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Prevalence of Bacterial and Fungal Infected Chronic Leg Ulcers at a Teaching Hospital in Ghana

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Abstract

Background: Chronic ulcers are responsible for considerable morbidity and significantly contribute to the escalation in the cost of health care. Chronic leg ulcers (CLUs) are susceptible to microbial infections and serious complications such as tissue necrosis and osteomyelitis, can result without the timely control of infections. Recent studies have also reported an increase in the association of fungal infections with chronic non-healing ulcers. **Aim:** To determine the prevalence of bacterial and fungal infections among patients reporting with chronic leg ulcers in participants without co-morbidities. **Methods:** A prospective cross-sectional study was conducted among patients with chronic leg ulcers at the National Reconstructive Plastic Surgery and Burns Centre, Korle-Bu Teaching Hospital (NRPS/BC-KBTH) and those who consented were enrolled. Characteristics of the wound as well as micro-organisms cultured from wound swabs were recorded. **Results:** A total of 50 participants were enrolled for the study with the mean (SD) age of 40.7 (10.7) years. Eighty percent of the participants presented with post traumatic leg ulcers with 80% being artisans and traders in the age group 31 - 50 years. There was no statistically significant association between sex and the organism cultured for post traumatic and cellulitis (p-value > 0.05). The prevalence of bacterial and fungal infection was 79.3% and 20.7% respectively. *Pseudomonas species* was the most isolated bacteria (61.5%) while *Aspergillus niger* was the most isolated fungi (41%).

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Conclusion: From this study, fungal infections should be included in managing chronic leg ulcers, especially among artisans, famers and gardeners even though there was a significantly higher burden of bacterial infections.

Keywords

Chronic Leg Ulcers (CLUs), Microbial Infections, Fungal Infections, Pseudomonas Species, Burn Surgery

1. Introduction

Chronic wounds affect about 1% of the world's population, with associated costs accounting for more than 2% - 4% of health care expenses worldwide [1]. A chronic leg ulcer is defined as a "defect in the skin below the level of the knee persisting for more than six weeks and showing no tendency to heal after three or more months" [2]. Chronic wounds often remain in the inflammatory stage for a long time [3]. They fall into three broad categories: diabetic ulcers, pressure ulcers and ulcers secondary to venous hypertension [4] [5]. Wound healing has three principal phases: inflammatory, proliferative, and remodelling [5]. It has been suggested that the three fundamental factors underlying chronic wound pathogenesis include cellular and systemic changes of aging, repeated bouts of ischaemia-reperfusion injury, and bacterial colonization with resulting inflammatory host response [6].

CLUs can persist for many months or even years, are often painful and debilitating, resulting in loss of quality of life [7] [8].

When bacterial bio burden moves from colonization to contamination through critical colonization and ultimately infection, wound healing becomes delayed or even halted [9].

Chronic wounds microbial flora encompasses a variety of organisms including gram positive and negative bacteria, as well as fungi [10]. Chronic ulcers represent an ideal environment for the formation of biofilms due to bacterial adhesion facilitated by the presence of necrotic tissue and debris. In fact, biofilms are present in 60% - 80% of patients with chronic wounds. The two most common biofilm generators are *Staphylococcus* and *Pseudomonas aeruginosa* [11].

The commonest aetiological factor for chronic lower limb ulcer is trauma which results from inadequate primary treatment of a trauma, or from complications during primary treatment. In most cases, chronic post traumatic leg ulcers develop as a result of initial under estimation of the soft tissue injuries that accompanies the primary trauma [12].

Traumatic ulcer can be either mechanical (example, road traffic accident), physical (example, electrical burn) or chemical as in application of caustics. In many countries, motor vehicle accidents rank first among all fatal accidents. These ulcers are seen in all age groups and more common in males. The most common part of the leg affected by these ulcers is the malleoli where skin is closely applied

to bony prominences [13].

In Ghana, some institutional based studies have been done to predict the prevalence of bacterial and fungal infections in some chronic wounds [14].

Chronic wounds are a major problem at the National Reconstructive Plastic Surgery and Burns Centre of the Korle-Bu Teaching Hospital. Data from the Out-Patient-Department records showed a steady rise of patients with chronic leg ulcers from 6% to 27% from January, 2003 to December, 2017. Theatre records between 2003 and 2016, showed approximately 20% - 22% of the surgeries performed annually were split skin graft for chronic wounds and approximately 2% - 5% of patients had re-grafting of their wounds. These poor healing ulcers have direct implication on medical cost, in-patient care, drugs and transportation. Additionally, these negatively impacts on the quality of the patient's life, as high number of cases become a burden to limited healthcare personnel at the Centre. The current clinical practice for evaluation of wound infection does not fully capture the extent of microbial burden and antimicrobial treatment as only bacterial cultures are obtained from wound swabs. Therefore, the need to study the prevalence of bacterial and fungal infected chronic leg ulcers.

2. Materials and Methods

2.1. Statistical Analysis

All data analyses were performed using SPSS (IBM SPSS Statistics 20).

2.2. Study Design

A prospective cross-sectional study was carried out between the months of April, 2019 to January, 2020 among chronic leg ulcer (CLU) patients at the National Reconstructive Plastic Surgery and Burns Centre of the Korle-Bu Teaching Hospital (NRPS/BC-KBTH).

2.3. Study Site/Area

The National Reconstructive Plastic Surgery and Burns Centre (NRPS/BC) of the Korle-Bu Teaching Hospital is a 68 bed facility and a major referral Centre for patients all over Ghana and neighbouring West African countries and provides reconstructive procedures for various injuries involving the skin as well as burns care. Approximately 280 patients are seen at the Out-Patients-Department annually with chronic ulcers alone and a total of approximately 300 patients with burns injuries are seen at the NRPS/BC-KBTH annually.

2.4. Inclusion Criteria

Patients included in the study were those who reported to the NRPS/BC seeking treatment for chronic leg ulcers. Medical records from patients were reviewed to include patients aged between 18 to 60 years without a diagnosis of diabetes, presence of ulcer on the leg, chronic wounds that occurred as a result of cellulitis and trauma and wounds that had been present for six weeks up to 18months. Patients

who were terminally ill, known to have sickle cell disease, neuropathic, diabetic, vascular, and neoplastic ulcers were excluded from the study. There are plans to include these in a follow up study. The aim of this study was to assess the microbiological profile of chronic leg ulcers in participants who are medically well. There are plans to include these groups in a subsequent study which will also include management protocols for various leg ulcer infections in participants with or without co-morbidities.

2.5. Recruitment Procedure

Patients with chronic leg ulcers who satisfied the criteria for inclusion were enrolled for the study using consecutive sample selection technique. For each consented participant, their demographic characteristics as well as wound characteristics (cause of wound, pain, exudate and odour) were recorded.

2.6. Specimen Collection

Two wound swabs were taken after debridement of necrotic material, eschar, purulent discharge and cleansing of the wound with sterile 0.9% saline. To ensure consistency and to minimize variation in sampling, a standardized protocol, Levine method for swab was applied throughout.

All samples were kept in an empty ice chest and transported within 30 minutes to the Medical Microbiological Laboratory of the University of Ghana Medical School in Korle-Bu Teaching Hospital.

2.7. Laboratory Analysis

Wound swab specimens were inoculated directly onto Sabouraud agar plate and incubated for 48 hours to seven days to determine the presence or absence of fungi. Cultures were stained with and lactophenol cotton blue and examined microscopically to identify fungi.

Bacterial agents were determined through aerobic culture by direct inoculation of wound swab specimen onto Blood, Chocolate and MacConkay agar plates, after which biochemical tests were used for identification of bacterial isolates.

2.8. Ethical Consideration

The study protocol was submitted to the Institutional Review Board of Korle Bu Teaching Hospital and approved in January, 2019 (Protocol Number: KBTH-IRB/000107/2018). Written informed consent were obtained from all patients recruited after explanation of the study. Refusal to participate from the onset or refusal to participate further after initial consent did not interfere with the treatment of the patients. The data obtained during the course of the study has been kept confidential. Participants were identified by codes throughout the study.

Characteristics such as sex, occupation, and smoking status were summarized as tables. Continuous data including age and wound size were summarized as means and standard deviation. Associations between categorical variables were

determined using Chi-square/Fishers Exact test. All p-values less than 5% was considered statistically significant.

3. Results

Fifty (50) participants were recruited for this study whose mean (SD) age was 40.7 (10.7) years with the minimum age being 19 years and maximum being 60 years. Majority 43 (86.0%) were 31 years and above and male participants form the majority enrolled into the study. Majority of the participants had post traumatic ulcers (80.0%). A significant proportion (70.0%) reported moderate pain and had unhealthy wound bed as shown in Table 1.

Table 1. Demographics and wound characteristics.

	Characteristics	Frequency (%)
Age	<20	2 (4.0)
	20 - 30	5 (10.0)
	31 - 40	18 (36.0)
	41 - 50	14 (28.0)
	>50	11 (22.0)
Gender	Male	31 (62.0)
	female	19 (38.0)
Drug History	Haematinics	14 (28.0)
	Analgesics	18 (36.0)
	Antibiotics	4 (8.0)
	None	14 (28.0)
Alcohol	Yes	17 (34.0)
	No	33 (66.0)
Smoking	Yes	1 (2.0)
	No	49 (98.0)
Wound Type	Post Traumatic	40 (80.0)
	Post Cellulitis	10 (20.0)
Presence of exudate	Heavy	22 (44.0)
	Moderate	21 (42.0)
	Minimal	7 (14.0)
	No discharge	0 (0.0)
Odour	Yes	12 (24.0)
	No	38 (76.0)
Pain	Severe	6 (12.0)
	Moderate	35 (70.0)
	Mild	9 (18.0)

Continued

Wound bed	Unhealthy Granulation	43 (86.0)
	Hyper granulation tissue	2 (4.0)
	Others	5 (10.0)
Wound slough/necrotic tissue	Moderate (++)	24 (48.0)
	Minimal (+)	18 (36.0)
	Excessive (+++)	6 (12.0)
	Absent (−)	2 (4.0)

Table 2 shows male artisans aged 31 - 50 years formed majority (64.7%) of the participants with post traumatic chronic leg ulcers. Professionals who had post traumatic ulcers also fell within the same age group of 31 - 50 years.

Table 2. Male wound type by occupation and age.

Occupation	Age	Post traumatic	Post cellulitis	*p*-value
		n (%)		
Professional	<20	0 (0.0)	0 (0.0)	1.000
	20 - 30	0 (0.0)	1 (33.3)	
	31 - 40	3 (75.0)	1 (33.3)	
	41 - 50	1 (25.0)	1 (33.3)	
	>50	0 (0.0)	0 (0.0)	
Artisan	<20	1 (5.9)	0 (0.0)	1.000
	20 - 30	2 (11.8)	0 (0.0)	
	31 - 40	5 (29.4)	0 (0.0)	
	41 - 50	6 (35.3)	0 (0.0)	
	>50	3 (17.7)	0 (0.0)	
Trader	<20	0 (0.0)	0 (0.0)	1.000
	20 - 30	0 (0.0)	0 (0.0)	
	31 - 40	1 (50.0)	0 (0.0)	
	41 - 50	0 (0.0)	1 (50.0)	
	>50	1 (50.0)	1 (50.0)	
Unemployed	<20	1 (50.0)	0 (0.0)	1.000
	20 - 30	0 (0.0)	0 (0.0)	
	31 - 40	0 (0.0)	0 (0.0)	
	41 - 50	0 (0.0)	0 (0.0)	
	>50	1 (50.0)	0 (0.0)	

Female traders aged 31 years and above formed majority of the female participants (91%). It was found that none of the female participants was unemployed (Table 3).

Table 3. Female wound type by occupation and age.

Occupation	Age	Post traumatic	Post cellulitis	*p*-value
		n (%)		
Professional	<20	0 (0.0)	0 (0.0)	1.000
	20 - 30	0 (0.0)	1 (100)	
	31 - 40	3 (100)	0 (0.0)	
	41 - 50	0 (0.0)	0 (0.0)	
	>50	0 (0.0)	0 (0.0)	
Artisan	<20	0 (0.0)	0 (0.0)	1.000
	20 - 30	0 (0.0)	0 (0.0)	
	31 - 40	1 (100)	0 (0.0)	
	41 - 50	0 (0.0)	0 (0.0)	
	>50	0 (0.0)	0 (0.0)	
Trader	<20	0 (0.0)	0 (0.0)	1.000
	20 - 30	1 (9.1)	0 (0.0)	
	31 - 40	3 (27.3)	1 (33.3)	
	41 - 50	4 (36.4)	1 (33.3)	
	>50	3 (27.3)	1 (33.3)	
Unemployed	<20	0 (0.0)	0 (0.0)	1.000
	20 - 30	0 (0.0)	0 (0.0)	
	31 - 40	0 (0.0)	0 (0.0)	
	41 - 50	0 (0.0)	0 (0.0)	
	>50	0 (0.0)	0 (0.0)	

Thirty-one (77.5%), 1 (2.5%), and 8 (20.0%) were positive cultures for bacterial, fungal and combined fungal and bacterial respectively for post traumatic chronic leg ulcers (**Table 4**).

Table 4. Culture positive samples.

		Bacteria	**Fungi**	**Fungi + Bacteria**	**Fisher exact test**	***p*-value**
			n (%)			
Post traumatic	Male	21 (84)	1 (4.0)	3(12.0)	3.10	0.213
	Female	10 (66.7)	0 (0.0)	5(33.3)		
Total		**31 (77.5)**	**1 (2.5)**	**8(20.0)**		
Post cellulitis	Male	3 (50.0)	0 (0.0)	3 (50)	3.75	0.153
	Female	3 (75.0)	1 (25.0)	0(0.0)		
Total		**6 (60.0)**	**1 (10.0)**	**3 (30.0)**		

Six (60.0%), 1 (10.0%), 3 (30%) were positive cultures for bacteria, fungi and combined fungi and bacteria respectively for participants with post cellulitic ulcers (Table 4).

There was no statistically significant association between sex and the organism cultured for post traumatic and post cellulitis wounds (p-value > 0.05).

Pseudomonas species were the highest bacteria isolated (61.5%) followed by *Proteus mirabilis* (12.3%). The least isolated bacteria were *Escherichia coli*, *Citrobacter species* and *Coliform*. *Aspergillus niger* was the highest fungal isolate (41.2%) while *Dermatophytes* and *Aspergillus flavus* were the least cultured fungi as shown in Table 5.

Table 5. Organisms cultured.

Organisms	*n* (%)
Bacteria	
Pseudomonas species	40 (61.5)
Proteus mirabilis	8 (12.3)
Enterobacter species	3 (4.6)
Escherichia coli	2 (3.0)
Citrobacter species	2 (3.0)
Klebsiella pneumoniae	5 (7.7)
Staphylococcus aureus	3 (4.6)
Coliform	2 (3.0)
Total	**65 (100)**
Fungi	
Aspergillus niger	7 (41.2)
Aspergillus flavus	1 (5.9)
Rhizopus	4 (23.5)
Dermatophyte	1 (5.9)
Yeast	4 (23.5)
Total	**17 (100)**

4. Discussion

Chronic leg ulcers are problematic and the major cause is trauma and compounded by infection or inappropriate treatment of acute traumatic wounds [2], similar to this current study as majority of the participants presented with post traumatic ulcers. According to Anning [15] the legs are exposed to injury of many kinds-kicks from the other foot, or that of other people, grazes, knocks, sprains and insect bites but women are less exposed to violence but their legs are not so well protected as those of men which may have accounted for the results of this study whereby women were the minority.

None of the participants had prior surgical treatment before presentation,

however, some took medications prior to presentation. 14 participants took haematinics, 18 participants took analgesics and 4 took oral antibiotics.

From the above discussion, occupation of both male and female may have contributed to their exposure to trauma. 64.7% of the male participants were artisans and 91% of the female participants were traders. This group of people are most often work in the open spaces which has soil and shrubs and may be exposed to all kinds of causes of trauma. Some of them also work bare footed in our environment. These cases of trauma were mostly neglected, untreated, self-treated or presented late resulting in cellulitis at time of presentation. Three of the five fungi seen in this study namely Aspergillus niger, Rhizopus and Aspergillus flavus usually live in soil, moist and humid environments (tropical and subtropical countries) accounting for the high prevalence of ulcers infected by the above three fungi (70.6% of all fungal infections). Trauma from various sources including thorns from plants and sharp objects in the soil can lead to infection by any of three listed fungi above. Dermatophytes and Candida can be normal commensals on human host on skin and hair respectively. 4 patients out of the 50 participants had self-medicated with antibiotics and possibly these could have led killing a range of bacteria leading to an overgrowth and subsequent infection by these two fungi. Mean age and preponderance of male participants is similar to the results of a study done by Jiburum and colleagues in Nigeria [16].

In this current study, bacterial isolates were the highest but significant fungi were also isolated. This finding indicates that fungi can coexist with bacteria and contribute to chronicity of leg ulcers and thus, should be considered when ulcers tend to be recalcitrant and non-healing. It was also found that the prevalence of fungal infection as well as both fungal and bacterial infection was comparable to a study done among type 2 diabetics [17] and also, in a hospital-based study by Krumpkamp and colleagues in the spectrum of antibiotic resistant bacteria and fungi isolated from chronically infected wounds in Ghana [18]. Similar results were obtained in previous studies by Dowd *et al.* and Raza *et al.* [19] [20]. This could be attributed to the fact that fungi and bacteria can coexist in chronic ulcers forming biofilms as reported by Watters *et al.* [10] contributing to the chronicity of ulcers.

The present study found 79% culture positivity for bacteria, similar to a Meta-analysis of wound infections done in Ethiopia [21].

Pseudomonas species were found to be highest isolated bacteria, a finding similar to a study done by Dadzie and colleagues in Ghana [22]. This could be attributed to the fact that *Pseudomonas species* is a ubiquitous organism which lives in the soil, water and can contaminate hospital equipment easily [23]. It could also be that since the leg is closer to the ground on which we walk, it is easier for the leg ulcers to be contaminated with *Pseudomonas species* as most of the participants come in public transport, or walk from a far distance to change their wound dressings and eventually get to the dressing area with very dirty wound bandages. It may also be due to where they had their wound dressings as *Pseudomonas* can

easily contaminate hospital equipment.

This study found *Pseudomonas aeruginosa* as the highest isolated organism followed by *Proteus mirabilis*, which is in contrast to a study done in Malaysia that found *Staphylococcus aureus* and *Pseudomonas aeruginosa* to be mainly implicated in chronic wound infections [24] which may be due to different hospital settings and the wound care practices employed.

Wound malodour is vital to both patients, care givers and clinicians. Wounds commonly associated with odour include exudating chronic wounds and is largely due to tissue degradation, necrosis or non-spore forming anaerobic bacteria that colonize cutaneous lesions, releasing compounds such as putrescine, cadavarine, unstable sulphur compounds and short chain fatty acids as metabolic end products. Foul odour is usually caused by gram negative bacilli. Different bacteria species have different odour: pungent and non-pungent [25]. From this study, only 24% participants had pungent smelling wounds but a significantly higher percentage (86%) had unhealthy wound bed (Unhealthy granulation is dark red in colour, often bleeds on contact, and may indicate the presence of wound infection. Excess granulation or over granulation may also be associated with infection or non-healing wounds) which could have accounted for the microorganisms cultured from the study [26].

The wound bed may be covered with necrotic tissue (non-viable tissue due to reduced blood supply), slough (dead tissue, usually cream or yellow in colour), or eschar (dry, black, hard necrotic tissue). Such tissue impedes healing. Since necrotic tissue can also harbour pathogenic organisms as evidenced from the results of this study, could have contributed to the microorganisms cultured [26].

Pain is a characteristic feature of many healing and non-healing wounds. Pain can be caused by both nociceptive and neuropathic stimuli. Intermittent pain is often related to dressing removal or recent application of new dressings and may necessitate the use of analgesia before the dressing is changed. Constant pain may arise as a result of the underlying condition, such as ischaemia, neuropathy, tissue oedema, chronic tissue damage, infection, or scarring [26]. Pain, is a common concern in patients with various types of chronic wounds as majority of the participants complained of moderate pain in their wounds [27].

5. Limitations

1) Anaerobic cultures from slough or wound swabs could not be done because the hospital laboratory in the study site didn't have that capability at the time this study was conducted.

2) Self-medication with antibiotics which was done by 4 participants could have altered the culture result of the would bed swab results and could have contributed to fungal infections like candida.

6. Conclusion

A significant number of post traumatic chronic leg ulcers had significantly high

burden of bacterial infections compared to fungal infections. The fact that fungi were cultured from some of the ulcers gives a strong indication that fungi also have a role in chronic ulcers and should also be considered in managing chronic leg ulcers especially in patients who work on soil, some bare footed. In summary, fungal infections should be considered and managed accordingly especially in this group of people.

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Conflicts of Interest

The researchers declare no conflict of interest with regards to the conduct and write-up of this manuscript.

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