ORIGINAL ARTICLE

Pseudomonas as trespassers in diabetic foot infections: More questions and fewer answers

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Abstract

Objective: To assess microbiological isolates from diabetic foot ulcers presented at a tertiary care teaching hospital.

Methods: The prospective observational study was conducted at department of Orthopaedics, Liaquat National Hospital Karachi, from January 2009 to October 2011, and comprised patients with diabetic foot ulcers (Wagner grading 1-4). Grade 0 and 5 were excluded. Wound swabs or tissue biopsies were taken before the induction of antibiotic prophylaxis according to the standard aseptic protocols. SPSS 16 was used for statistical analysis.

Results: Of the 250 patients in the study, 151(60.4%) were male with an overall mean age of the subjects of 46±8.1 years. Mean duration of diabetes was 14±6.7 years. Based on Wagner's grading, 90(36%) patients had grade 1, 58(23.2%) grade 2, 97(38.8%) grade 3, and 5(2%) grade 4. The most common isolates were polymicrobial in 90(36%) patients and pseudomonas aeruginosa in 87(34.8%). Resistance antibiogram for pseudomonas aeruginosa showed sensitivity against Imipenem, Aztreonam, Ceftazidime, Ciprofloxacin and Gentamicin.

Conclusion: The isolation of pseudomonas aeruginosa gives an idea about the recent microbial spectra from our region and should prompt the need for multi-centre studies from South Asia to devise treatment guidelines for this part of the world.

Keywords: Diabetic Foot, Infection/microbiology, Gram-negative bacteria. (JPMA 64: S-112 (Suppl. 2); 2014)

Introduction

Pakistan is among the top 10 sweet nations on World Health Organisation's (WHO) list with an estimated 5.2 million people with diabetes in year 2000 that makes up 12% of the adult population having diabetes.[1] Diabetes is associated with myriad complications with diabetic foot ulcers (DFUs) being a worrisome and costly complication in the present-day scenario. DFUs provide a niche for the exuberant growth of microbes that may lead to diabetic foot infections (DFIs) and, if not treated well, the amputation, which is considered the most common cause of non-traumatic lower extremity amputation.[2]

Lower limb amputation has variable prevalence across the globe. In the United States, the rate of lower extremity amputation is 8.6 per 1000 diabetic population per year, while in Pakistan two different studies reported 21% and 48%.[3,4] The French OPIDIA study found that risk factors like location and severity of wound along with osteomyelitis and peripheral vascular disease are poor prognostic factors and despite the rigorous medical treatment 48% DFUs will end up having an amputation.[5] Another study[6] while discussing the choice of empirical therapy for DFI concluded that severe infection should be treated with systemic therapy, coverage should be given to Methicillin Resistant Staphylococcus Aureus (MRSA), management should be targeted against gram-negative bacteria and ischaemic ulcer should have coverage against anaerobes. Patient education and good podiatry can decrease the incidence of DFUs and resultant DFIs by 50%[2] but in a developing nation like Pakistan the goal is difficult to achieve as DFU and DFI awareness is still in infancy.

It is well known that bacterial isolates differ in different patients, hospitals as well as geographical locations. Therefore, the choice of optimal initial therapy depends upon the knowledge of the microbiological spectra of the particular person or place. A study[7] found that gram-negative infections are three times common in diabetics compared to the non-diabetics. In particular, pseudomonas should not be considered an insignificant entity when considering DFIs as their knowledge may help in halting the progression to future amputations.[8] Most DFIs are polymicrobial in nature and the emergence of resistance is a major threat since developing countries have limited resources, and treatment of such infections pose a great threat to the already destroyed economies in developing world.

Due to the changing infectious aetiology from gram-positive to gram-negative organisms, particularly pseudomonas, in this part of the world, the current study was planned to assess the common isolates in our setup

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so that empirical therapy can be instituted in time to halt DFIs' progress towards amputations.

Patients and Methods

The prospective observational study was conducted at department of Orthopaedics, Liaquat National Hospital Karachi, from January 2009 to October 2011, and comprised patients (Wagner grade 1-4) with diabetes. After approval from the institutional ethical review board, both verbal and written consent was obtained from the participants. All patients had primary concern of active DFUs and were assessed for clinical infection according to the Infectious Diseases Society of America (IDSA) guidelines.[9] Wagner grading[10] was given to all patients by two separate examiners who were not involved in the study (degree of agreement= 0.90). Grade 0 and 5 were excluded. Swabs were taken for bacterial cultures before the induction of antibiotic prophylaxis with Metronidazole and Augmentin (Co-Amoxiclav). The swabs were taken after thorough washing with warm normal saline to avoid culture contamination with colonised bacteria. After all aseptic techniques, swabs were taken from deeper tissues as well as for all surgical debridement candidates, and the biopsied tissues were sent for cultures. The specimens were transported to the laboratory in sterile ice-cold environment. The cultures and susceptibility testing were done according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.[11-13] Mean and standard deviations (SDs) were calculated for age, duration of diabetes and duration of ulcers. Frequency and percentages were calculated for foot involvement, Wagner's grading, bacterial isolates and their relative susceptibilities to antibiotics. SPSS 16 was used for statistical analysis.

Results

Of the 250 patients in the study, 151(60.4%) were male with an overall mean age of the study subjects of 46±8.1 years. Mean duration of diabetes was 14±6.7 years. Right foot was involved in 167(66.8%) patients. Based on Wagner's grading, 90(36%) patients had grade 1, 58(23.2%) grade 2, 97(38.8%) grade 3, and 5(2%) grade 4. The most isolated species were polymicrobial in 90(36%) patients, pseudomonas aeruginosa in 87(34.8%)beta haemolytic streptococci 15(6%), and Klebsiella species in 12(4.8%).There were no isolates in 32(12.8%) patients (Table-1).

Of the patients with pseudomonas, 83(33.2%) had grade 3 diabetic foot, and 4(1.6%) had grade 4.

Resistance antibiogram for pseudomonas aeruginosa showed sensitivity against Imipenem, Aztreonam, Ceftazidime, Ciprofloxacin and Gentamicin (Table-2).

Table-2: Resistance Antibiogram of Pseudomonas aeruginosa.

Antibiotic	Number of Resistant Samples n=87	Percentage*
Ampicillin	87	100%
Co-Amoxiclav	87	100%
Piperacillin	58	66.67%
Imipenem	8	9.2%
Aztreonam	10	11.5%
Cefotaxime	81	93.1%
Ceftazidime	14	16.1%
Ciprofloxacin	11	12.6%
Gentamicin	43	49.4%
Amikacin	53	61%
Tobramycin	79	90.8%
Trimethoprim	87	100%
Sulphamethoxazole	87	100%

*Percentages were calculated by dividing the number of isolates resistant to certain antibiotic by total number of isolates (87).

Discussion

In our study, pseudomonas was the most common

Table-1: Common bacterial isolates and their relative frequencies in respective Wagner's grading.

Bacterial Isolate#	N+ (%)	Wagner Grading*			
		I	II	III	IV
Polymicrobial (Staphylococci++ and Streptococci± anaerobes)	90 (36%)	90	-	-	-
Pseudomonas aeruginosa	87 (34.8%)	-	-	83	4
Beta hemolytic streptococci	15 (6%)	12	3	-	-
Klebsiella species	12 (4.8%)	-	-	12	-
Escherichia Coli	7 (2.8%)	4	3	-	-
Bacteroides	6 (2.4%)	-	-	6	-
Actinomycesisraelli	1 (0.4%)	-	-	-	1
No isolates	32 (12.8%)	29	3	-	-

* The number in each column of Wagner grading indicates the number of patients (not percentages).
+ Indicates the number of patients affected with the respective bacteria.
++ indicates the S.aureus bacteria of staphylococcus family
# indicates the bacterial genera unless specified species is given.

monomicrobial isolate, while staphylococci were mostly found in polymicrobial infections. Our findings support the results of previous studies where gram-negative rods were the commonest isolates. A recent study from Kuwait[14] found that enterobacteracieae were the commonest isolates from DFIs and it found that polymicrobial infections were more common than monomicrobial infections. The study found pseudomonas and staphylococci as second and third common species in DFIs. In contrast, two recent Indian studies have shown preponderance of gram-negative aerobes.[15,16] One study[15] recovered 183 isolates from 80 ulcer specimen, of which 28.7% were gram-negative and 13.8 gram-positive organisms and the other study found gram-negative pathogens in 57.6%.[16] A Nepalese study[17] found S.aureus and P.aeruginosa as the most common isolates. In contrast, a recent western study[18] found that 40% isolates were polymicrobial. Gram-positives were more commonly isolated than gram-negative, and staphylococci were the most common monomicrobial isolates (29%). Another Kuwait study reported S.aureus to be the commonest isolate (38.4%) followed by P.aeruginosa.[19] The predominance of gram-negative rods might have resulted from the influence of sanitary habits like use of water for peri-anal wash after defecation which can contaminate the hands with faecal flora and thus are proposed to be responsible for increased gram-negative infections in our population.[20]

Treating a DFI with empirical antibiotics is influenced by severity of infection, route of administration, co-morbid and bacterial isolates, while specific therapy can improve the outcomes when directed against the isolated organism.[20] A recent systematic review[21] regarding treatment protocols have advocated that treatment should be devised in accordance with factors like the severity of the infection, recent gram stains and culture reports along with history of recent antibiotic treatment, previous infection with resistant organisms and patient factors, while this treatment should be modified according to the isolated organisms. It advocated the use of systemic broadspectrum antibiotics for moderate-to-severe infections and therapy should be directed against S.aureus, pseudomonas and other gram-negative rods keeping in mind their high resistance potential. One study[20] found the persistence of E.coli and pseudomona infections in third specimen for culture in 20%, which may be attributed to increasing resistance despite medical therapy. Another study[16] also isolated pseudomonas as the commonest isolates and 44% of these were multi-drug resistant. It found Colistin to be more than 90% sensitive against pseudomonas. Ciprofloxacin was found to be sensitive against P.aeruginosa in one study, similar to our findings.[18] Imipenem, Meropenem, and Cefepime were effective against gram-negative organisms.[19] It has been found that P.aeruginosa are dangerous pathogens and are commonly resistant to antibiotics.[15] Our study found pseudomonas to be sensitive against Imipenem, Aztreonam, Ceftazidime, Ciprofloxacin and Gentamicin. The presence of pseudomonas always implies severe infection and should never be taken lightly as they can cause sepsis and amputations since they are very difficult to treat due to their emerging resistance against a wide range of antibiotics.[22]

Previous studies[23] have found that male gender has greater propensity towards getting lower extremity amputation after DFI. The majority of the patients in our study were males. Though we did not follow the patients for amputations, most had advanced Wagner grading with an increased risk for getting an amputation. The mean age of our patients was 46 years and these patients were younger compared to those in other studies. One study[24] compared the risk factors in Germany, India and Tanzania, three different countries from Europe, Asia and Africa. It found that mean age was significantly higher in German patients (70.5 years), while Indian and Tanzanian patients presented in their 50s. The study found that this variation reflects the difference in age of onset of diabetes among these countries. The same study found that duration of diabetes was different among these countries. With Tanzanian patients having earlier onset of DFUs compared to German and Indian patients. This difference might reflect the quality of medical care being provided in these countries which delays the diabetic foot ulceration and further amputations. In our study the mean duration of diabetes was 14 years.

Majority of our patients were in Wagner grade 3, while a study from India found that most of their patients presented in grade 2[25] and a recent study[26] found that risk factors for lower extremity amputations like Ankle Brachial pressure Index (ABI), Albumin or Estimated Glomerular Filtration Rate (eGFR) were almost exclusively found in grade 3 patients.

In our study, 83 patients with pseudomona infection had grade 3 diabetic foot and 4 had grade 4, indicating that pseudomona infections were more severe and may make the patient prone to future amputations. Presentation of such severe cases showed the lack of liaison between multidisciplinary teams in primary and secondary care hospitals with additional factors like faith-healing and undetected diabetes aggravating the situation in resource-poor countries.

Since more severe grading was associated with

P.aeruginosa infections, we can say that pseudomona is associated with more severe infections and ultimately results in amputations. Diabetic foot is a real emergency and a good therapy in form of appropriate antibiotic coverage can halt progression to limb amputation. We still lack evidence as microbiological spectra change over time and this has left the medical community with a topic where many questions still have fewer or no answers.

Conclusion

Pseudomona is increasingly continuing to cause infection in diabetic foot ulcers in our part of the world. However, our single-centre observational study is not enough to develop treatment protocol for this serious pathogen eradication. More data is required in order to correctly address this problem so these gram-negative isolates can be cured in an appropriate manner.

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