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Original Research Article

Study of Co-Existence of Fungal Infection in Diabetic Foot Ulcers in Jharkhand Population: A Retrospective Study

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Abstract

Background: Foot is the most vulnerable part of injury in the body. The uncontrolled diabetic patients develop ulcers because of neglected sites of preference of neuropathy and ischemia, which develop into diabetic foot ulcers coexistence of fungal or bacterial flora.

Method: 94 (ninety-four) adult patients having diabetic foot ulcer were studied. A tissue biopsy was collected from a diabetic foot ulcer (DFU) and studied microbiologically. Gram stain and Gram's iodine tests were done; the LQCB test was done to rule out a fungal infection.

Results: The grades of foot ulcer were the highest at 42 (44.6%) grade III, followed by 32 (34%) grade II and 20 (21.7%) grade IV. The comorbidities were 36 (38.2%) hypertension, 27 (28.7%) CRF, 15 (15.9%) IHD, 4 (4.2%) tuberculosis, and 2 (2.1%) hypothyroidism. Out of 94 diabetic foot ulcers, 24 (25.3%) had fungal-positive patients, and 6 (6.3%) underwent amputation.

Conclusion: Majority of diabetic foot ulcer has 25.3% fungal infections. The positive study implies that mycological evaluation is mandatory in non-healing DFU. Apart from antibacterial therapy, foot care is compulsory to avoid the risk of amputation.

Keywords: Candidiasis, Grams Iodine, gram stain, LPCB, DFU.

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Introduction

Based on current trends >360 million will have diabetic in 2036. It is said that, presently, 62 million Indians are diabetic, and these numbers are likely to increase to 89.5 million by the year 2028. This will be 1/6th of the world's total diabetics [1]. There is evidence that the prevalence is increasing in the rural population; also, India is already the diabetes capital of the world.

The number of detected cases of DM reflects only the tip of the iceberg, because in India there is a larger number of undetected cases than detected cases [2]. The death of diabetes patients is due to its complications, which are common in the age group of 40-60 years, affecting both sexes equally. Complications are more prevalent among lower socio economic status because of negligence, illiteracy, and poverty.

Among the several chronic complications of uncontrolled diabetes, foot ulcer is quite common [3]. Approximately 15% to 20% of individuals with uncontrolled DM develop a foot ulcer (the great toe or metatarsophalangeal area is common). Type-III DM is the leading cause of non-traumatic lower extremity amputation in developing countries. The risk of lower limb amputation is 15-46 limes higher in uncontrolled diabetes. The foot is the most vulnerable part of the body for injury and also the most neglected; moreover, it is the site of preference for neuropathy and ischemia [4]. Hence, an attempt was made to evaluate the types of infections in the diabetic foot ulcers.

Material and Method

94 (ninety-four) adult patients aged between 35 to 70 years regularly visited the surgery department of Medinirai Medical College Hospital, Palamu, Jharkhand-822101, were studied.

Inclusive Criteria: DM patients with blood glucose under control Non-healing ulcers more than 2 months apart from antibiotics and wound care and who gave their consent for study in writing were selected.

Exclusion Criteria: Ulcer of the foot other than diabetic, like those secondary to venous disorders and diabetic feet with systemic infections; patients with allergies to antifungals; pregnant and lactating mothers. Immunocompromised patients were excluded from study.

Method: In every patient, the ulcer was cleaned with pivodine solution and sterile Normal saline samples for study were taken from the depth of the ulcers measuring around 0.5X0.5 cm. Transport media—Tissue samples were collected in plastic bottles (autoclavable) containing approx. 4.5 ml of normal saline in them. Tissue samples from the ulcers under aseptic precautions, sealed and labelled, and were taken to the microbiology laboratory within one hour.

Tissue blocks received were taken out from the sample collection bottle and were triturated, and then the tissue was ready for examination.

KOH 10% Preparation: The tissue specimen is placed on a clean slide, and 10% KOH is added and left in the incubator at 37°C for 2 hours. A cover slip is placed and examined under a microscope. The alkali digests the keratin tissue and other tissue materials, enabling the fungus elements to be seen clearly.

Potassium hydroxide, 10 mg, dissolved in 100 ml of distilled water first. Examination is done under low power with reduced light and fungal elements.

Gram Stain: With the triturated tissue material, a smear is made on a clean slide, which is then dried, heat-fixed, and stained. 1.0 gm crystal violet was added to a 5% sodium bicarbonate 1.0 ml solution and mixed, and the volume was made 100 ml by adding distilled water.

Gram's Iodine: 2 gm iodine crystals were added to freshly prepared sodium hydroxide solution (10 ml) and made the volume 100 ml with distilled water. Gram-stained smears are examined under 0.1 immersion for the presence of gram-positive budding cells and pseudohyphae. This was particularly important when Candida was suspected.

Cultural procedures were carried out, and samples were inoculated into 4 tubes containing Sabouraud's dextrose agar with antibiotics. Lactophenol cotton blue (LPCB) mounting was done for identification of fungal element growth.

The duration of study was from October 2024 to November 2025.

Statistical analysis: various degrees of DFU, duration of type, DM levels, RBS, HBA1C co-morbidity Bacterial and fungal infections were studied and classified with percentage. The statistical analysis was carried out in 2007 software. The ratio of males and females was 2:1.

Observation and Results

Table-1: Dermatographics of patients

- Duration of foot ulcer – 28 (29.7%) patients had from 3 to 12 months, 66 (70.2%) had 13 to 36 months
- Duration of type-II DM – 38 (40.4%) had 2 to 5 years, 56 (59.5%) had 6 to 12 years
- Random Blood Sugar levels – 44 (46.8%) patients had 200-320, 50 (53.1%) had 321-620
- HbA1c level – 27 (28.7%) had 6-7 % , 67 (71.2%) had 8-185%

Table-2: Grades of ulcers – 32 (34%) had grade-II, 42 (44.6%) had grade-III ulcer, 20 (21.7%) had grade-IV ulcer

Table-3: Co-morbidities of ulcer patients – 36 (38.2%) had HTN, 27 (28.7%) had CRF, 15 (15.9%) IHD, 4 (4.2%) had TB, 2 (2.1%) parathyroid

Table-4: Study of Bacterial flora in Diabetic food ulcer patients – 25 (26.5%) S. Aeurus, 17 (18%) pseudomonas, 15 (15.9%) Enterococus, 8 (8.5%) streptococlus, 4 (4.4%) proteus vulgaris, 4 (4.4%) Acimobacter, 2 (2.1%) Kleibeseelo

Table-5: Out of 94 patients 24 (25%) patents had fungal positive

- 10 (10.5%) had 5 to 6 years duration of type-II DM – 14 (14.8%) had 7 to 12 years.
- Duration of foot ulcer – 14 (14.8%) patients had 4 to 5 months, 10 (10.8%) had 6 to 10 months
- Duration of antibiotics – 17 (18.1%) started 15 to 20 days, 3 (6.3%) had 21 to 30 days
- Amputation were 6 (6.3%) in patients

Table 1: Dermatographics of patients

Sl. No	Details	No. of patients (94)	Percentage (%)
1	Duration of foot ulcer a) 3 months to 12 months	28	29.7
	b) 13 months to 36 months	66	70.2
2	Duration of Diabetes a) 2 years to 5 years	38	40.4
	b) 6 years to 12 years	56	59.5
3	Random Blood Sugar a) 200-320	44	46.8

	b) 321-670	50	53.1
4	HBN a) 6 – 7	27	28.7
	b) 8 – 15	67	71.2

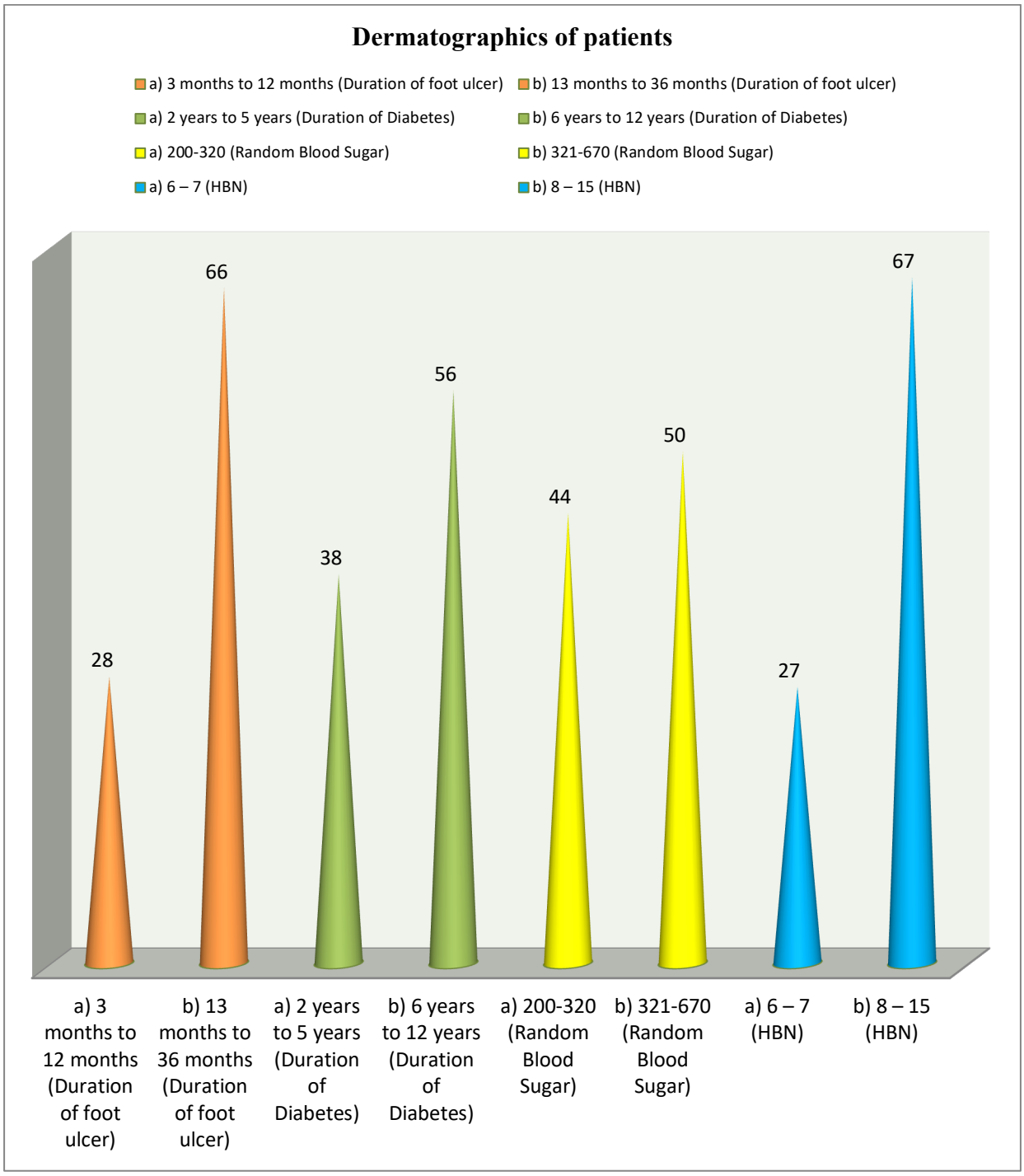

Figure 1: Dermatographics of patients

Table 2: Study of grades of foot ulcers

Sl. No	Grade of Ulcer	No. of patients (94)	Percentage (%)
1	Grade-II	32	34
2	Grade-III	42	44.6
3	Grade-IV	20	21.7

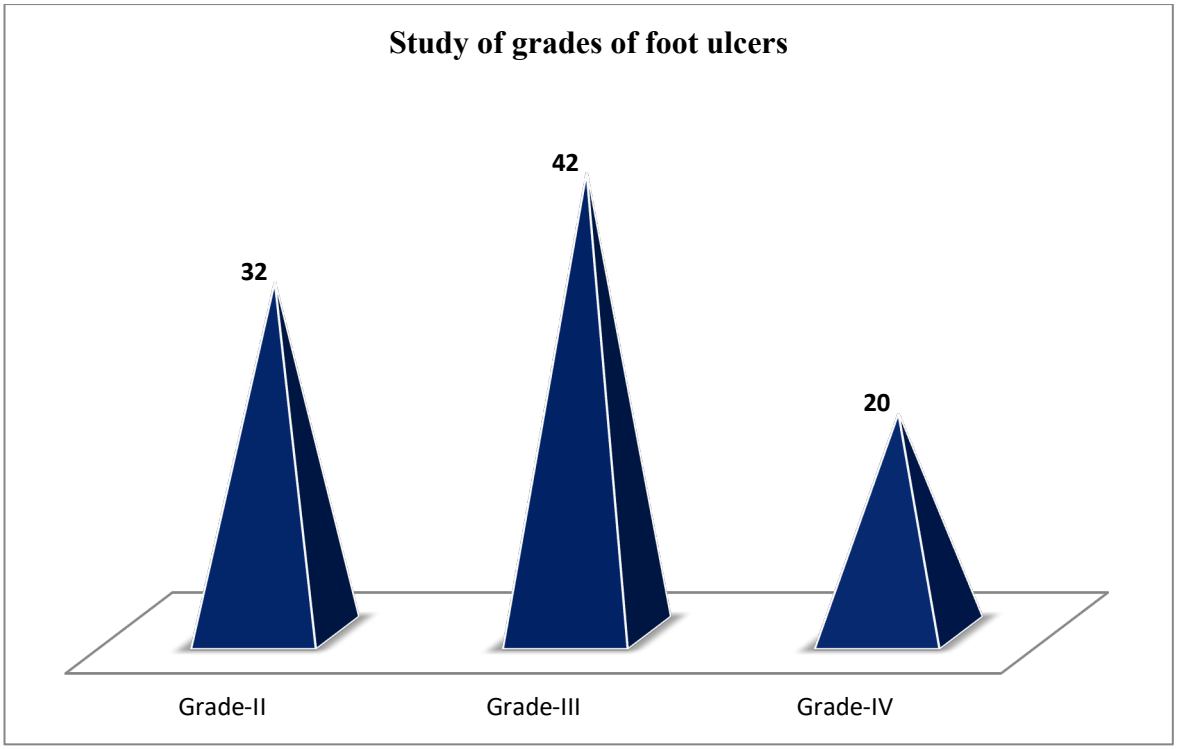

Figure 2: Study of grades of foot ulcers

Table 3: Study of Co-morbidities in Diabetic foot ulcer patients

Co-Morbidities	No. of patients (84)	Percentage (%)
HTN	36	38.2
CRF (chronic renal failure)	27	28.7
IHD	15	15.9
TB	4	4.2
Hypothyroid	2	2.1

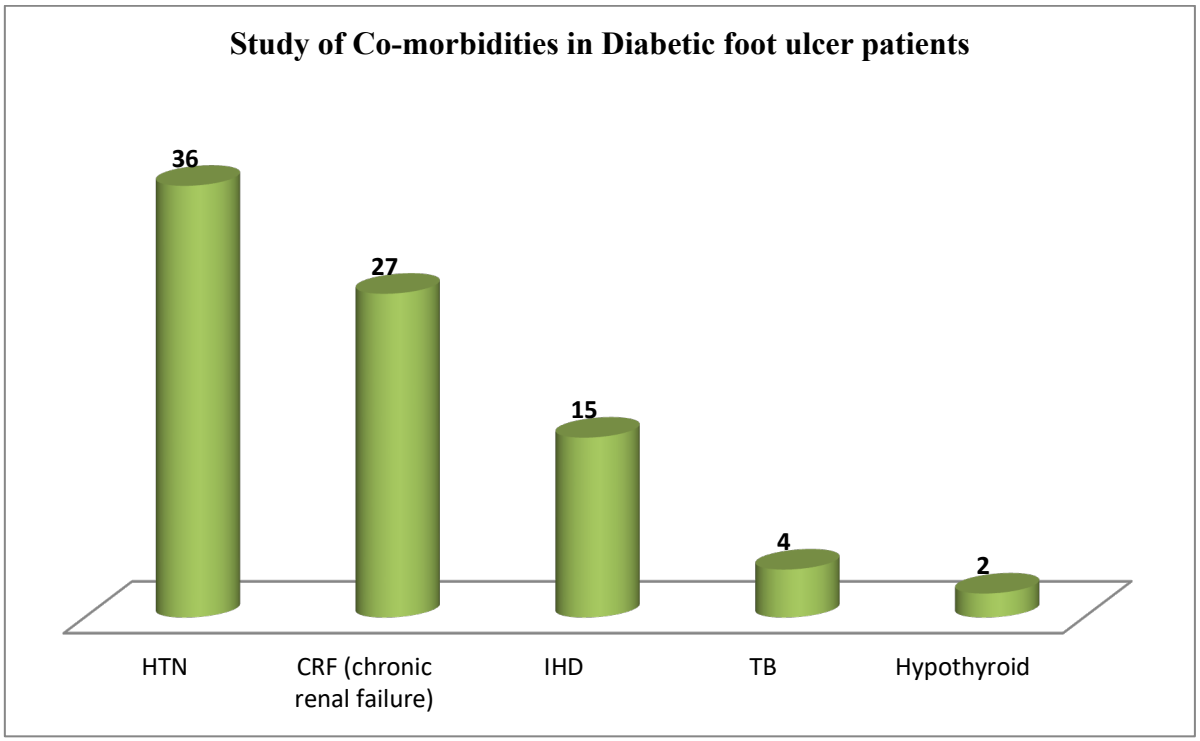

Figure 3: Study of Co-morbidities in Diabetic foot ulcer patients

Table 4: Spectrum of Bacterial flora in co-existence of fungal infections (Total No. of patients: 94)

Sl. No	Bacterial Flora	No. of patients (94)	Percentage (%)
1	Staphylococcus Aureus	25	26.5
2	Pseudomonas	17	18
3	Escheria coli	15	15.9
4	Enterobacter	9	9.5
5	Sterpococcus	8	8.5
6	Proteus vulgaris	4	4.2
7	Acinobacter	4	4.2
8	Klebsiella	2	2.1
9	Enterococcus	10	10.6

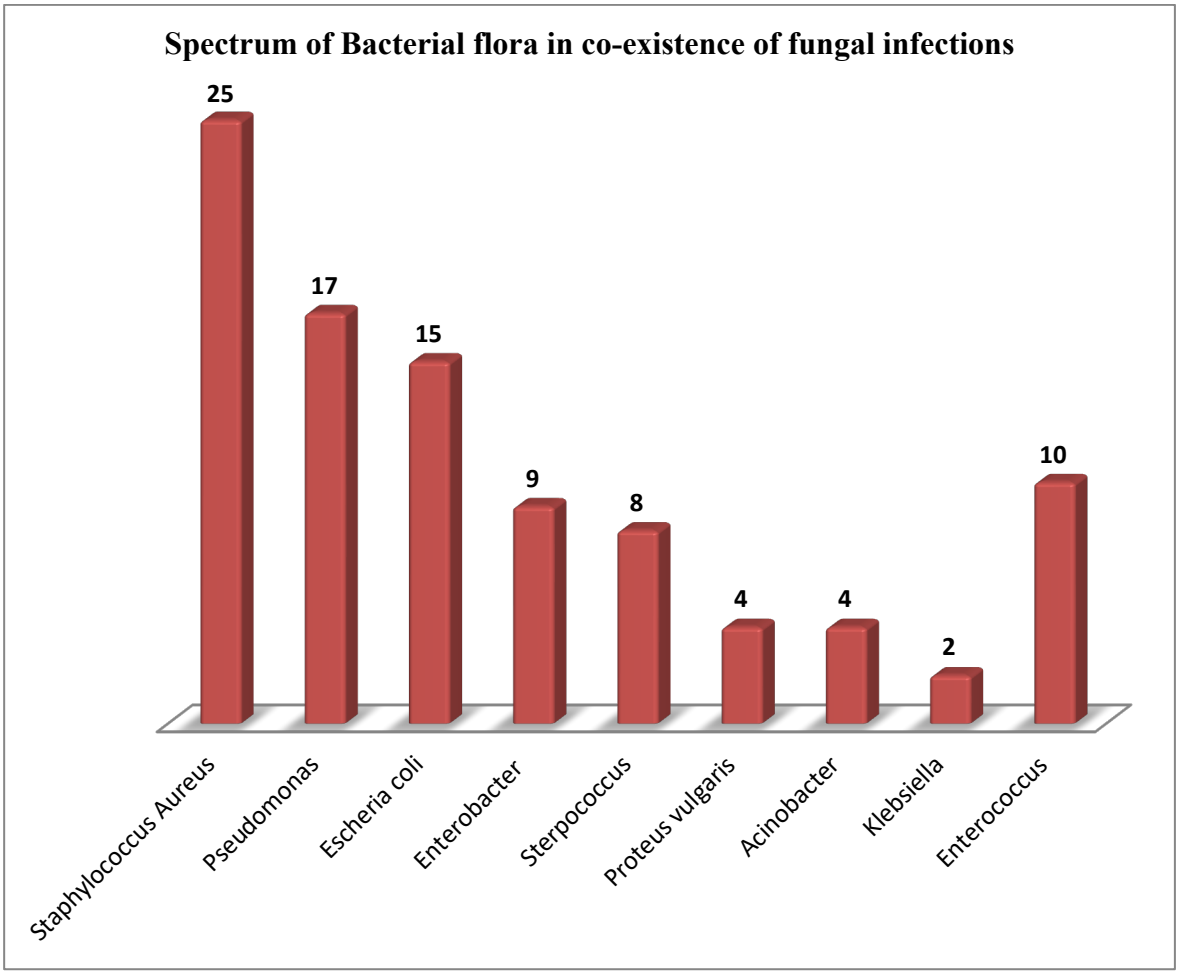

Figure 4: Spectrum of Bacterial flora in co-existence of fungal infections

Table 5: Study of Fungal positive patients (Total No. of patients: 24)

Sl. No	Details	No. of patients	Percentage (%)
1	Duration of type-II DM a) 5 – 6 year	10	10.6
	b) 7 – 10 year	14	14.8
2	Duration of foot Ulcer (in months) a) 4 – 5	14	14.8
	b) 6 – 10	10	10.6
3	Duration antibiotic a) 15-20	17	18
	b) 21-30 (days)	6	6.3
4	Amputation	6	6.3

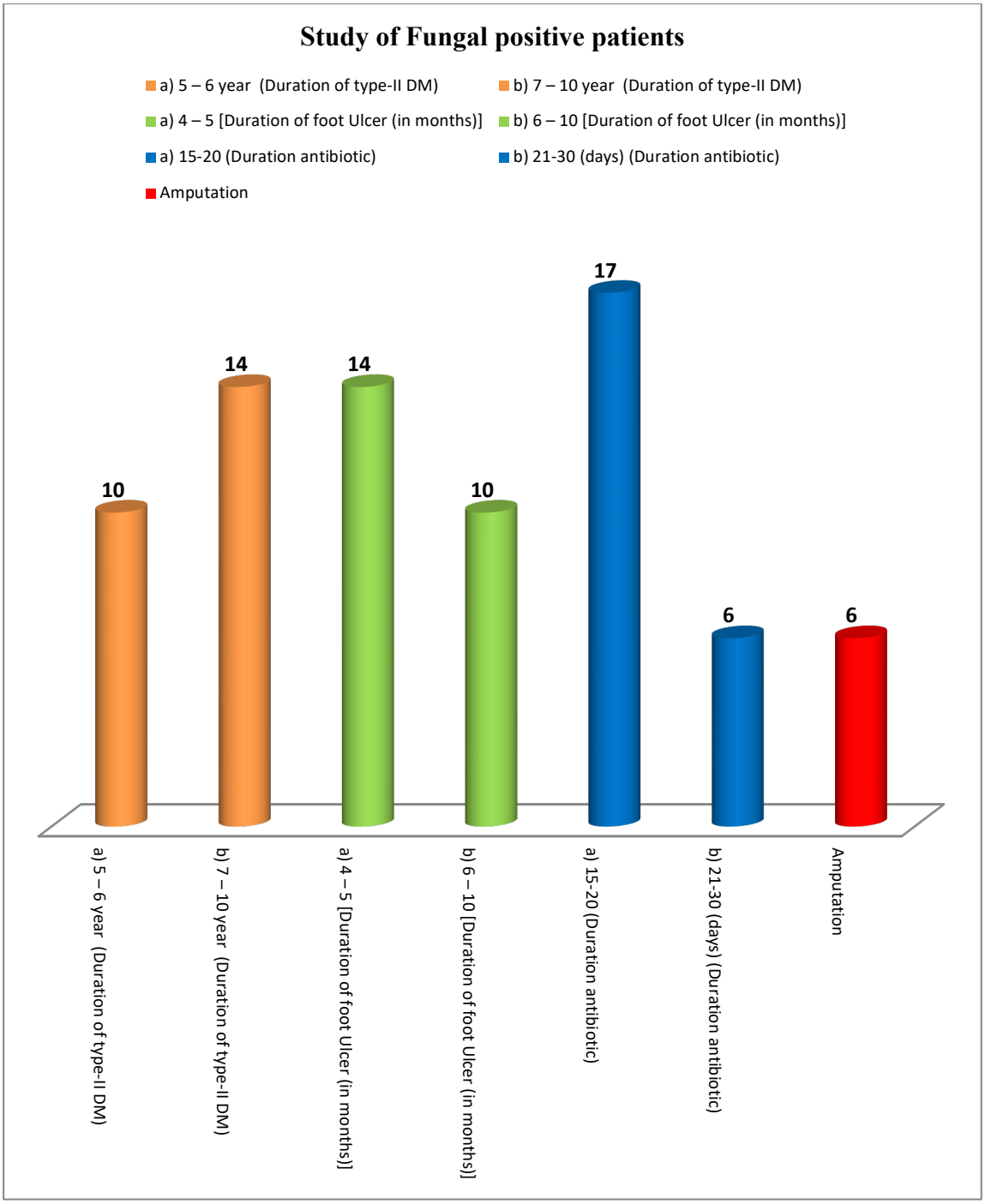

Figure 5: Study of Fungal positive patients

Discussion

Present study of co-existence of fungal infection in DFU I Delhi population. The dermatological profile had a profile. Duration of DFU: 28 (29.7%) had 3 months to 12 months, and 66 (70.2%) had 13 months to 36 months.

The duration of type-II DM was 38 (40.4%) from 2 to 5 years; 56 (59.5%) were 6 to 10 years. The RBS level was 44 (46.8%) with 200-300 and 50 (53.1%) had 321-670. The HbA1c level was 27 (28.7%) with 6-7% and 67 (71.2%) had 8-15% (Table-1). The grade of DFU was 32 (34%) with grade II, 42 (44.6%) with grade III, and 10 (21.7%) with grade IV (Table 2). Co-morbidity of DFU patients: 36 (38.2%) had HTN, 27 (28.7%) had CRF, 15 (15.9%) had IHD, 4 (4.2%) had TB, and 2 (2.4%) had hypothyroid (Table-3). In bacteriology flora study S. aureus was the most common, and the least was 2 (2.4%) Klebsiella, 17 (18%) pseudoamanas, 15 (15.9%) E. coli, 9 (9.5%) Enterobacter, 8 (8.5%) Enterobacter, 10 (10.6%) Enterococcus (Table 4), and 24 (25.2%) fungal infections positive were observed in DFU patients (Table 5). These findings are more or less in

agreement with previous studies [6,7,8]. When surgically treating diabetic foot ulcers, it is imperative to understand the type of wound that you are dealing with when choosing the particular way to classify an ulcer; it is important to know the depth of the ulcer and the level of ischemia and infection. A vascular examination should always be performed on any patient who presents with a diabetic foot wound. If the patient has palpable pedal pulses. Doppler should be utilized to listen for signals. The initial examination must be noninvasive vascular studies, including ankle-brachial indices and toe pressures. The pathogenesis of diabetic foot is highly complex, including peripheral neuropathy, peripheral vascular disease, compromised immunity, slower wound healing, trauma, and infection [9]. Complications are associated with the development of infection and diabetic foot syndrome, which are the main cause of morbidity, non-traumatic lower extremity amputations, and diabetic patients' mortality [10]. Bacterial infections of DFU are polymicrobial and mixed aerobic/anaerobic.

Fungal infections include Candida spp. and/or Candida albicans. It is reported that there is an increased incidence of fungal infections (dermatophytes) and candidiasis of interdigital spaces and nails in the toes of diabetic patients. These infections are associated with the development of severe and deep inflammatory processes in feet. It is also noted that candidiasis in DUF plays a secondary role, followed by bacterial flora in long-standing diabetic patients [11]. It is described that systemic antifungal therapy (flucytosine, fluconazole, itraconazole, or terbinafine administered orally at variable dosage and duration has a significant impact on revascularized DUF patients [12].

Summary and Conclusion

The present study of co-existence of fungal infection in DFU patients. It is polymicrobial in nature. DFU has predominantly Enterobacteriaceae and Pseudomonas bacteria with 25% positive fungal infections in long-standing diabetic patients. It is confirmed that fungal infections were observed only in chronic DFU patients. Treating such patients in revascularizes DFUs with a proper dosage of antifungal has faster wound healing capacity. It requires proper mycological evaluation to treat such patients. This study will help the clinician or dermatologist/surgeon to treat such DFU. But this study demands further pathophysiological, hormonal, nutritional, genetics, studies because the exact cause of incidence of fungal infection in polymicrobial DFU is still unclear.

Limitation of study: Owing to remote location of research centre, small number of patients, lack of latest techniques, we have limited findings and results.

This research was approved by Ethical committee of Medinirai Medical College, hospital, Palamu, Jharkhand-822101.

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