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Evaluation of Chlorhexidine and Delmopinol Application on Colonization of Candida In Complete Denture Wearers

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ABSTRACT

Background: Heat cure denture base resin material is the most commonly used material for the fabrication of complete denture, but its rough surface and surface porosity provides favorable site for harbouring bacteria of the oral flora and promote their multiplication including Candida albicans which is said to be one of the common etiological factor for denture sore mouth, which is the inflammatory condition of the oral mucosa. A routine denture cleaning regimen should be designed to remove and prevent reaccumulation of microbial plaque. Chemical denture cleansers (Antiseptic and Disinfecting agents) might be an important additive to mechanical cleansing, especially among geriatric &/or handicapped patients. 0.2% chlorhexidine gluconate has been successfully used in the treatment of Candia induced stomatitis. Another newer material in this category is morpholinoethanol derivative, Delmopinol which is a third generation of chemical plaque-control agents. The use of 0.2% delmopinol greatly reduces the plaque burden in gingivitis patients and also reduces potential for tooth staining which is a common disadvantage of chlorhexidine. This makes 0.2% delmopinol an attractive alternative to chlorhexidine for plaque control. But, here is lack of literature comparing the effect of Chlorhexidine and Delmopinol application on colonization of candida in denture wearing patients. So, this preliminary in vivo study was conducted with an aim of comparative evaluation of both of these antifungal agents on colonization of candida in denture wearers. The results of this study showed that the number of positive cultures after delmopinol application was very less (only one) as compared to the control and Chlorhexidine group. However, the difference between Chlorhexidine group and delmopinol group was not statistically significant.

Keywords: Chlorhexidine, Delmopinol, Oral hygiene practices.

INTRODUCTION

Poly methyl methacrylate heat cure denture base resin material is the most commonly used material for the fabrication of complete denture despite of the availability of newer materials. The reason for its popularity is ease of use, cost and acceptable aesthetic properties. However, rough surface of dentures and surface porosity of this material provides favourable site for harbouring bacteria of the oral flora and promote their multiplication. Great amount of tartar are observed frequently on the lingual flange of lower dentures that have not been polished properly[1]. Denture itself is considered to be a "plaque applicator," by holding plaque masses in contact with the oral mucosa for extended periods of time[2]. Due to this interaction, mucosal abnormalities like candidiasis are predictable in denture wearers.

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Candida albicans is a commensal organism found in the oral cavity of 20-60% of healthy individuals[3]. Candida albicans is said to be one of the common etiological factor for denture sore mouth, which is the inflammatory condition of the oral mucosa[4]. The adhesion of microorganisms to a surface is a prerequisite for the colonization of that surface. There are many studies on the adhesion of candida albicans to denture base acrylic resin causing opportunistic infection causing denture- induced stomatitis[5].

There are several factors which affect Candida albicans adhesion on acrylic surface; Quality and Quantity of saliva, wettability of acrylic surface, surface properties of acrylic, pH, Cell surface hydrophobicity (CSH), and Oral bacteria[6]. Candida associated stomatitis is commonest and because of the multifactorial nature of the disease, management is complex. Plaque- control, removal of denture at night, use of antiseptic and antimicrobial agents, antifungal agents and microwave irradiation are considered as treatment modalities for candida associated denture stomatitis[7].

Dental personnel have an increased risk of infection through constant exposure to debris, plaque, and saliva, which harbour pathogenic organisms that adhere to dental prosthesis. Dental personnel also may transmit potentially harmful organisms to the patients. These organisms pose an increase risk to elderly and medically compromised patients[8].

A routine denture cleaning regimen should be designed to remove and prevent reaccumulation of microbial plaque and also to remove mucin, food debris, calculus, and exogenous discoloration. In principle, mechanical cleansing is an effective means of improving denture cleanliness. The major disadvantage of mechanical cleansing is loss of surface details, which ultimately cause loss of denture retention. Chemical denture cleansers (Antiseptic and Disinfecting agents) might be an important additive to mechanical cleansing, especially among geriatric &/or handicapped patients. Antiseptic mouth rinse solutions are used in many clinical situations for different prophylactic and therapeutic purposes. It is difficult to decide which product is suitable for a particular purpose because of the variations of the antimicrobial efficacy and kinetics of the solutions [9].

0.2% chlorhexidine gluconate has been successfully used in the treatment of Candia induced stomatitis. The drug is a broad spectrum mouth rinse which is absorbed on the surface of microorganism, increasing permeability of cell membrane and causing precipitation of cytoplasmic contents. It was found that chlorhexidine bound to salivary pellicles as well as hard tissue in the oral cavity, resulting in chlorhexidine titres in saliva for 12 hours or more after rinsing [7].

The morpholinoethanol derivative Delmopinol is a third generation of chemical plaque-control agents. Plaque studies involving Delmopinol revealed that the nascent bio-film was loosely adherent and that there was a significant reduction in the proportion of dextran-producing Streptococci. The use of 0.2% delmopinol greatly reduces the plaque burden in gingivitis patients and also reduces potential for tooth staining which is a common disadvantage of chlorhexidine. This makes 0.2% delmopinol an attractive alternative to chlorhexidine for plaque control[10]. The surfactant action of delmopinol is greatest for heat cured acrylic resin than microwave-cured acrylic resin and lesser for cold-cured acrylic resin whether the acrylic resin specimens were treated with contamination before and after delmopinol application[11].

There is lack of literature comparing the effect of Chlorhexidine and Delmopinol application on colonization of candida in denture wearing patients. So, this preliminary in vivo study was conducted with an aim of comparative evaluation of both of these antifungal agents on colonization of candida in denture wearers. The null hypothesis for this study was, there is no statistically significant difference in the effect of Chlorhexidine and Delmopinol on colonization of candida in denture wearing patients.

METHODOLOGY

- 15 conventional complete denture wearer patients were randomly selected for cross-over in vivo study. Informed consent was taken from the subjects participating in this study. Completely edentulous patients wearing conventional complete dentures both in upper & lower arches and apparently healthy patients between the age group of 40-60 years were included in this study. Patients with Any history

of systemic diseases (diabetes), inflammation or mucosal lesions, subjects on antifungal/ antiviral/ antibacterial/antipsychotic drugs and having history of neutropenia & other blood disorders were excluded from this study. All the 15 patients were subjected to microbiological investigation for three times during the course of this study. The total time period for this study was 21 days. Patients were divided into 3 groups;

- Group 1- Control group
- Group 2- Chlorhexidine group
- Group 3- Delmopinol group

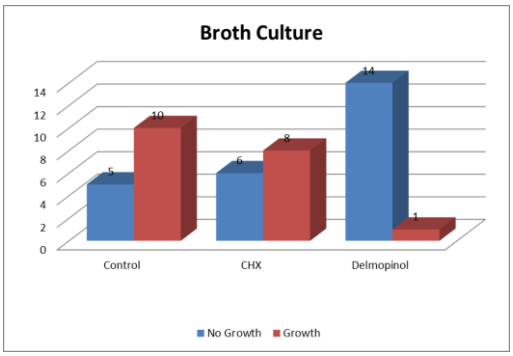
On 1st appointment- Maxillary denture from all the patients was collected and the intaglio surface was thoroughly washed with freshly prepared buffer solution aseptically. The liquid wash was collected in sterile container and subjected to centrifugation. The centrifuged collected was inoculated in the plates containing Hi- chrome differential agar as well as in Brain Heart Infusion broth (BHI) for 48 hours for the growth of candida. After 48 hours isolates were used to prepare slides from media and broth both. The slides were then stained with gram staining kit and observed under optical microscope at 40 X and 100 X magnifications. The patients were given 1st solution (0.2% Chlorhexidine). They were instructed to clean their upper dentures with fresh water and then dip the upper denture in 1:1 solution of water and chlorhexidine for the whole night. In the morning the denture was taken out, clean with water and used for the whole day. The patients were instructed to make fresh solution daily for every use. This procedure was repeated for 7 days. The mandibular denture was kept conventionally in normal water at night for the whole study period.

On 2nd appointment- The wash was collected from the maxillary denture –intaglio surface and subjected to the same microbial investigation. The cross- over period (window period) of 7 days was given before the use of 2nd solution so that the microbial flora can be re-established to the baseline level before the use of 2nd solution. During the cross- over period all the patients were instructed to clean both their dentures with normal water as they were doing routinely.

On 3rd appointment- The patient were given 2nd solution (0.2% Delmopinol) and instructed to use them in 1:1 concentration for same as that of 1st solution for seven days. The wash was collected from the maxillary denture and subjected to the same microbial investigation.

Table 1: Growth of Candida in the media (Hi-Chrome) and colony count in CFU (+ Growth present/ - No growth present).

Sr no	control	CHX group	Delmopinol group
1	-	+(spread)	-
2	-	-	-
3	+(3.3-10^3)	-	-
4	-	-	-
5	-	-	-
6	-	-	-
7	+(5.8-$10^{2)}$	+(2.4-10^2)	+ (1.860-10^3)
8	+(1.3-10^4)	+(1.8-10^2)	-
9	-	-	-
10	+(60)	+(1.0-$10^{2)}$	-
11	-	-	-
12	+(1.2-10^2)	+(20)	-
13	+(3.5-$10^{4)}$	-	-
14	+(6-10^2)	-	-
15	-	-	-

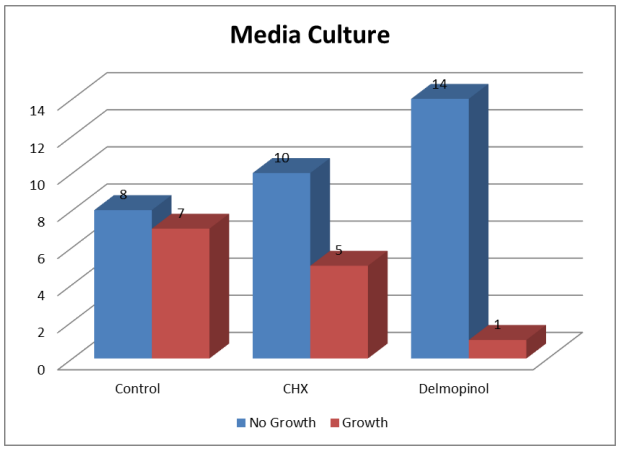


Graph 1: Broth culture.

Table 2: Growth of Candida in the media (Hi-Chrome) and colony count in CFU (+ Growth present/ - No growth present).

Sr no	control	CHX group	Delmopinol group
1	-	+(spread)	-
2	-	-	-
3	+(3.3-10^3)	-	-

4	-	-	-
5	-	-	-
6	-	-	-
7	+(5.8-10$^{2)}$	+(2.4-10^{2})	+ (1.860-10^{3})
8	+(1.3-10^{4})	+(1.8-10^{2})	-
9	-	-	-
10	+(60)	+(1.0-10$^{2)}$	-
11	-	-	-
12	+(1.2-10^{2})	+(20)	-
13	+(3.5-10$^{4)}$	-	-
14	+(6-10^{2})	-	-
15	-	-	-



Graph 2: Media culture.

DISCUSSION

Poly methyl methacrylate (PMMA) heat cure acrylic resin are the most commonly used material for denture base for partial and complete denture prosthesis as they provide acceptable color, esthetics, function and ease of processing. However, both in-vivo and in-vitro studies, regardless of simplicity or complexity of machinery or measurement method have constantly demonstrate the accumulation of plaque on denture surface [1, 2, 3,] which has been a matter of serious concern.

The process by which denture accumulates plaque stain and calculus is apparently similar to that process which take place on natural tooth surface[3]. Denture hygiene is an important element in maintenance of tissue health, which unfortunately, has always been a neglected aspect[9].

The initial stage of plaque formation involves attachments of microorganism to the acquired pellicle surface [12], followed by adherence of bacteria to each other, mediated by the component of the plaque matrix, causing the microorganism to form large polymicrobeal biofilm.

Chandra, et al[13] explained developm
on acrylic resins which happens in 3
initial stage – up to 11 hrs. From th
when some micro-colonies begin to be formed; intermediate stage – from 12 to 30 hrs. After colonization, when extracellular material begin to accumulate over colonies; and maturation stage – from 38 to 72 hrs. After colonization, when Candida colonies become totally involved by the extracellular matrix forming a biofilm.

Candida is most commonly associated with denture plaque [4, 14]. These yeasts are present in the normal microbial flora of oral cavity but display an affinity for adherence to acrylic resin and present in high titres in complete denture wearers.

Candida albicans is said to be one of the common etiological factor for denture sore mouth, which is the inflammatory condition of the oral mucosa[4]. There are many studies on the presence of candida albicans in patients with denture- induced stomatitis[5].

J. S. Landa[1] in 1960 discussed factors of oral hygiene, chemotoxicity, nutrition, allergy and conductivity. He conclude that rough surface of dentures as well as surface porosity of denture base materials provide a favorable sites for harboring bacteria which leads stomatitis. The management of denture stomatitis is complex and various mechanical and chemical methods of denture cleansing like tooth brushing, soaking in commercial cleansing tablet solution, combined method of brushing and soaking in tablet, soaking in commercial mouth-wash solution, irradiation by UV rays and soaking in distilled water[15] were used to clean the dentures but no single method is proven so much effective.

Attempts have also been made at modifying the materials and procedures of denture fabrication at various steps. Antimicrobials such as silver zeolite, triclosan [16], quaternary ammonium compounds[17] have been added to acrylic resins to inhibit the growth of microorganisms, but only with limited success. 0.2% Chlorhexidine gluconate has been successfully used in the treatment of Candia

induced stomatitis. Andrade IMD[18] evaluated the efficacy of complete denture biofilm removal using a Chlorhexidine solution in two concentrations: 0.12% and 2.0%. Results of this study showed that Immersion in 0.12% or 2.0% Chlorhexidine solutions can be used as an auxiliary method for cleaning complete dentures.

The use of 0.2% Delmopinol greatly reduces the plaque burden in gingivitis patients and also reduces potential for tooth staining which is a common disadvantage of Chlorhexidine. This makes 0.2% Delmopinol an attractive alternative to Chlorhexidine for plaque control. Moran et al [10] in 2007 did the meta-analyses of studies of 0.2 % Delmopinol mouth rinse as an agent to gingival health and plaque control measures and concluded that Delmopinol 0.2 % mouth wash is effective as an adjunct measure for reducing plaque burden and indices of gingivitis, whether or not it is used under supervision. The present in-vivo study was carried out to evaluate and compare the effectiveness Chlorhexidine and Delmopinol application on colonization of candida albicans in denture wearer patients.

In the present study, 0.2% Chlorhexidine and 0.2% Delmopinol were used as test chemical antiplaque agents because there is enough literature comparing the effect of these two in gingivitis and periodontitis patients but there is lack of literature comparing their effects in denture wearers. To determine the number of candida colony in the 10^{-3}, 10^{-4},10^{-5}, and 10^{-6} dilutions, replicate specimens 50 µL of the suspension were transferred to plates of selective media (Himedia manufacturers) sabouraud agar containing 5 µg/ml gentamicine. The plates were incubated at 37^{0} C for 48 hours. After incubation for 48 hours, candida colony of each plated sample were counted with naked eye & help of magnifying lenses and the number of the colonies according to the dilution ratio was calculated and defined as CFU/ml. CFU/ml = Dilution Factor x Total number of colonies on the plate

This technique of colony counting is advocated by Silva MM, Vergani CE, Giampaolo ET, Neppelenbroek KH[19]. A study by Ellepola ANB , Samaranayake LP[20] indicated that even a brief exposure to sub- therapeutic and sub-lethal level of Chlorhexidine gluconate may suppress the growth of Candida albicans, thereby minimizing the pathogenic potential of this opportunistic fungus.

This finding can also be supported by property of "substantively"[21]of Chlorhexidine which is the ability of a drug to absorb and bind to the soft and hard tissues and maintain effective concentration for prolong period of time.

A study was done by Ryalat S, Darwish R, Amin W[22] to evaluate the release of chlorhexidine as an antifungal drug from doped self-cured poly (methyl methacrylate) (PMMA) acrylic resin and the effect of the drug released on the growth of *Candida albicans.* Chlorhexidine leached steadily out of the acrylic resin into distilled water at mouth temperature, and the sustained drug release continued throughout the 28-day test period. The drug released also demonstrated antifungal activity against the resistant strain of *C. albicans.* The findings of this study support the use of chlorhexidine-impregnated self-cured PMMA chair-side resin as a new dosage form for the treatment of denture-induced stomatitis.

Montagner et al[23] evaluated the antifungal action of different agents on microwavable acrylic resin specimens, which were previously contaminated with *C. albicans*. They observed that sodium hypochlorite-based solutions and hydrogen peroxide are more efficacious disinfectants against *C. albicans* than 2.0% chlorhexidine solution and an effervescent agent. This lack of antimicrobial action of chlorhexidine 2.0% might be justified by the immersion periods used. The authors immersed the specimens in chlorhexidine for 10 minutes. In this study, the dentures were immersed in 2.0% chlorhexidine for 5 minutes, and the results showed that the solution was effective for biofilm removal. The difference in study design could be another reason for this discrepancy.

Similarly, Silva-Lovato and Paranhos[24] evaluated the effectiveness of disinfectant solutions (1.0% sodium hypochlorite, 2.0% chlorhexidine digluconate, 2.0% glutaraldehyde, 100% vinegar, sodium perborate-based tabs, and 3.8% sodium perborate) in the disinfection of acrylic resin specimens (n = 10/group) contaminated in vitro by *C. albicans, Streptococcus mutans*, *Staphylococcus aureus*, *Escherichia coli,* or *Bacillus subtilis* as measured by residual colony-forming units. The

acrylic resin specimens were immersed in the disinfectants for 10 minutes. It was concluded that 1.0% sodium hypochlorite, 2.0% glutaraldehyde, 2.0% chlorhexidine, 100% vinegar, and 3.8% sodium perborate are valid alternatives for the disinfection of acrylic resin.

Pavarina et al[25] also noted the effectiveness of chlorhexidine as a denture cleanser, though they used the chlorhexidine in a different concentration from that adopted in this study. In their study, the effectiveness of chemical agents (4.0% chlorhexidine gluconate, 1.0% sodium hypochlorite, iodophors, and alkaline peroxide) for cleansing and disinfecting removable dental prostheses was evaluated, and it was concluded that the 4.0% chlorhexidine gluconate, 1.0% sodium hypochlorite, and alkaline peroxide solutions were effective in reducing the growth of the microorganisms in the 10-minute immersion period.

Elworthy AJ et al[26] reported that Delmopinol appears to mediate its anti-plaque effect without causing a major shift in bacterial populations, although dextran-producing bacteria appear to be affected, which may have relevance to this agent's mode of action.

These findings can be correlated with the finding of Eley[27] that Delmopinol is truly a surfactant agent and has no antibacterial and antifungal effect.

Hase JC, Edwardsson, Rundegren, Attstrom, Kelty E[28] reported that total cultivable flora was affected to a certain extent by Delmopinol. However, Clear decrease in these counts was not observed until after 6 months. Considering the microscopic analyses, where a tendency of a shift towards a dental plaque dominated by Gram positive cocci was observed, it seems that Delmopinol mainly affected the anaerobic section of the plaque flora. The results are consistent with earlier studies on the effect of Delmopinol on the total microbial flora in developing dental plaque. In the Chlorhexidine group reductions over time were observed for both the total anaerobic and aerobic counts during the rinsing period, both in dental plaque and in saliva.

CIaydon N. et al[29] concluded that mouth rinsing with delmopinol, particularly at concentration of 0.2% provided adjunctive benefits to plaque control by mechanical methods. The most common local side effect of sensory disturbance on the tongue did not appear to affect patient compliance with long term use of the mouthrinse. Other local side effects, if anything, were less frequent and less intense than other plaque control agents particularly Chlorhexidine. This data, thus far, have provided positive evidence for Delmopinol as an antiplaque agent.

Though, care has been taken to simulate this in-vitro study to clinical conditions, but variation may be occurring in-vivo. Also, owing to a small sample size, the result may not truly represent the population and further in-vivo investigation in this field with a larger sample size may be required.

In the present in vivo study, the number of positive cultures after delmopinol application was very less (only one) as compared to the control and Chlorhexidine group. However, the difference between Chlorhexidine group and delmopinol group was not statistically significant.

Majority of patients complained altered taste (bitter) with Delmopinol application even after through washing of upper denture with water. This study also revealed that Delmopinol is not much effective against non- candida albicans candida (NCAC) species. Majority of microbial flora in group 1 and group 2 was mixed in nature similar to environmental or soil flora. Most of these patients were using hand pump water to clean the denture. The patient related outcomes suggested that normal water cannot repeal the potential source of Candidal contamination. So the effective denture cleansing regimen must be used. The cross over period in the present study was seven days, which can be increase to establish baseline flora before application of second solution. The sample size used in the present study is small to reach to a specific conclusion.

Further studies are needed to be carried out with a larger group of samples and a longer cross-over period for more definitive conclusions.

CONFLICTS OF INTEREST

The authors declare they have no potential conflict of interests regarding this article.

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