

A Study on Clinical and Microbial Culture Analysis in Diabetic Foot Disease

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Abstract

Background: To study the graphic includes of diabetic foot disease in relation with age, sex and blood glucose levels of diabetic patients at Owaisi Hospital & Research Centre.

Material and Methods: The present study is descriptive and cross-sectional study of diabetic patients with the diabetic foot lesions (Ulcer, cellulitis or osteomyelitis) of the patients or in patients of Medicine, Surgery and Orthopaedic departments at Owaisi Hospital & Research Centre. The Sample study was done at the Department of Microbiology, here at Deccan College of Medical Sciences from 1st October 2021 to 30th September 2022.

Results: A total of 76 microbes were isolated from 30 samples of pus and other executes. It was seen that there was high prevalence of infection males then in females. Almost all the cases studied showed high incidence of uncontrolled diabetes with blood glucose levels higher than 200 mg/dl. Age group affected was studied and it was found that the cases with age group of 65-75 years were highest affected (40%). Majority of samples collected were polymicrobial (nos .22-73.33%) and some also showed more than the microbes, the others were monomicrobial (nos 6-20%) and only two (6.67%) of the samples showed no growth for microbes. Aerobic bacteria showed prevalence in the sample collected (nos 54-71.05%) anaerobic bacteria were 22(28.95%) in number. A total of 29 cocci and 47 bacilli were isolated. Gram –straining showed that there was prevalence of gram-positive bacteria (nos.39) then gram negative was 37 in number.

Conclusion: Majority of diabetic foot ulcers were in farmers and people of other slums. These people of the slums showed diabetic foot ulcerations predominantly due to Trauma despite the fact that on the other hand the people of privileged class showed DFU's due to High incidence of uncontrolled diabetes with blood glucose levels higher than 200 mg/dl. Gram –straining showed that there was high prevalence of gram-positive bacteria than gram negative bacteria.

Keywords: Diabetic foot ulcers, Blood glucose, Microbes, Aerobic Bacteria, Cocci.

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Introduction

Diabetes is one of the most common diseases of the modern world, leading to several other secondary conditions like neuropathy, nephropathy, and cardiovascular disorders. Retinopathies and several others. Moreover this, this disease is constantly on the rise in the people of the modern of the world because health care's awareness. Diabetes and foot problems are almost synchronous.

The main concern for the today was the people getting amputated due to simple thorn prick on their foot and almost all of such poor patients were mostly having uncontrolled diabetes mellitus. Diabetes and foot problems are almost synchronous.^[1-4] Foot infections are seen become very common in patients with diabetes and are associated with high morbidity and a good risk of lower extremity amputation including the entire foot. Foot ulcers in diabetics are seen to be common mainly after a trauma which might be as small as just prick of the needle even without the oozing of blood. It has been seen through previous researchers that there is a trio of problems leading to the diabetic to the diabetic foot ulcers. The trio of problem leading onto the diabetic foot is neuropathy, vascular changes and infections which constitute the diabetic foot syndrome.^[5,6]

Peripheral neuropathy has a central role in the development of foot infection and it occurs in about 30-50% of the patients with diabetics. It has been seen in the patients with diabetes mellitus lose the protective sensations for temperature and pain, impairing awareness of trauma such as abrasions, blistering or penetrating of a foreign body. Motor neuropathy can result in foot deformities that contribute to local pressure from footwear, making skin ulceration even more likely. Once the skin broken, underlying tissues are exposed to Colonization by Pathogenic organisms. The resulting foot infection may begin superficially, but with delay in treatment and other impaired body mechanisms caused by neutrophil dysfunction and vascular insufficiency. It can spread to the contiguous subcutaneous tissues and to even deeper structures. Although most diabetic foot begins with ulcer, localized cellulitis and necrotizing facilities can develop in the absence of ulcer or traumatic injury”.

In addition to severe morbidities, they account for the largest number of bed-days and are the most common proximate and non-traumatic causes of amputations. Optimal management of diabetic foot infections can potentially reduce the incidence of major limb amputations. Unfortunately these infections and the squealer are also the most common cause of disability and the reason diabetic patients.

The foot of ulcers is the leading functions constitute a constantly growing and constantly growing and costly public Health concern. Unfortunately, these infections are frequently inadequately managed a most of hospitals amputations of foot. This may result from lack of understanding of current diagnostic and therapeutic approaches insufficient resources devoted to the problem, a lack of effective multidisciplinary collaboration.^[7]

Diabetic foot ulcers are estimated affect 15% of all diabetic during their lifetimes and proceed almost 85% of all foot amputations. Diabetics continues to be most common underlying cause of non-traumatic lower extremely amputations (LEA's)in the USA and Europe.

Commonly encountered problems with ulcers were, highly resistant organisms such as Staphyococcus aureus, Pseudomonas spp, etc which were resistant to many of the broad-spectrum antibiotics.

The Primary purpose of this study is to help reduce medical morbidity, psychological distress and financial costs associated with diabetic foot infections. The focus of the study is primarily of managing the diabetic patients with suspected or evident foot infection, because other published guidelines cover general management of the diabetic foot and foot ulceration moreover, the main input of the patient's in hospital with diabetic foot are the people of the rural who do not maintain proper hygiene of the foot.

Hence, this study is implicated for the identification of the microorganisms causing diabetic foot infections and selection of good anti-microbial therapy which would aid our doctors to give a good treatment for prevention of complications and spread of infection.

Aims & Objectives

- To study the graphic includes of diabetic foot disease in relation with age, sex and blood glucose levels of diabetic patients at Owaisi Hospital & Research Centre

Material and Methods

The present study is descriptive and cross-sectional study of diabetic patients with the diabetic foot lesions (Ulcer, cellulitis or osteomyelitis) of the patients or in patients of Medicine, Surgery and Orthopaedic departments at Owaisi Hospital & Research Centre. The Sample study was done at the Department of Microbiology, here at Deccan College of Medical Sciences from 1st October 2021 to 30th September 2022.

Inclusion Criteria

Included in the study were adult patients aged 18 years and above, previously diagnosed or newly diagnosed diabetic, presenting lower extremity infection, whose specimens were adequate enough to perform the cultural analysis (both aerobic and anaerobic).

Exclusion criteria

Iatrogenic and hospital acquired infections caused due to admission to hospital or injury at the hospital due to needle prick or other injuries caused due to surgery (plastic surgery grafts) and patients admitted for lower extremely lesions who did not report with diabetes mellitus and just with simple gangrene and necrosis were excluded.

Sample Size

- To estimate the prevalence of various aerobes and anaerobes from the sample of diabetic foot lesions collected with a high precision (+ 5%), thirty samples were collected.
- A detailed history taking and physical examination was done and the following data was obtained from the all of cases demographic data duration of diabetes, method of glycemic control, history of diabetes hypertension, cardiovascular disease previous amputations any anomalies of the lower extremity duration of the wound and history of antibiotic consumption prior to hospitalization.
- A Various diagnostic procedures included complete hemogram (complete blood Picture hemoglobin estimation), random blood glucose, urine glucose analysis on admission with regular blood glucose checkup. Complete urine analysis, culture and sensitivity of the puts or other excaudate of the infection. Other diagnostic procedure included were a 12 lead ECG, X-ray of the foot to rule out or other diseases whenever required.
- Sample Collection and Laboratory Techniques: The samples from diabetic foot patients were collected by sterile culture swabs and /or aspirates sterile syringes. The patient when presented with simple ulcers without bullae, the wound was first washed with normal saline, the exudate or an aspirate from exudate was taken using sterile syringes when the lesions were presented with loose tissue, the wound was first cleaned and unroofed by minor surgery then swab sample was taken. The collected sample was transported in sterile vials within an hour of collection and was also transported with extreme precautions when the chance of drying up or contamination of sample was presumed.
- After transporting to the Microbiology Laboratory at Deccan College of Medical Sciences, the following laboratory procedure were done as per the standard methods (Bailey and Scott's).
- Direct microscopy: Direct microscopy was done to observe for presence of pus cells, blood cells, bacteria, fungi etc.
- Grain staining: Grain straining of the culture isolates was done by the standard method by using saffranine, iodine and methylene blue as the reagents for gram staining for identification of bacteria.

RESULTS

A study of 70 microbes were isolated from 30 samples of pus and other exudates from the prevention of diabetic foot diseases from the outpatients and inpatients of the Medicine, Surgery & Orthopaedics Departments of Owaisi Hospital & Research Centre. All the samples were proficiently processed at the laboratory of Department of Microbiology here at the Deccan College of Medical Sciences. Male to Female Incidence:

Table 1: The Male-Female ratio

S.no	Sex	Number	Percentage
1	Males	20	66.67%
2	Females	10	33.33%

It was seen that there was a higher prevalence of the infection in males than females. Males were found to be more inclined to the infection and the ratio was found to be 2:1

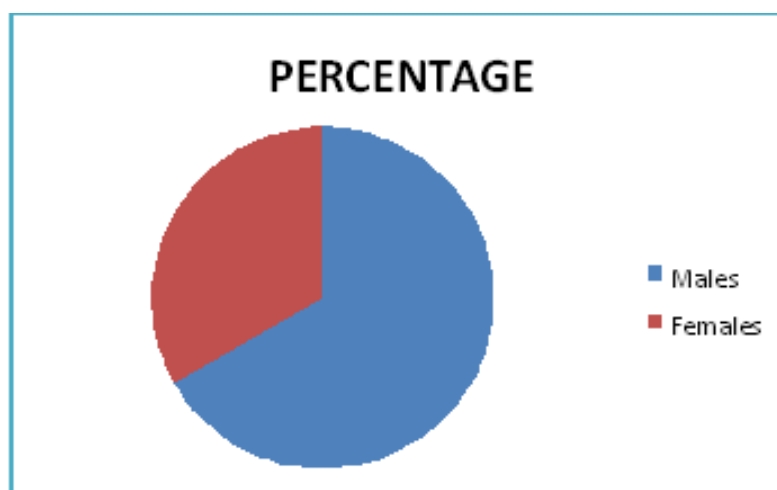


Figure 1: the male –Female ratio.

Blood Glucose Values

Table 2: Values of Blood Glucose in cases studied.

S.No	Glucose Level	Number	percentage
1	160-200	3	10%
2	200-250	8	26.67%
3	250-300	11	36.67%
4	300-350	8	26.67%
5	>360	0	0%

Almost all the cases studied showed high incidence of uncontrolled diabetes with blood glucose levels higher than 200 Mg/dl. Some patients also presented with undiagnosed and uncontrolled diabetes. Thus, this has also been studied to some extent and further can be.

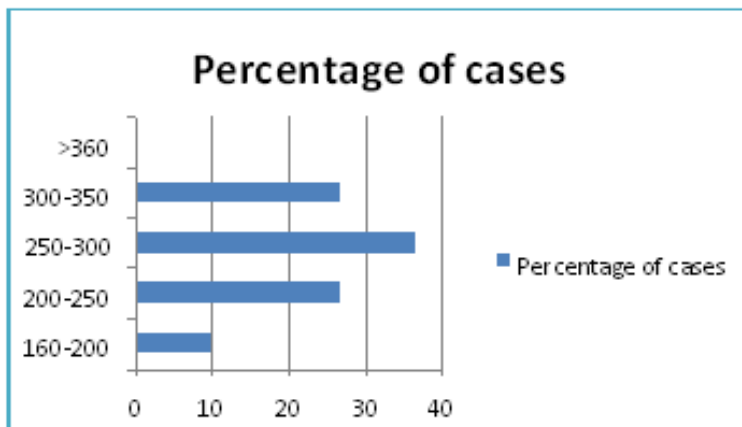


Figure 2: Values of Blood Glucose in cases studied

Microbial growth status: Majority of the samples collected were polymicrobial (nos, 22/76-28.95%) and some also showed more than three microbes, the other were monomicrobial (nos, 6-20%) and only two (6.67%) of the samples showed no growth for microbes
 Morphological prevalence study: Aerobic and Anaerobic Status:

Table 3: Aerobic and Anaerobic bacteria relation

S.No	Type	Number	Percentage
1	Aerobic	54	71.05%
2	Anaerobic	22	28.95%
	total	76	100%

Out of the total of 76 samples isolated from 30 patients aerobic bacteria showed prevalence in the sample collected (nos. 54-71.05%). Anaerobic bacteria were 22 (28.95%) in number.

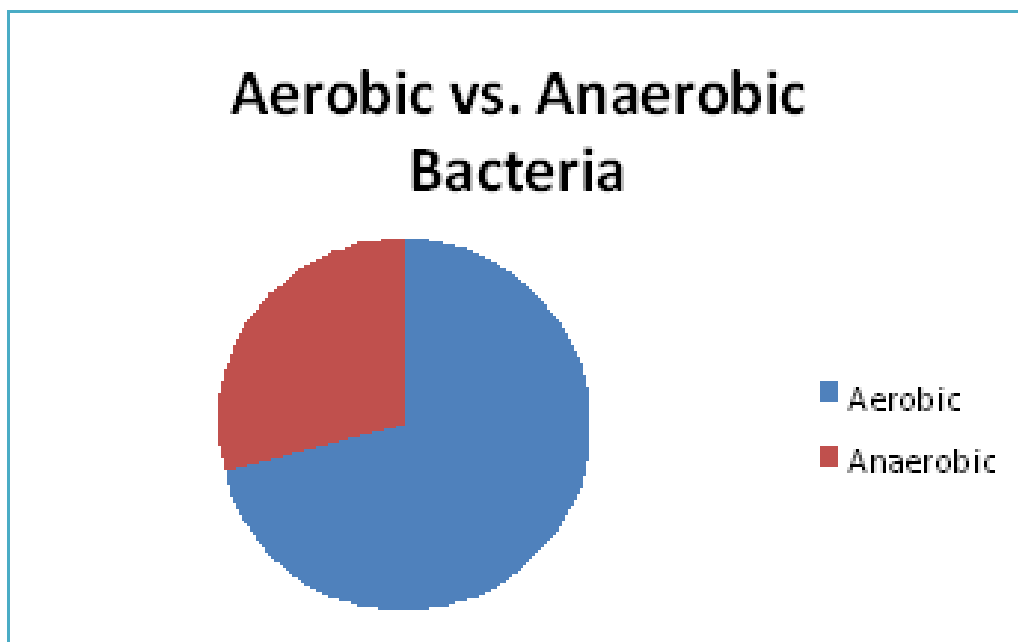


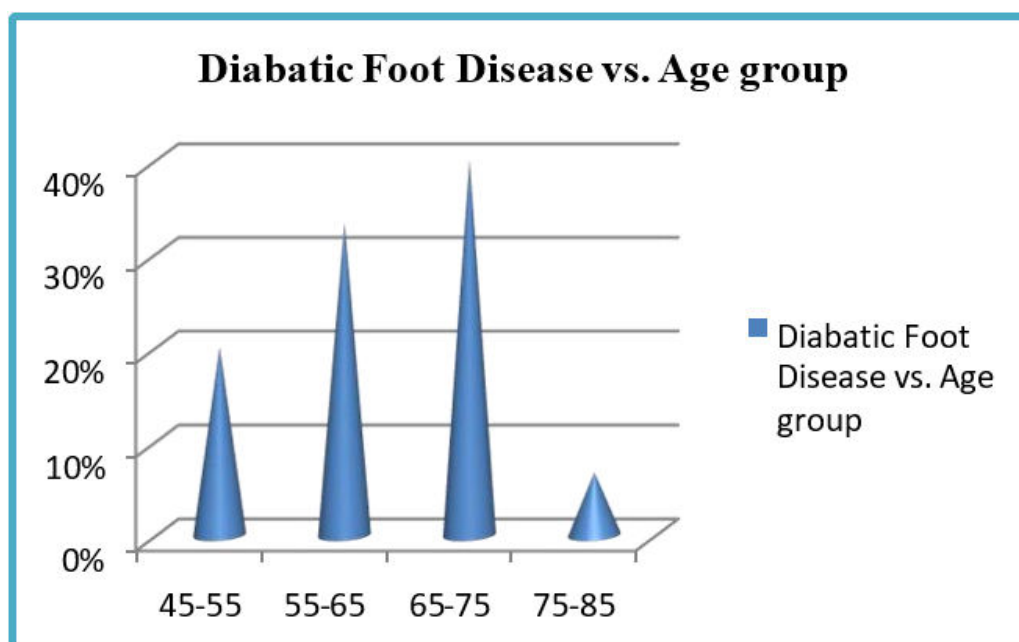
Figure 3: Aerobes vs Anaerobic bacteria in the isolates

Study of age specification prevalence:

Table 4. Diabetic foot in relation with age group

S.NO	Age group (Years)	Number	Percentage
1	45-55	6	20%
2	55-65	10	33.33%
3	65-75	12	40%
4	75-85	2	6.67%
	Total	30	100%

Age group getting affected was also studied and it was found that the cases with age group of 65-75 yrs were the highest (40%). The following by age group of 55-65% yrs (33.33%), 45-55 yrs (20%) and lastly 75-85 yrs (6.67%).

**Figure 4: Diabetic foot disease vs. Age Group****Table 5: Study of morphological prevalence of bacteria.**

S.No	Morphology	Number	Percentage
1	Cocci	29	38.16
2	Bacilli	47	61.84
3	Gram +ve	39	51.32
4	Gram -ve	37	48.68
	Total	76/76	100/100%

In terms of bacteria morphology and gram's-staining, the bacterial were studied. A total of 29 cocci and 47 bacilli were isolated. Gram's staining showed that there was prevalence of gram positive bacteria (no.39) than gram negative and they were 37 in number. However, it was seen that there was not much of different with gram straining classification but morphologically bacilli were highly numbered.

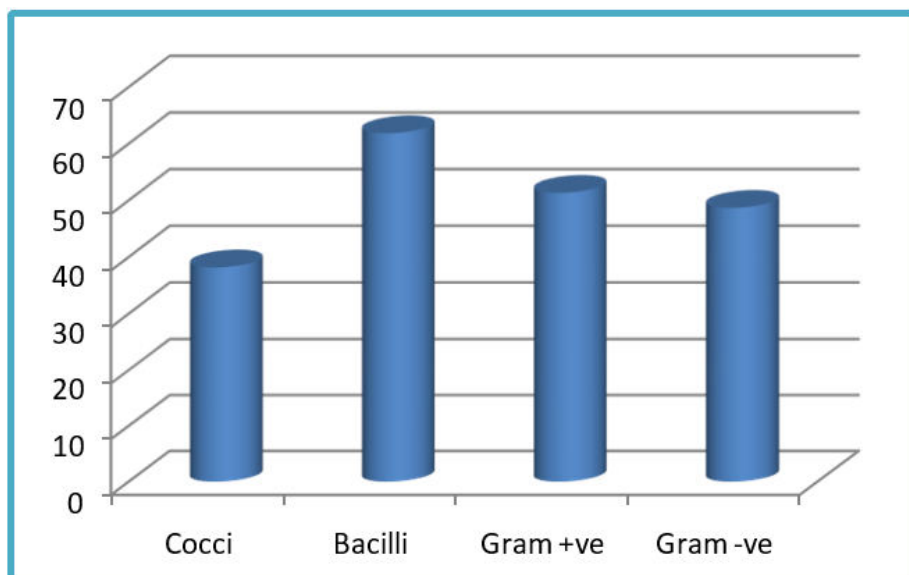


Figure 5: Showing relation of morphology features of isolates.

Study of Aerobic prevalence:

Table 6: Aerobics found in the Isolates

S.No	Organisms	Number	percentage
1	Staphylococcus aureus	19	35.19%
2	Staph. epidermidis	3	5.56%
3	Streptococcus spp	2	3.7%
4	Pseudomonas spp	10	18.52%
5	Proteus mirabilis	5	9.26%
6	Enterococcus spp	5	9.26%
7	Escherichia coli	7	12.96%
8	Klebsiella spp	3	5.56%
	Total	54	100%

From among the 54 aerobic samples 19 were Staphylococcus aureus (35.19%) followed by Pseudomonas spp (nos. 10-18.52%)

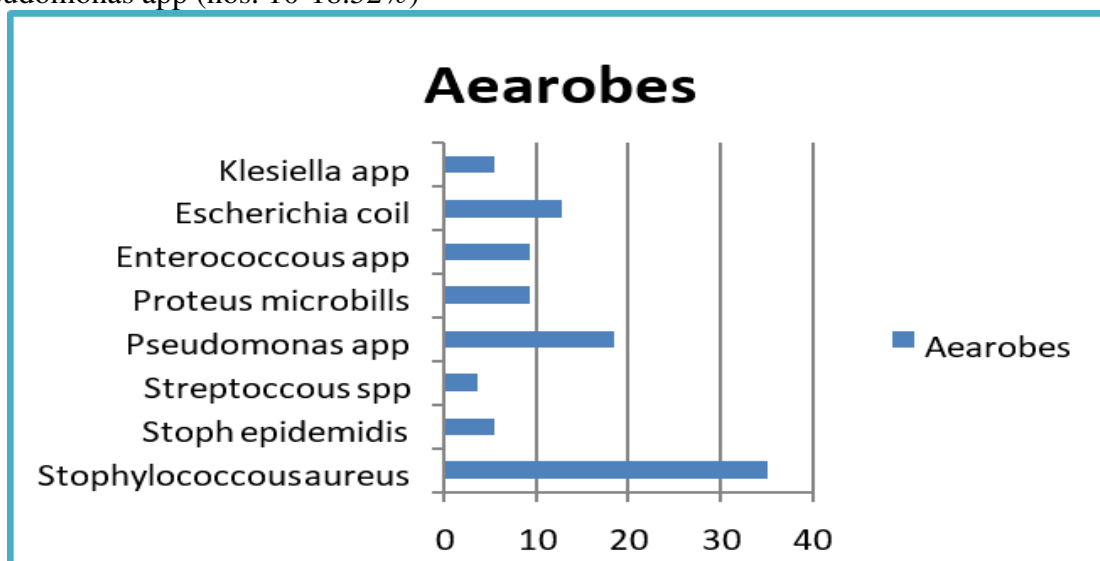
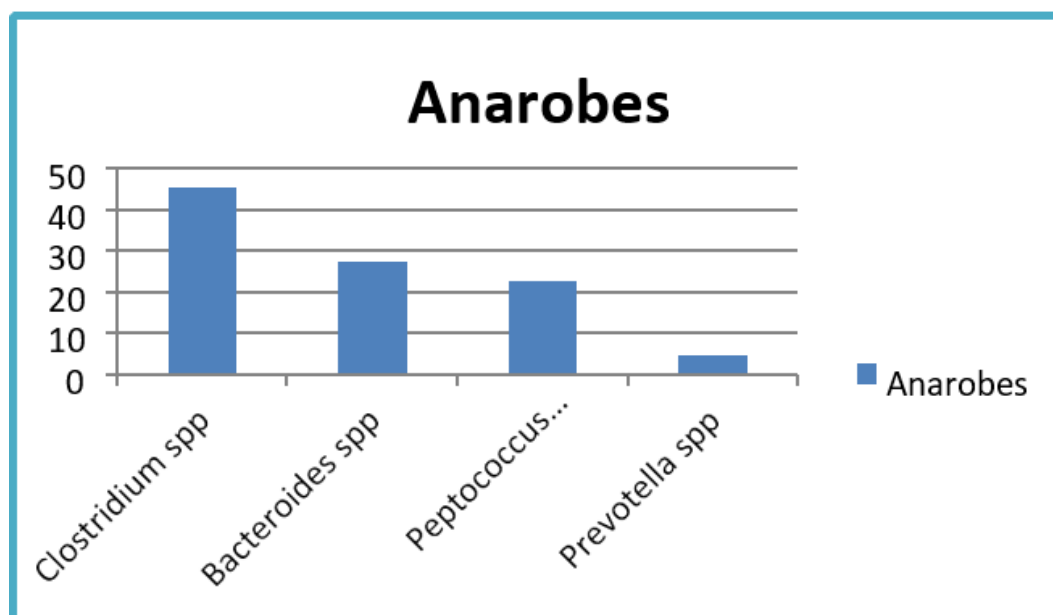


Figure 6: Showing the percentage of aerobes encountered in the isolates

Study of Anaerobic prevalence:**Table 7: List of anaerobes encountered in the isolates**

S.NO	Organisms	Number	Percentage
1	Clostridium spp	10	45.45%
2	Bacteroidesspp	6	27.27%
3	Peptococcus app	5	22.73%
4	Prevotellaspp	1	4.55%

From among the anaerobic bacteria the highest prevalence was seen by Clostridium spp (nos.10 -45.45), followed by Bacteroidesspp (nos.6-27.27%)

**Figure 7: Showing the percentage of anaerobes encountered in the isolates****DISCUSSION**

The total number of bacteria isolated from 30 patients was 76. The study was conducted in Deccan College of Medical Sciences, between the months of 1st October 2021 and 30th September 2022. Samples were collected from various departments but they were cultured and studied at the Microbiology Laboratory in OHRC Campus. Male: Female ratio:

Males were found to be more inclined to the infection and the ratio was found to be 21. Also it was seen that majority of diabetic foot ulcers were seen in farmers and people of other slums. These people of the slums showed diabetic foot ulcerations predominately due to trauma despite the fact that the other hand the people of privileged class showed DFUs due to secondary problems like neuropathy and vasculopathy and then trauma, also sum patients reported with spontaneous ulcers. Age Distribution:

Age group getting affected was also studied and it was found that the cases with age group of 65-75 yrs were the highest (40%). Then followed by age group of 55-65 yrs (33.33%), 45-55 yrs (20%) and lastly 75-85yrs (6.67%). Thus, it can be interpreted that higher age groups are most affected, probably due to inability to take care due to old age (it also one of the etiological factors) as they tend to easily overlook the minor sounds due to retinopathies.

Position of ulcers

Skuratoweiz –Kubica and colleagues and showed that in the study of ulcers were located mainly on the toes (52%) materials heads (19%) heel (9%)and in the 14% of the studied subjects phlegmon of the foot was diagnosed. His study further showed that bacteriological analysis of material obtained from the ulcers revealed mixed flora in 54% of specimens. One pathogen was found in 42% cases and in 2 subjects culture results were negative. Staphylococcus aureus was present in 30% of cases. Other bacteria included: Proteus mirabilis (17%). Enterococcus faecalis (15%), Escherichia coli (15%), Pseudomonas aeruginosa (10%) and anaerobic bacteria were present in 5% of ulcers.

In this study also mainly the ulcers were located at the toes and the heel. Cultures showed the prevalence of Staphylococcus aureus (35.19%). Whereas, this study showed that Pseudomonas spp. were also quite prevalent.

In another study by Gaur, Varma and Gupta, bacterial analysis showed prevalence of Enterococcus sp.(57.69%) followed by staphylococcus sp. Isolated in 40.9% of the patients out of 61% patients, 52 (85.24%) had polymicrobial infection and in the rest 9 patients 14.75% only single organism was isolated.^[8]

In this study, there was prevalence of polymicrobial infection (73.33%) confirming the above study.

Bacteria Encountered Profile

Ammadi et al. showed that in study from among 107 diabetic foot the ages were a mean of 43 and prevalence was seen in males (65.42%). The microbiologic profile showed prevalence of aerobes (76.58%). Anaerobes were 20.27% of all the isolates. E.coli showed the high prevalences (27.7%). Followed by Staphylococcus aureus and Klebsiella spp. 13.66% and lowest incidence was that of Enterococcus spp 7.33% from among the aerobes. Anaerobes showed highest prevalence by Clostridium perfringens (31.1%). Other Values among aerobes were Pseudomonas spp (11.3%), Proteus spp 16.9%), Enterobacterspp (9.66%)and among anaerobes were Cl. Sporogens (17.8%), Cl.tetanomophum (11.1%) and Prevotellaspp (13.3%).The Study showed highest prevalence aerobes to be staphylococcus relatively to the above study. The highest prevalence among was anaerobes was seen to be Clostridium spp. supporting the above study.^[8,9]

CONCLUSION

The present study concluded that there should be proper prescription of antibiotics to the patients of such severe disease, only after proper culture and sensitivity testing done extensively.

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