Use of chitosan mouth-wash in radio-chemotherapy induced oral mucositis: A case-control study

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Abstract

Background: Oral mucositis (OM) is a common dose-limiting and potentially serious complication of radio-chemotherapy which leads to atrophy and ulceration of the mucosa and an increased risk of infection in immunosuppressed patients. Chitosan is a cationic polysaccharide commercially extracted from the shells of shrimps and crab. Chitosan has beneficial biological and antimicrobial properties with high potential for wound healing. It is biocompatible, biodegradable, non-toxic, anti-microbial, anti-fungal, and a hydrating mucoadhesive agent.

Aims: To evaluate the efficacy and safety of chitosan mouthwash in the treatment of OM and if it could be used as a routine protocol for the treatment of OM.

Subjects and Methods: The study was conducted on 20 patients (10 cases and 10 controls) diagnosed with OM following radiochemotherapy. The study group received 1% freshly prepared chitosan mouthwash, and the control group received chlorhexidine mouthwash. Signs and symptoms were assessed using numerical rating scale (NRS), the WHO and OM Assessment Scale at 0, 10 and 20 days. Statistical analysis: Descriptive statistics, Friedman’s and Mann–Whitney U test were performed.

Results: Statistically significant differences were seen in the NRS (P = 0), ulceration (P = 0.007), and WHO (P = 0.029) scores.

Conclusions: Chitosan was found to be a more effective than chlorhexidine in the alleviation of symptoms of OM.

Introduction

Cancer is one of the leading causes of morbidity and mortality across the world. The number of people with cancer in 2030 may rise to 22.2 million, or 0.3% of the global population, from 12.7 million in 2008.[1]

Radio- and chemotherapy used in the treatment of cancer frequently causes oral mucositis (OM) characterized by painful mucosal ulceration accompanied by xerostomia and dysgeusia. It is characterized by atrophy of squamous epithelial tissue of oral mucosa, vascular damage and an inflammatory infiltrate concentrated at the basement region. Epithelial atrophy is usually followed by ulceration.[2] Pain, odynodysphagia, dysgeusia, malnutrition, dehydration are significant co-morbidities associated with OM and there is an increased risk for oral or systemic infections in immunocompromised patients.[3]

The risk of OM changes with the type of treatment modality used, with the least risk associated with chemotherapeutics such as gemcitabine and an increased risk with more aggressive agents such as 5-fluorouracil and cisplatin and/or radiation therapy.[4] OM can occur with cumulative radiotherapy doses as low as 1000-2000 cGy with therapy administered at a rate of 200 cGy/day.[5]

Radiochemotherapy induced mucosal injury comprises the following processes: Initiation by the production of reactive oxygen species resulting in DNA damage in basal squamous epithelial cells, with subsequent apoptosis and production of inflammatory cytokines ultimately resulting in epithelial basal cell injury and cell death. Activation of matrix metalloproteinase enzyme (MMPs) leads to the destruction of the collagenous subepithelial matrix, and the cell-membrane lipids are also hydrolyzed. Macrophages are activated which further activates MMPs and produces (tumor necrosis factor-alpha [TNF-α]). TNF-α activates the ceramide and capase pathways leading to tissue damage and also activates MMPs leading to direct tissue injury, ulceration with inflammation and healing.[6-8]
The Consensus Development Panel of the National Institutes of Health (Consensus Statement, 1990) stated that no drugs can prevent mucositis, an opinion that still holds to date. A systematic review of more than 100 studies in cancer patients undergoing radio- and/or chemotherapy suggested great diversity of mucositis management practices, many of which lack proven clinical efficacy. A number of agents including saline solution, sodium bicarbonate solution, topical anesthetics, mucosal coating agents, analgesics such as benzydamine hydrochloride, opioid analgesics, growth factors, cryotherapy, radioprotectors, antimicrobial and antifungal agents, chamomile, glutamine, interleukin-11 have been used in the management of OM. However, none of these treatment strategies has proven to be consistently efficacious in the treatment of OM.

Most of the commercially available mouthwashes and antimicrobials used for the treatment of OM contain alcohol and have an unpleasant taste which worsens the situation. Moreover, the flushing action of saliva rapidly washes away the drug from the mucosa. Also, there is a need for a non-irritative mucoadhesive agent that will form an occlusive film over the mucosa and enhance lubrication.

Chitosan, a copolymer of glucosamine and N-acetyl, is a cationic polysaccharide obtained by alkaline deacetylation of chitin. Chitin is commercially extracted from shrimp and crab shells. It is biocompatible, biodegradable, nontoxic, anti-microbial, anti-fungal and a hydrating mucoadhesive agent. Chitosan with beneficial biological and antimicrobial properties and high potential for wound healing is attractive for wound care.

Hence, a project was taken up to develop an effective occlusive mucoadhesive treatment system for the treatment of OM. With this objective in mind, chitosan based mouthwash was prepared to evaluate its efficacy in the treatment of OM and to assess the safety profile in patients. Furthermore, if found effective, chitosan mouthwash could be commercially produced and used for the treatment of OM.

**Subjects and Methods**

**Patient eligibility**

The study group comprised 20 subjects scheduled to receive radiation therapy at Regional Oncology Institute as out/in patients. Male and non-pregnant female subjects 18-80 years old diagnosed with OM following radiochemotherapy for cancer, willing to be a part of the study and give a signed informed consent were included in the study.

Terminally ill patients and patients who were unable to comply with chitosan mouthwash (as judged by the parent or investigator) were excluded from the study. Patients with the pre-existing oral disease, such as active oral infection, trauma to the oral mucosa or oral ulceration prior to chemotherapy were also excluded. Furthermore, patients with religious constraints for using chitosan-based mouthwash were excluded from the study.

**Study design**

A detailed history of selected participants was obtained, and they were preliminarily examined clinically. The general data, history and clinical findings were recorded in individual proformas designed especially for the study. The patients were divided into two groups - Group A: Study group consisting of 10 patients and Group B: Control group consisting of 10 patients.

Symptom score for OM was considered at baseline using numerical rating scale (NRS) ranging from: 0 (no oral discomfort) to 10 (worst imaginable oral discomfort).

The clinical signs of OM were measured at baseline using the WHO scale and OM Assessment Scale (OMAS). An oral examination was conducted, and atrophic and erosive changes were quantified based on severity and the number of sites involved. OMAS:

An intensity score for erythema ranging from 0 to 2 were used:
- Grade 0 = Normal
- Grade 1 = Not severe
- Grade 2 = Severe

The score for ulcerations were based on area of ulceration ranging from 0 to 3:
- Grade 0 = Normal
- Grade 1 = < 1 cm²
- Grade 2 = between 1 and 3 cm²
- Grade 3 = ≥ 3 cm².

Sixteen different oral sites were evaluated including buccal mucosa (right and left), labial mucosa (upper and lower), lateral aspect of tongue (right and left), dorsum of tongue (right and left), ventral tongue and floor of the mouth (right and left), maxillary gingiva (right and left), soft palate and hard palate. The scores for erythema and ulceration were obtained by summing the respective scores for these 16 sites and the total score for clinical signs were obtained by summing the erythema and ulceration scores.

The WHO mucositis scale was also used for assessment of OM in terms of degree of severity of mucositis affecting oral intake of food. The score ranges from 0 to 4.
- Grade 0 = No changes.
- Grade 1 = Soreness with erythema.
- Grade 2 = Erythema, ulcers, can eat solid foods.
- Grade 3 = Ulcers, liquid diet only.
- Grade 4 = Alimentation not possible.

These findings were then recorded in respective proformas. The subjects were blinded, i.e. the subjects were not aware of the nature of the drug they were receiving (the name of the drug was masked).

Group A: Study group – Consisting of 10 subjects, received 1% chitosan mouthwash. The mouthwash was prepared as a
freshly prepared solution of chitosan in distilled water at the Department of Pharmaceutics and sterilized at the Department of Microbiology. Patients were instructed to use the mouthwash in 1:1 dilution by swishing in the oral cavity for 1 min and spit out, thrice daily after meals for 20 days.

Group B: Control group – Consisted of 10 subjects who received commercially available chlorhexidine mouthwash in 1:1 dilution by swishing in the oral cavity for 1 min and spit out, thrice daily after meals for 20 days.

Patients were asked to report immediately in case they encounter any adverse effects.

The following clinical parameters were assessed during follow-up:
1. Pain: NRS
2. Erythema and ulceration: OMAS, the WHO scale
3. Side effects.

The patients were followed up after 10 and 20 days. Readings for all clinical parameters for each patient from baseline to subsequent visits were recorded. The patients were enquired for side effects if any.

These findings were then recorded in respective proformas. The data collected were then entered into the computer using Microsoft Excel and were analyzed using SPSS software for windows (version 16).

The data were tabulated and subjected to the following statistical analysis.
1. Descriptive statistics
2. Wilcoxon signed rank test

Results

As shown in Table 1, there were no significant differences in gender, age of the patient, site of cancer, total radiation dose, number of chemotherapy cycles or clinical data between the two groups.

Study group (chitosan)

Statistically significant results were seen in the values of all four parameters – NRS (*P* = 0.005), erythema (*P* = 0.004), ulceration (*P* = 0.016) and WHO (*P* = 0.046) scale [Table 2]. There was no incidence of candida infection in any of the patients in the study group. No adverse events were noted in the study group.

Control group (chlorhexidine)

Statistically significant values were seen in NRS (*P* = 0.004) and erythema scores (*P* = 0.007). Ulceration (*P* = 0.317) and WHO (*P* = 1) scores were insignificant [Table 3]. 4 out of 10 patients developed concurrent *Candida* infection.

Comparison of the study and control group

Mann–Whitney *U* test was used to test the significant differences between baseline and second follow-up in both the groups. As shown in Table 4, the difference in the NRS (*P* = 0), ulceration (*P* = 0.007) and WHO (*P* = 0.029) scores were statistically significant. The difference of erythema (*P* = 0.11) scores between the study and control group was not statistically significant.

### Table 1: Characteristic demographic data of the included subjects

<table>
<thead>
<tr>
<th>Characteristic demographic data</th>
<th>Chitosan group (n=10)</th>
<th>Control group (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>35–83 years (mean=61)</td>
<td>40–71 years (mean=59)</td>
</tr>
<tr>
<td>Gender</td>
<td>4 males (40%)</td>
<td>6 males (60%)</td>
</tr>
<tr>
<td>Area of cancer (%)</td>
<td>6 females (60%)</td>
<td>4 females (40%)</td>
</tr>
<tr>
<td>Pharyngeal</td>
<td>5 (50)</td>
<td>4 (40)</td>
</tr>
<tr>
<td>Laryngeal</td>
<td>2 (20)</td>
<td>2 (20)</td>
</tr>
<tr>
<td>Oral mucosa</td>
<td>3 (30)</td>
<td>4 (40)</td>
</tr>
<tr>
<td>Total dose of radiation</td>
<td>2100 cGy in 15 divided fractions over a period of 3-week</td>
<td>2100 cGy in 15 divided fractions over a period of 3-week</td>
</tr>
<tr>
<td>Number of chemotherapy cycles</td>
<td>2-5 cycles</td>
<td>2-5 cycles</td>
</tr>
</tbody>
</table>

### Table 2: Values of all four parameters in study group at baseline and second follow-up

<table>
<thead>
<tr>
<th>Study parameters</th>
<th>Baseline median (IQR)</th>
<th>Second follow-up (median)</th>
<th><em>P</em> value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NRS</td>
<td>5.5 (3.5-7.5)</td>
<td>1 (0-2.25)</td>
<td>0.005</td>
</tr>
<tr>
<td>Erythema</td>
<td>2 (1-2)</td>
<td>0 (0-1)</td>
<td>0.004</td>
</tr>
<tr>
<td>Ulceration</td>
<td>1 (0-2.25)</td>
<td>0</td>
<td>0.016</td>
</tr>
<tr>
<td>WHO scores</td>
<td>3 (2.75-3)</td>
<td>2 (2-3)</td>
<td>0.046</td>
</tr>
</tbody>
</table>

NRS: Numerical rating scale, IQR: Interquartile range

### Table 3: Values of all four parameters in control group at baseline and second follow-up

<table>
<thead>
<tr>
<th>Study parameters</th>
<th>Baseline median (IQR)</th>
<th>Second follow-up (median)</th>
<th><em>P</em> value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NRS</td>
<td>4 (2-6)</td>
<td>3 (1.3-4.25)</td>
<td>0.004</td>
</tr>
<tr>
<td>Erythema</td>
<td>2 (1.75-2)</td>
<td>1 (1-1)</td>
<td>0.007</td>
</tr>
<tr>
<td>Ulceration</td>
<td>1 (0-1.25)</td>
<td>1 (0-1.25)</td>
<td>0.317</td>
</tr>
<tr>
<td>WHO scores</td>
<td>3 (2-3)</td>
<td>2 (2-3)</td>
<td>1.000</td>
</tr>
</tbody>
</table>

NRS: Numerical rating scale, IQR: Interquartile range

### Table 4: Comparison between study and control groups at baseline and second follow-up

<table>
<thead>
<tr>
<th>Study parameters</th>
<th>Baseline median (IQR)</th>
<th>Second follow-up (median)</th>
<th><em>P</em> value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NRS</td>
<td>4.5 (2.5-5.25)</td>
<td>1 (0.5-1)</td>
<td>0.000</td>
</tr>
<tr>
<td>Erythema</td>
<td>1 (1-2)</td>
<td>1 (0.75-1)</td>
<td>0.11</td>
</tr>
<tr>
<td>Ulceration</td>
<td>1 (0-2)</td>
<td>0 (0-0)</td>
<td>0.007</td>
</tr>
<tr>
<td>WHO scores</td>
<td>0 (0-1)</td>
<td>4.5 (3-5)</td>
<td>0.029</td>
</tr>
</tbody>
</table>

NRS: Numerical rating scale, IQR: Interquartile range
**Discussion**

Although radio-chemotherapy is highly effective for the cancer treatment, painful OM and taste disturbances occur frequently, which results in a difficulty in oral intake of food and thus necessitates the need for parenteral or intravenous nutrition. In a study,[14] a review was done to find out the incidence of radiation-induced OM in head and neck cancer patients and the results showed that the incidence rate is over 80%. In our study, mild to moderate OM was found in all patients in 3 weeks of the initiation of radiation therapy, with a cumulative dose of 2100 cGy in both the study and control group patients. The amount of radiation exposure at which ulcers first appeared in the oral mucosa was similar in both treatment groups, i.e., up to 2500 cGy.

Studies have been done on a number of compounds to evaluate their efficacy in the prevention of radiation- or radiochemotherapy-induced OM; however, most of them have failed to show a definitely positive and unswerving protective action.

The four-point mucositis scale was used for the assessment in our study because of its simplicity, because it scores ulcerations and erythema separately, and because it is limited to objective anatomic-pathologic findings and excludes associated evaluation of functional symptoms. In contrast, the WHO Index scores signs (mucosal lesions) and symptoms (alimentation behavior) concomitantly. In addition, pain, the primary symptom of mucositis, i.e., pain, was measured separately in the present trial using NRS pain scale.

The study demonstrated that use of chitosan mouthwashes significantly and effectively reduced the morbidity associated with chemoradiotherapy-induced OM. Chitosan, which is capable of accelerating the healing processes at molecular, cellular, and systemic levels showed good biocompatibility and positive effects on wound healing. The acceleration of the wound healing process, along with bioadhesiveness and hydrating properties made up for a good occlusive dressing, thereby, rapidly healing ulcerations and alleviation of pain.

Chitosan can accelerate the repair of different tissues and facilitates contraction of wounds and regulates secretion of the inflammatory mediators such as interleukin 8, prostaglandin E, interleukin 1, and others. It provides a non-proteinaceous matrix for three-dimensional growth of the tissue and initiates macrophage actions for tumoricidal activity. Cell proliferation is promoted and it aids in histoarchitectural tissue organization. Chitosan is a hemostat, which aids in natural clotting of blood reduces pain by blocking nerve endings,[15] thereby yielding significant NRS scores in our study. Chitosan gradually depolymerizes to release N-acetyl-D-glucosamine, the following which fibroblast proliferation is initiated, thereby aiding in ordered collagen deposition and increases the synthesis of natural hyaluronic acid at the wound site. It helps in rapid healing of wounds and scar prevention.[16] Significant WHO scores were seen owing to the same properties, indirectly aiding patients in eating and drinking.

No adverse events were seen in patients as it is non-toxic, biodegradable and has a very good biocompatibility. In a study, the oral mean lethal dose of chitosan in mice was found to be in excess of 16 g/day/kg of body weight, which is higher than that of sucrose.[12] Due to these properties, it shows good biocompatibility, is non-toxic and also shows positive effects on wound healing.

The significant improvement in erythema scores and no incidence of secondary infections were seen in our study owing to its antimicrobial activities.

Chitosan interacts with the negatively charged membrane of bacteria and alters its surface morphology, thereby increasing the permeability of bacterial cell membrane, leading to leakage of intracellular substances, or reduces the cell membrane functions, precluding nutrient transport.[16]

None of the patients in the chitosan group developed a concurrent fungal infection in comparison to 4 out of 10 patients in the control group owing to its fungistic activity. The anti-fungal activity if Chitosan is through permeabilization of yeast cells and efflux of potassium ions, along with an increased uptake of calcium ions, and inhibition of respiration, fermentation, and viability of the cells.[17] A study[18] was done to assess the antifungal and post-antifungal effects of chlorhexidine, fluconazole, chitosan and its combinations on Candida albicans in patients with OM and chitosan was found to be effective to inhibit the concentrations of Candida. Chitosan is a bio-adhesive or a mucoadhesive polymer and forms an occlusive dressing over the mucosa which inhibits the settling of secondary infection and also aids in the protection of the wound from secondary injuries.

**Conclusion**

Chitosan is an excellent candidate for the treatment of OM, offering not only the palliative effects of an occlusive dressing owing to its bioadhesive and antimicrobial, fungistic, wound-healing and pain relieving activities but also has the potential for delivering therapeutic compounds. The results of this study support routine use of chitosan mouthwash in patients with carcinoma receiving a variety of RT and CT regimens.

**References**
