EVALUATION OF CANDIDA AND ORAL MICROBES WITH OR WITHOUT USE OF DENTURES AMONG PROSTHODONTIC PATIENTS IN LAGOS, SOUTH WESTERN NIGERIA

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ABSTRACT

BACKGROUND: The presence of oral microbes in individuals that wear denture has been associated with denture stomatitis that affects the mucosa of the hard palate in contact with complete or partial dentures.

OBJECTIVES: To compare the dominant oral micro-flora between denture wearers and non-denture wearers and to analyze the bacterial composition of denture wearers.

METHODS: A cross-sectional study was conducted with the total number of 94 subjects who met the inclusion criteria were grouped into denture wearers and non-denture wearers. Mucosa swabs were taken from the subjects using sterile swab; and placed immediately in Sabouraud's dextrose (SD) agar and sent to the microbiology laboratory for analysis. Data obtained were analyzed using Statistical Package for Social Sciences (SPSS). Association between variables was carried out using Chi-square test. The significance level adopted was 0.05.

RESULTS: The mean ages among the NDW and DW were 46.4±18.5 and 57.4±18.2 respectively. The highest oral microbes 6(12.8%) isolated among the DW was Candida albicans whereas the highest 15(31.9%) oral microbes isolated among NDW was Staphylococcus aureus. There was a statistically significant association between the number of subjects who presented with Candida albicans and microbial load among the denture wearers and non-denture wearers (P=0.001) and (P= 0.047) respectively

CONCLUSIONS: The dominant oral micro-flora among denture wearers was Candida albicans. The bacterial composition of the denture wearers and non-wearers were Streptococci mutans and Staphylococcus aureus respectively.

KEYWORDS: Candida Albicans, Oral Microbes, Denture wearers, Denture Stomatitis

INTRODUCTION

The oral cavity is a complex environment that is continually exposed to numerous opportunistic microbial pathogens.[1] These are preserved by immune factors to maintain a healthy oral environment and prevent development of disease. Researches have been done focusing on oral diseases such as caries, gingivitis and periodontitis affecting dentate patients but limited research that involved denture related disease. In view of improvements in oral health worldwide, the rate of edentulism varies from 7% to 69% of the world's adult population[2] and this is reflected in the western countries like UK and US populations experiencing use of removable denture.[3]

This high prevalence called for researchers to develop an understanding of the implications of dentures on oral and systemic health. The presence of oral microbes in relation with denture wear has been linked with a common inflammatory condition, denture stomatitis that affects the mucosa of the hard palate in contact with complete or partial dentures.[4] Denture stomatitis is a primary condition suffered by prolonged denture wearers. It is the inflammation of the oral mucosa and pathological changes associated with wearing of dentures.[5] The prevalence of denture stomatitis ranges from 10% to 75% of denture wearers[6]. In comparison with the type of denture, it was found that complete dentures cover a large palatal area with increased risk of denture stomatitis[7] whereas in partial dentures a prevalence of 1.1% to 36% denture stomatitis has been reported[8].

Candida has been implicated as the cause of infection in relation to denture wear, however there has been increased evidence of polymicrobial disease among bacterial and fungal interactions in the disease pathogenesis.[8] Candida –associated denture stomatitis is considered the most frequent form of oral candidiasis.[9] Some factors have been implicated with the adhesion of Candida species on dentures such as salivary proteins, proteins and glycoproteins, low salivary ph, decrease in salivation, and oral bacteria[10]. Some studies have isolated bacteria directly from the surface of dentures using standard microbial culture technique, primarily streptococci and staphylococci species.[11] However, these culture-based methods give inadequate representation of polymicrobial population containing 10^{11} microbes per milligram of denture plaque.[10]

Moreover, it has been shown that Candida species adhere with different species of oral bacteria such as Streptococci, and increase their potential colonization of dentures.[12] Streptococcus mutans is a common inhabitant of natural teeth, and acrylic denture plays an important role in biofilm formation by providing binding sites for bacterial colonization and promotes adhesion of Candida species. The effect of biofilm virulence is increased with the association between Streptococcus mutans and Candida albicans by increasing production of exopolysaccharides with Candida and inducing the expression of virulence genes in S. mutans.[13]

However, limited studies have been carried out in our sub-region regarding the association of oral micro-flora and dentures, despite the increasing population of denture wearers. The purpose of this study was to compare the dominant oral micro-flora between denture wearers and non-denture wearers, to analyze the bacterial composition of the oral microbiome of denture wearers and to assess the changes in the diversity and composition of microbiome against Candida.

METHODOLOGY

A correlational, cross-sectional study conducted in the Prosthodontic clinic, Restorative Dentistry department at the Dental Centre of the Lagos State University Teaching Hospital, Ikeja in the cosmopolitan hub of Lagos State, Nigeria.

The subjects included in the study were the patients who attended the clinic, regardless of gender, aged 18 years old or older. The study also included denture wearers and non-denture wearers who were healthy. Subjects excluded were patients with obvious candida infections, systemic conditions or have had courses of antibiotics, antifungal, antimalarial drugs or immunosuppressive drugs in the past 1 week.

Clinical Procedure

The ethical approval for this study was taken from the Research and Ethics Committee of Lagos State University Teaching Hospital, Ikeja. A total number of 94 subjects who met the inclusion criteria were recruited according to non-probability consecutive sampling for the study and consents were taken. The selected subjects were grouped into denture wearers (DW) and non-denture wearers (NDW) and using the modern diagnostic kit among prosthodontics patients in South Western Nigeria

Firstly, the questionnaire was self-administered but guided by one of the researchers especially with the explanation of terminology. Then, dental examination was conducted in the prosthetic clinic under adequate lightning, comfortable supine positioned dental chair and a chaperon. Subjects were required to open his/her mouth for examination with dental mirror and denture wearers were asked to leave in their dentures in the mouth. Mucosa swabs were taken from the palatal mucosa of subjects of NDW, and from the tissue bearing area on the fitting surface of the upper dentures of DW using sterile cotton wool swab, which is done by wiping the palatal area and the fitting surface of denture respectively. These swab samples are placed immediately in Sabouraud's dextrose agar (SDA) containing gentamycin (2mhs/dl) and chloramphenicol (5mg/dl), which were subsequently sent to microbiology laboratory.

Laboratory procedure

The samples were incubated for 48 hours at 370C and isolation of oral microbes and candida species was done. The samples were identified by pick-up and stain by Gram's and the Lactophenol blue to observe the candida species and other microbes their morphology (shape, colour and size of the yeast and bacterial colonies grown on agar), and then subjected to a battery of biochemical test. Further tests were done on identification of candida through studying surface growth in Sabouraud's dextrose broth, carbohydrate assimilation test, carbohydrate fermentation test, serum germ tube test and studying chlamydospores formation on Corn meal agar.

Data Analysis

Data obtained were analyzed using Statistical Package for Social Sciences (SPSS). Data presentation was done using frequency distribution and tables. Association between variables was carried out using Chi-square test. The significance level adopted was 0.05. Binary logistic regression was used to predict the presence of Candida albicans among denture wearers.

RESULTS

A total number of 94 subjects; 47 non-denture wearers (NDW) and 47 denture wearers (DW) participated in this study. Among the NDW, the majority of the subjects 19(40.4%) were 40years and below, while the majority of the subjects 18(38.3%) among the DW were between the age group of 61-80 years. The mean ages among the NDW and DW were 46.4±18.5 and 57.4±18.2 respectively. More males 24(51.1%) were NDW, while more females 30(63.8%) were DW (Table 1).

Table 1: Demographic Characteristics of the Subjects

Variable	Non-Denture Wearers Freq (N=47)	Non-Denture Wearers Percentage (%)	Denture Wearers Freq (N=47)	Denture Wearers Percentage (%)
Age Group (Yrs)				
≤ 40	19	40.4	11	23.4
41 _ 60	17	36.2	16	34.0
61 – 80	10	21.3	18	38.3
>80	1	2.1	2	4.3
Age range	*19 - 82*		*18 – 84*	
Median Age	*47*		60	
Mean ± SD	*46.4 ± 18.5*		*57.4 ± 18.2*	
Sex				
Male	24	51.1	17	36.2
Female	23	48.9	30	63.8

The highest oral microbes 6(12.8%) isolated among the NDW was Staphylococcus aureus whereas the highest oral microbes 15(31.9%) isolated among the DW was Candida albicans. Among both groups (NDW and DW), the highest majority of the subjects presented with no microbial growth 32(68.1%) and 23(48.9%) respectively. Equal number of subjects 4(8.5%) presented with Streptococcus mutans isolates among both the 2 groups. Pseudomonas was not isolated from NDW and no Staphylococcus was isolated from DW (Table 2).

Table 2: Microbial Composition of the Oral Microbiome of the Subjects

Microbial Isolates	Non-Denture Wearers Freq (N=47)	Non-Denture Wearers Percentage (%)	Denture Wearers Freq (N=47)	Denture Wearers Percentage (%)
Candida albicans	2	4.3	15	31.9
No Growth	32	68.1	23	48.9
Staphylococcus aureus	6	12.8	0	00.0
Streptococcus mutans	4	8.5	4	8.5
Streptococcus spp.	3	6.4	3	6.4
Pseudomonas	0	00.0	2	4.3

The prevalence of Candida albicans among DW was 88.20% (Figure 1).

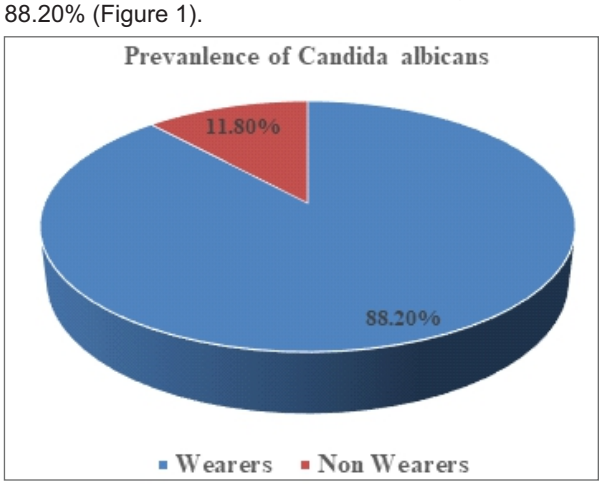

Fig 1: Prevalence of Candida albicans among Denture wearers and Non-denture wearers

There was a statistically significant association between the number of subjects who presented with Candida albicans among the DW and NDW (P=0.001). There was also a statistically significant association between the number of subjects who presented with Staphylococcus aureus among the DW and NDW (P=0.013) (Table 3).

Table 3: Association between Sociodemographic and Microbial Isolates Among Denture Wearers and Non-Denture Wearers

Variable	Denture (Freq (%) Non-Wearers (n=47)	Wearers (n=47)	X^2	Df	P-Value
Age grp(yrs)					
≤ 40	19(63.3)	11(36.7)	4.807	3	0.171
41 – 60	17(51.5)	16(48.5)			
61- 80	10(35.7)	18(64.3)			
Above 80	1(33.3)	2(66.7)			
Sex					
Male	24(58.5)	17(41.5)	2.120	1	0.106
Female	23(43.4)	30(56.6)			
Candida albicans					
Yes	2(11.8)	15(88.2)	12.136	1	0.001*
No	45(58.4)	32(41.6)			
Pseudomonas					
Yes	0(00.0)	2(100.0)	2.043	1	0.247
No	47(51.1)	45(48.9)			
Streptococcus mutans					
Yes	4(50.0)	4(50.0)	0.001	1	0.643
No	43(50.0)	43(50.0)			
Streptococcus spp					
Yes	3(50.0)	3(50.0)	0.001	1	0.661
No	44(50.0)	44(50.0)			
Staphylococcus aureus					
Yes	6(100.0)	0(00.0)	6.409	1	0.013*
No	41(46.6)	47(53.4)			

Df= Degree of freedom, X2 = Chi Square,
**= Statistically Significant*

Generally, there was more microbial growth isolated from the wearers of Denture than the Non-denture wearers. There was a statistically significant association between the microbial load isolated among the wearers and non-wearers (P= 0.047) (Table 4).

Table 4: Association between Number of Microbial Load among Denture Wearers and Non-Denture Wearers

Variable	Microbial load (Freq (%) No Growth	Growth	X^2	Df	P-Value
Denture Therapy					
Wearers	23(48.9)	24(51.1)	3.550	1	0.047*
Non-Wearers	32(68.1)	15(31.9)			

Df= Degree of freedom, X2 = Chi Square,
**= Statistically Significant*

Binary logistic regression revealed that those who wear denture are 10.6 times more likely to present with Candida albicans (P= 0.003, CI (2.253- 49.367) (Table 5).

Table 5: Binary Logistic Regression Predicting the Presence of Candida albicans among Denture wearers

	B	Sig.	Odd Ratio	95% C.I. for OR Lower	Upper
Denture	2.356	.003	10.547	2.253	49.367

DISCUSSION

The presence of dentures or any other prosthetic appliances in the oral cavity alters its micro environment through the enablement of Candida colonization and proliferation.[14] Denture stomatitis has been a major common problem associated among denture wearers. The primary factors behind this issue have been linked to ill-fitting or poorly-fitting denture prostheses, which lead to trauma to the underlying mucosa, or inadequate maintenance practices by the wearer.[15,16] In other part of the world, around 65%–70% of individuals who wear dentures have been recognized as experiencing Candida-associated denture stomatitis (CADS).[17] However, the presence of Candida does not regularly cause disease in healthy individuals, but may contribute to the colonization of oral and denture surfaces, acting as a likely source of infection.

In this study, the mean age among denture wearers was 57.4±18.2 years similar to a study by Szalewski et al.[18] who reported 58.5±9.7 years as the mean age in their study population. A large number of denture wearers, particularly dependent elderly persons, do not clean their dentures in an appropriate manner exposing the dentures to different biofilms. A prevalent misconception suggests that dentures are solely meant for older individuals. Although tooth loss becomes more likely as one ages, it's important to recognize that dentures are not limited to any particular age demographic. The average age at which individuals begin wearing dentures is influenced by a range of factors, including health, genetic predisposition, economic status, and geographical location.

The placement of prosthesis in the mouth brings about significant alteration in the surrounding conditions. This is because the prosthesis becomes colonized with oral microorganisms, hindering the underlying mucosa from benefiting from the tongue's mechanical cleaning action and the saliva's natural antimicrobial properties.[19] These result in an increase rate of candida carriage and infection by this yeast among DW which is also reported in this present study; the highest oral microbes 15(31.9%) isolated among the denture wearers was Candida albicans and the prevalence of Candida albicans among DW was 88.20% and binary logistic regression revealed that those who wear dentures are 10.6 times more likely to present with Candida albicans. These observations of the aforementioned sentence have been documented for many years[20,21] with further illustration that the yeasts tend to disappear from the oral cavity after tooth extraction, but the oral cavity is repopulated when dentures are worn.[22] These yeast adheres to complement receptors and some sugar residues that are present on host surfaces such as denture and oral mucosa.[17] Several studies[17,23-25] have documented Candida albicans as the most frequent microbial growth being isolated among DW.

This present study showed more and different microbial growths among the DW; Candida albicans, Streptococcus mutans, Streptococcus spp. and Pseudomonas. This can be attributed to the presence of Candida albicans in the host relies on the creation of a specific environment within diverse bacterial communities. Thus, the interaction (congregation or co-adhesion) between Candida albicans and oral bacteria plays a vital role in the colonization and long-term presence of this yeast within intricate microbial biofilms.[26,27] Moreover, the mouth consists of various microenvironments that encompass different surfaces where microbial cells can attach and build up, including teeth, oral mucosal tissues, and prostheses like dentures.[26,27]

The prevalence of Streptococci mutans and its other

species in both denture and non-denture wearers are the same in this study, despite the difference in percentage of Candida albicans among the two groups. This might be due to the presence of microbial constituent from dental plaque among the NDW. However, the interactions between yeast and streptococci appear to be essentially synergetic, with streptococci not only providing adhesion sites but also releasing lactate, which can serve as a carbon source to support yeast growth.[28] Candida albicans, meanwhile, not only reduces oxygen levels to those favored by streptococci but also potentially generates growth stimulatory factors for the bacteria through nutrient metabolism.[27]

While the debate over the existence of staphylococci in the mouth persists, some studies have identified the presence of Staphylococcus species in oral samples and is a normal flora of the body which can be found in nose, skin and respiratory tracts. In this study, Staphylococcus aureus was not isolated from the dentures, but isolated (12.8%) in the oral cavities among the NDW. Several local infections may be caused by Staphylococcus aureus, including angular cheilitis, endodontic infections, parotitis and mucositis in the elderly patients.[29] The present study contradicts the evidence of increase in this particular microorganism count among denture wearers,[29] and further research might be carried out to look into it. Studies[30,31] have shown increase in Staphylococcus aureus count among denture wearers which are in agreement with this aforementioned evidence.

Our findings validated that there is higher prevalence of combination of Candida spp. and bacteria that are harbored in dentures. The association of this fungus and bacteria within polymicrobial microfilms has been demonstrated to increase their adherence to the acrylic surface of dental prostheses.

CONCLUSION

The dominant oral micro-flora among denture wearers was Candida albicans. The bacterial composition of the oral microbiome of denture wearers and non-denture wearers were Streptococci mutans and Staphylococcus aureus respectively. Candida albicans were found to the most frequent micro-flora on the dentures, followed by Streptococci mutans and other Streptococci species. Therefore, all patients that are denture wearers need regular dental visits and good oral hygiene maintenance (both tooth structures and dentures) and practice to prevent development of oral diseases.

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