

EFFECTIVENESS OF NYSTATIN INCORPORATED IN TISSUE CONDITIONERS IN THE TREATMENT OF DENTURE INDUCED STOMATITIS

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ABSTRACT

Background and Objectives: Denture induced stomatitis (DIS) is quite common (up to 65%) among complete denture (CD) wearers and is generally associated with candida albicans (CA). The management of candida associated DIS in elderly is complex due to poor compliance on the part of patient. In this in vivo study, Nystatin was incorporated into tissue conditioner (TC) for the treatment of denture induced stomatitis and investigated the effectiveness of this combination against candida albicans.

Methods: It was an interventional, quasi experimental study in which tissue conditioner with Nystatin (500,000 units) and without Nystatin was applied on the fitting surface of upper denture. Effect of this medication against candida albicans was observed. Thirty six (36) patients were selected by purposive non-probability sampling. Upper complete denture was divided into right (Rt) and left (Lt) sides for medication. Right side was the control side i.e., tissue conditioner alone was applied while left side was interventional side i.e., tissue conditioner incorporated with Nystatin (500,000 units) was applied. Samples were taken from the palatal mucosa of both sides before the application of the tissue conditioner and 3 days after medication; candida colonies were detected and counted.

Results: The reduction in the number of colonies on both sides was significant ($p < .05$) but it was highly marked on the interventional side than on the control side.

Conclusion: Nystatin (500,000 units) when combined with tissue conditioner was effective against candida albicans and can be an effective treatment in non-compliant DIS patients.

Keywords: Candida albicans, Antifungal, Denture stomatitis, Nystatin, Tissue conditioners, Denture wearers.

INTRODUCTION

The pathological reaction of the denture – bearing mucosa caused by trauma from ill – fitting dentures is called denture stomatitis. If the yeast Candida is involved, the term denture stomatitis is used with the prefix Candida – associated.¹⁻³

The prevalence of denture stomatitis is about 65% among complete denture – wearers. The diagnosis of Candida – associated denture stomatitis is confirmed by the isolation of Candida specie in higher numbers from the lesion. (> 50 Colonies).^{1,4}

Candida albicans is asexual diploid fungus. It reproduces by budding and produces pseudohyphae both in culture and in tissues. It may be found in yeast form (blastospore) or mycelial form (pseudohyphae). Yeast form is found in denture wearers with normal mucosa while mycelial form is seen in denture stomatitis patients. Like other yeasts, Candida albicans is gram positive.⁵

The management of Candida associated denture

stomatitis is complex due to its multi-factorial etiology. Current treatment includes following options:

- Meticulous oral and denture hygiene.
- Removal of dentures at night or prolonged rest periods.
- Antifungal therapy.
- Correction of minor denture faults.
- Tissue conditioners application on the fitting surface of the denture.^{1,6}

Dentists have different opinions for the treatment of Candida – associated denture stomatitis.^{1,7} Some of them recommend that the denture should be worn less regularly or left out altogether for a time, but this option is inconvenient and understandably unpopular with patients.⁶ An alternative option is to apply a soft polymeric gel (a conditioner) on the fitting surface of the denture. This conditioning gel relieves the trauma caused by the denture as it is able to flow and adapt as the underlying tissues heal. This soft gel which is cal-

led tissue conditioner can be discontinued once the infection has subsided.⁸

Tissue conditioners are resilient materials which are used to treat inflamed, distorted tissues and to record functional impressions. They consist of acrylic resins which form a gel without undergoing cross-linking reactions. This gel acts as a viscoelastic medium which flows under a steady load. It also acts like a cushion under the dentures. This property of tissue conditioners reduces the stresses borne by underlying unhealthy tissues. The proprietary brands of tissue conditioners are many but generally they consist of polymer and co-polymers with polyethyl methacrylate as the main component and a mixture of ethanol and ester plasticizer.⁹

The implementation of topical antifungal therapy is difficult in geriatric patients due to multiple factors like cognitive impairment, reduced motor activity and memory loss.^{1,6} In addition to this maintenance of an effective topical antifungal dose in the oral cavity is difficult. These agents do not adhere and remain in contact with oral mucosa due to regular and constant wash out by the salivary flow.¹⁰ To overcome this problem antifungal agents can be incorporated in tissue conditioners to investigate their effectiveness against *Candida albicans*.¹¹ This concept of combining tissue conditioners with antifungal agents is not new and a number of studies have been reported in the literature. Different combinations and concentrations of tissue conditioners and antifungal agents have been tested. But most of the studies are in vitro and very less has been carried out in vivo.¹²⁻¹⁸

Thomas and Nutt did an in vitro analysis of the antifungal effect of viscogel alone and viscogel combined with Nystatin and Amphotericin B and found that viscogel and Nystatin combination was effective in the treatment of denture stomatitis.¹³

Schneid carried out an in vitro analysis to investigate the sustained - release delivery system for the treatment of denture stomatitis with four different antifungal agents (chlorhexidine, clotrimazole, fluconazole, and nystatin) in combination with a tissue conditioner (Lynal) at zero (control), low, medium, and high concentrations. It was concluded that all drugs showed release from the tissue conditioner matrix and inhibited the growth of *C. albicans* that was either total or dose - related.¹⁹

More recently different herbal oils, photo-catalysts and magnesium oxide has been mixed with tissue conditioners as antifungal agents for the inhibition of growth of *Candida albicans*.²⁰⁻²²

Keeping in view the usefulness of combination of antifungal agents and tissue conditioners the present in vivo study was conducted. The objective of this study was to compare the number of candida colonies before and after three days of one application of tissue conditioners with and without Nystatin on two halves

of the palatal mucosa in the same patient.

It was hypothesized that the mean number of colonies will not be the same as the mean number of colonies before and after application of proposed medication.

METHODS

Setting: Department of Prosthodontics, FMH College of Dentistry, Lahore and Microbiology Lab, Fatima Memorial Hospital, Lahore.

Duration of Study: 6 months.

Sample Size: Samples were taken depending on the previous record of the patients visiting the hospital with denture stomatitis. 36 patients were included who fulfilled the inclusion criteria.

Sampling Technique: Purposive, non-probability sampling.

Sample Selection

Inclusion Criteria: Complete denture wearers of all age groups with history of denture stomatitis.

Exclusion Criteria: Patients taking topical or systemic antifungal drugs for the treatment of denture stomatitis and medically compromised patients.

Study Design: It was an interventional, quasi experimental study.

Data Collection

Patients with type II and type III denture induced stomatitis (Fig. 1, 2) were selected by purposive non-probability sampling according to inclusion and exclusion criteria of the study.

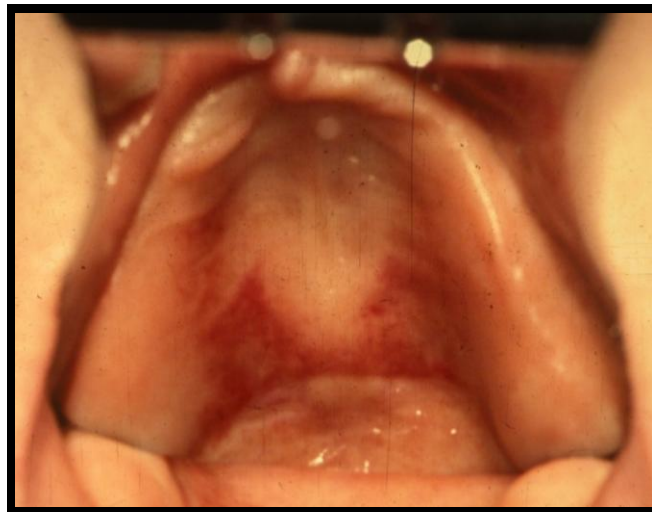


Fig. 1: Type II Denture induced stomatitis.

All the records of the patients were taken i.e., name, age, sex, address, and contact number and since how long the patient was wearing the complete denture. The sampling procedure and tissue conditioners application was described to the patient. Patients con-

sent and approval was obtained for their participation in the study.

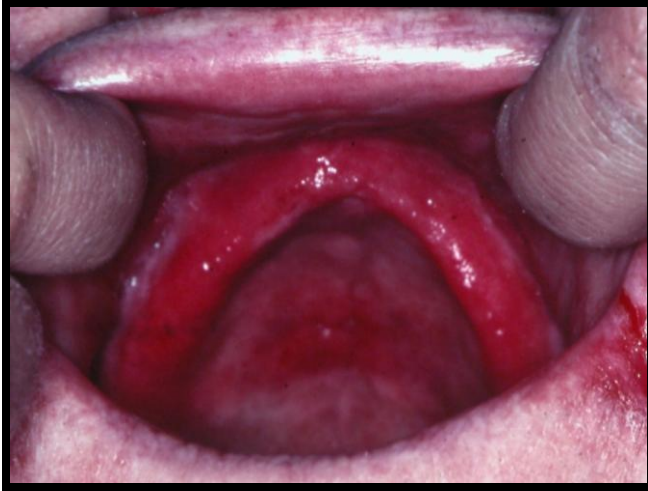


Fig. 2: Type III Denture induced stomatitis.



Fig. 3: Samples being taken from Rt. Sid.

After removal of the upper denture, a palatal scraping with the help of sterile cotton swab was obtained from the palatal mucosa of the patient from right and left sides (Fig. 3, 4) and these were collected into the sterilized saline solution in a "biju bottle". These biju bottles containing palatal scraping / swabs were sent to the microbiology laboratory for the confirmation and count of *Candida* colonies.

The palatal surface of the upper denture was roughened and cleaned for the application of the medication and was divided into right and left halves. On the right half, which acted as the control side, tissue conditioner alone was applied and on the left half that acted as interventional side tissue conditioner plus Nystatin 500,000 units was applied. To maintain the equal thickness of the medication on the both sides, wax stops were placed on the three sites on the fitting

surface of the denture i.e., two stops on palatal slopes on the right and left sides and one in the anterior region of the palate on the midline (Fig. 5). The patient was advised to avoid extreme temperature foods to prevent the changes in wax stops during three days application.



Fig. 4: Sample being taken from Lt. Side.



Fig 5: Three wax stops were placed.

The patient was then advised to return after 3 days. On the next appointment, the upper denture was again removed and samples were taken from the right and left halves of the palatal mucosa and sent to the microbiology laboratory for *Candida* colonies evaluation as before. These samples were taken from the pala-

tal slopes only avoiding any effect of the antifungal agent on the control side.

In the laboratory Sabouraud's dextrose agar plates (Laboratorios Britania S.A. Los Patos, Argentina.) were prepared. Twenty – five (25) microlitre saline solution was taken from the biju bottles and spreaded on to the prepared plate of dextrose agar. For this purpose, a Pasteur spreader was red heated over a flame to ensure sterilization. It was cooled in the air. The spreader was dipped in saline solution. The specimen was inoculated on the culture plate. For the sake of standardization, same size of Pasteur spreader was used for all cases. After inoculation, the culture plate was covered with sterile covers.

The plates were transferred to incubator. They were left there for 48 hours at 37°C in aerobic condition. This allowed the growth of candida if present.

After 48 hours, the culture plates were observed for CFUs. The CFUs of candida were identified by their morphologic characteristics and specific odor. The CFUs in the culture plate were counted and noted in patients' data collection form.

Data Analysis

SPSS software 17 was used to analyze the data. Statistical analysis was performed by using the students' t-test to compare the difference in the reduction of colonies on the control side and on the interventional side after medication. Repeated t-test was also used to compare the colonies within the control and interventional side and colonies count was also compared in between the both sides before medication.

Statistical tests were paired samples and at the 5% significance level.

RESULTS

Initially 36 patients were selected. One patient did not return after the first visit. The samples of three patients were contaminated with bacteria and Klebsiella and were not included in the study results.

The results of the study show that a total of 32 patients completed the treatment regimen and follow up according to the protocol. The mean age of the patients was 65 years while denture – induced stomatitis was more prevalent in males than females. Tests were carried out for the gender distribution of the subjects and a male predilection was found according to this study.

There was reduction in the number of colonies on the both sides of the palatal mucosa after medication and the results were statistically significant ($P < .05$; T-Test). But the reduction in the number of colonies was greater on the interventional side than on the control side (Table 2). The distribution of the Candida colonies on the control (right) side alone was also evaluated and it showed significant reduction. ($P < .05$; T-Test).

The distribution of Candida colonies on the Inter-

Table 1: Gender Distribution of the Subjects ($n = 32$).

Gender	N (%)
Male	21 (65.5)
Female	11 (34.4)
Total	32 (100)

Table 2: Reduction of Candida Colonies on the Control (Right) Side and Interventional (Left) Side after Medication ($n = 32$).

Sides of the Palatal Mucosa	Mean \pm SD
Control (Right)	481.8 \pm 159.5
Interventional (Left)	330.5 \pm 127.1

$P < .05$

Table 3: Distribution of Candida Colonies on the Control (Right) Side ($n = 32$).

Tissue Conditioner	Mean \pm SD
Before application	691.6 \pm 229.2
After application	481.8 \pm 159.5

$P < .05$

Table 4: Distribution of Candida Colonies on the Interventional (Left) Side ($n = 32$).

Tissue Conditioner with Nystatin	Mean \pm SD
Before application	704.9 \pm 243.9
After application	330.5 \pm 127.1

$P < .05$

Table 5: Distribution of Candida Colonies on the Control (Right) Side and Interventional (Left) Side before Medication ($n = 32$).

Sides of the Palatal Mucosa	Mean \pm SD
Control (Right)	691.6 \pm 229.2
Interventional (Left)	704.9 \pm 243.9

$P > .05$

ventional (left) side alone was also found to be statistically significant ($P < .05$; T-Test) but reduction was greater on this side of the palatal mucosa than on control side (Table 3 and 4). The distribution of the Candida colonies on both sides before medication was also evaluated and compared between both groups (Table 5) but it was not a statistically significant result ($P > 0.82$; T-Test).

DISCUSSION

The results of the study clearly reveal that if antifungal agents are incorporated into the tissue conditioner then efficacy of the tissue conditioner is markedly increased in eliminating the candida colonies from the palatal mucosa. This helps in treating candida associated denture stomatitis.

(Table 2) reveals reduction of candida colonies on the both sides of the palatal mucosa but it is more marked on the interventional side than on the control side. This result for the interventional side is in comparison with many *in vitro* studies.^{12-14,23-27}

(Table 4) show that incorporation of Nystatin into the tissue conditioners markedly increases the efficacy of tissue conditioner against candida albicans and hence quick results can be achieved.

The Incorporation of antifungal agents into tissue conditioners (Table 2 and 4) is a promising method of drug delivery to overcome compliance problem in geriatric denture patients. The major advantages of this incorporation are:

- i) Avoidance of side effects of using local antifungals.
- ii) Patient compliance is not required.
- iii) Simultaneous treatment of inflamed tissues and candidal infection.
- iv) Application frequency is reduced.
- v) Cost effective as only a fraction of the antifungal agent is used compared to conventional topical therapy.^{7,11,26}

Results for the control side (Table 3) of this study are not in agreement with some of the studies^{11,13,23} which state that tissue conditioner alone is not effective in inhibiting candida growth. Thomas and Nutt¹³ in their *in vitro* study concluded that tissue conditioner visco-gel alone was ineffective and would therefore only be used with nystatin in the treatment of a denture stomatitis. Falah-Tafti et al²³ also reported that tissue conditioner alone is not effective rather has a supportive role for the growth of candida albicans. On contrary to this Marin Zuluaga⁷ and Iqbal et al²⁷ found tissue conditioner effective against candida albicans.

The reason for inhibition of candida colonies by the tissue conditioners in the present study could be that only initial three day result has been presented. As the tissue conditioner acts as cushion and relieves trauma to the tissues, healing starts and infection subsides initially.^{1,7,9} When tissue conditioner becomes older and monomer leaches out, it becomes porous and adherence of candida albicans becomes evident as reported by the above studies.

The effect of Nystatin incorporation into the tissue conditioner is vital in the dimensional stability of tissue conditioner, like how much antifungal should be incorporated to get effective results and to avoid any dimensional changes of the tissue conditioner. Schneid¹⁹ reported that combination of antifungals and

tissue conditioners did not alter the mechanical properties of the tissue conditioners. This found the basis of the study and method of investigation. Recently studies have been carried out to see the effect of mixing different medications with tissue conditioners.^{16,20,28} Srivatstava²⁰ and colleagues in their study investigated the antifungal effect and mechanical properties of a tissue conditioner by adding organum oil. Organum oil at varying concentrations was incorporated into a poly (methyl methacrylate) based tissue conditioner, and its antifungal activity against *Candida albicans* was evaluated. The adherence of *Candida albicans*, surface roughness, tensile strength, and bond strength of the tissue conditioner with an optimized concentration of this oil were evaluated. They concluded that organum oil can be added to tissue conditioner to reduce the adherence of *Candida albicans* without any effect on its roughness and bond strength to heat-polymerized acrylic resin. Another study by Urban et al¹⁶ resembling the current study investigated the combination of Nystatin with a tissue conditioner for ultimate tensile strength. The ultimate tensile strength of a tissue conditioner was evaluated without nystatin incorporation (GI-control group) and the same tissue conditioner modified by the addition of nystatin in two concentrations: GII-500,000 International Units (U) and GIII-1,000,000 (U). It was suggested that the addition of Nystatin concentrations below 1,000,000 U had no effect on its ultimate tensile strength.

On the basis of above studies 500,000 (U) of Nystatin were added into the tissue conditioner in present study to avoid any dimensional instability. Fungicidal effects of tissue conditioner alone (Table 3) and its incorporation with Nystatin (Table 4) were seen clinically. Although *in vitro* studies on the release of antifungal agents from tissue conditioners have been done,^{7,10,14,26,29-31} but only a few *in vivo* research^{17,39} on the topic could be found. Skupian et al⁶ carried out a systematic review on the prevention and treatment of *Candida* colonization on denture liners. It was recommended in that review that the incorporation of Nystatin in tissue conditioners or denture liners is able to treat or prevent oral candidiasis. However, it was pointed out that most of the studies were *in vitro* and reliable evidence is insufficient to truly provide recommendations regarding whether the addition of antifungal agents is worthwhile. It was advocated that well-designed randomized controlled trials are needed to provide answers to this. This finding and recommendation by Skupian et al⁶ made the basis to do a clinical study. Authors found only two *in vivo* studies^{17,39} to check the amount of candida colonies after the treatment. Geerts et al¹⁷ in 2008 conducted an *in vivo* pilot study to investigate the effect of an antifungal denture liner on the saliva yeast count in patients with denture stomatitis. They determined *in vivo* the salivary yeast count after an antifungal agent was released from a tis-

sue conditioner. Forty edentulous patients with denture stomatitis caused by *Candida albicans* were included in this study and they were divided in two groups. In Group 1 (control) tissue conditioner was used only. In Group 2 (case) a tissue conditioner and Nystatin (500,000 U) combination was used. Both groups were asked to perform oral rinses before treatment and every second day during treatment for a period of 14 days. Total yeast counts of the oral rinses were performed and the averages and standard deviations for both groups' calculated and logarithm – transformed data of the counts over time were statistically analyzed using the Wilcoxon signed – rank test. In the control group the average oral rinse yeast count was decreased up to day 4. After that period the number of colonies increased till the end of the test time. It was concluded that a Nystatin – containing short-term soft tissue conditioner can significantly decrease the salivary yeast count of patients with denture stomatitis compared with a conditioner without Nystatin. In this study oral rinses were performed by both the groups before and after the treatment for candida colony counts. But in present study the effect was seen intra-orally. It is evident from the methodology of the current study that it is copying the exact clinical situation and oral environment. Swabs for microbiology testing were taken from the patients' mouth making the results more reliable. Almost same methodology was used by Catalan et al³⁹ in their in vitro and in vivo study. Some effect of the medications mixing on the control and interventional palatal side was inevitable in current study because both test and control samples were taken from the same patient. An effort was made to make the samples from the ridge crest and palatal slopes avoiding the center of the palate.

Findings of the Geerts et al¹⁷ and FalahTafti et al¹⁰ suggest that *Candida* colonization reduction takes place at its peak in the initial 3 – 4 days. After that this effect is lost as the days are increased. Results of the present in vivo study exactly confirm these findings. So it can be safely recommended that tissue conditioners containing Nystatin used under the denture should be replaced every three to four days.

With tissue conditioners as the drug release mechanism, the drug is administered once and need not be added to daily, as recommended.^{18,19,32} This study showed that the antifungal agents released caused marked reduction in the number of colonies on the fourth day (Table 4) as compared to premedication number of colonies (Table 5).

(Table 1) demonstrates the sex predisposition of DIS patients. It reveals that males are more affected by the DIS than females. Association of gender with DIS is frequently reported.^{3,33-38} Shaikh³ found that the incidence of generalized DIS was more in male patients. Kulak and Arikan³⁴ also reported higher incidence of DIS in males than females. Davenport³⁷ found

higher sex incidence of DIS in males to females in the ratio 1:4. These studies^{3,34,37} support the present study. However, Alkumru and Beydemir³⁵ in their study concluded that candidal carrier rate was greater in females than males. This was supported by Webb et al.³⁶ Ghani⁴ also found higher DIS incidence in females. The reasons for the male predilection in the present study can be small sample size of 32 patients only and not selecting the patients randomly. A separate study to investigate sex predilection in new DIS patients can be conducted.

RECOMMENDATIONS

The findings of the present study suggest that tissue conditioners are good vehicles for the release of anti-fungal agents and do not seem to hinder drug release. Long-term effects of this treatment modality are not considered in this study, as there is evidence of recurrences after the treatment has been discontinued.^{10,17} This effect can be further investigated in an in vivo study in future.

It is **concluded** that within the limitations of this study the following observations can be made: Nystatin when combined with tissue conditioner is effective against *Candida albicans*.

Tissue conditioner alone can also decrease the number of candida colonies on the palatal mucosa of DIS patient in initial phase of treatment.

Tissue conditioners containing Nystatin used under the denture should be replaced every three to four days.

500,000 units of Nystatin mixed with GC Soft Liner can be recommended safely in DIS patients where compliance of the patient cannot be relied upon and an effective and early reduction in the DIS is desired.

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CONTRIBUTION OF AUTHORS

Z. I. as main researcher. M. R. K. as main researcher. Z. M. as reviewer and proof reading of the research. N. Y. as supervisor of the study.

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