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Adherence of *Candida albicans* to Soft Denture Liners in Controlled Diabetic Patients.

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ABSTRACT

This study investigated the adhesion potential of *Candida albicans* on heat cured silicone based soft liner and acrylic resin-based liner in controlled diabetic patients. Twenty controlled male diabetic edentulous patients were precisely selected and thoroughly examined in an attempt to reduce human variables and eliminate any factor or habit that might adversely affect the results of this study. The patients were randomly divided into two equal groups: Group 1: Patients in this group were rehabilitated with maxillary heat cured acrylic dentures lined with silicone based soft liner "Molloplast B". Group 2: Patients in this group were rehabilitated with maxillary heat cured acrylic dentures lined with acrylic based soft liner "VERTEX soft". Sampling the tissue surface of the denture following the swab technique assessed the effect of each of the previously mentioned lining materials on candida counts. The swabs were taken from the fitting surface of denture base. The colonies were quantified according to the scale developed by Oslen and modified by Bergendal et al. The germ tube test was carried out to confirm the presence of *Candida albicans*. Samples were collected at time of denture insertion, one, three, six, and nine months after denture insertion. Data were tabulated and statistically analyzed. A significant increase in the calculated means of *Candida albicans* growth was evident 3 months after denture insertion in the two studied groups. Three, six, and nine months after denture insertion confluent growth score (3) was detected in 100% the two studied groups. Statistical analysis of the data revealed higher significant values among cases wearing maxillary dentures lined with Molloplast B <0.05 compared to cases wearing maxillary dentures lined with liner "VERTEX soft".

Keywords: Soft liner, *Candida Albicans*, Diabetic patients

INTRODUCTION

Diabetes Mellitus is a metabolic disorder of multiple etiologies characterized by hyperglycemia, with disturbance of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both [1]. The classical triad of Diabetes symptoms is Polyuria, Polydipsia, Polyphagia, and weight loss may occur [2,3].

Oral complications of diabetes include delayed wound healing by inhibiting fibroblastic activity and collagen formation. Gingivitis, periodontitis, hypertrophy of filiform papillae in controlled diabetic and loss of filiform papillae in uncontrolled diabetic patients. Xerostomia has been reported to be a common complaint, reduction in the salivary flow makes both the hard and soft tissues of the mouth more prone to infection, dental caries, in addition to the changes in the oral mucosa as candidal infection as well as burning of mouth cheilosis. Increased level of glucose in serous saliva of the parotid gland [4-8].

Tooth loss and increased alveolar bone loss due to reduction in bone formation and matrix apposition rate due to impaired osteoblastic activity [6,9]. Diabetes mellitus is considered as one of the major systemic factors involved in the process of residual ridge resorption and osteoporosis [9,10]. The complications of diabetes are far less severe in people who have well-controlled blood sugar levels. In fact, the better the control, the lower the risk of complications [11].

Conventional heat cured acrylic denture is intolerable for many diabetic patients because the supporting mucosa of the alveolar ridge is sensitive to pressure from hard prosthetic materials due to alveolar ridge resorption and non-resilient mucosa covering knife edge ridges, ridges with bony prominences, sharp mylohyoid ridges, and severe tissue undercuts because of their viscoelastic properties [12].

The use of soft liner proved to be an effective treatment modality for the management of diabetic patients due to the cushion effect of the material. Resilient lining materials have been used to provide a cushion between the denture base and the supporting tissues, and allow for a more uniform distribution of stress at the mucosa lining interface, reduce the impact force on the denture supporting structures and preserve the residual oral structures as much as possible [13,14].

Resilient denture lining materials are advantageous when treating patients with residual ridge atrophy or resorption, relatively thin and non-resilient mucosa, bony undercuts, bruxism, xerostomia, and when the dentures oppose natural dentition. In principle, retention of the denture can be improved and pressure on the mucosa can be reduced by using resilient soft liners [15].

The resilient denture lining materials are broadly classified according to their chemical composition into acrylic based liners and silicone based elastomers. Acrylic based resilient denture liners are plasticized polyethylmethacrylate polymer. They are characterized by their bonding strength to acrylic resin base; however their major drawbacks are high water sorption and low resiliency [16,17].

Silicone-based resilient liners are poly-dimethyl siloxane polymer. Silicone liners are color stable, and more resilient than acrylic based liners. The polymer is an elastomer, which does not require an external plasticizer and is, therefore, more stable over time [18].

Dootz and his associates found that heat-polymerized acrylic resin-based resilient liner materials have the greatest hardness. Vertex Soft liner and heat-polymerized silicone-based Molloplast-B liner are harder than the interim auto-polymerized acrylic resin-based liner and definitive auto-polymerized silicone-based liner [19].

The clinical use of resilient lining material has been associated with many problems. Among these are staining, color change, porous surface texture, degradation and decreased resiliency with time. The porous surface texture as well as debonding between the denture and the resilient liner favor the accumulation of food debris and encourage bacterial growth. Loss of surface integrity and surface roughness can irritate the denture bearing area and create an environment for the colonization of oral micro-organisms [13,20,21]. Radford found that contamination of resilient denture liner materials with microorganisms, particularly *Candida albicans*, is a common clinical problem reported with hard denture base acrylic resin and silicone-based resilient liner materials [21]. It has been shown that Molloplast B was more hydrophobic than acrylic resin, and therefore Molloplast B would have a lower surface free energy than acrylic resin [22,23].

In vivo investigations of colonization of denture bases with *C. albicans* show that soft-lining materials in clinical situations become colonized with *C. albicans* more frequently than does acrylic resin. The association with Molloplast B is widely accepted; however, this may be more due to the common use and frequent investigation of this material rather than to its specific susceptibility to colonization by *C. albicans*. Colonization by the organism is not a problem unique to the oral cavity and has for instance also been described on silicone rubbers [23].

Candida species are normal oral commensals present in 17% to 60% of apparently healthy persons. Multiple factors have been implicated to predispose to Candidal growth and colonization. Wearing of dentures is among the most

important risk factors affecting Candidal carriage. The presence of an intraoral appliance produces alteration in the ecological environment of the prosthesis covered area. Such ecological changes promote colonization and adhesion of *Candida albicans*, which was found to flourish in denture wearers [24].

The association between diabetes and pathological changes in the oral cavity has been the subject of many reports. Awareness of the susceptibility of denture base liner to *Candida albicans* colonization should be an important factor in their use to preserve and maintain the health of oral mucosa in diabetic patients. Accordingly this study investigated the adhesion potential of *Candida albicans* on heat cured silicone based soft liner and the soft acrylic denture liner in controlled diabetic patients.

MATERIALS AND METHODS

Twenty Completely edentulous male controlled diabetic [type II diabetic] patients were included in this study. Age of the selected patients ranged between 60-70 years, the residual alveolar ridges exhibited adequate height and width, with no bony exostosis or, undercuts and covered with firm fibrous mucoperiosteum. With no signs of inflammation, ulceration, or hyperplasia. no history of parafunctional habits, nonsmokers and had no previous denture experience. Patients were free from any systemic disease other than diabetes to avoid any adverse effects on the process of bone and soft tissue.

Detailed information about the treatment was given to all the patients. All the patients were motivated to the treatment and were informed that they will be a part in a study that needs their best co-operation. All the patients agreed to share and follow the instructions given to them in the form of signed consent. As well as laboratory investigations, complete blood picture, blood sugar level [fasting and post prandial] and erythrocyte sedimentation rate were made.

All patients participating in this study were rehabilitated by mucosa supported maxillary and mandibular complete dentures. The upper dentures for all the patients were lined by resilient denture liner. According to the type of the lining material, patients sharing in this study were randomly divided into two equal groups.

Group 1: Patients in this group were rehabilitated with maxillary heat cured acrylic dentures lined with heat cured silicone-based liner [Molloplast B] *.

Group 2: Patients in this group were rehabilitated with maxillary heat cured acrylic dentures lined with heat cured acrylic-based liner [Vertex soft acrylic] **

Complete dentures were constructed to all patients following the same basic principles. Border tracing was done using medium consistency rubber base impression material * and the secondary impressions were taken using light consistency rubber base impression material with light finger pressure. Centric occlusion was developed at centric relation. Non-anatomic cross-linked acrylic teeth *** were arranged following the monoplane concept of occlusion. The waxed up denture was tried in the patient's mouth to ensure proper facial contour, aesthetic, even contact between all the posterior at the predetermined vertical dimension of occlusion.

Maxillary dentures were flaked and wax eliminated. Tin foil of 2-3mm thicknesses was adapted over the residual ridge and the palatal area, 2mm shorter than the depth of the vestibule fig1.



Fig. (1) Trial packing with tin foil spacer

*Molloplast B® [heat-cured silicone elastomer] MB Karl Huber GmbH & Co. Germany Single component paste.

**VertexTMSOft [heat-cured acrylic resin] VX Dentimex BV, Holland Powder and liquid *

For Group 1, heat cured acrylic resin was packed; the flask was placed under the bench press for 10-15 min at 100KP. Per polymerization of the heat cured acrylic resin was carried out for 30 min at 100° C. Flasks were opened, spacer were removed and primer adhesive was applied on the fitting surface of the heat cured resin bases. Heat cured soft liner [Molloplast-B] was applied on the fitting surface of the stiff heat cured acrylic resin. Flasks were closed and bench-pressed for 10 – 15 minutes. Dentures were reprocessed for two hours at 100°C to allow for curing of the liner.

For Group II, Heat cured acrylic resin was packed. The flask was placed under the bench press for an hour to allow the resin to be firm enough to resist distortion during packing of the acrylic resin. Dentures flasks were opened; spacers were removed to pack [Vertex] soft acrylic lining material. The recommended powder/liquid ratio [12gm/10ml] was stirred thoroughly, when the mixture reaches the dough stage consistency; it was applied evenly on the fitting surface of the dough stage of the conventional heat cured acrylic resin. The [Vertex] soft acrylic was polymerized in a water bath curing tank for 1½ hour at 70°c and for another ½ hour at 100°c.

Laboratory remounting was done before decasting of the dentures and occlusal discrepancies were adjusted. Dentures were stored in tap water for 24 hours before delivery. Any necessary adjustments were done and post insertion instructions were given to the patient. Patients were educated and instructed to maintain strict oral and denture hygiene measures. Frequent follow-up appointments were scheduled to ensure proper oral and denture hygiene. Follow up visits were scheduled at time of denture insertion, three, six, and nine months after denture insertion for collection of the samples and to evaluate the condition of the denture supporting structures.

Oral candidial count was assessed before denture insertion, three and six, and nine months after denture insertion using the swab technique. The swabs were taken by rubbing the fitting surface of the maxillary denture vigorously using sterile ordinary swabs. The swabs were placed into vials containing 2 ml transport medium [nutrient broth]. Each sample was carefully homogenized by vortexed for 30 seconds, serial dilution [1:10, 1:100, 1:1000] were made in sterile saline then 0.1 ml from each dilution was smeared to be inoculated on the surface of Sabouraud's agar plates by plate streak method. The plates were inoculated at 37°C for 48 hours. Candidal colonies appeared to be white to creamy in color, smooth and glistening Fig [2].



Fig. (2) Positive growth of Candida Albicans .

The colony forming units of organisms were quantitated according to the scale developed by Olsen 1974[25] as follows: No colonies = 0; 100 colonies = 1; more than 100 colonies = 2; confluent growth = 3

Smears from the colonies were prepared, stained with gram stain and examined by oil –immersion lens of the microscope for the presence of budding oval gram positive yeast cells and pseudohyphae of candida. The colonies were subcultured on Sabouraud's agar slopes with chloramphenicol to subject dextrose to germ tube test for identification for Candida albicans. The presence of germ tubes is characteristic for Candida albicans. The data was collected and statistically analyzed.

RESULTS

In this study, data of Candida albicans colonies were coded, edited, collected and analyzed as means and standard deviations for the studied groups at time of denture insertion, three, six, and nine months follow up periods. Statistical analysis was carried out using Microsoft Excel 2010 program. While testing significance was performed using SPSS® 20 [Statistical package for scientific studies, SPSS, Inc., Chicago, IL, USA] and Minitab® statistical software Ver. 16. Paired t-test was used to test the significance of Candida albicans colonies within each group during the follow-up period. A probability level of $P \leq 0.05$ was considered statistically significant.

Three, six and nine months after denture insertion confluent growth score [3] was detected in 100% the two studied groups.

The calculated means of candida colonies their levels of significance for patients rehabilitated with upper denture lined with [Molloplast-B] are presented in tables [1,2]. The mean of number of colonies at time of insertion, three, six and nine months were [0], [23.67], [30.57] and [31.86] colonies respectively, as listed in table [1]. Comparing the mean values candida albicans growth among the follow up period between each two consecutive measures, table 2 revealed that a significant increase was evident during the interval 0 – 3 months by [23.67] colonies as P-value <0.05, while there was insignificant increase from 3- 6 months by [6.9] colonies and from 6 - 9 months by [1.29] colonies as P-value > 0.05. A total significant change of [31.86] colonies was evident at the end of the follow up period.

Table (1): Mean and standard deviation for the effect of Molloplast -B on Candidal growth (group I)

Group I Maxillary denture lined with Molloplast-B	Follow-up periods	Mean (colony)	Standard deviation
	At insertion	0	0
	3 rd month	23.67	11.33
	6 th month	30.57	12.08
	9 th month	31.86	10.16

Table (2): Mean difference and Paired T- test for the effect of Molloplast -B on Candidal growth (group I):

Group II Maxillary denture lined with Vertex	Follow up periods	Mean (colony)	Standard deviation
	At insertion	0	0
	3 rd month	13.57	1.51
	6 th month	19.00	1.83
	9 th month	24.14	1.95

*insignificant difference **significant difference

The calculated means of candida colonies their levels of significance for group of patients rehabilitated with upper denture lined with Vertex soft acrylic are at the time of denture insertion and at each recall appointment are represented in tables [3,4] The mean of number of colonies at time of insertion,three, six, and nine months follow up periods were [0], [13.57], [19.00] and [24.14] colonies respectively, as listed in table [3].

Table (3): Mean and standard deviation for the effect of Vertex on Candidal growth (group II):

Interval	Mean difference	SD	P value
0 - 3 months	23.67	11.33	0.0001**
3 – 6 months	6.9	0.75	0.188*
6 – 9 months	1.29	1.92	0.852*
0-9 months	31.86	10.16	0.0001**

To compare between the mean values candida albicans growth among the follow up period between each two consecutive measures, paired T test was applied and is shown in table [4]. A significant increase in the calculated means of candida albicans growth was evident during the interval 0 – 3 months by [13.57] colonies as P-value < 0.05 while there was insignificant increase from 3- 6 months by [5.43] colonies and from 6 - 9 months by [5.14] colonies as P-value > 0.05. A total significant change of [24.14] colonies was evident at the end of the follow up period.

Table (4): Mean difference and Paired T- test for the effect of Vertex soft on Candidal growth (group II):

	Mean difference	SD	P value
0 - 3 months	13.57	1.51	0.0001**
3 – 6 months	5.43	0.32	0.649*
6 – 9 months	5.14	0.12	0.905*
0-9 months	24.14	1.95	0.0001**

*insignificant difference **significant difference

To compare between the effects of studied lining materials during the follow up period on the count of candidal colonies, paired- T test was applied and the results are shown in table [5]. The calculated mean of the CFU[Colony Forming Unit] three month after denture insertion was found to be [23.67 and 13.57 for group I [patients rehabilitated with maxillary denture lined with Molloplast B] and group II [patients rehabilitated with maxillary denture lined with Vertex] respectively. The means of the CFU scores showed higher significant $P < 0.05$ values among cases wearing maxillary dentures lined with Molloplast B.

Table (5): Mean, standard deviation and Student –T test of colony forming units count for group I and group II patients during the follow up period.

	At insertion		3 rd month		6 th month		9 th month	
	Mean (colony)	SD	Mean (colony)	SD	Mean (colony)	SD	Mean (colony)	SD
Group I Molloplast B	0.00	0.00	23.67	11.33	30.57	12.08	31.86	10.16
Group II Vertex	0.00	0.00	13.57	0.51	19.00	1.83	24.14	1.95
T value	00		32.100*		36.84*		43.45**	

*insignificant difference **significant difference

At the end of the six months follow up period, the calculated mean of the CFU was found to be 30.57 and 19.00 for group I [patients rehabilitated with maxillary denture lined with Molloplast B] and group II [patients rehabilitated with maxillary denture lined with Vertex] respectively. Statistical analysis of the data revealed higher significant values among cases wearing maxillary dentures lined with Molloplast B $P < 0.05$.

The calculated mean of the CFU at the end of the nine month follow up period was found to be 31.86 and 24.14 for group I [patients rehabilitated with maxillary denture lined with Molloplast B] and group II [patients rehabilitated with maxillary denture lined with Vertex] respectively. The difference between the two studied groups was statistically significant $P < 0.05$.

DISCUSSION

This study investigated the adhesion potential of *Candida albicans* on heat cured silicone based soft liner and the soft acrylic denture liner in controlled diabetic patients. Two commercially available, silicone-based heat-cured soft liner "Molloplast B" and acrylic based soft liner "VERTEX soft", were selected for their biological evaluation. Controlled diabetic patients were selected for this study as they are challenging situation presenting an absolute indication for soft denture liners. In an attempt to carry out a fair comparison between the effects of the two tested materials, patients in this study were precisely selected and thoroughly examined in an attempt to reduce human variables and eliminate any factor or habit that might adversely affect the results of this study. Only type II controlled diabetic patients were selected in the study as it was proven by many authors that they are more or less similar to normal individuals [26].

Patients were free from any systemic disease other than diabetes to avoid any adverse effects on the results of this study. Patients were nonsmokers, to eliminate the effect of smoking on the integrity of the resilient lines, as well as on *Candida albicans* growth [27].

Centric occlusion is in harmony with centric jaw relation in the most closed position of the teeth. The vertical dimension of the occlusion is within the physiological tolerance of the patient as continuous traumatic occlusal contact could increase the frequency of denture stomatitis due to inflammatory reaction as a result of denture trauma. Consequently, inflammation due to trauma may create an environment favorable to microorganisms found in denture stomatitis.

It is well known that microbial adherence is highly affected by surface roughness. The selection of heat processed lining materials rather than room-temperature polymerized ones, was based on the fact that surface roughness is significantly higher on room-temperature polymerized resilient liners [13,17]. In an attempt to achieve optimum flexibility and to compensate for the materials tendency to stiffen with time, 2-3mm thickness, of the resilient liners under investigation were packed in a space, created during trial closure stage [13].

All patients received dentures that were constructed following the same procedures, using the same materials and having the same occlusal scheme. Strict oral and denture hygiene measures were emphasized at time of denture

insertion and maintained during frequent follow up visits in an attempt to exclude the effect of improper oral and denture hygiene on the results of this study.

Swabbing technique was selected in this study to isolate *C.albicans* due to its simplicity, efficiency and as it is frequently used in the literature for oral yeast isolation. Swab was taken by rubbing the fitting surface of maxillary dentures to ensure more accurate and standardized isolation procedure .The irregularity of the fitting surface of maxillary denture act as a reservoir for *C.albicans* and it is considered the most suspected area for bacterial colonization [25].

Although patient's selection and the technique followed in denture construction were directed mainly to minimize the possibility of Candidal infection, *Candida albicans* was isolated in all the cases of the two groups after three months. The marked increase in both the frequency of *Candida albicans* carriers and density of colonization three months after denture insertion in both the studied groups agrees with the results of other studies which reported that resilient denture lining materials often show *Candida albicans* colonization with aging, which affect longevity and serviceability of these materials [28,29].

Candida survival within the oral ecosystem depends on balance between the host tissues and the constituting micro flora, which is usually disturbed in diabetic patients. Imbalance of the oral microbial flora and to adversely affect tissue tolerance, resulting in increased mucosal inflammation with the use of dentures. *Candida albicans* may return into complex structures, often encapsulated within a matrix of exopolymeric material that favors a strong adhesion to biotic and abiotic surfaces when the balance of normal oral microflora is disrupted if such a condition occurs *Candida* species can easily proliferate [30,31].

In addition,the increase of Candidal count is due to the fact that denture insertion causes changes in the ecology of the oral cavity and provides a saprophytic environment, which encourages *Candida albicans* growth. Also, the bacterial denture plaque that rapidly forms on the denture-fitting surface promotes *Candida albicans* colonization. Moreover, the inevitable presence of porosities and roughness in the fitting surface of the dentures favor the mechanical retention of denture plaque to which *C.albicans* adhere, with subsequent colonization [32].

It was shown with Molloplast- B-groups generated the most suitable growth environment for *Candida albicans*. The difference in the degree of *Candida albicans* colonization between three groups was most probably due to the different physical properties of the materials, which determine the pattern of *Candida albicans* adherence and hence its density of colonization.

In fact, prolonged exposure of the silicone elastomers to such media may have more implications in vivo regarding the functional lifespan of silicones since contacting host surfaces would be more heavily colonized with initial microorganisms, when opportunist pathogens such as *Candida albicans* form of colonization could be due to the variations in surface roughness [33]. The least *Candida* count in acrylic based soft liner was attributed physical property of the material which maintains its viscoelastic properties for long periods of time without being adversely affected [34].

This provides long term shock-absorbing cushion, reducing trauma to the underlining supporting tissues and consequently reduce the candida colonization and can account for this finding.

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