Original Research Paper

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Estimation of Adherance of Candida Albicans to Different Denture Base Materials and its Relationship with Denture Stomatitis : An in Vivo Study.

Abstract:

Background - Candida albicans is the most common human oral fungi frequently recovered from the denture surface causing denture stomatitis. Modern treatment approaches suppress the normal host defense mechanisms, alter the normal human microbial flora& allow non bacterial species to become more prevalent in commensal flora.

Aims & Objectives - The objective of the present study was to evaluate the prevalence & incidence of Candida albicans in DPI &Lucitone 199 heat cure acrylic denture wearers & its relationship with denture stomatitis.

Materials & Methods – The study was conducted at our institutionwherein 52 edentulous patients enrolled in the study .These patients were randomly assigned to two groups DPI and Lucitone 199 . Dentures were made using the two materials . At three months follow up swab samples were taken from the denture surfaces and sent to lab for culture .

Result– The prevalence of Candida albicans in denture wearers was found to be 23%. The incidence of denture stomatitis was 27%. Prevalence of Candida albicans& incidence of denture stomatitis was less with Lucitone 199 heat cure acrylic dentures.

Conclusion - Any treatment that we do should not be cause of other pathology. Denture stomatitis falls into such category. Follow up should be done on a mandatory basis to evaluate the changes & performing necessary modifications.

Key Words –Candida albicans , edentulous, dentures , DPI , Lucitone 199 heat cure acrylics

Introduction:

Oral cavity is home to multitude of microorganisms. Teeth & denturesurfaces are covered by complex biofilms consisting of coccoid& rod shaped bacteria, yeasts & desquamated epithelial cells. Candida albicans is the most common human oral fungi frequently recovered from the denture surface causing denture stomatitis. Around 40-75% of denture wearers have Candida in their oral cavity .[1] There are 150 species of genus Candida. But only a few cause diseasein humans. The human pathogens are C. albicans, C .guilliermondii, C. Krusei,C. parapsilosis, C.trepicalis, C. Kefys, C. lusitaniae, C. dubliniensis& C. glabrata. Modern treatment approaches suppress the normal host defense mechanisms, alter the normal human microbial flora& allow non bacterial species to become more prevalent in commensal flora. [2] In developed countries it is the most common nosocomial pathogen.

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Regardless of the cleaning efforts by denture wearers , plaque & calculus are often found on denture surfaces. The probable reason for improper cleaning of denture can be wrong choice of cleansing agent or wrong technique employedwhich can be because of differences in action of various cleansing agents . Ultrasonic bath should be used for 90sec for dislodgement of denture plaque. [1] The presence of denture plaque acts as a harbor for growth of Candida albicans& the differences in the surface free energy of denture base materials accounts for

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varying adherence of Candida albicans to different denture base materials .

The differences in the prevalence of Candida albicans& denture stomatitis have been reported due to the variations in choice of study population , diagnostic criteria & methodology. Thus ,the objective of the present study was to evaluate the prevalence & incidence of Candida albicans in DPI &Lucitone 199 heat cure acrylic denture wearers & its relationship with denture stomatitis.

Materials & Methods:

An in vivo study was conducted in Dept. of Prosthodontics ,of our institute where 75 patients were treated with complete dentures & 52 (46 males, 6 females) patients cooperated in completing the study. Standard treatment protocol was followed for all the patients . 26 patients were treated using DPI heat cure acrylic resin & 26 with Lucitone199 acrylic resin .

StandardisationProtocol Followed For AcrylisationOf Dentures –

Procedure for curing Lucitone 199 Heat cure acrylic resin

Waxed up dentures wereflasked using two pour technique. Wax was softened by placing flask in boiling water for approx. 6 min. Separated flasks were flushed with boiling water to remove any residual wax. Next, cold mould seal was applied to areas of warm mould that contact the resin.Any excess was removed using cotton pellet & also from teeth .for a single arch 21 gm (32 cc) powder to 10 ml liquid was added & mixed for 15 seconds to assure wetting of all powder particles. Mixing jar was covered & material was allowed to reach packing consistency (dough stage , approx. 9 min at room temp.). Resin was placed in dough stage into the mould& condensed with finger pressure. The procedure was done following manufacturer sinstructions .The two halves of flask were closed & placed in a hydraulic bench press to ensure complete closure of flask(metal to metal contact of flask) for curing , flasks were removed from bench press & held with clamp in water at 73 C for 1 ½ hrs, followed by ½ hr in boiling water .Then the clamped flasks were bench cooled at room temperature . Later on deflasking was done &dentures were retrieved.

Procedure ForDPI Heat Cure Acrylic:

Waxed up dentures were flasked using two pour technique . Clamped flasks were immersed in boiling water for 6 min . Flasks were opened & flushed with boiling water . Cold mould seal was applied to all plaster surfaces & master cast .Any excess around teeth were removed using cotton pellet. Powder& liquid were mixed in a ratio of 3"1 by volume. Mixed resin was covered & allowed to reach dough stage. Then resin was packed into mould& covered with a moistened separating sheet for trial closure . Flask opened & separating sheet was removed & excess was trimmed with a sharp knife. Flasks were then assembled & placed in hydraulic bench press to ensure complete closure of flask .Flasks were then fastened in clamps & placed in acryliser in water .Temperature was slowly raised to boil in not less than 30 min, & then for next 30 min at boiling temperature. After curing procedure , flasks were bench cooled & then deflasked to retrieve dentures. The procedure was done following manufacturer sinstructions .

Laboratory remounting was done followed by finishing & polishing according to standard protocol to eliminate the chances of Type I denture stomatitis. [1]

Dentures were inserted & post insertion instructions were given to patients. Patients were instructed to clean dentures every night after meal using soft tooth brush & mild soap. Any use of detergents /toothpastes was not advocated. Patients were asked to immerse dentures at night in water. [3]

For 3 months post insertion check up patients were recalled for evaluation regarding denture stomatitis & presence of candida albicans infection (Fig 1). Specimen from the impression surface of maxillary denture was collected. Using Cawson technique, a sterile cotton swab was used to collect the swab from the intaglio surface of maxillary denture & then transferred to Sabouraud s Dextrose Agar on Mac Courtney s bottle .[4]

Fig 1 – Clinical presentation of C .albicans3months post denture insertion

Sample collection for Candida from oral cavity can be either by swab or by saliva collection. If Candida albicans is present in the oral cavity, a swab of the oral tissues has much greater probability as a positive source of isolation of the yeast than a random sample of saliva. Improved technical procedures , including the use of the agar plates & 2 week incubation of the isolation broth, further increases the probability of a positive isolation. [5]

At this point the specimen was transferred to a diagnostic research centre, Jaipur for evaluation by laboratory technician. In laboratory ,the specimens were incubated at 35 0C for 2 weeks . Identification of Candida sp. was based on colony &microscopic morphology.

Candida colonies are creamy white , smooth &with a yeasty odour (fig 2). Candida albicans can be identified from other candida sp by growth characteristics. A smear layer of colony was formed over glass slide & staining was done using gram staining technique. After staining, slide is observed under oil immersion lens (1000x magnification) . Candida cells show budding under Gram staining . (fig 3).

Fig 2 - Creamy white , smooth Candidal colonies with a yeasty odour seen in Sabouraud s Dextrose Agar on Mac Courtney s bottle

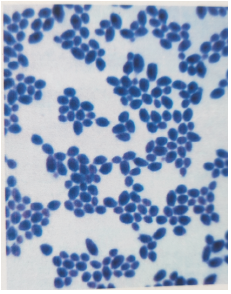


Fig 3 - Candidacells show budding under Gram staining

After this rapid serum test was employed for confirmation of Candida sp.rapid method of identifying candida albicans is based on its ability to form germ tubes within two hours when incubated in human serum at 37 0 C (Reynolds Braude phenomenon)[6]. After microscopic morphology identification, Germ tube test method was followed for further confirmation of Candida albicans .According to the study by Shepard , Germ tube test is 100% specific for Candida identification , so it provides a fast & convenient method for its identification.

Germ tube test standard operating procedure

It is based on the principle that germ tubes are formed within 2 hrs of incubation in Candida albicans sp. & not in other sp. of this genus.

Procedure

1. A small portion of an isolate colony of the yeast to be tested is suspended in a test tube containing 0.5 ml of human plasma/serum.
2. The test tube is incubated at 35 degree centigrade for no longer than 2 hrs.
3. A drop of yeast serum suspension is placed on a slide. Overlaid with a coverslip & examined microscopically for the presence of germ tubes .
4. The test is not valid if examined after 2 hrs.

Data gathered was utilized for tabulating the results & conducting statistical analysis.

Results:

The results obtained were tabulated & sent for statistical analysis . Chi square test was performed.

Table 1

Age (yrs)	Females	Males	Total
<60	3	13	16
>60	3	33	36
Total	6	46	52
Mean ±SD	59.17 ± 8.77	62.67 ± 9.47	62.27 ± 9.38

Table 2

	< 60 yrs (total -16)	>60yrs (Total – 36)	p value
Candidal Presence	3	9	0.6215
Count of other sp.	6	7	0.1652
Stomatitis presence	3	11	0.3757

Table 3

	Candidal Presence			p value
Denture stomatitis	No	Yes	Grand Total	0.1892
No	31	7	38	
Yes	9	5	14	
Grand Total	40	12	52	

Table 4

	Stomatitis Presence			p value
Denture	yes	no	total	0.211
DPI	9	17	26	
Lucitone	5	21	26	
Total	14	38	52	

Table 5

	Candidal presence			P value
Denture	Yes	no	Total	0.188
DPI	8	18	26	
Lucitone	4	22	26	
Total	12	40	52	

Discussion:

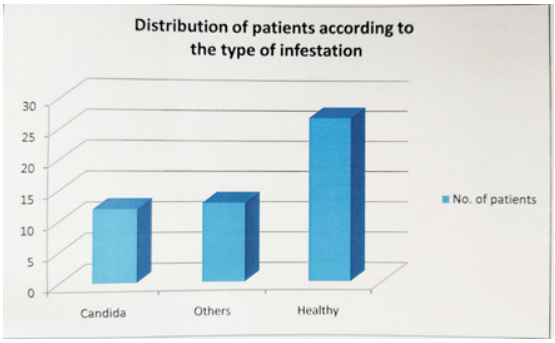
Oral diseases take a huge toll on the finances & place an economic & social burden on the population in terms of pain ,suffering& lost productivity. The additive effects of periodontal disease , caries & trauma result in the likelihood of tooth loss with age . Such patients are mainly left with the option of complete dentures. Complete denture wearers inspite of maintaining good oral & denture hygiene face the problem of denture stomatitis.

Candida albicans is reported to be an oral commensal in 20-50 % of normal subjects &upto 60% of denture wearers[5,7]. Candida is an asexual diploid fungus with more than 150 different species. It either adheres directly or to the plaque biofim on the denture acrylic .

The prevalence is high in institutionalized patients where little attention is paid to denture hygiene.[7] The sample population for this study were patients reporting to Dept. of Prosthodontics of our institution. The patients reporting to the institution were a part of the rural population , belong to low economic status , have less awareness about importance of denture hygiene along with increased usage of tobacco & poor nutrition status in elderly.

Various factors found to be associated to denture stomatitis are denture trauma ,denture cleanliness,allergic & primary irritant reactions to denture base materials, dietary factors, candidal infections, systemic predisposing factors etc.

A total of 52 patients were included in the study with mean age of 62.27 yrs (Table 1). In <60 yrs age group the Candidal presence was 5.76% &> 60 yrs age group 17.3% . So 23% of individuals were found positive for Candidal infections (Table 2)(Graph 1).

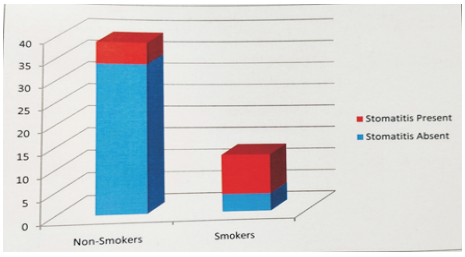


Graph 1 Shows distribution of patients according to the type of infestation

A higher incidence of Candidal presence was found in patients from higher age groups . This is in accordance with the study done by Lockhart et al [8] he reported that frequency & intensity of colonization of yeast increases with age in elderly.

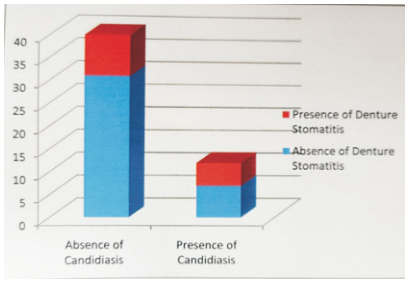
Prevalence of denture stomatitis in this study was found to be 27% in 52 subjects. In this institutionalised population 17.3% (n=14) denture wearers were affected with denture stomatitis & 13.5% (n=12) were positive for presence of Candida albicans. The correlation between presence of Candida & denture stomatitis shows that only 9.6% denture wearers (n=5) reported positive for both denture stomatitis &candida (Table 3). Chi square test showed a statistically insignificant relation between these two entities (p value =0.1892). No direct relation has been established between both as Candida fails to fulfill Kochs postulates. [9]

A striking relationship was found between smokers & denture stomatitis .Out of 13 smokers , 9 were found positive for denture stomatitis i.e. 69% (p value < 0.05)(Graph 2).



Graph 2 Shows presence of stomatitis in smokers & non smokers

Out of the total sample population , 26 patients were wearing dentures made with DPI heat cure acrylic resin & remaining 26 were wearing Lucitone199 acrylic resin dentures . From the 14 denture stomatitis cases (17.3%) , 9 were wearing DPI heat cure acrylic resin dentures& only 5 were wearing Lucitone199 acrylic resin dentures. Also that 64% of stomatitis cases belonged to DPI group, but no statistically significant interpretation could be drawn (p value > 0.05).(Table 4)(Graph 3)



Graph 3 Shows the distribution of patients associated with denture stomatitis &candiasis

A similar finding was seen when two different resins were evaluated for presence of Candida . 2/3rd of the patients positive for Candida were wearing DPI dentures. 84.6% denture wearers (n=22) from Lucitone group did not show any sign of Candidal presence whereas 30.8% DPI denture wearers were positive for Candidal presence (p value >0.05) .(Table 5). Denture base surface properties , as surface charge , surface free energy, hydrophobicity & surface roughness affect the initial adhesion of microorganisms.

Kalla et al 2011[10] concluded that heat cure poly methylmethacrylate denture base resins showed less adherance of Candidal cells compared to self cure& light cure resins. Also that Lucitione 199 showed the least adherence of Candidal cells compared to DPI resins. In this study we found that though the results were statistically insignificant, but the presence of Candida was lower in Lucitone 199 group. These findings are supported by the study done by Kalla 2011.

There is sufficient evidence that Newton´s (1962) Type I denture stomatitis is caused by trauma only. This condition is seen less frequently in patients with satisfactory alveolar ridges compared with those with flat ridges where there is greater degree &frequency of denture trauma . Samaranyake, McCourtie&McFarlane 1980 suggested that an inflammatory exudate leaking from traumatised palatal mucosa might promote candida colonisation .[11]Continuous denture wearing increases the local injury & may precipitate denture stomatitis. In this study , we have made sure that treatment was followed according to protocol to prevent trauma . Manufacturer instructions should be strictly followed while maintaining the denture base material as it helps in decreasing the incidence of denture stomatitis .

Budtz Jorgensen & Bertram 1970 [12]established a significant relationship between poor denture cleanliness & severe inflammation in denture stomatitis. A true allergy to denture base materials or its constituents seldom occurs , the incidence of such reactions is low but a primary irritant reaction to high concentrations of free residual monomer leaching out from the denture base resin may occur.

Many systemic diseases have been reported with denture stomatitis like diabetes mellitus ,anaemia, hypoparathy roidism, nephritis , mercury intoxication , bulluspemphigoid, kidney & urinary tract disease.

All the above etiologic factors should be eliminated. The treatment of oral candidiasis varies depending upon the type & extent of lesion. For angular chelitis Miconazole 2% cream, Clotrimazole 1% cream, Ketaconazole 2% cream &Nystatin ointment 100,00 units /gm can be applied topically twice a day for 2-4 weeks. For intraoral candidiasis Nystatin oral suspension 100000 units/ml, Amphotericin B 100 mg/ml is used to swish for 2 min 3-4 times a day. For systemic involvement Ketoconazole 200mgtab once daily, Fluconazole / Itraconazole 100 mg tab once daily.

Another preventive technique is incorporation of antimicrobial agents into the denture base resin itself . The absence of salivary statherins&histatin on the denture base has been attributed to the lack of anionic charge in polymethylmetha acrylate (PMMA) polymers. Thus , the lack of surface charge may be responsible for the decreased protective function of the acquired pellicle on denture. [13]Incorporation of materials have been tried to increase the surface charge of PMMA polymers. Park et al found that 10% methacrylic acid incorporation into monomer siugnificantly reduced the surface area of adherent Candida albicans .[13] Whereas Dhir et al in their study found that incorporation of phosphate containing monomer facilitated the addition of anionic charge on denture base resin without altering the physical properties of denture base resin. [15] In those cases of denture stomattits where there is no systemic involvement , mechanical elimination of the denture plaque alone will often effectively treat this condition.

Conclusion

This study states the prevalence of Candida albicans in denture wearers as to be 23% & the incidence of denture stomatitis to be 27%. Prevalence of Candida albicans& incidence of denture stomatitis was less with Lucitone 199 heat cure acrylic dentures.

Any treatment that we do should not be cause of other pathology. Denture stomatitis falls into such category. Every possible care should be exercised to eliminate incorporation of errors that may be hazardousto the patient on a later date. Beginning from the diagnosis , to material selection , material manipulation, proper maintenance instructions& regular followups, all play an important rolein prognosis of the treatment. Followup should be done on a mandatory basis to evaluate the changes & performing necessary modifications.

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