Microbial Infections Associated with Diabetic Foot Ulcer in Nasiriyah City

Muna F. Jahad[1], Mohammed Hashim AL-Yasiri[2]

[1]Research Scholar, Ministry of Health, Al-Nasiriyah, Iraq ,
[2]Assist Prof. Dr., Department of Biology, College of Science, University of Thi-Qar, Iraq

Abstract

Diabetic foot ulcers (DFUs) are a major complication of diabetes lead to development of gangrene associated with reduced patient quality of life, lower-extremity amputation, hospitalization, and high morbidity and mortality. This study was aimed to identify the pathogenic microorganisms in diabetic foot ulcers and to test their antibiotic susceptibility. A total of 134 patients with diabetic foot ulcers (DFU) attending to Diabetic center/ Thi-Qar province, South of Iraq were randomly selected. Samples were cultured for isolating bacteria species and Fungi species. Antibiotic sensitivity testing for bacteria species was done by Kirby Bauer disc diffusion method. Elderly diabetic males were frequently observed to develop foot ulcers higher than female. The most of patients 56 (41.79%) were in the age group 51 to 60 years followed by the 36 (26.86%) patients were in age group 41 to 50 years. Out of 162 isolates, bacteria species was 116 (71.6%) more than Fungi species 46 (28.4%). The most isolated bacteria was *Proteus mirabilis* 25 (15.43%) followed by *Enterobacter cloacae* 22 (13.58%) and both *Escherichia coli* and *Staphylococcus epidermidis* 17 (10.49%). Diabetic foot infections are life-threatening. Many pathogens associated with diabetic foot ulcers.

***Keywords**: Diabetic foot, Microbial infection, Foot ulcer*

Introduction

Diabetes is a global public health problem that is a major burden on the economy and society. Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia resulting from irregularities in the secretion and action of insulin (1). During the life of diabetics may develop foot ulcers, which are a serious complication of diabetes. Approximately 25% of diabetics have a lifelong cumulative risk of foot ulcers with increased exposure to infection in 40-80% of cases (2). DFU refers to a breach in the skin epithelium involving full thickness or beyond, but not reaching the ankle joints, in a person with diabetes (3) . Clinically, diagnoses of diabetic foot infections are based on classic findings of inflammation. The infections are classified as mild, moderate, and severe infections (4) . There is variation in the prevalence of isolated pathogens. The most mild foot infections caused by mono microbe, while more severe infections are caused by poly microbes (5-6) .

Many previous studies confirmed the presence of aerobic and anaerobic bacteria in diabetes foot infections. Moreover, high rate of antibiotic resistance in these pathogens (7-8).The infection may be caused by pathogenic bacteria that arise from the external environment as well as by bacteria that forming the physiological microflora of the skin (9). A wide range of fungal species are found in infected foot ulcers, with *Candida* species being the most common strains isolated (10). Therefore, the study aimed to increase knowledge of the diverse and abundance of microorganisms that cause diabetic foot infections for patients who admit to the Diabetes Center in Nasiriyah city, Thi-qar province, southern Iraq.

Materials and Methods

Sample collection and cultivation

A total of 134 foot ulcer swab samples were collected from diabetic patients attending to the Diabetic Center in

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Corresponding auther,
Una F. Jahad,
Research Scholar Ministry of Health, Al-Nasiriyah, Iraq

Thi-qar province, which includes both sexes of different ages. These samples were collected using sterile swabs after scrubbed thoroughly with normal saline to remove surface colonizers. All collected samples were inoculated on suitable sterile culture media (such as Nutrient agar, Blood agar, MacConkey's agar, Mannitol salt agar and Brain heart infusion media) for the detection of aerobic bacteria and fungi species in the clinical microbiology laboratory of Public Health Laboratory. The plates were incubated at 37°C for 24 and 48 hrs. The purified bacterial isolates were preserved in the refrigerator at 4°C until they were used (30). Identification of bacterial isolates was done according to cultural characteristics on Blood agar, MacConkey agar, and Mannitol salt agar. Also diagnosed with results of different biochemical tests including Oxidase test, Catalase test, Coagulase test, gram staining properties and IMVIC test for gram negative bacteria. The diagnosis was confirmed with Api-20E System (BioMerieux, France) (11).

Fungal Culture

The specimens were inoculated into Sabouraud dextrose agar and Malt Extract Base agar supplemented with chloramphenicol and incubated at 30°C for 3-5 days. Fungi colonies were identified by macroscopic appearance of colonies on Chromogenic agar compared with standard color photographs supplied by manufacturer (12) .

Statistical Analysis

Data were analyzed using Excel-Data analysis tools as available in Excel software 2010. Descriptive statistics were applied to analyze all data and to calculate Mean and Standard deviation. The T-test and Chi-Square were applied to assess the significant differences (p-value ≥ 0.05) between different parameters used in this study.

Results

Samples culture was positive to 116 (71.60%) bacterial isolates and 46 (28.40%) fungal isolates. The identification of 116 bacteria isolates showed *Proteus mirabilis*, *Enterobacter cloacae*, *E. coli*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas*, *Serratia*, *Citrobacter*, *Streptococcus*, *Acinetobacter* and *Pantoea*. Fungal identification of 46 isolates was showed four *Candida* species and *Rhodotorula*. Fungal species were identified with microscopic examination as well as their characteristics on chromogenic agar. *Candida krusei* 13(8.02%) was the most prevalence yeast species followed by *Rhodotorula* 10(6.17%), *C. glabrata* 9 (5.56%) and both *C. albicans* and *C. tropicalis* 7 (4.32%).

Table (1) Numbers and Percentages of Bacterial and Yeast Isolates

NO	Genus	Number	%
1	Proteus mirabilis	25	15.43
2	Enterobacter cloacae	22	13.58
3	Escherichia coli	17	10.49
4	Staphylococcus epidermidis	17	10.49
5	Staphylococcus aureus	12	7.41
6	Klebsiella pneumoniae	7	4.32
7	Pseudomonas	5	3.09
8	Serratia marcescens	5	3.09
9	Citrobacter freundii	2	1.23
10	Streptococcus	2	1.23

***Cont...* Table (1) Numbers and Percentages of Bacterial and Yeast Isolates**

11	Acinetobacter baumannii		1	0.62
12	Pantoea agglomerans		1	0.62
Total Bacterial Isolates			116	71.60
13	Candida krusei		13	8.02
14	Candida glabrata		9	5.56
15	Candida albicans		7	4.32
16	Candida tropicalis		7	4.32
17	Rhodotorula		10	6.17
Total Yeast Isolates			46	28.40
Total Microbial Isolates			162	100.00
CalX2=34.95	Tabx2=26.3	DF=16	p-Value =0.004	

Discussion

Diabetic foot ulcer (DFU) is a serious and common complication of diabetes mellitus (DM). Impaired wound healing in diabetic foot patients considers a great challenge for human healthcare worldwide. The leading problem that hamper the wound healing of DFUs are peripheral vascular disease, neuropathy, and impaired immune response, which render the diabetic patients predispose to microbial foot infections (14) .

In this study, we have noticed significant variations in certain variables such as gender and age of diabetics as well as spectrum of microbial infections. Study samples were collected from patients between the ages of 28 and 75 years. Most of the patients were in the age group from 51 to 60 years, which is normal because diabetes is more common in elderly people (5,15) . The elderly age of presentation can be explained on the basis of the fact that DFU is a chronic complication of diabetes.٦ elderly patients had major risk to develop foot ulcer due to peripheral neuropathy, vasculopathy and poor vision (16) .

The predominant infections were in males (57.46%) more than females (42.53%). This was in agreement with previous study (17). Male predominance in DFU could be linked to factors such as gender related differences in life styles such as smoker which as strong risk factor for peripheral arterial disease which major risk factor of diabetic foot ulceration (18).

In present study, the bacterial species were predominantly cultured (71.6%) compared with fungal species (28.4).This result come to an agreement with previous study (10,19). The major *Candida* species isolated in our study were *C. krusei* and *C. glabrata.* This confirmed the emergence of non-albicans *Candida* over *C. albicans* (20). *C. krusei* was the major species isolated in this study has become an important cause of candidemia (21).

In current study, Gram-negative bacteria were more predominant than Gram-positive bacteria. This result is similar to several other studies in Brazil and Malaysia (22-23). Several studies have shown that *Enterobacteriaceae* members to be consistently associated with diabetic foot ulcers (9,6). Among *Enterobacteriaceae* members, we found that *Proteus and Enterobacter* were dominant genera, followed by *E. coli and Staph*ylococcus respectively. Many reasons, such as source of infection, use of antibiotic drug for treatment, sample collection method and different type of infections can influence of pathogens diversity in DFI (24).

Both gram positive and gram negative bacteria isolated from diabetic foot ulcers were resistant to different classes of antibiotics. The highest resistance 100% was observed in *S. aureus* for Amoxicillin – clavulanate (20/10 µg) AMC, Cefoxitin (30 µg) FOX, Oxacillin (1µg) OX, Nalidixic acid (30µg) NA, and Cephalothin (30µg) CEP. Methicillin-resistant *S. auerus* (MRSA) strains are having a major impact worldwide, and due to their resistance to all β-lactams (29) . Eighty-Five of Gram negative bacterial isolates were resistant to the most antibiotics, such as the resistance was 100% for Ampicillin (10µg) AMP, Amoxicillin-clavulanate (20/10µg) and Cefoxitin (30µg). The development of resistance has several risk factors linked to the severity of the infection and that resistance is associated with increased mortality (25-26).

The effective of Imipenem and Amikacin were reported against aerobic Gram-negative bacteria (5). The high resistance to Ampicillin and Amoxicillin-clavulanate should warrant careful consideration of these drugs requires experimental treatment. Another critical observation of our study was the *Acinetobacter* spp. had already developed resistance to most of the common antibiotics (AK, CIP and IMP). In Maniple, *Acinetobacter* was documented as multidrug resistances (27) . We found an increasing trend in the prevalence of multi-drug resistance bacterial pathogens, highlighting the need for surveillance programs that guide appropriate empirical treatment and thereby reduce the risk of DFI complications. In present study agree with another study in Malaysia focus on the selection of empiric antimicrobial therapy depends on many factors such as infection severity, medication allergies ,overall patient condition, antibiotic activity, previous antibiotic, toxicity and excretion, and glycemic control (28) . Therefore, early diagnosis and proper identification of lesions with causative agents, prompt initiation of appropriate antimicrobial therapy are essential for controlling the infection and management of complication of diabetic foot infections and improving the quality of life.

Conclusion

Diabetic foot infections are polymicrobial in nature. Proteus mirabilis in Gram negative bacteria followed by Staphylococcus sp.in Gram positive organism and Candida spp in fungal were the predominant pathogens. Empirical antibiotic selection should be followed by culture guided adjunctive therapy. Imipenem and Amikacin and would be appropriate for empirical treatment.

Ethical Clearance : Taken from University of Thi-Qar ethical committee

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Conflict of Interest : Nil

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