CLINICOMICROBIOLOGICAL STUDY OF DIABETIC FOOT ULCERS

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ABSTRACT

BACKGROUND

Type 2 diabetes mellitus is a chronic disease caused by a combination of lifestyle and genetic factors affecting all organs in the body. Foot ulcers are one of the common and serious long-term complications of diabetes leads to recurrent and chronic infections, which results in limb loss when treatment is delayed.

The aim of this study is to find out the clinical outcome and microbiological profile in patients admitted with diabetic foot ulcers.

MATERIALS AND METHODS

The study conducted between November 2008 to November 2009 over 50 patients with history of foot ulceration and diabetes. 50 patients were admitted with diabetic foot ulcer over a period of one year between November 2008-2009. They were studied after getting written consent. A predesigned pro forma was used to get the parameters comprising age, gender, duration, type of diabetes mellitus, presence of neuropathy, nephropathy (serum creatinine, urine albumin), retinopathy (screening funduscopy by ophthalmologist).

RESULTS

Among 50 patients admitted and treated for diabetic foot ulcers with mean stay of 18 days, 29 (58%) had complete healing on conservative management, 18 (36%) underwent minor amputation (toes), 3 (6%) had major amputation (below knee/above knee). No mortality among the study groups encountered. Gram-negative aerobes E. coli (36%), Pseudomonas (52%), Klebsiella (28%), Proteus vulgaris (20%) and Acinetobacter (16%) were most frequently isolated followed by gram-positive aerobes MRSA (14%), Enterococcus (6%), Strep pyogenes (4%) and no anaerobic growth.

CONCLUSION

Diabetic foot infections are frequently polymicrobial and predominantly gram-negative aerobic bacteria at presentation. Multi-drug resistance pseudomonas aeruginosa and MRSA in diabetic foot ulcer is at its emergence and life threatening. Initial aggressive multimodal approach with surgical intervention, culture specific and sensitive targeted combined broad-spectrum antibiotics decreases the morbidity and mortality. Early screening for diabetes, tight glycaemic control decreases the prevalence of risk factors for diabetic foot ulcer related amputations and enhances the quality of life.

KEYWORDS

Diabetic Foot, Foot Ulcers, Diabetic Ulcers, Diabetic Foot Infection.

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BACKGROUND

Diabetes mellitus is a chronic disorder affecting a large segment of population and is a major public health problem.[1] The most common skin and soft tissue manifestation of diabetes mellitus are protracted wound healing and skin ulcerations, especially in the lower extremities and are almost synchronous with disease.[1] Diabetic ulcers are the most common cause of non-traumatic lower extremity amputations in the industrialised world. Fifteen percent (15%) of people with diabetes will develop a foot ulcer at some time during their life and 85% of major leg amputations begins with a foot ulcer.[2] The risk of lower extremity amputation is 15 to 46 times higher in diabetics than in persons who do not have diabetes mellitus.[3] They are the major cause of morbidity and mortality in developing countries such as India and have increased dramatically in recent years.[4] The peripheral sensory neuropathy interferes with the normal protective mechanism and allows the patient to sustain repeated minor trauma mostly without their knowledge.[5] The peripheral arterial disease and poor wound healing impede resolution of minor breaks allowing them to become enlarged and infected. 41% of patients with Peripheral Arterial Disease (PAD) have diabetes.[6] Early recognition of lesions and prompt initiation of appropriate therapy as well as aggressive surgical debridement of

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necrotic tissues and a modification of host factors like hyperglycaemia, concomitant arterial insufficiency are equally important for successful outcome.[7] Initial antimicrobial therapy in treating diabetic foot ulcers is empiric as there is variability in prevalence of common bacterial pathogens isolated in different studies. It is well documented in literature that diabetic ulcers are polymicrobial in nature. Lately, there are reports of increase in the multi-drug resistant organism both in hospital settings and in community. Appropriate care of diabetic foot requires recognition of the most common risk factors for limb loss. Many of this risk factors can be identified based on specific aspects of the history and a brief, but systematic examination of the foot. Recognition of risk factors, preventive foot maintenance and regular foot examinations are essential in preventing foot ulcers in patients with diabetes. When foot ulcers develop despite preventive measures, a systematically applied regimen of diagnosis, classification, coupled with early and appropriate treatment, should help to reduce the tremendous personnel and social burden of diabetes-related amputations.[8] This clinical study is being undertaken to find out the most common causes and clinical manifestations and common organisms of patients with diabetic foot ulcers admitted in our institution.

Aims and Objectives

1. To find out the clinical outcome in patients with diabetic foot ulcers.
2. Microbiological study to find out the common organisms in diabetic foot ulcers.

MATERIALS AND METHODS

50 patients were admitted with diabetic foot ulcer over a period of one year between November 2008-2009. They were studied after getting written consent. A predesigned pro forma was used to get the parameters comprising age, gender, duration, type of diabetes mellitus, presence of, neuropathy, nephropathy (serum creatinine, urine albumin), retinopathy (screening funduscopy by ophthalmologist). Detailed history, clinical examination including the ulcer, infection (redness, oedema, discharge, gangrene) along with relevant baseline blood investigations (Hb, PCV, urea, creatinine, RBS, FBS, PPBS, HbA1c, ECG, urine routine and microscopy and radiography of the infected part to rule out osteomyelitis.

Inclusion and Exclusion Criteria

To be included in the study, subjects were required to be 18-80 years of age and to have a history of foot ulceration, a diagnosis of diabetes, the ability to provide informed consent and ankle brachial indices >0.79 to 1.1. Subjects were excluded if they are non-diabetics had open ulcers or open amputation sites, active osteoarthropathy, postoperative open wounds, traumatic wounds, severe peripheral vascular disease (ABPI ≤0.79) dry gangrene, dementia or other conditions that would preclude active participation based on the investigators judgment.

Microbiology- After admission, the infected site is cleaned with saline, pus aspirated using 14 gauge needle and send for anaerobic culture. Wound swab for aerobic culture taken after incision and drainage. In patients with ulcer, the surface cleaned with saline and debrided tissues were sent for cultures.

The culture specimens were inoculated in 5% sheep blood agar and Mac-Conkey agar and incubated for 24 hours. Antibiotic sensitivity testing done using Kirby-Bauer disc diffusion method following CLSI guidelines 2007. The used antimicrobial discs are penicillin (15 µg), gentamycin (10 µg), Amikacin (30 µg), cefepime (30 µg), chloramphenicol (30 µg) cefotaxime (30 µg), ceftazidime (30 µg), piperacillin (100 µg), imipenem (10 µg), ciprofloxacin (5 µg), tetracycline (30 µg) and vancomycin (30 µg). MRSA detection done using cefoxitin (30 µg) disc, a zone of more than 20 mm inhibition is considered significant and less considered in significant, ESBL production in gram-negative organisms detection done using ceftazidime (30 µg) disc.

Neuropathic foot assessment using the 10-g Semmes-Weinstein monofilament. The monofilament is applied to various areas on the foot mainly at the dorsum of the great toe just proximal to the nail bed and plantar surface of the big toe, metatarsal heads and heel. Enough pressure is applied to bend the nylon filament. Patients are asked to identify the location of the filament with their eyes closed. Those who cannot feel the monofilament are diagnosed to have the loss of protective sensation and sensory neuropathy.

Ethical Consideration- Ethics committee approval obtained.

RESULTS

Condition	Male (n=34)	Female (n=16)
Hypertension	30	10
Renal disease	31	15
Retinopathy	21	8
Neuropathy	19	8
Cardiovascular disease	6	1
Cerebrovascular disease	4	2

Table 1. Comorbid Conditions Associated with Diabetes (n=50)

Mild (ABPI 0.8-0.9)		Moderate (0.5-0.79)		Severe (≤0.5)	
Male (n=34)	Female (n=16)	Male (n=34)	Female (n=16)	Male (n=34)	Female (n=16)
3	0	0	0	0	0

Table 2. Peripheral Vascular Disease

PVD- Peripheral vascular disease; ABPI- Ankle brachial pressure index.

Parameters	Male (n=34) Mean ±Σ (SD)	Female (n=16) Mean ±Σ (SD)
Age (years)	53.64 ± 10.38	51.29 ± 9.77
Body mass index	25.01 ± 4.41	28.48 ± 5.44
Duration of diabetes	7.76 ± 03.91	7.30 ± 3.65

Systolic blood pressure	128.39 ± 20.84	138.14 ± 23.24
Diastolic blood pressure	78.86 ± 10.94	82.47 ± 12.80
Serum creatinine (mg/dL)	1.57 ± 0.38	1.69 ± 0.59
Fasting plasma glucose (mg/dL)	193.42 ± 86.43	182.18 ± 76.65
Random plasma glucose (mg/dL)	247.08 ±86.63	234.18 ± 78.87
HbA1c	10.13 ± 2.67	9.77 ± 2.47

Table 3. Mean and Standard Deviation of Physical and Biochemical Parameters by Gender

∑-Standard deviation; HbA1c-Gylcated haemoglobin.

Age Group (Years)	No. of Cases	Male (n)	Female (n)
18-30	0	0	0
31-40	3	2	1
41-50	13	12	1
51-60	16	06	10
61-70	14	11	3
71-80	4	3	1

Table 4. Age Frequency

Sex	Total	Type of Diabetes
Male	34	Type 2 DM
Female	16	Type 2 DM

Table 5. Sex Ratio and Type of Diabetes

Duration (Years)	No. of Cases	Surgery	Conservative
<6 months	3	0	2
6 months-1 year	0	0	1
1 year-5 years	14	13	1
6 years-10 years	21	19	2
11-15 years	10	9	1
16-20 years	2	2	0

Table 6. Duration of Diabetes and Outcome

	Wagner's Grade					
	0	1	2	3	4	5
18-30	-	-	-	-	-	-
31-40	-	-	-	2	1	-
41-50	-	-	4	6	2	-
51-60	-	2	4	10	4	1
61-70	-	-	3	5	2	-
71-80	-	-	-	2	2	1

Table 7. Age and Grade of Ulcer (n=50)

Gram-Positive Prevalence, (n)%	Gram-Negative Prevalence, (n)%
MRSA (7) 14%	Pseudomonas aeruginosa (26) 52%
Staph aureus (12) 20%	E. coli (18) 36%
Enterococcus (3) 6%	Proteus vulgaris (10) 20%
Strep. pyogenes (2) 4%	Acinetobacter (8) 16%
Alpha-haemolytic streptococci (1) 2%	Klebsiella pneumonia (14) 28%

Table 8. Bacterial Pathogens Isolated from 50 Diabetic Foot Ulcers Number of Isolates and Percentage

Antibiotics	S. aureus n (%) n=Number of Sensitive Strains
Amikacin	4 (33.33)
Amoxicillin + clavulanic acid	3 (25)
Cefepime	3 (25)
Chloramphenicol	10 (83.33)
Ciprofloxacin	2 (16.67)
Gentamycin	4 (33.3)
Penicillin	0 (0)
Tetracycline	1 (8.34)
Gatifloxacin	8 (6.67)
vancomycin	6 (50)

Table 9. Antimicrobial Susceptibility Pattern of Staphylococcal Isolates from Diabetic Foot Ulcers (n=12)

Antibiotics	E. Coli (n=18)	K. Pneumonia (n=14)	P. Vulgaris (n=10)	Pseudomonas (n=26)
Ciprofloxacin	9 (50)	7 (50)	6 (60)	7 (26.92)
Ceftazidime	11 (61.1)	4 (28.6)	6 (60)	12 (46.15)
Piperacillin	12 (66.7)	6 (42.9)	5 (50)	23 (88.46)
Cefotaxime	10 (55.6)	3 (21.4)	4 (40)	4 (15.38)
Amikacin	11 (61.1)	9 (64.3)	3 (30)	15 (57.69)
Imipenem	15 (83.3)	9 (64.3)	5 (50)	26 (100)
Chloramphenicol	4 (22.2)	11 (78.6)	-	9 (34.62)
Gentamycin	10 (55.6)	7 (50)	4 (40)	4 (15.38)
Gatifloxacin	11 (61.1)	9 (64.3)	4 (40)	18 (69.23)
Tetracycline	2 (11.1)	-	-	3 (11.53)

Table 10. Antibiotic Sensitive Pattern of Aerobic Gram-Negative Bacteria

Organisms	Penicillin	Gentamycin	Amikacin	Amoxyclav	Cefoperazone	Ceftazidime	Imipenem
Staph. Aureus	100%	37.5%	-	87.5%	-	-	-
Pseudomonas aeruginosa	-	60%	80%	-	100%	100%	0%
Escherichia coli	-	25%	25%	25%	-	-	0%

Acinetobacter Spp.	-	33.3%	33.3%	-	-	-	0%
Proteus mirabilis	-	0%	0%	0%	-	-	0%

Table 11. Antimicrobial Resistance Pattern of Common Isolates from Diabetic Foot Infections

Outcome	Number (n) = 50
Complete healing	29 (58%)
Amputation	18 (36%)
Not healed	3 (6%)
Patient death	0 (0%)

Table 12. Clinical Outcome of in Diabetic Foot Ulcer Patients (n=50)

DISCUSSION

Diabetes currently affects more than 194 million people worldwide and the figures expected to reach 333 million by 2025 with maximum burden falling upon developing countries.[9] India is considered as the "diabetic capital of the world." Currently, India alone accounts for 35 million people harbouring the disease. Diabetic foot ulcers are the most common complication requiring hospitalisation among diabetic patients. The aetiological agents and the antibiotic susceptibility pattern is important to treat patients.

Comorbid Conditions- Diabetes-related comorbid condition (Table 1) decreases the quality of life and increases the morbidity. Most diabetics have one coexisting condition and 40% have at least three or more. The common comorbidities, hypertension (80%), obesity, neuropathy (54%), nephropathy (90%), retinopathy (58%), stroke (12%) and cardiovascular disease (14%) are concordant conditions increases the risk of poor outcomes. Nearly, 32% of the patients had irregular treatment for diabetes mellitus. Out of 50 patients, known diabetics were 35 (70%). 70% patients among them were on oral hypoglycaemic medications, 20% on insulin with oral hypoglycaemic agents. As much as 10% were unaware and not on treatment for diabetes previously.[10] Screening and timely intervention for parameters like blood pressure, blood glucose, renal function, body mass index, glycated haemoglobin (HbA1c) as done in this study and optimising them to desired levels will bring down the morbidity related to micro and macrovascular disease[11] (Table 3).

Age/Socioeconomic Status- Peak incidence of complications due to diabetes occurred in 51-79 years age group. Maximum number of patients in this group underwent amputation. It is surprising to know that lesion occurred even in young people with diabetes mellitus. There were 3 such cases in 31-40 years group. All Wagner grade 1 and 2 lesions treated conservatively. 92% patients in this study belonged to rural poor socioeconomic group.[12] This is similar to the reports from other studies done in rural South India.

Diabetic Peripheral Neuropathy- More than 60% of diabetic foot ulcers are the result of underlying neuropathy.[13] In this study, using non-invasive quantitative assessment with Semmes-Weinstein monofilaments, 27 (54%) patients were found to have neuropathy. The male-to-female distribution was 70.37% (19) and 29.82% (8), respectively. These patients with neuropathy varied from 35-80 years of age. Majority had a history of diabetes more than 7 years.[14] This shows that the peripheral neuropathy is common in longstanding diabetic patients. In South India, a similar high prevalence of peripheral neuropathy 61.9% among the diabetics was revealed by Ramachandran et al.[15] Neuropathy is also more prevalent among people who had higher systolic and diastolic blood pressure.[16]

Peripheral Vascular Disease- The prevalence of peripheral arterial disease associated with diabetes is 3.2% to 18.1% shown in the studies conducted in India by Mohan et al[17] and Agarwal et al.[18] In our study, the presence of peripheral arterial disease has been cited by many authors as a risk factor for amputations in diabetics. But, several other independent risk factors like severe soft-tissue infection,[19] joint infections, osteomyelitis, wet gangrene, digital deformity, renal disease, neuropathy, fasting blood sugar >200 mg/dL[20] and HbA1c >10.[21] History of previous amputation[22] are cited in various studies as individual risk factors for lower extremity amputation in absence of PVD with inconsistencies in the results. However, our study does not have significant association of amputations with PVD as we excluded the patients who had Ankle Brachial Pressure Index (ABPI) less than 0.79 on initial screening and among the inclusion patients 3 (6%) had mild arterial disease with ABPI (0.8-0.9), they were predominantly male.

Microbiology- In our study of 50 patients, 101 bacterial isolates were obtained. Gram-negative bacteria were frequently found than gram-positive isolates. The most prevalent aerobic gram-negative isolates were Pseudomonas aeruginosa (26) 52%, E. coli (18) 36%, Klebsiella pneumonia (14) 28%, Proteus vulgaris (10) 20% and Acinetobacter (8) 16%. Aerobic gram-positive isolates were Staphylococcal aureus (12) 20%, MRSA (7) 14%, Enterococcus (3) 6%, Streptococcus pyogenes (2) 4% and Alpha haemolytic streptococci (1) 2%. In our study, the infection is significant with mixed and polybacterial isolates. A study conducted by Ravisekar et al[23] in AIIMS, New Delhi, showed that gram-negative aerobic bacteria predominate in the isolates. According to their study, 72% of the isolates were multidrug-resistant organisms. The ratio of gram positive to gram negative was found to be 2:3.

Another study conducted in south India by Anandi et al on 107 patients with diabetic foot lesions showed polymicrobial aetiology in 69 patients and single aetiology in 21. They also found Pseudomonas as predominant isolate. A study done in Pondicherry has shown 22.2% MRSA prevalence among diabetic foot ulcers.[24] Another study done on ESBL producing E. coli and Klebsiella pneumonia in diabetic foot infections at Raheja Hospitals, Mumbai, over a

period of one year showed that out of 103 isolates, 40% were E. coli and 59.7% were Klebsiella pneumonia, among them only 21.13% were ESBL producers of which E. coli was 48.38% and Klebsiella pneumonia was 51.61%.[25] In our study, the polymicrobial aetiology is consistent with various studies conducted in India and abroad. Our study showed an average of 1.57 isolates per case, which is similar to study conducted by Ekta et al,[26] which is higher than 1.52 per case as reported by Vishwanathan et al.[27]

Increasing antimicrobial resistance is a problem in India and in this study has shown that presence of MRSA 7 (14%), out of 12 (20%) of Staphylococcal aureus strains. All MRSA strains are 100% resistant to penicillin, 87.5% resistant to amoxicillin and clavulanic acid, 37.5% resistant gentamycin, however, all strains are sensitive to vancomycin and imipenem is in coherent with the Indian study done by Murugan et al,[28] which enables to consider these drugs in treatment of all resistant MRSA strains. Another study by Anandhi et al show ESBL producers were 48.8% among the gram negative and 23.13% by Mani et al. None of the isolates in our present study were found to be ESBL producers. Pseudomonas aeruginosa was predominate isolate among the gram negatives in our study and were only sensitive to imipenem. In the present study, maximum number of patients belong to Wagner's grade 3 and 4.

All patients treated under surgical team comprising of diabetologist, vascular surgeon and plastic surgeon.[29] The team approach has important role in managing the ulcers. All patients were administered insulin ranging from 3 units to a maximum of 40 units. The requirement of insulin does not correlated with severity of ulcer grade. But, other studies in Portland, Oregeon[30] has showed that multiple daily insulin or intravenous insulin infusion therapy has improved the wound healing and less mortality. Similarly, hypoglycaemia is associated with poor wound healing.

The mean duration of stay in the hospital was 18 days and it was more than 31 days for patients who had major amputation. The mean duration of stay is 7 days and increased to 4 times if amputation occurs as per the analysis of Apelavist et al, 1995.[31] In German population, the average duration of hospital stay is 92 days and in India 118 days. The relative shorter duration in our setup maybe due to the fact the early and timely conversion from conservative to definitive surgical management.

Treatment- In our institution, a broad-spectrum antibiotic amoxicillin with clavulanic acid and metronidazole were administered parenterally after taking swab for Gram stain, culture and sensitivity. Antibiotics were changed according to culture and sensitivity pattern of microbes, 57.4% (23) patients managed conservatively. These patients constituted with cellulitis and trophic ulcers. The abscess was managed with incision and drainage. Daily Eusol cleaning and dressing is efficient along with proper wound debridement in our patients. Other wound care measures like hyperbaric oxygen therapy, negative-pressure wound therapy, use of advanced wound care products have not shown any encouraging data on the efficacy and cost-effectiveness of these procedures in the studies.[32] 15 patients underwent minor amputation and 3 underwent major (below-knee amputation) with split thickness grafting. Split thickness graft cover applied to 9 healing foot ulcers with 100% uptake. No mortality among the study groups.

Risk Factors- Presence of previous amputation was 6% in overall patients and 2% of them underwent re-amputation in the course of current illness. In multicentre prospective studies, sensory neuropathy was the most frequent component causing ulceration in diabetic patients.[33] Sensory neuropathy in diabetic causes, sensory loss numbness, dysesthesia, night-time pain, followed by loss of proprioception, callus ulcers and amputation. Most of the patients will succumb to the complications of diabetes at this stage and undergo amputation. Those who survive and when mechanisms are poorly understood and factors like hyperglycaemia, ulcers are inadequately managed the neuropathy is generally progressive produces motor neuropathy. Presence of foot deformities like hammer toes, hallux valgus and Charcot joints were 2% in our patients who were on irregular control of diabetes 15 (30%) compared with the studies conducted in tertiary referral centres like DRC, Chennai-2 (0.5%), GRH (Madurai)- 7 (2%), CMC (Vellore)- 19 (7.5%)[34] when compared to western studies 24%.[35]

None of the patients in our study were vision impaired to blindness as per the WHO criteria. When compared with risk factors along with the grading of the ulcers, it is clear that more number of risk factors more is the grade of ulcer. Only 7 patients had the awareness of specialised footwear for diabetes and all of them had grade 1 Wagner ulcer. The rate of amputation is 38% in our study. In India, it is about 18% less in USA (5%) and Vietnam (30)%. The poor socioeconomic status (80%) and lack of adequate knowledge (20%) of diabetic foot care maybe the reason for high rates amputation.

Among the studied patients, 25 (50%) had no previous treatment for diabetes, 15 (30%) patients with irregular control of diabetes and unaware about diabetic complications, 10 (20%) had a history of previous amputation.

CONCLUSION

Diabetic foot infections are frequently polymicrobial and predominantly gram-negative aerobic bacteria sat presentation. Multidrug-resistant Pseudomonas aeruginosa and MRSA in diabetic foot is at its emergence and life-threatening. Initial aggressive multimodal approach with surgical intervention culture specific and sensitive targeted combined broad-spectrum antibiotics decreases the morbidity limb loss eventually the mortality. Early screening for diabetes, tight glycaemic control decreases the prevalence of risk factors for diabetic foot ulcer related amputations and enhances the quality of life. The present study helped us to identify the common pathogens associated with diabetic ulcers. The susceptibility pattern would help in formulating antibiotic policy for empirical

treatment. Grading diabetic foot lesions according to the Wagner classification helps in correlating appropriate treatment to proper grade of lesion with better outcome. The poor knowledge of foot care among the diabetic patients, lack of proper foot care teams, delayed referral of patients with foot infections to specialists are some of the factors responsible for the poor quality of foot care at present. Education of patients and healthcare professionals regarding diabetic foot care is an urgent priority in order to reduce the number of amputations.

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