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COMPARATIVE MYCOBIOME PROFILING OF ORAL MUCOSA IN DIABETES PATIENT

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ABSTRACT

Background: Nearly 1.4 million people in India are diagnosed with diabetes mellitus each year, making it one of the most prevalent endocrine illnesses in the world. The illness may cause the immune system to deteriorate and raise the risk of infctions and other microbial illnesses.

Aim: Using molecular and phenotypic identification of years isolated, the current study sought to evaluate the mycobiome profile of the yeast in the oral cavity of people with or without type 2 diabetes mellitus as well as any potential correlation between oral candida species and HbA1c levels in diabetics.

Methods: 128 participants between the ages of 18 and 70 were split into two groups according to their glycemic status: 68 were diabetic and 60 were not. For determination of various fungal species using morphological and molecular identification after isolation from mucosal oral cavity in Indian diabetic and non-diabetic subjects.

Results: In both diabetics and non-diabetics, the study's findings showed nine common species. Diabetics have higher levels of isolated fungus than non-diabetics. The most prevalent species was Candida albicans, which was followed by K. marxsianus.

Conclusion: The current study provides important information on the variety and distribution of commensal yeast in Indian individuals' oral cavities as well as the risk of infection transmission due to shifts in the microbial species composition in relation to health problems.

Keywords: Candida, diabetes, microbiota, phylogenetic analysis, yeast

INTRODUCTION

The most prevalent endocrine condition, diabetes mellitus (DM), is characterized by malfunctioning beta cells in the pancreatic islets of Langerhans, which may lead to persistently elevated glucose plasma levels. Diabetes is the fastest-growing illness in the world, affecting millions of people worldwide and posing a serious danger to human health. Following the statistics from 2021, literature research indicates that the prevalence of diabetes is lower in female individuals than in male people. Diabetes has also been linked to changes in the oral environment, which in turn affects the colonization of microorganisms in the oral cavity.[1]

The oral cavity, which is coated with mucosal cells and frequently contains saliva released by the salivary glands, is one of the main entrance sites for bacteria into the body. Several beneficial microorganisms' processes are activated by the oral mucosal cavity. Because of their varied physiological activity and capacity for adhesion to the oral mucosa, bacteria and fungus are among the microbiota found in normal oral mucosa. Health and illness are governed by these microbiotas. The network of oral microorganisms and the host immune response, however, are not well documented in the literature.[2]

The bacterial community has been used to characterize the oral microbiota in the majority of the research. Oral microbiome has only been found in a small number of investigations, though. Although fungi, particularly yeast, can colonize the oral cavity without affecting the host's health, they can serve as opportunistic infections by altering the balance of the local microbiota and immune system. Candida species in the oral environment can endanger a number of organs in people who are very sick or have impaired immune systems.3

The oral myobiota is constantly evolving during its life cycle and is influenced by a number of host lifestyle characteristics as well as associated factors such as food choices, immune responses, and personal hygiene practices. In healthy individuals exhibiting a specific indication or symptom, Candida species often make about 60% of the overall oral flora. Although several species of candida have been identified from the oral cavity as commensals, their frequency varies in non-diabetic patients because to elevated salivary glucose levels that lead to pathogens and yeast infections. However, 30% of persons without diabetes mellitus and 56% of subjects with the disease had candida albicans, which is thought to be more common among diabetics. 4 There is a dearth of information in the literature currently available on yeast flora and the causes of yeast infections in both diseased and healthy Indian patients.

Therefore, based on genetic and phenotypic identification of the years isolated, the current investigation sought to evaluate the mycobiome profile of the yeast in the oral cavity of people with or without type 2 diabetes mellitus as well as any potential correlation between oral candida species and HbA1c levels in diabetics.

MATERIALS AND METHODS

Using molecular and phenotypic identification of the years isolated, the current descriptive study sought to evaluate the mycobiome profiling of the yeast in the oral cavity of subjects with or without type 2 diabetes mellitus as well as any potential correlation between oral candida species and HbA1c levels in diabetics. The research participants came from the Institute's Department of Medicine. Prior to their involvement in the study, all individuals gave their written and verbal informed consent.

128 participants in the current study, who were of both sexes and ranged in age from 25 to 70, visited the institution during the designated study time. There were two groups of these subjects: 68 individuals with a verified clinical diagnosis of type 2 diabetes mellitus (36 females and 32 males) and a control group of individuals without the disease (36 females and 24 males).

Blood samples were taken from each participant in both groups in order to estimate their HbA1c (glycated hemoglobin) levels using the absorption spectroscopy technique, which shows how much glucose the erythrocytes had been exposed to during the last ninety days. A fiber-based single beam spectrophotometer was used to record the absorption spectra of HbA1c (glycated hemoglobin) in the 200–850 nm range. Glycated hemoglobin values were assessed following its estimation using spectrum for absorption.

Following final inclusion, a pre-made structured questionnaire was used to collect data from all subjects. This questionnaire recorded demographic information such as age, name, gender, medical history, smoking, symptoms of candida disease, and whether or not they had received antifungal or antibacterial therapy in the previous month. Every participant in both groups did not smoke. Following sterilization, samples were taken from each subject's oral cavity using swabs. After being promptly transferred to sterile test tubes, the samples were taken to the laboratory. After that, swabs were cultivated in Sabouraud dextrose agar (SDA) medium. For two weeks, cultured materials were incubated at 37°C with frequent evaluations until fungal colonies formed or a negative result was seen. After that, pure colonies were cultivated on candida medium in order to isolate and identify the species of candida. Colony colors were cultured and recorded.[5] Colonies having varying colors or morphological features were transferred in SDA slants and stored for molecular identification.

Purified entire genomic DNA was extracted and isolated in accordance with the manufacturer's instructions. Before being amplified using PCR, DNA samples were stored at minus 20 degrees. The amplified DNA's PCR products were chosen for sequencing. ANOVA, chi-square test, student's t-test, Fisher's exact test, Mann Whitney U test, and SPSS software (IBM Corp., Armonk, NY, USA) were used to statistically evaluate the collected data. A p-value of less than 0.05 was taken into consideration for the significance threshold.

RESULTS

Using the absorption spectroscopy technique, the assessment of HbA1c (glycated hemoglobin) in the blood samples from the two groups was determined to be between 6.5% and 15% for the participants with diabetes and between 4% and 6.3% for the subjects without the disease.

Results were evaluated from 128 oral swabs taken from research participants who did not exhibit any signs of a fungal infection. Different species were isolated from the oral cavity mucosa of both diabetic and non-diabetic patients, according to the results. 90% of those with diabetes at lunchtime and 97% of those with diabetes had Ascomycota, primarily candida species, with Wickerhamomyces anomalus being the only Basidiomycota. Upon clinical oral examination, none of the subjects in either group showed signs of subclinical colonization.

According to the study's findings, both diabetics and non-diabetics had different species isolated from their mucosal oral cavities; however, non-diabetic participants had nine more species than diabetic patients. Yarrowia sp., Nakaseomyces glabratus, Yarrowia sp., Kluyveromyces sp., Kluyveromyces marxianus, Candida Africana, Candida dubliniensis, and Candida albicans were among the nine species. When Candida species were identified, yeast species were seen. Additionally, all sequenced fungal isolates were shown to cluster with the material type of known particular fungal specimens, according to phylogenetic analysis. Every species of Candida split off from other fungal species in a monphylogenetic clade and grouped according to specimen type. There were 948 colonies of Candida albicans, 60, 142, 264, and 622 of Naganishia sp. for the total number of colonies. The number of colonies in non-diabetics was essentially equal. Compared to diabetics, who had seven species of yeast colony, non-diabetic participants had nine species.

The same species were seen in both men and females when evaluating the impact of gender on the organization of the community over time, with the exception of extra species in females, such as candida Africana. C. albicans was most prevalent in diabetics (45%), followed by Kluyveromyces marxianus in men with diabetes mellitus (16%), K. marxianus in males with diabetes mellitus (16%), and C. albicans in females with diabetes mellitus (7%). With seven species, the yeast community was more varied in female diabetes individuals. Wickerhamomyces anomalus was most common (29%), followed by Naganishia sp. However, yeast species in men were less diversified . 15% of the species included were Naganishia sp., followed by 13% of Kluyveromyces marxianus.

DISCUSSION

According to the study's findings, the absorption spectroscopy technique was used to estimate the amount of glycated hemoglobin (HbA1c) in the blood samples from the two groups. The results indicated that the HbA1c ranged between 6.5% and 15% for the patients with diabetes and between 4% and 6.3% for the people without the disease.

The findings were evaluated from 128 oral swabs taken from research participants who did not exhibit any signs of a fungal infection. Different species were isolated from the oral cavity mucosa of both diabetic and non-diabetic patients, according to the results. 90% of those with diabetes at lunchtime and 97% of those with diabetes had Ascomycota, primarily candida species, with Wickerhamomyces anomalus being the only Basidiomycota. Upon clinical oral examination, none of the subjects in either group showed signs of subclinical colonization. These findings were comparable to those of earlier research by Nigotia P et al. (2022) and Magalhães J et al. (2022), where the authors similarly reported findings for species isolation comparable to the current study in their separate investigations.

Even though different species were isolated from the mucosal oral cavity in both diabetics and non-diabetics, it was shown that non-diabetic participants had nine more species than diabetic ones. Yarrowia sp., Nakaseomyces glabratus, Yarrowia sp., Kluyveromyces sp., Kluyveromyces marxianus, Candida Africana, Candida dubliniensis, and Candida albicans were among the nine species. The findings of the current study were in line with those of Ibrahim SL et al. (2022) and Al Anbagi RA et al. (2019), who indicated that more species were isolated from non-diabetic people than from non-diabetic participants.

Yeast species were observed in relation to the identification of Candida species. Additionally, all sequenced fungal isolates were shown to cluster with the material type of known particular fungal specimens, according to phylogenetic analysis. Every species of Candida split off from other fungal species in a monphylogenetic clade and grouped according to specimen type. 948 colonies of Candida albicans were established, followed by colonies of Naganishia sp., Candida dubliniesis, Candida albicans, and Kluyveromyctes marxianus, in that order. The number of colonies in non-diabetics was essentially equal. Compared to diabetics, who had seven species of yeast colony, non-diabetic participants had nine species. These results were consistent with those of Chyad SA et al. in 2024 and Nouraei H et al. in 2021, where the authors reported a number of yeasts that were identical to the current study in their separate investigations.

The study's findings also shown that when evaluating how gender affects community organization over time,

C. albicans was most prevalent in diabetics (45%), followed by Kluyveromyces marxianus in men with diabetes mellitus (16%), K. marxianus in males with diabetes mellitus (16%), and C. albicans in females with diabetes mellitus (7%). With seven species, the yeast community was more varied in female diabetes individuals. Wickerhamomyces anomalus was most common (29%), followed by Naganishia sp. Male yeast species, on the other hand, were less varied, consisting of only five species. 13% of Kluyveromyces marxianus was followed by 15% of Naganishia sp. The present study's findings were consistent with those of Russell CM et al. (12) and Al Anbagi RAA et al. (13), who revealed similar findings about the effect of gender on years community structure.

CONCLUSION

Notwithstanding its limitations, the current study provides important information about the distribution and diversity of commensal yeast in Indian subjects' oral cavities as well as the risk of infection transmission due to shifts in the microbial species' composition with regard to health problems.

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Species	Mean	Sum	S (Richness)	E (evenness)	H (Shannon's diversity index)	D (Simpson's diversity index)
Naganishia sp.	1.25±5.29	79	2	0.92	1.29	0.69
Yarrowia sp.	0.42±2.82	26	1	0.73	0.50	0.32
Wickerhamomyces anomalus	1.37±6.09	87	5	0.64	1.26	0.67
Kluyveromyces sp.	0.70±4.01	44	1	1.00	0.67	0.48
Kluyveromyces marxianus	5.40±18.31	345	14	0.64	1.82	0.78
Nakaseomyces glabratus	0.21±1.86	13	1	0.00	0.00	0.00
Candida dubliniensis	2.28±11.29	145	2	0.76	1.07	0.59
Candida africana	1.18±6.22	75	2	0.66	0.93	0.55
Candida albicans	7.64±20.93	490	25	0.66	2.22	0.85
Mean	2.27±8.53	146.5	7.2	0.67	1.08	0.54

Table 1: Diversity of the fungus as assessed in study subjects