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ORIGINAL ARTICLE

ASSESSMENT OF CANDIDA SPECIES IN PATIENTS WITH ORAL MALIGNANCIES

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ABSTRACT:

Background: Infections is one of the important causes of cancer, causing approximately one in five malignancies worldwide. Three of the main infectious agents causing malignancies are The three main examples are infection with the bacterium Helicobacter pylori leading to an elevated risk of developing gastric adenocarcinoma and gastric lymphoma, infection with particular types of human papilloma virus (HPV) leading to cervical cancer, tonsillar carcinoma and some cases of oral squamous cell carcinoma (OSCC) and chronic hepatitis B and C infections leading to hepatocellular carcinoma. Hence; we evaluated the Candida species in OSCC patients. **Materials & Methods**: Histopathologically proven OSCC patients were included in the present study. Total no. of patients is 30 in which (25 males and 5 females) of clinically (Stages III and IV) . Age, systemic conditions, site of the lesion, and alcohol consumption were not taken into consideration due to the shortage of time while collecting the samples; 22 were well differentiated OSCC patients, 7 were moderately differentiated OSCC patients, and 1 was a poorly differentiated OSCC patient. The present study was performed to isolate the presence and quantification of different *Candida* species from the individuals of a random age group. The growth appeared in 48 h as cream/white‑colored, smooth, and pasty colonies. Gram staining was performed to confirm the growth of yeast. Once the colonies were confirmed, a colony count was done by a Digital Colony Counter and expressed as colony‑forming unit (CFU)/mL of saliva. Further identification and speciation were done by the germ tube test, sugar assimilation test, corn meal agar morphology, and other standard tests. The data were analyzed using the Statistical Package for Social Sciences (SPSS) statistical software. The values of these parameters were expressed as mean ± standard deviation and the levels of significance were determined by employing the Student's *t* test. **Results:** The mean CFU/mL of saliva for healthy group was 1420.5 while for the squamous cell carcinoma group was 7428.8. Statistically significant results were obtained while comparing the mean CFU/mL of saliva for the presence of candida species. **Conclusion: S**ignificant increase in candidal carriage in OSCC patients is seen. Salivary parameters offer the scope for detailed future research on their applications in the screening, diagnosis, and management of cancer.

Key Words: Candida, Oral squamous cell carcinoma

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INTRODUCTION

One of the important causes of cancer is the infections, causing approximately one in five malignancies worldwide.[1, 2] The three main examples are infection with the bacterium Helicobacter pylori leading to an elevated risk of developing gastric adenocarcinoma and gastric lymphoma, infection with particular types of human papilloma virus (HPV) leading to cervical cancer, tonsillar carcinoma and some cases of oral squamous cell carcinoma (OSCC) and chronic hepatitis B and C infections leading to hepatocellular carcinoma.[1-4] Literature quotes less evidence of an etiological association between fungal infection and cancer, even though studies are present which stress on the role of Candida spp in various epithelial cancers. For dysplastic cervical lesions or cervical carcinoma, candidal infections does not appear to be a risk factor and most interest in Candida and carcinogenesis is related to oral and esophageal carcinoma. Development of oral or esophageal carcinoma developing in

immunocompromised patients with chronic mucocutaneous candidiasis and often with autoimmune polyendocrinopathy candidiasis- ectodermal dystrophy is supported by various reports from the literature.[5-8] Hence; we evaluated the Candida species in OSCC patients.

MATERIAL AND METHODS

In this study histopathologically proven OSCC patients were included total no. of patients is 30 in which (25 males and 5 females) of clinically (Stages III and IV). Age, systemic conditions, site of the lesion, and alcohol consumption were not taken into consideration due to the shortage of time while collecting the samples; 22 were well differentiated OSCC patients, 7 were moderately differentiated OSCC patients, and 1 was a poorly differentiated OSCC patient. All patients were tobacco chewers/smokers (>10 years) with a mean age of 50.00 ± 13.25 yrs. The control group included 30 healthy controls (29 males and 1 female) of a random age group without any age limit taken into consideration, who were free of any oral lesions, habits, and systemic illness such as diabetes mellitus, hypertension, or immunocompromised status; none of them were on antibiotics, anti fungals, radiotherapy, or chemotherapy. Their mean age was 34.34 ± 6.06 yrs. Informed consent was obtained from all the patients prior to the study. Detailed histories of all the patients were taken with emphasis on tobacco and alcohol use. Individuals from both the groups showed no evidence of oral candidal infection on clinical examination. The present study was performed to isolate the presence and quantification of different *Candida* species from the individuals of a random age group. The unstimulated whole saliva was analyzed in this study. Saliva samples were collected from 10 AM to 12 PM, or 2 h according to the method of Navazesh (1993). The timing was such that it was 2 h after the subjects' usual breakfast time. The subject was asked to rinse his/her mouth thoroughly with distilled water to remove any food debris and then after 10 min was directed to spit into a sterile plastic container. The subjects were instructed not to spit forcibly so as to avoid blood contamination.

This was to ensure the variability in salivary flow and the minimization of compositions due to diurnal variation. Once 2 mL of saliva was collected, the container was placed in an ice carrier box and transferred to the laboratory for the biochemical analysis and candidal estimation. The sterile unstimulated saliva was vortex-mixed for 30 s for optimal disaggregation. Then, 50 ml of saliva was pipetted from the container and inoculated onto the dry Sabouraud dextrose agar (SDA) culture plate; the sample was spread all over the plate using sterile inoculating loop and incubated at 37°C for 48 h. The growth appeared in 48 h as cream/white-colored, smooth, and pasty colonies. Gram staining was performed to confirm the growth of yeast. Once the colonies were confirmed, a colony count was done by a Digital Colony Counter and expressed as colony-forming unit (CFU)/mL of saliva. Further identification and speciation were done by the germ tube test, sugar assimilation test, corn meal agar morphology, and other standard tests.[10,11] The data were analyzed using the Statistical Package for Social Sciences (SPSS) statistical software. The values of these parameters were expressed as mean ± standard deviation and the levels of significance were determined by employing the Student's *t* test.

RESULTS

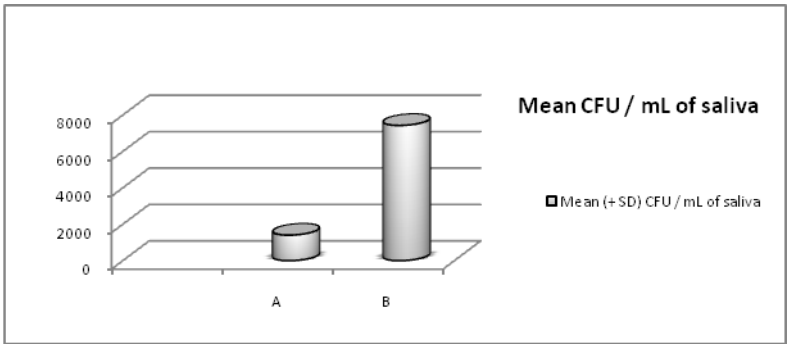
Table 1 shows the p-value for mean CFU/ml of saliva for the healthy group and squamous cell carcinoma group. The mean CFU/ml of saliva for healthy group was 1420.5 while for the squamous cell carcinoma group was 7428.8. Statistically significant results were obtained while comparing the mean CFU/mL of saliva for the presence of candida species. Graph 1highlights the comparison of mean CFU/ml of saliva for the detection of candida species.

Table 1: p-value for mean CFU/ml for the two study groups

Groups	No. of cases	Frequency – Candida isolation (percentage)	Mean (± SD) CFU / ml of saliva	p-value
A	60	48	1402.5 ± 1648.8	0.005
B	60	72	7428.8 ± 5245.2	

CFU= Colony Forming units

Graph 1: Mean CFU/ml for the two study groups



Oral cancer is one of the ten most prevalent cancers worldwide. More than 90% of malignancies being squamous cell carcinomas originating from the oral mucosa.[12, 13] *Candida* species are common members of the oral microflora and are generally regarded as being commensals. However, they are able to cause a range of opportunistic infections, referred to as candidoses.[14,15] The prevalence of diseases caused by *Candida* spp. has increased in recent years, mainly due to the increasing number of immunocompromised patients. *Candida albicans* is still the predominant species isolated, and it has the potential to infect virtually any tissue within the body. However, it is predominantly found on the oral and vaginal mucosa.[16,17] The possible association between *Candida* spp. and oral neoplasia was first reported in the 1960s, with later reports suggesting a link between the presence of *C. albicans* in the oral cavity and the development of oral squamous cell carcinoma (OSCC).[18,19] Hence; we evaluated the candidal species OSCC patients. In our study, 13 cases (43.3%) were found to be culture-positive. In the mycological investigation carried out by salivary culture technique among cancer subjects, 21 cases (70%) were found to be culture-positive. Our finding of increased candidal isolation in OSCC patients is in accordance with the majority of findings by other researchers. There was a definite increase in the candidal carriage rate in OSCC patients when compared to healthy controls. As observed in the present study, the candidal carriage rate increased in OSCC individuals. Thus, there is mounting evidence to suggest that there is an interaction between oral infections with yeasts, in particular *Candida* species, and the development of neoplasia. Jahanshahi et al detected Candida albicans in oral squamous cell carcinoma patients by fluorescence staining technique. They found that in 74 % of the cases of OSCC, *Candida was* present. From the results, they concluded that since the fluorescence technique had a higher accuracy in the identification of *Candida* and it was nearly evident in two-third of the samples, the role of fungi as a primary cause is suggested to be studied in future investigations.[20]

CONCLUSION

From the above results, we conclude that the use of saliva as a diagnostic tool is increasing widely due to its non invasiveness nature and ease of its collection. Saliva is a convenient biological fluid for the diagnosis of various diseases. We also observed a significant increase in candidal carriage, especially *C. albicans*, in OSCC patients. Salivary parameters offer the scope for detailed future research on their applications in the screening, diagnosis, and management of cancer.

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