ORIGINAL RESEARCH PAPER

Medical Science

ISOLATION AND SPECIATION OF CANDIDA FROM THE SALIVA OF TYPE II DIABETIC PATIENTS USING CHROM AGAR

KEY WORDS: CHROM Agar, Candida tropicalis, Oral cavity, Prevalence

Arunkumar.p — Second Year MBBS, Saveetha Medical College And Hospital, Thandalam, Chennai- 602105

Dr. S. S. M. Umamageswari * — Professor, Department Of Microbiology, Saveetha Medical College And Hospital,Thandalam, Chennai- 602105. *corresponding Author

Dr. Rajavani. I — (B.S.M.S) (m.sc Medical Microbiology III Year, Department Of Mcrobiology Saveetha Medical College And Hospital,Thandalam, Chennai- 602105.

G. Mahalakshmi — M. Sc, Medical microbiology III year, Department Of Mcrobiology Saveetha Medical College And Hospital,Thandalam, Chennai- 602105.

ABSTRACT

Introduction: Infection of the Candida species in the oral cavity is increased to a higher rate. Several immune alterations have been described in diabetes with cellular immunity being more compromised and with changes in monocytes and lymphocytes. Hence, there is increased infection occurs in Diabetic than non-diabetic patients.
Aim: To isolate different Candida species from the saliva of diabetic individuals and compared it with non-diabetic individuals.
Materials and Methods: The duration of the microbial study is 2 months. From diabetic and non-diabetic patient with consent oral sample has been collected and processed in microbiology department in Saveetha Medical College and Hospitals, Chennai. The Candida species will be identified and isolated with CHROM Agar under standard technique.
Result: There is a higher occurrence of Candida species namely, Candida albicans, Candida glabrata, Candida tropicalis except for Candida Parapsilosis in the diabetic group when compared to healthy individuals. Most commonly identified species is Candida tropicalis. Conclusion: C. tropicalis is now considered as one of the significant causes of candida infection in oral cavity. The increased virulence will affect the global burden of Candidiasis as few treatment option are available for this new pathogen.

INTRODUCTION

Infections of the mouth are the most common among children less than one month old, the elderly and those with weak immune system include HIV/AIDS, the medications used after organ transplantation, diabetes and the use of corticosteroids. Diabetes mellitus is a group of metabolic disorder characterized by high blood sugar levels over a prolonged period [1]. Type II diabetes is characterized by high blood sugar, insulin resistance, relative lack of Insulin and it is caused due to lack of exercise and obesity. Most of the patients with Diabetes mellitus are having Candida species in their oral cavity and it is frequently isolated [6]. The most common Candida species isolated is Candida albicans; it more often affects the patients with less insulin production [2]. The less commonly identified species are Candida tropcalis, Candida glabrata, Candida parapsilosis, and Candida krusei [5].Identification and differentiation of both albicans and non-albicans Candida species is important because they differ both in their potential to cause disease and their response to Antifungal drugs.

The culture medium used in this study is **SDA agar** for the isolation and growth of the fungi and **Germ tube test** was used to detect *candida albicans*. **CHROM agar** is used for speciation which helps in isolation and differentiation of some important species of *Candida* [7]. Enzymes produced by Candida species react with substrates of CHROM agar and it results in production of different colored colonies on agar.

This study was undertaken to isolate different Candida species in diabetic patients and to identify the most common species other than Candida albicans and to compare the prevalence of it in diabetics with that of non-diabetic individuals.

MATERIALS AND METHODS

The prospective study was conducted at the Department of Microbiology, Saveetha Medical College, Chennai after taking ethical approval. The study was carried out for duration of 2 months, written consent was taken from the participants before their inclusion in the study. In this study, two group of individuals were taken. 30 individuals with Type II Diabetes [Random Blood Sugar (RBS) ≥ 200 mg/dl and Fasting Blood Sugar (FBS) ≥ 126 mg/dl] were taken as GROUP-1 and 30 individuals with any other lesions and having normal blood glucose level were taken as GROUP-2. Participants were instructed not to eat or drink anything for at least 1 hr before the collection of saliva sample [4]. Participants were asked to rinse their mouth thoroughly 10mins before collection to avoid collection of food debris and 2-3 ml of saliva was collected by allowing the participant to drool or gently expectorate into clean, sterile test tube. The test tube was closed and sent to lab within 30mins of collection.

Samples were stored at 4°C before analysis. The solution was centrifuged at 5000 rpm for 5mins to make it concentrated, pellets remained at bottom of the tube and it was streaked in CHROM agar plates [3] and incubated at 37°C for 3-4 days. CHROM agar was visualized daily at 24hrs, 72hrs. Candida Speciation was done based on the different coloured creamy colonies appeared [8] on CHROM culture media compared with color chart shown ina Tble-1.

Fig-1 showing different species of Candida in CHROM agar

COLOUR	SPECIES
Light Green	C.alibicans
Cream	C.parapsilosis
Blue	C.tropicalis
Purple	C.glabrata

Color chart showing Candida species [Table-1]

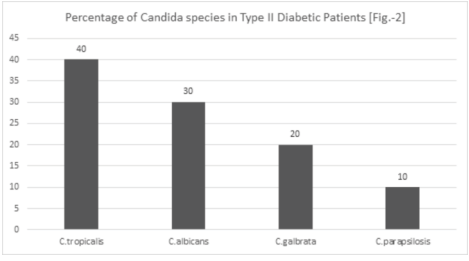
Different colored colonies were appeared on CHROM agar cu the fig-1[10,11].

STATISTICAL ANALYSIS

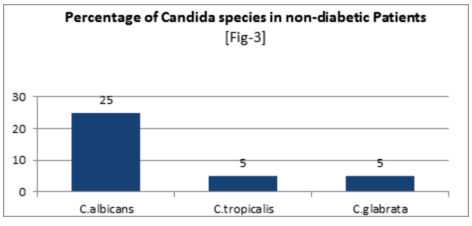
The mean value of all the *Candida* spp. was calculated for two groups, namely, Type II diabetic group and the healthy group. Statistical analysis for comparison between these two groups was performed by **Student's t-test. A p<0.5** was considered statistically significant.

RESULTS

This study includes 60 patients, out of which 30 patients had Type II diabetes without any oral lesion, and 30 were healthy individuals. The species of *Candida*, namely, *C.albicans, C.tropicalis, C.galbrata, C.parapsilosis* showed a significantly higher occurrence in the diabetic group compared to the healthy group. In diabetic individuals, *C.tropicalis* had the highest occurrence of (40%) where the *C.albicans* was found to be 30% shown in [Fig.-2]. The other species are *C.galbrata (20%), C.parapsilosis (10%).*



In non-diabetic individuals, C.albicans had the highest occurrence of 25%.



The other species are C.tropicalis (5%), C.galbrata (5%), shown in [Fig.-3]

DISCUSSION

Diabetes mellitus (DM) is a group of metabolic disorders characterized by high blood sugar levels over a prolonged period. This may be due to either pancreas not producing enough insulin, or the cells not responding properly to the insulin produced or the *Candida* infection is the major among the less immune people [9] [14]. The concomitant occurrence of DM with candidiasis has been established since long, but this association is still to be questioned [12].

A higher colonization of *Candida* species was seen in the diabetic individuals when compared to the control group and it is found to be statistically significant. Mohammadi F etal, in 2006 and Lydia Rajakumari M et Al in their study found that oral candidiasis frequency in diabetic patients in relation to non-diabetic one was more due to factors that promote oral Candida flora in diabetic patients [13].

In study by Mohammadi F et al and Lydia Rajakumari, there is a increased frequency of candida found in the diabetic individual than the control group which is found in accordance with our study [15]. Both the study show there is a increased frequency of C.albicans , but in our study C.tropicalis is found in increased number, this shows that there is a change in trend of candida infection.

CONCLUSION

Candidiasis has been known to be an opportunistic infection in the oral cavity [10] [11] since a long time, among which C.albicans being the known the leading cause. But there is a change in the trend, C.tropicalis has now currently emerged as a human pathogen. There is a need to develop targeted antifungal agents against it. The currently available diagnostic aid are providing further information about the virulence of C.tropicalis.

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