs) MBChB, MPH, MGCP
Prof. Thomas Jonghans
Prof. Fred Binka
School of Public Health, Disease Control and Epidemiology ,Accra –Ghana
University of Heidelberg
College of Sciences University of Ghana.

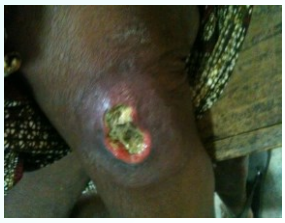
### BACKGROUND

Buruli ulcer is a neglected chronic, indolent, necrotizing disease of the skin caused by *Mycobacterium ulcerans*. It is characterized by a painless nodule, papule, plaque or oedema evolving into a painless ulcer with undermined edges. It leads to functional disability if not diagnosed and treated early.

Surgery has previously been used as the main mode of treatment, but antibiotics are now being used. We therefore examined the response of the various categories of Buruli ulcer lesions with respect to the WHO-recommended treatment with IM streptomycin and oral rifampicin



CATEGORYI
A single lesion 0 < 5 cm



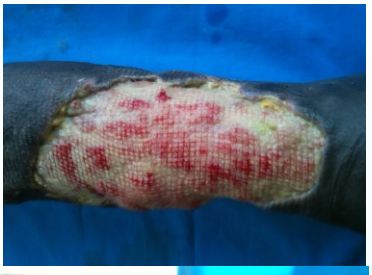
CATEGORY II
A single lesion between 5 – 15 cm

### STUDY OBJECTIVE

The study objective was to examine the response of various categories of lesions with respect to antibiotic treatment

### METHODS

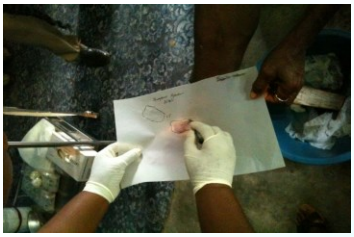
- Study Area: Akwapem South and Suhum-Kraboa-Coalter Districts
- We recruited 68 laboratory confirmed Buruli ulcer patients.
- They were given the WHO recommended 8-week treatment of a combination of streptomycin and rifampicin.
- Clinical history and physical examination performed to assess the form and severity of the disease and to rule out other systemic diseases.
- Wounds were dressed on daily basis and observed bi-weekly for reduction in size. The longest diameter of lesions were measured and photographed in a standardised manner in order to monitor changes in sizes
- Lesions were grouped according to WHO classification of lesions by size and site, namely: category I, II, III single and III-multiple lesions.
- Statistically significant differences were declared at p-value of <0.05



CATEGORYIII
A single lesion greater than 15cm, multiple lesions, critical site lesion or osteomylitis

### RESULTS

- Duration of primary wound healing for category I lesions is 29 (14-84) days for; 52 (42-168) days for category II lesions and 65 (42-188) days for category III single lesions and 43 (28 – 139) 139 days for category III-multiple lesions.
- Using one way analysis of variance for the categories of lesions there is a significant difference between the categories and their respective duration of healing with a p-value = 0.003.



Tracing of lesion with tracing paper by Principal investigator

### CONCLUSION

Early detection and treatment of Buruli ulcer lesions with the recommended antibiotic regimen and and proper wound management significantly improve the healing of wounds.

### RECOMMENDATIONS

- All health providers should be trained in the diagnosis of Buruli ulcer and proper wound management so that Bu would be diagnosed and treated early to save the country's scarce resources
- Behavioral change communication should be undertaken in BU endemic communities for people to report early