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The effect of some antifungal agents and chlorhexidine on *Candida albicans* adherence on acrylic resin denture base surface (*In vitro* study)

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ABSTRACT

The adherence of *Candida albicans* on acrylic resin denture base surface is considered as the first step in the pathogenicity of candida–associated denture stomatitis which is the most prevalent form of oral candidiasis in denture wearers. This study was an *in vitro* study aimed to evaluate the anifungal effect of fluconazole, nystatin and chlorhexidine and their anti-adherent effect on *Candida albicans* cells that attached on the fitting surface of denture base. It was determined, using broth microdilution method at two concentrations for each drug, and the results were determined using spectrophotometer. All drugs had a highly significant antifungal effect on *Candida albicans* isolate at their two concentrations.

The effect of saliva on the adherence of *Candida albicans* on the fitting surface of denture base was also considered. This study concluded the importance of saliva in increasing the microbial adherence; i.e., the mean number of adherent cells on acrylic resin samples in the absence of saliva was 7.4 $\pm$ 1.9 cell/mm^2 and with the presence of saliva the number was 29.1 $\pm$ 2.7 cells/mm^2. The number of remaining adherent cells was counted using fluortescent microscope. The mean number of remaining cells which adhered on acrylic resin samples after the immersion in first concentration fluconazole, nystatin and chlorhexidine for one hour respectively were 4, 5 and 2.6 cells/mm^2 and for the second

الخلاصة

يعتبر التصاق المبيضات البيضاء على سطح قاعدة الطقم من الراتنج الاكريلي هو الخطوة الأولى في إحداث حالة التهاب الفم المرتبطة بارتداء الطقم وهي الأكثر شيوعاً لدى مرتدي طقم الأسنان. لقد استهدفت هذه الدراسة التقييم المختبري للتأثير المضاد والقاتل لبعض الأدوية وهي الفلوكونازول والنستاتين والكلورهيكسيدين، وكذلك تأثيرها المضاد على إلتصاق خلايا المبيضات على السطح الداخلي لقاعدة الطقم؛ وقد استخدمت طريقة التخافيف الدقيقة في الوسط السائل لتقييم التأثير القاتل للأدوية وبواقع تركيزين لكل دواء. قُرئت النتائج باستخدام جهاز المطياف الضوئي، فقد تبين أن جميع الأدوية لها تأثيراً معنوياً قاتلاً على عزلة المبيضات البيضاء عند كلا التركيزين.

لقد أخذت هذه الدراسة بنظر الاعتبار تأثير اللعاب على التصاق المبيضات البيضاء على سطح قاعدة الطقم وتم التوصل إلى الدور المهم الذي أظهره اللعاب في زيادة عدد الخلايا الملتصقة حيث كان عدد الخلايا الملتصقة بعدم وجود اللعاب ٧.٤ $\pm$ ١.٩ خلية / ملمتر مربع وفي حالة وجود اللعاب ارتفع العدد إلى ٢٩.١ $\pm$ ٢.٧ خلية / ملمتر مربع. وعند استخدام الأدوية كان عدد الخلايا الملتصقة بعد غمر النماذج في التركيز الأول لمدة ساعة هو ٤ ، ٥ ، ٢.٦ خلية /

concentration/1 hour the numbers were 3.2, 4.1 and 1.7 cells/mm^2 compared to the normal number of adherent cell 29.1 $\pm$ 2.7 at $p<0.01$.

Key Words: Antifungal drugs, adherence, *Candida albicans*.

ملمتر مربع واحد لمحاليل الفلوكونازول والنستاتين والكلورهيكسيدين على التوالي وعند التركيز الثاني وبنفس الترتيب كان عدد الخلايا الملتصقة ٣.٢ ، ٤.١، ١.٧ خلية/ ملمتر مربع واحد من سطح الراتنج الاكريلي مقارنة مع العدد الطبيعي ٢٩.١ $\pm$ ٢.٧ عند مستوى معنوية ٠.٠١.

INTRODUCTION

The colonization of *Candida albicans* on the fitting denture surface is widely recognized as the main cause for the developing of denture stomatitis, which is the most prevalent form of oral candidiasis among denture wearers. [1–3]

The colonization depends on several factors including: Adherence of yeast cells, interaction with oral commensal bacteria and the surface properties of acrylic resins.[4]

As dentures are the potential sources of infection and reinfection of the oral soft tissues by *Candida albicans*,[5] denture plaque is considered as another important factor in the pathogenicity of candida–associated denture stomatitis which can be enhanced by a number of factors stimulating yeast propagation such as poor oral hygiene, high carbohydrate intake, reduced salivary flow and continuous denture we-aring.[6] Therefore, the use of antifungal agents has been shown to play an impor-tant role in the control of denture plaque and prevention of denture stomatitis.[7]

This study was designed to evaluate the effect of saliva and antifungal drugs and antiseptic on the adherence and colonization of *Candida albicans* on acrylic resin denture base surface.

MATERIALS AND METHODS

The methodology in this study includes two parts: The prosthetic and the mycological methods.

In the first part, a total of 108 specimens from heat cured acrylic resin were fabricated from wax–plate of dimensions 10×10×3 mm $\pm$ 0.5 mm.[8] The prosthetic methods include: Wax–elimination, dough stage mixture, packing, curing and cooling[9] followed by conditioning and disinfection of acrylic resin samples before the adhesion assay.[10, 11]

While in the second part, the isolation of *Candida albicans* was carried out taking swabs from the inner surface of upper den-ture in addition to the palatal mucosa for 8 patients selected randomly. Those were wearing their dentures for more than one year[12] and they were attending Prostho-dontic Department/College of Dentistry at Mosul Universal Hospital. Swabs were cultured on Sabouraud's dextrose medium at 37 ºC for 24–48 hours[13] and identified according to culture characteristics, microscopic appearance and germ tube formation.[14]

Antifungal drugs solutions were prepared according to their potencies. The fluconazole solution was prepared according to its potency (8, 64 µg/ml, respectively). The weighted amounts of fluconazole tablet were allowed to dissolve in distilled water (DW) immediately at the time of work.

While for nystatin, the drug was used as a solution with two concentrations (10,000; 100,000 IU). This is according to Banting *et al*.[15] who had reported that nystatin drug can be used as a denture soaking solution by dissolving 48 ml of nystatin liquid (suspension)/100,000 IU in 432 ml of DW to produce 10,000 IU/ml of nystatin solution. So, in this study, 10,000 IU/ml solution is considered as the lowest concentration of drug solution, while the higher concentration was 100,000 IU/ml of nystatin suspension (oral drops).

This solution of nystatin in a concentration of 10,000 IU/ml had been prepared immediately and must be changed every 48 hours, because it is affected by long exposure to light, air and heat.[15, 16]

The chlorhexidine gluconate was used as a solution in two concentrations, the higher concentration was 2%. This is according to Kulak *et al*.[17] and Banting and Hill[18] who reported that chlorhexidine could be used at a concentration of 2% as a denture soaking solution or denture clea-nsing agent.

The lowest concentration of chlorhexidine solution was 0.2% according to Gage and Pickett[19] who reported that chlorhexidine can be used as a mouth rinse in a concentration of 0.2%.

Chlorhexidine supplied as 5% weight/ volume by manufacture. The dilution was done with DW according to Summerlin.[20]

Chlorhexidine solution must be changed every two days and should be diluted immediately.[18]

All the prepared drug solutions were kept at 25 $\pm$ 1 ºC. This temperature was chosen as a parameter in this study.[21]

The unstimulated whole saliva was collected from 30 volunteers using spitting method.[22] The collected saliva then was pooled, clarified using refrigerated centrifuge.[23, 24] For the adherence experiment, the acrylic resin samples were divided into two groups. The first group was treated with saliva, the second was not. A standardized *Candida albicans* suspension (2 ml) was added to each specimen, incubated for 1 hour at 37ºC,[10, 25] then washed with phosphate buffer saliva to remove the loosely adherent cells. Fixation and staining of the remaining adherent cells to the surface was achieved.[26, 27]

The number of adherent cells was examined using flourescent microscope (Olympus, Japan) for at least 3 replicates for each variable was taken.[10, 11]

The statistical analysis was carried out using one way analysis of variance (ANOVA) and Duncan's Multiple Range Test at levels of significance 1% and 5%.

RESULTS

The antifungal Effect of the Drugs (Against *Candida albicans* Isolate)

For the first group of each drug (fluconazole 64 µg/ml, nystatin 100,000 IU and chlorhexidine 2%) (Table 1), the mean absorbance values in nanometer (nm) of the replicates were measured and compared with the control group by the Duncan's Multiple Range Test.

Table (1): Duncan's Multiple Range Test for the antifungal effect of the first group of different drugs against *Candida albicans*

First Concentration of Drug Solution	Absorbance Mean $\pm$ SD	Duncan's Grouping*
Control Positive	0.62 $\pm$ 0.2	A
Fluconazole 64 µg/ml	0.15 $\pm$ 0.05	B
Nystatin 100,000 IU	0.05 $\pm$ 0.004	B
Chlorhexidine 2%	0.26 $\pm$ 0.06	B

*Different letters mean significant difference exists.
SD: Standard deviation.

The results of this table indicated that all drug solutions at their first group had antifungal effect which was significantly different from the control group although there was no significant difference among them.

The same results were obtained with the second group (low potencey of drug solutions: Fluconazole 8 µg/ml, nystatin 10,000 IU and chlorhexidine 0.2%) on their antifungal effect against *Candida albicans* isolate (Table 2).

Table (2): Duncan's Multiple Range Test for the antifungal effect of the second group of different drugs against *Candida albicans*

Second Concentration of Drug Solution	Absorbance Mean ± SD	Duncan's Grouping*
Control (+ve)	0.62 ± 0.2	A
Fluconazole (8 µg/ml)	0.12 ± 0.04	B
Nystatin (10,000 IU)	0.01 ± 0.001	B
Chlorhexidine 0.2%	0.06 ± 0.07	B

*Different letters mean significant difference exists.
SD: Standard deviation.

The Effect of Saliva on the Adherence of *Candida albicans* Isolate on Acrylic Resin Samples

The results of this experiment showed that the number of *Candida albicans* cells/mm^2 adhered to saliva coated acrylic resin samples which indicated that saliva had a high significant effect on increase in the number of *Candida albicans* cell/mm^2 to acrylic resin sample (Table 3) as shown in Figure (1).

Table (3): Duncan's Multiple Range Test for the effect of saliva on adherence of *Candida albicans* on acrylic resin samples

Acrylic Resin Samples	Number of *Candida albicans* Cells/mm^2 Mean ± SD	Duncan's Grouping*
Saliva +ve •	29.1 ± 2.7	A
Saliva –ve º	4.7 ± 1.9	B

*Different letters mean significant.
SD: Standard deviation.
•Saliva coated samples
ºSaliva uncoated sample.

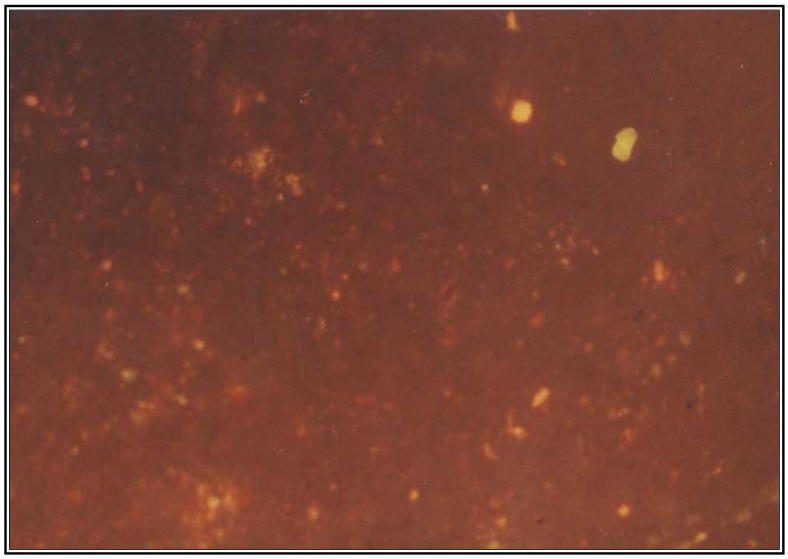

Figure (1): The adherent cells of *Candida albicans* on acrylic resin samples at (×) magnification power

The Antiadhered Effect of Antifungal Drugs and Chlorhexidine on *Candida albicans* Isolate

The analysis of variance for the data revealed that the three drugs; fluconazole, nystatin and chlorhexidine in their high and low potencies at different times of immersion had a highly significant anti–adherent effect on both saliva coated and saliva uncoated acrylic resin samples different from control group when compared at level of significance $p<0.01$, and the duration of immersion was effective in reducing the number of *Candida albicans* cells adhered to saliva uncoated and saliva coated acrylic resin samples. However, there were no statistical significant differences among them as shown in Tables (4), (5), (6), (7), (8) and (9).

Table (4): The antiadherent effect of first and second groups of fluconazole on *Candida albicans* on saliva uncoated samples

		Time of Immersion (Hour)	Number of *Candida albicans* Cells/mm^2 ± SD	Duncan's Grouping*
		Control Time 0 Hour	7.4 ± 1.9	A
Saliva Uncoated (–ve) Acrylic Resin Samples	First Concentration	1 Hour	0.7 ± 0.6	B
		24 Hours	0.3 ± 0.1	B
		48 Hours	0.2 ± 0.1	B
	Second Concentration	1 Hour	0.6 ± 0.5	B
		24 Hours	0.39 ± 0.52	B
		48 Hours	0.1 ± 0.06	B

*Different letters mean significant difference exists; SD: Standard deviation.

Table (5): The antiadherent effect of first and second groups of fluconazole on *Candida albicans* on saliva coated samples

		Time of Immersion (Hour)	Number of *Candida albicans* Cells/mm^2 ± SD	Duncan's Grouping*
		Control Time 0 Hour	29.1 ± 2.7	A
Saliva Coated (+ve) Acrylic Resin Ssamples	First Concentration	1 Hour	3.28 ± 0.79	B
		24 Hours	2.8 ± 0.8	B
		48 Hours	2.5 ± 0.6	B
	Second Concentration	1 Hour	4.0 ± 1.0	B
		24 Hours	3.70 ± 0.95	B
		48 Hours	3.54 ± 0.9	B

*Different letters mean significant difference exists; SD: Standard deviation.

Table (6): The antiadherent effect of first and second groups of nystatin on *Candida albicans* cells on saliva uncoated samples

		Time of Immersion (Hour)	Number of *Candida albicans* Cells/mm^2 ± SD	Duncan's Grouping*
		Control Time 0 Hour	7.4 ± 1.9	A
Saliva Uncoated (–ve) Acrylic Resin Samples	First Concentration	1 Hour	0.6 ± 0.07	B
		24 Hours	0.54 ± 0.09	B
		48 Hours	0.4 ± 0.08	B
	Second Concentration	1 Hour	0.4 ± 0.07	B
		24 Hours	0.3 ± 0.05	B
		48 Hours	0.25 ± 0.03	B

*Different letters mean significant difference exists; SD: Standard deviation.

Table (7): The antiadherent effect of first and second groups of nystatin on *Candida albicans* cells on saliva coated samples

		Time of Immersion (Hour)	Number of *Candida albicans* Cells/mm^2 ± SD	Duncan's Grouping*
		Control Time 0 Hour	29.1 ± 2.7	A
Saliva Coated (+ve) Acrylic Resin Ssamples	First Concentration	1 Hour	5.08 ± 0.3	B
		24 Hours	4.96 ± 0.4	B
		48 Hours	4.8 ± 0.5	B
	Second Concentration	1 Hour	4.1 ± 0.3	B
		24 Hours	3.9 ± 0.2	B
		48 Hours	3.6 ± 0.2	B

*Different letters mean significant difference exists.
SD: Standard deviation.

Table (8): The antiadherent effect of first and second groups of chlorhexidine on *Candida albicans* cells on saliva uncoated samples

		Time of Immersion (Hour)	Number of *Candida albicans* Cells/mm^2 ± SD	Duncan's Grouping*
		Control Time 0 Hour	4.7 ± 1.9	A
Saliva Uncoated (–ve) Acrylic Resin Samples	First Concentration	1 Hour	0.39 ± 0.1	B
		24 Hours	0.27 ± 0.1	B
		48 Hours	0.2 ± 0.08	B
	Second Concentration	1 Hour	0.1 ± 0.03	B
		24 Hours	0.008 ± 0.01	B
		48 Hours	0.06 ± 0.01	B

*Different letters mean significant difference exists.
SD: Standard deviation.

Table (9): The antiadherent effect of first and second groups of chlorhexidine on *Candida albicans* cells on saliva coated samples

		Time of Immersion (Hour)	Number of *Candida albicans* Cells/mm^2 ± SD	Duncan's Grouping*
		Control Time 0 Hour	29.1 ± 2.7	A
Saliva Coated (+ve) Acrylic Resin Ssamples	First Concentration	1 Hour	2.68 ± 0.2	B
		24 Hours	2.48 ± 0.2	B
		48 Hours	2.2 ± 0.1	B
	Second Concentration	1 Hour	1.78 ± 0.2	B
		24 Hours	1.6 ± 0.2	B
		48 Hours	1.3 ± 0.1	B

*Different letters mean significant difference exists.
SD: Standard deviation.

DISCUSSION

In the present study, the isolation of *Candida albicans* was achieved more frequently from the inner or fitting surface of denture base rather than from corresponding mucosa, because *Candida albicans* colonization on the fitting denture surface is heavier than on the palatal mucosa due to the fact that microorganisms are partly eliminated with shedding of the epithelial cells.[28]

The result of this study demonstrated a significant effect of saliva on increasing the number of *Candida albicans* cells adh-

ered to acrylic resin samples, due to the adsorption of certain components of saliva by the plastic surface which enhance the attachment of *Candida albicans*.[29] Thus, this chemical substance (saliva) acts as acceptor for microbial adherence.[30] Furthermore, the aging of the material and biological fluids of the host promote the yeast colonization on thermocycled prosthetic material.[31]

The antifungal and chlorhexidine drugs which had been used in this study showed a high significant anti–adherent effect on *Candida albicans* cells that adhered on acrylic resin samples in spite of their concentrations and duration of immersion. The second group (i.e., low concentration) of each drug and the least time of immersion (i.e., one hour) was preferable.

The anti–adherent effect of fluconazole is due to its effect on reducing the production of phospholipase by *Candida albicans* isolate.[3, 32] The same results were obtained for both nystatin and chlorhexidine because both of them were effective in the elimination or eradication of hyphal form of *Candida albicans* which reduce the number of the yeast cells.[33]

CONCLUSION

Soaking of acrylic resin samples in antifungal agents and chlorhexidine was an effective mean of reducing surface adhesion by *Candida albicans*. This appro-ach to fungal control is believed to be a practical for home care of denture.

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