Abstract: This study investigated the antifungal effects of low-molecular-weight chitosan solution on Candida albicans in denture stomatitis in comparison with nystatin suspension. This randomized, single-blind clinical trial included 40 patients diagnosed with denture stomatitis. Patients were divided into two groups, wherein one was treated with chitosan and the other with nystatin for 2 weeks. Changes in the erythematous area were recorded during and after treatment. A palatal smear was obtained for each patient before and after treatment to determine the number of blastospores and mycelia of C. albicans. The results were compared using the Mann-Whitney U test, revealing that the chitosan solution significantly decreased the erythematous surface area, burning sensation, time required for clinical improvement, and number of blastospores and mycelia. The antifungal efficacy of chitosan along with its inherent biocompatibility makes it a promising candidate for use as an antifungal mouthwash.

Keywords: Candida albicans; chitosan; oral infection; denture stomatitis.

Introduction

Candida albicans is an opportunistic fungus that causes an infection known as candidiasis or candidosis. Other members of the Candida species are C. guilliermondii, C. parapsilosis, C. krusei, and C. tropicalis; these are found in the oral cavity but rarely cause disease (1-3). C. albicans exists in a yeast form that is relatively safe and a hyphae form that is usually associated with invasion of host tissue (2-4). At least three factors determine the clinical existence of a fungal infection: the immune status of the host (1,2,5), the status of the oral cavity (1,2), and the fungal species involved (1,3). Candida infections have various clinical forms based on interactions between the host and the organism; these range from superficial mild involvement of the mucosa to a disseminated fatal disease in immune-compromised patients (1,2).

The term denture-induced stomatitis is used for chronic mucosal inflammatory changes associated with denture usage characterized by palate and alveolar ridge erythema. C. albicans is an etiological factor for denture stomatitis. Bacteria, mechanical microtrauma, and poor denture hygiene are predisposing factors for this disease (2,6). The medical treatment of Candida infection depends on the severity and location of the lesion as well as patient’s immune status (6-9). Treatment of denture stomatitis includes cleaning the dentures, avoiding overnight use of dentures, relining or replacement of prosthesis, and use of topical or systemic antifungal medications. The use of all methods of treatment is more
effective than just one (7,10).

The most common antifungal agents are polyenes and azoles. Polyene agents such as nystatin and amphotericin are remedial treatments for primary oral candidosis (1,9,11). Nystatin binds to ergosterol within the cell membrane of *Candida* and creates pores in the fungal cell membrane, resulting in leakage of fungal cell contents and potassium ions, which results in cell death (7,12,13). Polyenes are not absorbed from the gastrointestinal tract, and drug-induced resistance is extremely rare (9,12). However, topical use of nystatin produces an unpleasant taste in the mouth and, if swallowed, can cause symptoms such as nausea, vomiting, and diarrhea. Azoles may be systemically used for primary deep candidosis such as denture stomatitis and chronic hyperplastic candidosis; however, one harmful effect of the topical application of azoles is their absorption through the gastrointestinal tract (1-3,9).

Chitosan is a biodegradable and biocompatible natural polymer derived from the outer shell of crustaceans (5,14,15). The antibacterial and antifungal properties of chitosan make it appropriate for use in various medical applications (16-19). It is biologically safe and has been recommended as an oral mucosal bioadhesive (20). Chitosan oligomers inhibit the growth of fungal cells through diffusion into hyphae and interfering with enzymes responsible for their growth (20-22). The present study evaluated the antifungal effects of low-molecular-weight chitosan solution on *C. albicans* in denture stomatitis. The antifungal properties of oligochitosan were also compared with those of nystatin as a commercially available antifungal oral suspension.

**Materials and Methods**

In this clinical trial, chitosan with a low molecular weight of 5000 D was purchased from Hangzhou New Asia International Co. (Hangzhou, China), and nystatin oral drops of 100,000 U/mL were purchased from Jaber Ebne Hayyan Pharmaceutical Co. (Tehran, Iran). Chitosan solution (1 wt%, pH = 5) was prepared by dissolving it in distilled water.

Patients referred to dental clinics were selected for participation. A participant should have type 2 denture stomatitis, should have worn dentures for at least 6 months, and should not have a systemic disease that compromises the immune system such as diabetes. Participants should not have any disorder that causes muscle weakness, such as Parkinson’s disease, or that limits cognitive functioning, such as dementia or psychosis (23). Patients should not have a history of use of antifungals, antibiotics, or drugs that reduce saliva production (23). Patients were informed of the purpose and course of the study, and they signed written consent forms before participation in the study. Demographic data was collected in a written questionnaire. A total of 40 patients participated in the study; there were 12 women and 8 men in the chitosan group and 17 women and 3 men in the nystatin group. The average patient age was 54.5 (±12.5) in the chitosan group and 59.7 (±20.5) in the nystatin group; no significant differences were noted in patient age between both groups (*P* = 0.322). Patients were randomly selected, and statistically, no significant gender differences were noted between the groups (*P* = 0.077). The duration of denture usage in the chitosan and nystatin groups was 8.3 (±4.8) and 17.2 (±14.8) years, respectively, with no significant differences (*P* = 0.202). Patients had been diagnosed with denture stomatitis (Newton type II) of the palate. All steps were approved by the Ethics Committee of Kerman University of Medical Sciences (AR.KMU.REC.1394.118).

Patients were randomly divided into two groups. One group was treated with chitosan solution and the other with nystatin. Patients were trained to use 2 mL of mouthwash for 2 min, 4 times a day. The method of application was the same for both groups. Patients were also taught methods of dental hygiene, including removal of dentures at night, immersion of dentures in hypochlorite solution (1 wt%), and cleansing using a toothbrush during the day (24).

For both groups, oral examination to evaluate palatal lesions was performed at the dental unit on days 1, 7, and 14. Photographs were recorded at every visit. Moreover, as part of the clinical assessment, the size and location of the erythematous area were measured, recorded, and evaluated on days 1, 7, and 14. The level of pain was measured using a visual analog scale (VAS).

Palatal smears of the infected areas were collected to obtain epithelial cells for microbiological investigation. Four smears were collected using sterile cotton swabs before and after treatment (total: eight smears). A clean glass slide that had been passed twice over the blue flame of a burner was used for each sample. The samples were transferred to the slides by rotation to prevent any destruction of epithelial cells. The slides were air dried, and two of these received application of a few drops of isopropyl alcohol (90%). The remaining slides were fixed using a fixator spray, Padtanteb Co. (Tehran, Iran). These were stored in a special container to prevent contamination with dust and harmful insects.

Gram staining was used, and the slides were observed using YS100 light microscope (Nikon, Tokyo, Japan) under 100× magnification by using an immersion oil.
The number of yeast cells with or without buds, in a vegetative form, and the presence of mycelium or pseudomycelium were reported.

**Statistical analysis**

Data were analyzed using the Shapiro-Wilk test. Either the parametric t-test or the nonparametric Mann-Whitney test was used to compare the results. Statistical analysis was performed using SPSS for Windows (Version 16.0, SPSS Inc., Chicago, IL, USA) at a significance level of 0.05.

**Results**

Table 1 summarizes the average erythematous surface area recorded at treatment initiation (with no significant differences between the groups in terms of size of the initial area, \( P > 0.05 \)), at 1 week after treatment onset and at the end of treatment. Both treatment modalities significantly decreased the erythematous surface area \( (P < 0.001) \). Moreover, erythematous surface measurements were compared pairwise between groups for different posttreatment durations (Table 2). Although the nystatin group revealed a higher percent decrease in size of the erythematous surface area (Table 1), no significant differences were noted between the nystatin and chitosan groups when compared pairwise for different durations (Table 2, \( P > 0.05 \) for all durations).

Figure 1 indicates the significant differences in the duration of clinical improvement between the two groups. Four patients from the chitosan group (20%) showed prominent improvements in the initial 7-14 days, and 16 patients (80%) showed clinical improvement in >14 days. In the nystatin group, 11 patients (55%) showed significant improvement during the initial 7-14 days of treatment, and the recovery period for the nystatin group was shorter than that for the chitosan group \( (P = 0.024) \).

Furthermore, the pain and burning sensation experienced by patients were measured for both groups by using VAS. None of the nystatin-treated patients reported pain or discomfort, but two patients from the chitosan group reported a burning sensation that had ceased on the 5th day after treatment.

The average numbers of mycelia and blastospores collected from specimens in both groups before and after treatment are summarized in Table 3. Excluding
the baseline effect (before treatment), the decrease in the number of mycelia and blastospores in the nystatin group was greater than that in the chitosan group. Moreover, the decrease in the average number of mycelia and blastospores within each group was statistically significant ($P < 0.05$).

To consider the baseline effect, the average difference in the number of mycelia and blastospores before and after treatment in both groups was evaluated (Table 4). The mean difference in mycelia was 0.65 in the chitosan group and 3.15 in the nystatin group, and the mean differences in the number of blastospores in the chitosan and nystatin groups were 3.15 and 7.40, respectively. No significant difference was noted between both groups ($P > 0.05$).

### Table 4 Average differences in numbers of mycelia and blastospores before and after treatment with either chitosan or nystatin

<table>
<thead>
<tr>
<th></th>
<th>Chitosan</th>
<th>Nystatin</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycelium</td>
<td>0.65 (±1.69)</td>
<td>2.3 (±5.6)</td>
<td>0.157</td>
</tr>
<tr>
<td>Blastospore</td>
<td>3.15 (±5.58)</td>
<td>7.40 (±12.48)</td>
<td>0.089</td>
</tr>
</tbody>
</table>

Numbers in parentheses represent standard deviation.

Discussion

The present study was designed to evaluate the clinical and microbiological antifungal effects of a solution of 1% chitosan with a molecular mass of 5000 Da for the treatment of denture-induced stomatitis and to compare the results with those of nystatin. No side effects were reported during the study in either group. Chitosan significantly decreased the erythematous surface area, burning sensation, duration for clinical improvement, and the number of blastospores and mycelia (Table 3). Moreover, the decrease in the average number of mycelia and blastospores within each group was statistically significant ($P < 0.05$).

Chitosan lead to its possible use in food, cosmetic, pharmaceutical, and biotechnology industries (22,32,36,37). The tendency to use natural materials has increased recently. Chitosan is an amino polysaccharide with antifungal effects derived from chitin by alkaline decatylation. Chitin is the second most abundant polymer in nature after cellulose (25-30) and has a safe biological profile for patients. It exhibits a therapeutic effect on diabetes along with cholesterol-lowering, wound-healing, antitumor, antifungal, and antimicrobial effects (31,32). Chitosan is a cationic polysaccharide with a positive charge that can react with the negatively charged cell walls of microorganisms and can damage the targeted cells, causing loss of cell membrane. It prevents the development of fungal diseases by preventing formation and maturation of biofilm and preventing the attachment of *C. albicans* to human mucosal cells (33-35). The positive electrical charge of chitosan that targets the negative charge of the fungal membrane, increases the permeability of the membrane, and eventually leads to leakage of cells and their death. In addition, chitosan acts as a chelating agent and limits access of the fungi to nutritional components present in the environment. Another mechanism is the ability of chitosan to penetrate the cell membrane of fungi and bond to DNA, which inhibits mRNA transcription and inhibits protein and enzyme synthesis in the cells. Chitosan can also bond to metals, which are critical toxic products involved in the growth of microorganisms (22,26). These characteristics of chitosan lead to its possible use in food, cosmetic, pharmaceutical, and biotechnology industries (22,32,36,37).

It has been reported that a decrease in the molecular weight of chitosan is associated with an increase in its antifungal efficacy (24,31,35,38-42). Low-molecular-weight chitosan is water soluble, whereas high-molecular-weight chitosans are soluble in acidic water solutions. Water is the most common base carrier solution. It is nontoxic, nonirritant, tasteless, and inexpensive. It should be noted that the soluble form is the oldest pharmaceutical formulation and has numerous advantages over other pharmaceutical formulations; these include high absorption of pharmaceutical products as well as speed and homogeneity of the solution, which makes use of the medicine possible without shaking beforehand. The use of a water-based solution is the advantage of this study.
and, to the best of our knowledge, no similar clinical studies have been reported in the literature.

One criterion for the evaluation of clinical efficacy was healing time, which was defined as 7-14 days or >14 days. Significant differences were observed between the groups, and nystatin appeared to have a shorter improvement duration compared with chitosan (Fig. 1). However, small macular lesions (1 × 1 mm and 0.5 × 0.5 mm) were observed in the oral mucosal surfaces of patients treated with chitosan, which was considered as an imperfect total improvement in these patients. These small lesions were clinically negligible and did not appear to reflect deficiency in treatment.

The present study introduces a new and less aggressive treatment modality for denture stomatitis by using low-molecular-weight chitosan solution. The results indicate that the chitosan solution provides an alternative medication, which is clinically comparable with nystatin in treatment of stomatitis. The results may lead into the production of a promising biocompatible and safe antifungal mouthwash.

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Conflict of interest
None declared.

References


