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Research Article

Sensitivity of microorganisms isolated from diabetic sores to antibiotics.

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Abstract:

Introduction: One of the complications of diabetes mellitus (DM) is peripheral neuropathy which among other effects causes loss of coordination of muscle groups in the foot, formation of callus which separates and an unclear easily infected by microorganisms appears.

Objective: An attempt to establish the relationship between the bacteriology of the diabetic sores, the diabetic status of each patient, and the effect of the duo on sore healing was investigated.

Method: Swab specimens of sores from 48 patients of both sexes (38 diabetic and 10 none-diabetic) was obtained for culture and sensitivity analysis. Every patient was placed on therapy which involved daily sore dressing, oral antimicrobial administration, and appropriate individualized anti-diabetic treatment. Each patients sore was evaluated two weeks after cessation of antimicrobial therapy.

Results: A total of 48 isolates were recovered consisting of Staphylococcus aureus (62.5%), Echericia coli (20.8%), and Pseudomonas aeruginosa (16.7%). Antibiograms showed microbial resistance to ampicillin, penicillin G, tetracycline; partial sensitivity to chloroamphenicol, gentamycin, erythromycin, and septrin. Ciprofloxacin and ofloxacin were the most effective with as much as 100% sensitivity in vitro. The study found no disparity between diabetic sores and non-diabetic sores with regards to identity and sensitivity profiles of the isolated organism. The results of therapy and subsequent follow-up showed an overall 86.9% resolution of the sores 100% in the non-diabetic and 83.3% in the diabetics. A culture of the unresolved diabetic sores yielded no microbial growth indicating a corroboration between in vitro and in vivo sensitivity of isolated microorganism. Two of the diabetic with unresolved sore had attained normoglycermia.

Conclusion: The study revealed that although infection and inappropriate wound care impede sore healing, hyperglycemia was undoubtedly the Achilles' heel of patients with diabetic sores and concludes that optimum blood glucose control, effective wound care, and combating infection with antibiogram – based antibiotic therapy, are collectively of immense importance in the resolution of diabetic sores.

Keywords: Sensitivity, Microorganisms, Diabetic Sores, Antibiotics.

INTRODUCTION

Diabetic mellitus describes a group of disorders of varying etiology and pathogenesis usually characterized by elevated blood glucose concentration (hyperglycemia), reduced insulin action or insulin deficiency [1]. It is associated with both abnormalities of glucose, lipid, and protein metabolism and the development of both acute and long term complications [1]. Although multiple etiological factors are implicated in the disorder, the common denominator remains its association with insulin deficiency [2]. Insulin is the hormone secreted by the *beta* cells of the islets of Langerhans in the pancreas. In diabetic mellitus, insulin deficiency could be the context of co-existing insulin resistance[2]. Lack of insulin plays a primary role in the metabolic derangements linked to diabetes and hyperglycemia which when poorly controlled is responsible for diabetes – related pathological endpoints and complications [2,3,4]. World Health Organization (WHO) put the present population of diabetic patients at 140, million and predicts a doubling of the population by 2025[4]. The diagnosing of diabetes is best done with the fasting plasma

glucose after an overnight fast not less than eight hours. A positive diabetic value is ≥ 140mg /dl. However a random plasma value taken any time of the day with value ≥ 200mg/dl is positive [5].

Peripheral sensory neuropathy, peripheral vascular disease, and infections are the predominant factors in the development of sores in diabetic patients. Neuropathy result from vascular disease, occluding the vasa nervorum, deficiency of myinositol – altering myelin, and diminishing ATPase activity which is important in energy metabolism [6]. Ulcers develop because such patients lack protective sensation to warn them of injury to the foot, as a result, the foot may be subjected to repeated stress, puncture wounds may go unnoticed, foreign bodies may remain in subcutaneous tissues, and poor fitting shoes may continue to be worn until pressure necrosis develops. Neuropathy also causes loss of coordination of muscle group in the foot. Repetitive and excessive pressure leads to formation of callus, which eventually separate from the underlying dermis and an ulcer appears which easily gets infected with microorganism. In addition, hyperglycemia compromises the body's immunologic defense. Granulocyte adherence, chemotaxis, phagocytosis and bactericidal function are enhanced in the euglycemic state [6].

The Food and Drug Administration (FDA) of the United State defines an ulcer as a wound that has failed to proceed through an orderly and timely series of events to produce a durable structural, functional and cosmetic closure [7]. Diabetic foot ulcers fit squarely into the FDA definition. Various wound classification system are used that attempt to encompass different characteristics of a wound or ulcer such as site, depth, presence of neuropathy, infection, ischemia etc. Poor clinical outcomes are generally associated with infection, peripheral vascular disease and increasing wound depth [7,8].

Microbial infection is one of the key factors in the pathophysiology of diabetic foots sores. Infected wounds do not heal, and an understanding of the bacteriology of an infected wound is important in guiding antibiotic selection[7]. The diagnosis of infection in the diabetic foot is often subtle and difficult to reach. Since most patients with diabetic ulcers have various underlying degree of neuropathy, peripheral vascular disease and abnormalities of the immune system, the classical findings of serious infections such as inflammation, pain, fever, and elevated white blood count, are usually greatly diminished or absent. However, infection is suspected when there is purulent discharge together with some other local signs like warmth, erythema, lymphadenopathy, fever, a complaint of pain in an insensate foot or a sudden loss of glycemic control[8]. Generally, most infections of the diabetic foot are polymicrobial in nature. Smelling drainage and the presence of gas in the tissues often predict a mixed polymocrobial flora [9]. A combination of pathogens to be found at the site of infections include gram-negative and gram-positive aerobes as well as anaerobes [9].

Treatment of diabetic sores involves first, the treatment of the diabetic itself, the optimal management of other systemic factors (e.g. hypertension, hyperlipidemia, heart disease, renal insufficiency), combating infection with appropriate antibiotics, and proper wound care.

Microbial infection of wounds, sores, ulcers, etc is inimical to their healing and this applies to all of them irrespective of etiology [7,9].

In the case of diabetic sores, the situation is aggravated by such concomitant factors as neuropathy and vasculopathy which are offshoots of the diabetics [9,10].

Previous studies on this subject did focus primarily on microorganisms isolated from diabetic sores and their sensitivity to antibiotics. Such studies fell short of any comparisons between diabetic sores and non-diabetic sores under similar conditions [11,12]. The present study was aimed at investigating the identity and antibiotic sensitivity patterns of microorganism isolated from diabetic and non-diabetic sores. Hopefully, it will proffer some basis for the empirical use of delayed sores healing often associated with diabetics especially as this phenomenon relates with microbial and sensitivity patterns of isolated microorganism.

MATERIALS AND METHODS

Recruitment of patients.

Patient were recruited after satisfying standard criteria of the study which include a foot sore of ≥4cm in diameter with a significant level of pathogenic bacteria with or without concomitant hyperglycemia. The patients also had no history of intolerance to the drugs used in the study. They were able to take oral medication and gave a written consent to participate in the study after explanation of what the study will entail.

Forty eight patients (30 males and 18 females) aged between 38 – 56 years with foot sores attending hospital for the first time between January and September 2016 were identified by a physician consulting in the University of Uyo Teaching Hospital. The diabetes status of each patient was determined and a swab specimen of the sore taken. Vital signs: body temperature, body weight not less than 60kg, and blood pressure of each participant was recorded before commencement of treatment.

Drugs, Chemicals and Reagents

All drug products were obtained direct from the manufacturer's representative here in Uyo, and they were less than one year from the date of manufacture. The chemicals: MacConkey, Chocolate and blood agar were freshly prepared after the manufacturer's instruction.

Blood sugar determination.

The Fasting Blood Glucose levels was established by finger prick using lanset and the blood dropped on dextrostix reagent pad of one touch ultra strip inserted into microprocessor digital blood glucometer and the readings were noted. Two determinations were done but on separate days [13].

Culture, isolation, and identification of microorganisms from sores.

Swab specimen of sore was taken and a small area of each culture plate inoculated with it. A sterilized platinum loop was used to inoculate the specimen into each medium. The plates were incubated at 37^{o}C for 24 hours and inspected for microbial growth. If any plates showed no growth, a re-incubation for a further 24 hours was done. Organism growing on culture media were identified once isolated. Criteria for isolation and identification included colonial appearance, Gram stain reaction and other standard methods fully described by Cheesbrough, Cowan and Reginald[13,14,15].

Sensitivity testing

The plate diffusion technique was used. Isolates were streaked to cover a culture plate surface, allowed to dry, and sensitivity discs placed at spots on the agar surface. The plates were incubated overnight at 37^{o}C and reading of the inhibition zone diameters (IZD) were taken[13,14,15].

Follow-up

All patients in the study received the same standard of wound care with daily dressing using normal saline solution. Each patient also received ciprofloxacin 500mg orally twice daily for 14 days[16,17]. The diabetics received optimum individualized antidiabetic therapy in addition. Diabetic in the study were advised on the chronic nature of the disorder and the need for continuous therapy and routine checkups[17].

Statistical analysis

The data obtained were expressed as mean ± standard deviation (SD). Student t-test was used to assess statistical significance, values of $p<0.05$ were considered to be significant.

Ethical considerations

The study was conducted between January and September 2016 at the University of Uyo Teaching Hospital, after approval by the Ethics Committee of the University. The ethics approval number is UUTH/EC/vol.3/239 of January 8th. 2016.

RESULTS

Fasting plasma glucose test: Diabetic was defined according to the American Diabetic Association (ADA) criterion of Fasting Plasma Glucose (FPG) value of 126mg/dl or more on more than one occasion[18]. The fasting plasma glucose distribution on two different days for the patients is as summarized in table 1.

Table 1: Fasting Plasma Glucose (FPG) on two separate days

FPG	1st day result	2nd day result
70 – 12mg/dL	10	10
140 – 200mg/dL	24	20
>200mg/dl	14	18
Total Number of Patients	48	48

Microbial culture:

A total number of 48 isolates were obtained with each specimen yielding one microbial specie. There were no poly-microbial growths. *S. aureus* accounted for 24 of the isolate obtained from the 38 diabetic sores (63.2%), *E. coli* accounted for 8, (21%) and *P. aeruginosa* accounted for 6, (15.8%). For the 10 none-diabetic sores, *S. aureus* was 6 isolates (60%), *E.coli* 2 isolate (20%); and *P aeruginosa* 2 Isolate (20%). Table 2

Table 2: Microorganism isolates and percentage frequency

	Frequency	%Frequency
Diabetics		
S. aureus	24	63.2
E. coli	8	21
P. aeruginosa	6	15.8
Non –Diabetic		
S. aureus	6	60
E. coli	2	20
P. aeruginosa	2	20

***In vitro* sensitivity test.**

All the isolates of *S. aureus* were 100% sensitive to the flurorquinolones antibiotic ciprofloxacin and ofloxacin, 33.3% of *S. aureus* were sensitive to erythromycin but with reduced IZDs.

All *E. coli* isolate were sensitive to ciprofloxacin and ofloxacin. Sensitivity to gentamycin, septrin and erythromycin were 60%, 40% and 20% respectively. IZDs for the non-quinolones were less than for quinolones

All isolates of *P. aeruginosa* were sensitive to ciprofloxacin and ofloxacin. Sensitivity to gentamycin, chlroramphenicol, erythromycin and septrin were 50%, 25%, 25% and 29% respectively, Table 3. Table 4 gives a summary of the inhibition zone diameters (IZDs) to the various antibiotics.

From tables 3 and 4, it can be adduced that ciprofloxacin and ofloxacin were the antibiotic of choice for us in the circumstances under investigation. Ciprofloxacin was used in treatment of the patients.

Patient's treatment and follow-up

Thirty six diabetic and ten non-diabetic completed the study. Two diabetic patient had abandoned midway for no obvious reasons. All 10 non-diabetic and 30 diabetic sores were completely resolved during the study period. Resolution was based on the FDA standard of structural, functional and cosmetic closure [7]

The six unresolved diabetic sores yielded no microbial growth on culture. This implies corroboration between *in vivo* and *in vitro* sensitivity of isolated microorganisms and also indicated that no new wound infections had occur within the period of the study.

It was observed that four of the diabetic patients with unresolved sores had attained normolglycemia sequel to therapy which was ongoing. Only two diabetic still had

glycemia problems and investigation showed that they were slack in their dietary habit. They benefited least from the therapy. The non-resolution of the sores of the four patients who had attained normal blood glucose level and the two patient with hyperglycemia bring to question the reversibility of the pathophysiological scenario that precipitated the complications. The result of the follow-up are summarized in table 5.

Table 3:Microbial sensitivity as % of total number of isolates of each specie

Organism	Total number of isolates	Antiobiotics									
		AM	**PE**	**CH**	**TT**	**GE**	**ST**	**SP**	**OF**	**ER**	**CP**
S. aureus	45	Nil	Nil	Nil	Nil	Nil	Nil	Nil	100	33.3	100
E. coli	15	Nil	Nil	Nil	Nil	60	Nil	40	100	20	100
P. aeruginosa	12	Nil	Nil	25	Nil	Nil	Nil	20	100	25	100

Table 4: Sensitivity and inhibition zone diameters (IZD) in mm to the various antibiotics

Organism	Antibiotics									
	AM	**PE**	**CH**	**TT**	**GE**	**ST**	**SP**	**OF**	**ER**	**CP**
S. aureus *(IZD)*	R	R	R	R	R	R	R	S 19±0.82	S 11.20±0.90	S 19±0.80
E. coli *(IZD)*	R	R	R	R	S 10.8±0.56	R	S 11±0.64	S 17.6±1.36	S 9.8±0.75	S 17±1.36
P. aeruginosa *(IZD)*	R	R	S 8±2.12	R	S 13±0.70	R	S 13±0.70	S 18±0.70	S 16±0.70	S 17.5±1.12

Key: R = Resistant, S= Sensitive: Antibiotics: AM =Ampicilin, PE= Penicillin G, CH = Chloramphenicol, TT = Tetracycline, GE = Gentomycin,ST = Streptomycin,SP = Septrin , OF = Ofloxacin, ER = Erythromycin CP = Ciprofloxacin

Table 5: Follow-up results

Patients	**Number**
Sores were completely resolved	
Diabetics	30
Non-diabetics	10
Unresolved cases (Diabetics)	6
Abandoned treatment (Diabetics)	2
Total in the study	48

The culture of swabs taken from the six unresolved diabetic sores at the end of the 14 days monitoring period yielded no microbial growth after incubation for 24 hours at 37^{o} using the same medium (MacConkey agar).

DISCUSSION AND CONCLUSION

Infected foot ulcers are a common complication of diabetes mellitus. This study recorded a 100% infection prevalence rate in the study groups. Similar studies in whites have reported various data including a rate of 95% [19]. The high incidence in the present study can be attributed to poverty, ignorance and poor hygiene among the patients studied. Diabetic foot disorders are reported to find expression mostly in the low socioeconomic class [20] .

The isolation of *Staphlococcus aureus* (62.5%), *E coli* 20.8% and *Psendomonas aeruginosa* (16.2%) is in consonance with bacterial flora typical of infected wounds, sores, ulcers and boils etc including diabetic sores [13]. Previous studies of diabetic sores revealed a poly-microbial morphology with the isolation of *Proteus* and *Klebsiella* species alongside those found in the present study [9] . However, and to the contrary, our study found a mono-microbial pattern of infection and no differences existed between isolates from diabetic and non-diabetic patients in terms of morphology

The microbiological features of diabetic sores vary according to the tissue infected but the distribution of organisms is the same as in an individual without diabetes except in chronic osteomyelitis. In chronic osteomyelitis, a sequestrum and involucrum form representing islands of infected bone. Bone fragments that are isolated are devoid of blood supply and administered antibiotic drugs cannot penetrate the devascularized fragments. Therefore antibiotic therapy alone cannot cure patients with chronic osteomyelitis without surgical debridement to remove these isolated infected elements [9,21].

Ciprofloxacin and ofloxacin (both fluroquinolones) were the most active agents tested with *in vitro* activity against all isolates in the present study, there was significant reduction $p<0.05$. This finding is in agreement with some earlier studies and recommendations [11]. The microorganisms were resistant to commonly used antibiotic. Ramani reported a similar experience which necessitated the use of gentamycin and metronidazole to optimize results[20].Commonly used antibiotics such as ampicillin, tetracycline, septrin, chloramphenicol etc are readily available and more affordable and so are widely subject to unguided use. This could be responsible for the emergence of resistant microbes to them [20]. With regards to susceptibility to antibiotics tested, this

study found no difference between isolates from diabetic and non-diabetic patients.

The response of patients to treatment which encompassed daily wound dressing, oral administration of ciprofloxacin (500mg bid) for 14 days, and optimum glycemic control (for the diabetics) was quite remarkable and showed a relationship between the diabetic status of a patient and the possible outcome of foot infection. Within the 14 days surveillance period, the study discovered that 40 of the patients (30 diabetics, all 10 non-diabetics, had a complete healing of their lesions. Of the remaining eight diabetics, six had problem bordering on inadequate glycemic control because of allergy to insulin and poor response to oral hypoglycemic drugs. Their sores did not heal. This was remarkable and particularly so when cultures of the unhealed sores done after the surveillance period yielded no microbial growth. The finding that the swab specimens from the yet to be healed diabetic sores cultures yielded no growth implied a corroboration between the *in vitro* sensitivity of the organisms and clinical response. Another unmistakable implication was the fact that microbial infection alone could not be responsible for the non-resolution of the sores but that the pathophysiology of diabetes played a critical role.

Earlier studies fell short of any comparison between diabetic and non-diabetic sores. The common denominator in the present study is neither the flora nor the susceptibility patterns of the pathogens isolated but the glycemic status of the patients involved.

This study had revealed that the restoration and/or maintenance of the euglycemic status of a patient are of paramount importance in wound healing. Infections in patients with diabetes generally are more severe and take longer to cure than equivalent infections in other people and these include wound infections or sores. Infections in diabetics are difficult to treat because these patients have impaired microvascular circulation which limits the access of phagocytic cells to the infected area and result in poor concentration of antibiotics in the infected tissues [9,22] . The present study confirmed not only the delayed healing associated with infected sores including those of diabetics, but also the similarity in the bacteriology of both diabetic and non-diabetic sores.

The similarity was however jettisoned when the diabetic remained uncontrolled and even when adequate wound care and antimicrobial therapy are mounted. The treatment of the diabetic remain a sine-qua-non for sore resolution in the diabetics.

Based on these findings, the study recommends prompt initiation of therapy with ciprofloxacin or ofloxacin, coupled with adequate wound care and tight blood glucose control for diabetics who have developed sores. At present, a total reversal of the chronic complications of diabetes mellitus can neither be achieved nor guaranteed. It is wisdom to pursue measures to abort the development of any such complications.

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