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Prevalance and distribution of candidia species from diabetic foot ulcer in tertiary care centre, Jamnagar, Gujarat

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ABSTRACT

Introduction: Diabetes Mellitus is a chronic disease which may cause diabetic foot ulcer, which is a major cause of morbidity and mortality, it may also lead to foot amputation due to gangrene, and may cause cellulitis, abscess etc.
Aims & Objectives: To study prevalence of candidiasis in diabetic foot ulcer in a tertiary care centre, Jamnagar.
Materials and Methods: 32(10.66%) isolates that were recovered from wound discharge samples (300 samples tested) from November 2017 to September 2018. All isolates were visualized under direct microscopy, cultured, & sugar assimilation tests were performed.
Results: Amongst 300 samples 32(10.66%) were positive for fungal culture, in which major isolates was C. albicans (50%), C. tropicalis (18.75%), C. dubliniensis (9.37%), C.krusei (9.37%), C. glabrata (6.25%), C. parapsilosis (6.25%).
Conclusion: This study shows that in Diabetic foot ulcer most common fungal pathogens were C. Albicans, C. tropicalis, C. dubliniensis, etc.
Early identification of organism can help in early treatment and early recovery.

1. Introduction

Diabetes Mellitus affect globally, about 463 million people had diabetes worldwide as of 2019.[1] Diabetes mellitus have major 3 types (I) Type I: Insulin dependent diabetes mellitus (IDDM), where pancreases produces decreased amount of insulin (Insulin deficiency). (II) Type II: Non- Insulin dependent diabetes mellitus (NIDDM) or Adult diabetes mellitus, where body cells do not respond to insulin (Insulin Resistance). (III) Type III: Gestational Diabetes occurs in pregnant women due to high sugar level. Among these type 90% cases were Type II Diabetes mellitus.

Diabetes mellitus has multi-system affliction and causes long term complications like cardiovascular diseases, Diabetic retinopathy, nephropathy, neuropathy and diabetic-related foot ulcers.[2–4] In the history of diabetes treatment there has been a wide range of modern treatments available to control it, in the near future we may expect a complete cure.[5,6]

In non- traumatic lower limb amputation most common cause was diabetic foot ulcer. Amputation leads to morbidity and disability or discomfort in routine physical activity. Diabetic foot ulcer infection is poly-microbial and multidrug resistant. Several studies and research which were conducted showed that aerobic and anaerobic bacterial infection were of primary importance. Due to the lack of mycological importance, fungal infections were ignored to be a cause of diabetic ulcer.

The present study was conducted to isolated the fungal pathogen from diabetic foot ulcer wound and in which

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candida species were more common. All fungi constituting the genus Candida belongs to the yeast like fungi because they exhibit a mycelium as well as a yeast form. The yeast cells are unicellular, small, oval, 3-5 μm in diameter and exhibit budding forms called blastospores or blastoconidia. The mycelia forms are of two types, pseudo mycelium and true mycelia.[7,8] Candida infections were most common in foot ulcers because of widespread use of empirical antibiotic and any medical devices.

Proper identification of fungi may help for the better outcome and prevents their complications.

2. Aims and Objectives

1. Prevalence of candida species in diabetic foot ulcer.
2. Identify non albicans candida species by using phenotyping methods.

2.1. Ethics statements

The study was approved by Institute ethics committee, M. P. shah Govt. Medical College and Guru Gobindsingh Hospital, Jamnagar with Ref. No. IEC/CERTI/113/2017.

3. Materials and Methods

In this retrospective study total 300 pus samples were collected from Guru Gobind singh Government Hospital, Jamnagar. Diagnosis of a yeast infection is done by direct microscopic examination, culturing, further diagnosis done with serological, molecular methods and other newer rapid diagnostic tests are available like Flurogenic Tests, Platelia Candida antigen test, Rapid trehalose assimilation test, Cand Tec Ramco labs.[9]

3.1. Collection of the specimen

On the basis of clinical history and finding, samples were collected as per laboratory protocol. The ulcer site from diabetic foot exudate was collected by sterile thin cotton wool swabs, aseptically. Then sample were immediately transported to laboratory for processing.

3.2. Diagnostic methods

All the samples were processed in the following manner:

A. Direct microscopy

Primary smear, including Gram's stain: Yeast cells and pseudo hyphae are stained dark blue.

B. Culture

1. Sabouraud Dextrose Agar (SDA): within 2-3 days of incubation Cream coloured, Pasty and smooth colonies appear.
2. Corn Meal Agar: At 25°C large, highly refractile, thick walled, terminal chlamydospore were noted.
3. CHROM Agar: The medium consists of specially selected peptones and artificial substrates called chromogens, which release differently coloured compounds upon degradation by specific enzymes, permits the differentiation of different species of Candida like C.albicans- light green to blusih green, c. dubliniensis- dark green, C.parapisolosis- cream coloured, C.krusei- Pinkish to purplish, C.glabrata- Pink to purple.

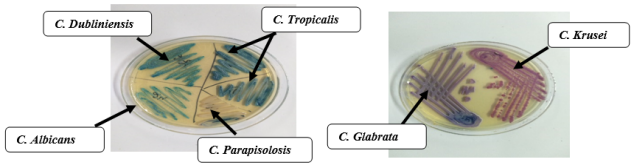

Fig. 1: Different Species of candida on CHROM Agar)

3.3. Germ tube test:[10,11]

Candida species treated with normal human serum and incubated at 37°C for2-4 hours, shows long tube-like projection extending from mother yeast cells and no constriction at the point of attachment. Only C. albicans and C.dubliniensis produce germ tube.

3.4. Sugar Assimilation test:[12]

Shows ability of yeast to use particular carbohydrate utilization by presence of halo zone around disc.

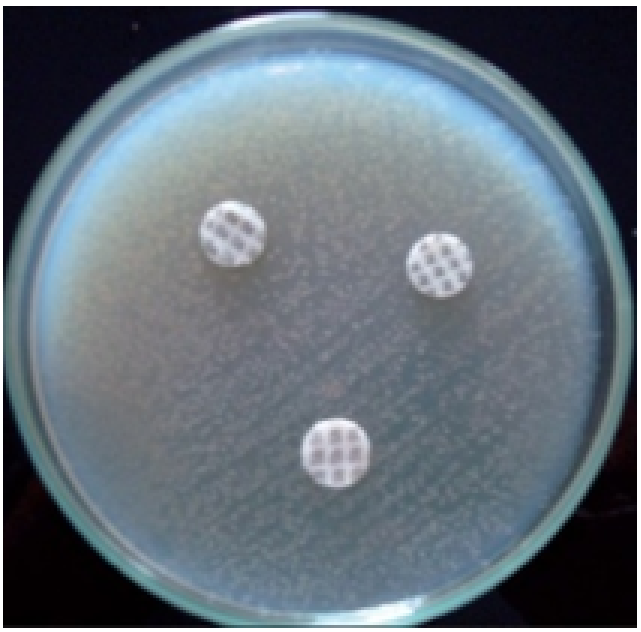

Fig. 2: Sugar assimilation test

3.5. Sugar fermentation test: [13]

Gas production in durham's tube with colourless to pink colour changes shows sugar fermentation test positive.

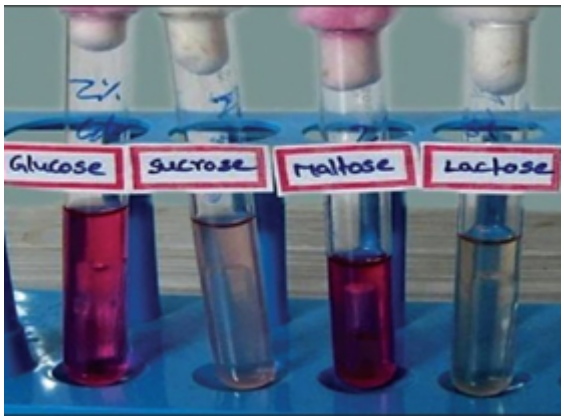

Fig. 3: Gas production with carbohydrate assimilation

4. Result

In present study out of 300 samples 32 samples shows presence of Yeast cells on direct smear examination, so the isolation rate of positive cases was about 10.67%, sex wise prevalence of positive cases showed that there is higher prevalence in male (59.38%) than female (40.62%). It is more common in patients of age group between 51-60yr (28.1%), followed by 61-70 yr (25%),41-50 yr (18.8%), 31-40yr (12.6%), 21-30 yr & >70yr shows (6.2%)and 11-20yrs (3.1%).

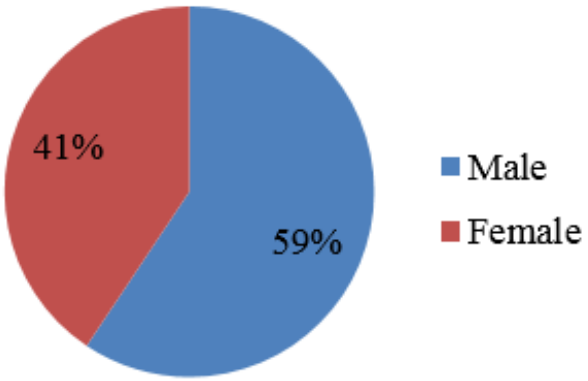

Fig. 4: Sex wise distribution of Candida

Candida isolated among positive samples which include C.albicans (50%), C.tropicalis (18.75%), C.dubliniensis (9.37%), C.krusei (9.37%), C.glabrata (6.25%) & C.parapsilosis (6.25%).

The chi-square statistic is 0.068. The p-value is 0.999434. The result is not significant at $p < 0.05$.

Table 1: Age group wise distribution of Candida

Age (year)	Total No.	Percentage
11-20	1	3.1%
21-30	2	6.2%
31-40	4	12.6%
41-50	6	18.8%
51-60	9	28.1%
61-70	8	25%
>70	2	6.2%

The chi-square statistic is 0.0098. The p-value is 0.999988. The result is not significant at $p < 0.10$.

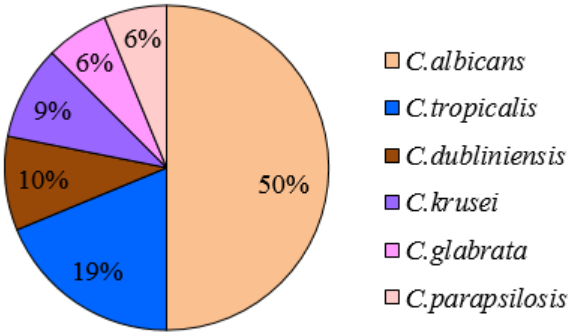

Fig. 5: Candida Spp. differencing on CHROM Agar

5. Discussion

Diabetes foot ulcer may be associated with some predisposing risk factors like smoking, alcoholism, trauma, previous ulcer, prior amputation, previous ulcer leading to amputation, neuropathy, etc. Ulcer may be due to diabetes any of its complication, which may include fungal isolates may show different patterns, which may affect treatment.

In fungal infection identification of the fungal agent species were most important than the isolation. In candida species CHROM Agar was used as a differential medium due to its ability to detect mixed culture of yeast from clinical specimens for presumptive identification. It is used for the definitive identification because the phenotypic method was time consuming and unable to discriminate C. albicans and C. dubliniensis. [18,19]

In present study, we have tested 300 samples of pus. On direct microscopy, smears were examined for pus cells or any fungal elements (yeast cells). All the samples were tested for fungal culture & biochemical test.

A Study conducted by Sanniyasi S et al [14](72.4%), J Nithyalakshmi et al [15](67.6%), Abhilash et al [16](66.7%) showed prevalence of candida species more common in male than female which was same as present study (59.38%).

In our study, among the positive samples the highest number of isolation were c.albicans (50%), C.tropicalis (18.75%), C.dubliniensis (9.37%), C.krusei

Table 2: Comparison of positive cases and sex wise distribution in different studies

Total	Sanniyasi S et al (2015)[14]	J Nithyalakshmi et al (2014)[15]	Abhilash et al (2015)[16]	Present Study
Positive cases	15.23%	15.49%	18%	10.67%
Male	72.4%	67.6%	66.7%	59.38%
Female	27.6%	32.4%	33.3%	40.62%

Table 3: Comparison of various isolates in different study

Organism	J Nithyalakshmi et al (2014)[15]	Abhilash et al (2015)[16]	Emilija M M et al (2005)[17]	Present study
C.albicans	64%	49%	18.2%	50%
C.tropicalis	18%	23%	22.7%	18.75%
C.dubliniensis	4%	5%	9.1%	9.37%
C.krusei	5%	5%	4.5%	9.37%
C.parapsilosis	9%	18%	36.4%	6.25%

(9.37%), C.parapsilosis (6.25%) which compared with J Nithyalakshmi et al[15] (64%), Abhilash et al[16] (49%), but study by Emilija M M et al[17] shows C. parapsilosis (36.4%) followed by c.tropicalis (22.7%) as its highest isolation.

6. Conclusion

Early diagnosis of the patients on clinical ground as well as diagnosis of the causative organism and to know its effective treatment is of much importance for the positive outcome.

In this study non albicans candida species was found to be equally responsible for this clinical condition. Treatment failure is common with candida non-albicans, because of its high resistance and low susceptibility to azoles. Therefore accurate identification of different species of Candida is essential.

For prevention, proper personal hygiene along with awareness of cleanliness may help the situation. Strict hospital ward and operation theatre cleanliness is also required. Frequent changing of antiseptic solution bottles and judicious use of antibiotics are important.

7. Limitation

As candida albicans can be seen as normal flora it was difficult to differentiate both the pathogen and non pathogen forms.

As we had only limited resources it was difficult to differentiate the candida non albicans upto species level.

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