ORIGINAL ARTICLE

Randomized Controlled Trial for Comparative Assessment of Effect of Artificial Saliva Versus Virgin Coconut Oil on Salivary Proteolytic Activity & Radiation Mucositis in Patients undergoing Radiotherapy for Head and Neck Cancers

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ABSTRACT

Introduction: Oral mucositis is considered as the most debilitating and unavoidable side effect of chemoradiotherapy for head and neck cancers. Keratin is one of the protective factors present in the superficial layers of the oral epithelium. Break in the integrity of the epithelial tissue seen in radiation mucositis is suggestive of production of proteolytic enzymes which help to dissolve the keratin in the epithelium. **Methods:** This randomized controlled trial was conducted among eighty head and neck cancer patients scheduled to undergo radiotherapy. Patients were divided into control group (40 Patients) who received artificial saliva(carboxymethyl cellulose base)and the study group (40 Patients) who received virgin coconut oil in the form of oral rinses 4 times daily during the entire course of the radiotherapy. Salivary proteolytic levels were measured in optical density units, spectrophotometrically using modification of Erlanger et al.'s (1961) method along with clinical grading of the radiation mucositis. **Results:** The study showed high degree of correlation between salivary proteolytic levels and radiation mucositis. The levels of salivary proteolytic activity and radiation mucositis were lower in the patients who received virgin coconut oil as compared to those who received artificial saliva. **Conclusion:** The study showed better therapeutic efficacy with virgin coconut oil as compared to artificial saliva in the management of radiation mucositis in head and neck cancer patients.

Keywords: Salivary proteolytic activity, Radiation mucositis, Spectrophotometry, Randomized controlled trial

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INTRODUCTION

Oral mucositis is considered as the most debilitating and unavoidable side effect of chemoradiotherapy for head and neck cancers (1). Radiotherapy is a multidisciplinary approach to the head and neck cancer patients and to whom surgery and radiotherapy is the only treatment solutions (2).Oral Mucositis is one of the most common side effects of radiotherapy in cancer patients (3,4). Radiation mucositis is one of the main limiting factors affecting the success and compliance of this therapy (5). The onset of radiation mucositis occurs in the second week of the radiotherapy (3). It is a combined effect of several factors like damage to the basal epithelial cells of the oral mucosa (6), destruction of the salivary glands, alteration of the oral microbial flora, and the loweredpH of the saliva.

The severity of the radiation mucositis increases with the increasing dosage of radiation over the six weeks of radiotherapy. This results in dysphagia, dysphasia, difficulty in chewing, inability to tolerate any spices, adversely affecting their quality of life (4). In the moderate and severe stages, there is ulceration of the oral mucosa. For this to occur there must be a destruction of the protective keratin layer of the oral mucosa. Since keratin is a protein by nature, we hypothesized that for its destruction to take place there must be probable action of the proteolytic enzymes on the keratin layer of the oral mucosal epithelium.

Several factors like the reduction of salivary flow, with its resultant reduced washing action, altered salivary composition in the form of its reduced buffering action, decrease in Salivary IgA, acidic pH, and development of a predominantly anaerobic environment are believed to play a synergistic role in the pathogenesis of radiation mucositis. The loss of the protective keratin layer is followed by the seepage of bacterial endotoxins through the breached epithelium along with super infection by

candida organisms result in the clinical manifestations of the radiation mucositis.

The present study is an attempt to assess the levels of the salivary proteolytic enzymes produced by the oral microflora in head and neck cancer patients undergoing radiotherapy for malignancies; to check if there is any correlation between the salivary proteolytic activity and the degree of radiation mucositis. The study was done to make a comparative assessment of the effect of artificial saliva versus virgin coconut oil on the levels of salivary proteolytic enzymes in patients undergoing radiotherapy for head and neck cancers and their possible correlation with the severity of degree of radiation mucositis.

MATERIALS AND METHODS

A total of 80 head and neck cancer patients between the ages of 30 to 70 years scheduled to undergo teletherapy as a part of their normal treatment protocol were recruited for the study from the Indian Red Cross Society Cancer Hospital. The patients were allocated to the study group and control group by computerized block randomization. One group scheduled to receive the standard(carboxy methyl cellulose based) artificial saliva (40 patients)were designated as the control group & the other group scheduled to receive virgin coconut oil (40 patients)was designated as the study group.

Salivary proteolytic activity was measured in each person in both groups on the 1st day before starting the radiotherapy and at the end of every week, during the course of the radiotherapy. 2ml of unstimulated whole saliva was collected from each of the patients between 10 a.m. to 11:00 a.m. to avoid diurnal variations. Modification of Erlanger et al.'s method was used for assessment of salivary proteolytic activity (7).

Institutional ethical committee clearance was obtained for the study, and the study was registered in Clinical Trials Registry of India. The present study has been ethically approved by Institutional Ethics Committee, Narayana Medical College, Nellore, India dated 30th April 2018 and Trial Registered Prospectively vide Ref No. CTRI/2019/05/019319 dated 24th March 2019.

RESULTS

Table I shows the age and gender distribution of the patients. In the control group, 67.5% of the head and neck cancer patients were males and 32.5% of the head and neck cancer patients were females. In the study group, 60% of the head and neck cancer patients were males and 40% of the head and neck cancer patients were females. This study population showed a higher tendency of head and neck cancers in the males in the control group and the study group.

Table II shows the salivary proteolytic activity of the

Table I: Age & Gender Distribution of Patients

Age	Males	Females
30-40yrs	2	4
40-50yrs	4	0
50-60yrs	34	17
60-70yrs	11	8
Group	**Male**	**Female**
Control Group	67.5%	32.5%
Study Group	60%	40%

subjects. In the control group, at the end of the 1st week of the radiotherapy, 14 patients (35%) developed radiation mucositis grade 1 with mean salivary proteolytic activity corresponding to 0.28 O.D., units while 26 patients (65%) were asymptomatic with mean salivary proteolytic activity corresponding to 0.26 O.D. units.In the study group 40patients were asymptomatic with mean salivary proteolytic activity corresponding to 0.26 O.D. units.

After finishing ofthe 2nd week of RT, in the control group, 16 patients (40%) were asymptomatic with mean salivary proteolytic activity corresponding to 0.28 O.D. units and 24 patients(60%) developed grade 1radiation mucositis with mean salivary proteolytic activity corresponding to 0.34 O.D. units. In the study group, at the end of the 2nd week of RT, 28 patients (%) developed grade 1 radiation mucositiswith mean salivary proteolytic activity corresponding to 0.34 O.D. units while12 patients (%) were asymptomatic with mean salivary proteolytic activity corresponding to 0.26 O.D. units.

After finishing of the 3rd week, in the control group,8 patients (%) were asymptomatic with mean salivary proteolytic activity corresponding to 0.26 O.D. units, 22 patients (%) had grade 1 radiation mucositis with mean salivary proteolytic activity corresponding to 0.36 O.D. units, 10 patients (25%) had grade 2 radiation mucositis with mean salivary proteolytic activity corresponding to 0.38 O.D. units. After finishing of the 3rd week, in the study group, 32% had grade 1 radiation mucositiswith mean salivary proteolytic activity corresponding to 0.32 O.D. units and the rest of the 8 patients (5%) did not show any signs of radiation mucositis with mean salivary proteolytic activity corresponding to 0.28 O.D. units.

After finishing the 4th week, in the control group, 18% patients had grade 1 radiation mucositis with mean salivary proteolytic activity corresponding to 0.34 O.D. units, 22% had grade 2 radiation mucositis with mean salivary proteolytic activity corresponding to 0.36 O.D. units. After finishing of the 4th week in the study group, 34% of the ad grade1 radiation mucositis with mean salivary proteolytic activity corresponding to 0.32 O.D. units and rest of the 6% patients did not show any signs of radiation mucositis with mean salivary proteolytic activity corresponding to 0.28 O.D. units.

Table II: Salivary proteolytic activity of patients

Radiation Mucositis Score	0	1	2	3	4
Baseline Salivary Proteolytic Activity in O.D. (Number of Patients)	0.25 (80)	0	0	0	0
End of 1st Week of RT					
Salivary Proteolytic Activity in O.D.(C.G.) (Number of Patients)	0.26 (26)	0.28 (14)	0	0	0
Salivary Proteolytic Activity in O.D.(S.G.) (Number of Patients)	0.26 (40)	0	0	0	0
End of 2nd Week of RT					
Salivary Proteolytic Activity in O.D.(C.G.) (Number of Patients)	0.28 (16)	0.34 (24)	0	0	0
Salivary Proteolytic Activity in O.D.(S.G.) (Number of Patients)	0.26 (12)	0.34 (28)	0	0	0
End of 3rd Week of RT					
Salivary Proteolytic Activity in O.D.(C.G.) (Number of Patients)	0.26 (8)	0.36 (22)	0.38 (10)	0	0
Salivary Proteolytic Activity in O.D.(S.G.) (Number of Patients)	0.28 (8)	0.34 (32)	0	0	0
End of 4th Week of RT					
Salivary Proteolytic Activity in O.D.(C.G.) (Number of Patients)	0	0.34 (18)	0.36 (22)	0	0
Salivary Proteolytic Activity in O.D.(S.G.) (Number of Patients)	0.28 (6)	0.32 (34)	0	0	0
End of 5th Week of RT					
Salivary Proteolytic Activity in O.D.(C.G.) (Number of Patients)	0	0	0.30 (24)	0.34 (8)	0.36 (8)
Salivary Proteolytic Activity in O.D.(S.G.) (Number of Patients)	0.30 (4)	0.28 (24)	0.32 (12)	0	0
End of 6th Week of RT					
Salivary Proteolytic Activity in O.D.(C.G.) (Number of Patients)	0	0	0	0.40 (28)	0.42 (12)
Salivary Proteolytic Activity in O.D.(S.G.) (Number of Patients)	0	0.30 (12)	0.36 (16)	0.38 (12)	0

O.D.= Optical Density units, C.G.= Control Group, S.G.=Study Group

After finishing of the 5th week, in the control group, 24% had grade 2 radiation mucositis with mean salivary proteolytic activity corresponding to 0.30 O.D. units, 8 patients (%) had grade 3 with mean salivary proteolytic activity corresponding to 0.34 O.D. units and 8(%) had grade 4 radiation mucositis with mean salivary proteolytic activity corresponding to 0.36 O.D. units. After finishing of the 5th weekin the study group, 24 patients (%) had grade1 radiation mucositis with mean salivary proteolytic activity corresponding to 0.28 O.D. units, 12 patients (%) had grade2 radiation mucositis with mean salivary proteolytic activity corresponding to 0.32 O.D. units, and rest of the 4 patients (%) did not show any signs of radiation mucositis with mean salivary proteolytic activity corresponding to 0.30 O.D. units.

After finishing of the 6th week, in the control group, 28 patients (%) had grade 3 radiation mucositis with mean salivary proteolytic activity corresponding to 0.40 O.D. units and 12 patients (%) had grade 4 radiation mucositis with mean salivary proteolytic activity corresponding to 0.42 O.D. units. In the study group, at the end of the 6th week, 12% had grade1 radiation mucositis with mean salivary proteolytic activity corresponding to 0.30 O.D. units, 16% had grade2 radiation mucositis with mean salivary proteolytic activity corresponding to 0.36 O.D. units, and 12 patients (%) had grade 3 radiation mucositis with mean salivary proteolytic activity corresponding to 0.38 O.D. units.

DISCUSSION

Various medications have been tested by various investigators in their search for an ideal agent for prevention and management of radiation mucositis including natural substances like manuka honey (8), combinations of drugs (lidocaine, diphenhydramine and sodium bicarbonate in normal saline) (9). Another research shows that there is positive effect of alcohol-free povidone-iodine mouthwash on oral mucositis due to radiotherapy in head and neck cancer patients (10). Heydarirad G et al. did RCT using traditional Persian medicine preparation of hollyhocks and common mallow and opined that this should be considered as a suitable treatment for xerostomia and improving QOL in HNC patients with radiation-induced xerostomia (11, 12). However, none of these medications are prescribed universally nor has any universally acceptable protocol has been evolved till date and the search for ideal therapeutic agents is still going on (4,8). Vinicius et al. did a metanalysis to assess effectiveness of low level laser in prevention of radiation mucositis and concluded that low level laser prevented radiation mucositis in

28% of the patients receiving radiation therapy and suggested that more work should be done on low risk of bias so a higher scientific evidence can be obtained (13). Chattopadhyay et al. show about reduction of duration of grade 3 and 4 mucositis by using glutamine in the head neck cancer patients (14).According to Parkhil(15) mucositis has a very bad impact on the patient and the healthcare systems because of its dose-limiting toxicities for these urgent studies are needed to develop effective strategies for prevention and treatment.

Artificial saliva containing carboxy methyl cellulose is a good moisturizer which helps keep the mucosa moist and soft. It further helps in providing a lubricant action and helps the patient in mastication, bolus formation and speech to a considerable extent and has therefore been used since a considerable period of time as a suitable option to help offset the problems associated with xerostomia to some extent (16). However, it does not help the patient to overcome the pain and the burning sensation which adversely affects the quality of life of these patients.

The carboxymethyl cellulose which is one of the major components of the artificial saliva needs to be imported from outside but now days the import related issues are one of the major problems to the artificial saliva manufacturers of the India and many other parts of the world (17).

This results in worsening the suffering of the head and neck cancer patients who undergo radiotherapy for its treatment. A few patients have allergy to some of the components of the artificial saliva which precludes the usage of artificial saliva in such patients.

Maryam Baharvandet al. in their review article stated that it was noticed some herbal plants can improve the mucositis through different interventions (18). In this scenario, virgin coconut oil assumes a lot of clinical importance on account of its being a totally natural and indigenous product with many beneficial properties likeanti-inflammatory, analgesic, antifungal, antimicrobial properties apart from its ability to be better lubricant and moisturizer. It additionally also has antioxidant properties which are very beneficial for healing of the lesions and better and quicker recovery of the patients (19).

Inflammatory reactions maybe mediated by proteolytic enzymes like metalloproteases released by neutrophils and macrophages. These enzymes may cause degradation of the cell membrane bound proteins thus contributing to the development and progression of inflammatory reaction. The main cause ofradiation mucositis isconcomitant changes in the tissues by a combination of alteration of the normal oral microflora (5).Salivary Proteolytic activity maybe associated withgram negative bacteria in the oral cavity along with contributions from leukocytes and macrophages.

Alterations in salivary flow changed the oral environment and lowered the pH level which promotes colonization of thegram-negative organisms. The proteolytic enzymes produced by these bacteria apart from playing a role in initiation and progression of inflammatory reaction and may also help in breaching the integrity of the mucosa through their action on the mucosal keratin (20).

Our study has shown a mild elevation in the salivary proteolytic activity levels suggestive that this may also be contributing to the development of the radiation mucositis. This elevation was a little more pronounced in the control group patients receiving the artificial saliva than the study group patients receiving virgin coconut oil. Virgin coconut oil hasanti-inflammatory property, modulator effects on the neutrophils and the macrophages,and it is also an inflammatory mediator along with its antimicrobial properties maybe helpful in mitigating the severity of the radiation mucositis.

CONCLUSION

The study shows a slight correlation between the salivary proteolytic activity and the degree of severity of the radiation mucositis, with increase in salivary proteolytic activity corresponding to the severity of the radiation mucositis. Further research on the underlying mechanisms of actions of virgin coconut oil on the inflammatory mediators, reactive oxygen species and other factors likely to influence the onset of radiation mucositis will help us in improving our understanding and developing a better protocol for management of radiation mucositis.

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