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International Journal of Current Research
Vol. 6, Issue, 03, pp.5760-5764, March, 2014

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

ASSESSMENT OF ORAL LEUKOCYTE LEVELS IN CHEMO-RADIOTHERAPY INDUCED ORAL MUCOSITIS DURING IMRT FOR HEAD AND NECK CANCER

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ARTICLE INFO

Article History:
Received 21st December, 2013
Received in revised form
24th January, 2014
Accepted 15th February, 2014
Published online 25th March, 2014

Key words:

Oral mucositis,
Oral neutrophil count,
Chemoradiotherapy,
radIotherapy,
Chemotherapy.

ABSTRACT

Background: Oral mucositis is a common, dose limiting and potentially serious complication of both radiotherapy and chemotherapy of head and neck cancer. Homeostasis of both malignant and normal host cells are affected. This study is conducted mainly to evaluate application of oral leukocytes as an indicator of mucositis progression during the course of chemo-radiotherapy (CRT).

Methodology: 30 patients who were diagnosed as having malignancy and were undergoing CRT as treatment option using Intensity Modulated Radiation Therapy (IMRT) were selected and followed up through course of treatment. The procedure involved clinical scoring, collection of oral washings and preparation of buccal smears from both study group and control group.

Results: The results revealed a significant occurrence of oral mucositis in almost all patients during followup. There was a significant increase in percentage of oral leukocytes in patients when compared to controls ($P<0.005$). There was a significant decrease in blood leukocyte level when compared to control ($P<0.005$). The changes in the oral neutrophil counts correlated with WHO clinical score.

Conclusion: Assessment of oral leukocyte especially neutrophils can be considered for evaluation and prediction of mucositis development during CRT. Therefore, this assessment may be useful in research studies aimed at preventing mucositis and can also be used as an adjunct in tracking mucositis development and plan therapy.

INTRODUCTION

The treatment of Head and Neck carcinoma has gone through various leaps and progress in the past few decades with the advent of molecular biology and non-surgical therapies. Mucositis and xerostomia are the most common oral complications of the nonsurgical therapy of cancer. Mucositis is particularly disabling and seen almost invariably in cancer chemotherapy as well as in Radiotherapy for Oral Cancer(1). Both these therapies are non-specific, interfering with the cellular homeostasis of both malignant and normal host cells. An important effect is the loss of rapidly proliferating epithelial cells in the oral cavity, gut and in the bone marrow. Within the mouth, the loss of these cells leads to mucosal atrophy, necrosis and ulceration (Parulekar *et al.*, 1998). Oral mucositis induced by irradiation is defined as a reactive inflammatory-like process of the oropharyngeal mucous membranes. During the radiation therapy severe stages of mucositis may develop, such as the formation of pseudomembranes (3 to 4 weeks) and ulceration. The severity of mucositis is determined by radiation modalities as dose per day, cumulative dose, volume of irradiated tissue and type of ionizing radiation (Spijkervet *et al.*, 1989).

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Mucositis induced by antineoplastic drugs is an important, dose limiting and costly side effect of cancer chemo-therapy (Sonis, 1998). Direct toxicity to the oral epithelium is perhaps the most obvious drug induced cause. This usually occurs within 5 to 10 days post administration of medication. The other mechanism, indirect toxicity can occur during the period of drug induced neutropenia. During this time patients are prone to infections that can manifest as mucositis. Microbial culturing is critical at this point to differentiate chemotherapy induced mucosal toxicity from mucosal neutropenic infectious complications caused by bacterial, fungal or viral microorganisms (Jackson, 2000).

Radio-chemotherapy regimens induce high levels of acute toxicity, significantly higher than for radiotherapy alone. The addition of chemotherapy introduces systemic toxicity and can exacerbate local tissue reactions when used concurrently with radiotherapy. Mucositis is recognized as the principal limiting factor to further treatment intensification in such situations (Bensadoun *et al.*, 2001). As new agents become available and as combinations of radiotherapy and multiple drug chemotherapy are used concurrently, reports of apparent interaction are appearing frequently in the literature (Phillips and Fu, 1976). This study is aimed at evaluating the use of oral leukocytes especially the neutrophils to assess the oral

mucositis severity and comparing them with the clinical grading through the period of chemo-radiotherapy.

MATERIALS AND METHODS

Source of data: 30 patients who are diagnosed as having Head and Neck malignancy undergoing chemo-radiotherapy as treatment option at the Department Of Radiation Oncology, Father Muller Hospital, Mangalore were selected and followed up through the course of treatment. The study group consisted of 30 patients who were diagnosed with having malignant condition of the body who are to undergo chemo-radiotherapy as treatment option. The samples were taken in week 1,2,3 and 4. The control group were 30 normal healthy persons who were systemically well and not under any medication or without any adverse habits. Exclusion criterias were: patients with any oral mucosa defect, patient who needs any obturator or prosthesis, treatment with antibiotics for an infection in the 2 week period before the start of therapy, oral candidiasis or acute periodontitis and patients with naso-gastric tube at the start of treatment.

The procedure involves clinical scoring, collection of oral washings and preparation of buccal smear from both study group and control group. For the study group, clinical procedures were done on the first day prior to the commencement of therapy. Then the next samples were taken at 7th, 14th and 21st day. If the patient experiences any complications, weekly samples will be collected till the oral mucosa returns to normal. Only the initial single samples were collected for healthy controls as per the inclusion and exclusion criteria.

I. Oral mucositis scoring

Patients who were to undergo chemotherapy were clinically evaluated for mucositis and scoring will be done based on WHO scale (8). i.e. grade 0 – no change, grade 1 – soreness/ erythema, grade 2 - erythema/ ulcers/ can eat solids, grade 3 – ulcers/ requires liquid diet only, grade 4 – alimentation not possible.

II. Oral Washings

To prepare oral washings patients were asked to gargle /rinse their mouth with 10ml sterile saline for 15seconds and asked to spit into a glass beaker. Oral washing was centrifuged (ACSW-163 centrifuge machine, Atul chemicals and scientific works) and the centrifugate cells were obtained. The cells are suspended in 1ml of RPMI 1640 (Hi media Lab Pvt Ltd, Mumbai, India) medium containing fetal calf serum (Hi media Lab Pvt Ltd, Mumbai, India) 5%. A micropipette (SC – single channel, Atul chemicals and scientific works) was used to obtain 50µl of suspension was incubated for 15 minutes with acridine orange (Hi media Lab Pvt Ltd, Mumbai, India) and diluted with phosphate buffer saline and were examined by fluorescence microscopy. The number of leukocytes were determined.

III. Buccal smear

The buccal scrapings from representative sites were prepared and fixed using ether alcohol after preparing the smear and stained using Papanicolaou stain. Epithelial cell morphology and differentiation were studied under the light microscope. There were remarkable difference in the amount of oral neutrophils present on the smears from week 1 to week 4. Blood investigations were done on the study group on the day of sample collection. Total count, differential counts were estimated and level of blood leukocytes and oral leukocytes were tabulated. Results were statistically evaluated using “t test”, Wilcoxon signed rank test.

RESULTS

The study group consisted of 30 persons aged between 38 to 78 years who received chemotherapy as well as radiotherapy.. The radiation dose received by the fourth week were between 999 rads to 4200 rads. The gender wise distribution consisted of 25 males (83%) and 5 females (17%). Major forms of carcinomas included were CA tongue (21%) followed by CA buccal mucosa (10%), CA oropharynx (10%) and CA tonsil (10%). Comparision of WHO mucositis grading from week 1 to 4 showed that there was a significant increase in incidence and severity of oral mucositis starting from week 2 (figure 1). A severe form of mucositis development was observed by the end of fourth week. In week 3 mean frequency of patients exhibiting grade 2 mucositis increased slightly and none of the patients were under grade 0 anymore. As the patients entered week 4 considerable numbers of patient exhibited grade 3 type of mucositis. Statistics showed p-value (< 0.0005) between week 1 and 2, week 1 and 3 and also week 1 and 4 (Table 1, 2). Blood leukocyte level showed statistically significant difference (p – 0.26) between the study and the control group while oral leukocyte levels showed insignificant difference (p – 0.73) at the beginning of therapy. As the cancer therapy progressed, the level of oral leukocyte exhibited gradual increase (t-15.51) on comparing week 1 and 4 (Table 4). Blood leukocyte did not exhibit much difference in the first week but showed a statistically significant (p – 0.036 & p – 0.004) fall at the third and fourth week respectively.

On comparing the cells of the buccal smears stained using PAP to obtain the maturity of buccal epithelial cells, the visual examination also revealed high level of neutrophil counts The WHO mucositis score corresponded with viable cell count and showed earlier change when compared to WHO grading (Figure 2,3).

DISCUSSION

The rate at which the radiation is delivered and the total dose delivered are important variables in radiation-related mucositis. Chemotherapy on the other hand, can either produce a general mucosal or a site specific problem that is related to the agents, the dose, and the sequence of administration. Complications can be direct, caused by toxic action of treatment agents on the proliferative mucosal lining of the mouth, or indirect, the result of hemopoeitic shut down (Toth *et al.*, 1990). Severe courses of oral mucositis are observed during simultaneous radio-chemotherapy, which affects virtually all patients with head and neck cancer who receive this therapeutic modality. However, up to 40% of patients treated with conventional chemotherapy and more than 70% of patients undergoing

Table 1: The Wilcoxon signed rank test comparing WHO mucositis grading

Statistical Analysis	WHO mucositis week 2-week 1	WHO mucositis week 3-week 1	WHO mucositis week 4-week 1
Z	-4.873	-4.890	-4.928
p-value	<.0005	<.0005	<.0005

Table 2: Comparison of Oral Leukocytes and Blood Leukocytes with controls

Group	N	N	Mean	Std. Deviation	Mean Difference	t	p-value
Oral Leukocyte Week 1	Test	30	.8550	.13219	-.01333	-.350	.727
	Control	30	.8683	.16108			
Blood Leukocyte Week 1	Test	30	8233.33	2126.245	-613.333	-1.145	.257
	Control	30	8846.67	2012.732			

Table 3: Comparison of oral leukocyte level from week l to week 4

Group categories		Mean	Std. Deviation	Mean difference	t	p-value
Pair 1	Oral leukocyte week 1	.8550	.13219	-.31333	-4.773	<.0005
	Oral leukocyte Week 2	1.1683	.30129			
Pair 2	Oral leukocyte week 1	.8550	.13219	-.59000	-8.629	<.0005
	Oral leukocyte Week 3	1.4450	.33742			
Pair 3	Oral leukocyte week 1	.8550	.13219	-1.24667	-15.512	<.0005
	Oral leukocyte Week 4	2.1017	.40204			

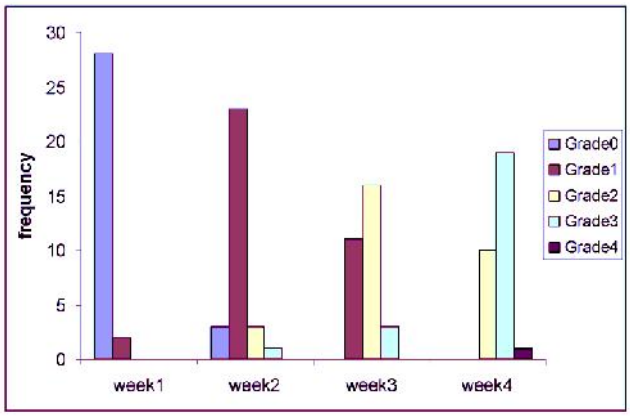

Figure 1: Frequency table comparing the incidence of oral mucositis grading from week 1 to week 4

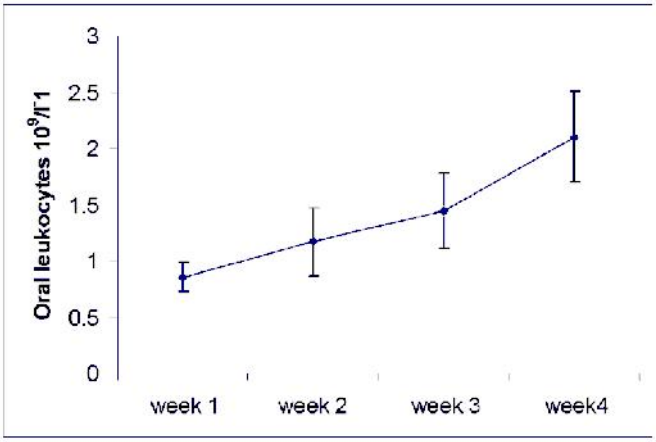

Figure 2: Comparison of Oral leukocyte level from week 1 to week 4

conditioning therapy for bone marrow transplantation also experience oral treatment-related complications (Köstler *et al.*, 2001). A study was developed to assess and quantify oral neutrophils in periodontal diseases that include obtaining oral rinse in order to quantify the severity (Bender *et al.*, 2006). One study validated a noninvasive oral rinse assay to potentially help in the management of pediatric patients undergoing HSCT that enables a noninvasive assessment of neutrophil tissue delivery by measuring the level of neutrophils in oral tissues in patients recovering from HSCT (Cheretakis *et al.*, 2005). Our present study also utilizes such assay to evaluate the use of oral neutrophils for oral mucositis progression.

In the study by Wymenga to assess oral mucositis, an in-vitro assay utilized the oral rinse method to count the oral neutrophil levels during & after high-dose chemotherapy (Wymenga *et al.*, 1997). The study also well correlated with the previous study that indicated the beneficial effect of G-CSF in the kinetics of oral mucosal neutrophil recovery in addition to the effect of G-CSF to accelerate peripheral blood neutrophil recovery (Lieschke *et al.*, 1992). The diagnosis of grade I mucositis is based on the presence of asymptomatic mucosal erythema, evaluated on clinical grounds, and need no treatment. It has to be differentiated from erythematous candidosis, a common infection during head and neck radiotherapy and antineoplastic chemotherapy, which needs antifungal treatment (Sonis *et al.*, 1999 and Sonis *et al.*,1990).

Grade II mucositis, with small foci of ulcers, is also diagnosed based on the clinical presentation, and it has to be differentiated from an early intraoral herpetic infection or from a superimposed candidosis. The most distressing

grade III and IV mucositis is diagnosed based on its clinical presentation of superficial ulcerations covered by pseudomembranes, that are very painful to be rubbed off. These pseudomembranous ulcerations are to be differentiated from pseudomembranous candidosis, consisting of whitish, easy to rub off, pseudomembranes. Again, the laboratory isolation of yeasts from smears, taken from the lesions, may be helpful, but is not critical for diagnosis. Pseudomembranous candidosis may be superimposed on the pseudomembranous ulcerations of mucositis and, in these cases, the differentiation is difficult. Severe mucositis is important to be differentiated from the clinically identical, herpes simplex virus-1 reactivation and infection in neutropenic patients. Herpetic infection, if not diagnosed and treated promptly, may further aggravate mucositis and delay may further aggravate mucositis and delay healing, thus compromising the antineoplastic protocol (Sonis *et al.,* 1999). The correlation co-efficient of the head and neck cancer oral stomatitis domain and the WHO scale was 0.58 and hence being commonly use (Sylvester, 1980). In our present study the WHO mucositis grading system was utilized which showed that every patient had a significant increase in the gradation of mucositis mostly starting from the 2nd week of treatment and by the end of the 4th week, grade III or grade IV mucositis (Figure 1). The Wilcoxon signed rank test showed that there was a statistically significant difference between the clinical grade of mucositis between week 1 and week 2 (Z-4.873), week 1 and week 3 (Z-4.890) and also week 1 and week 4 (Z-4.928) as per Table 1. Our present study consists of 30 patients of whom 25 were males and 5 females consisting of 83% and 17% respectively. Carcinoma of tongue, carcinoma alveolus and carcinoma of buccal mucosa were major carcinoma. In our study, approximately 63% of patients developed severe form of mucositis as per the WHO mucositis grading scale. In the total of 18 patients who underwent chemo-radiotherapy about 10% of patients developed grade III mucositis by the end of the 3rd week of therapy.

The authors believe that this is due to the different mechanism of action of chemotherapy and radiotherapy. While chemotherapy has a discontinuous effect due to different regimes of administration, radiotherapy is given in continuous cycles for a period of 6- 7 weeks. In the present study of chemo-radiotherapy, the combined toxicity of the two modalities are studied and thus the significant increase of oral leukocyte where according to our study an increase in more than 50% was considered as minimum detectable change. The chemo-radiotherapy induced oral mucositis has been extensively evaluated in patients receiving HSCT to be observed in up to 99% patients and that in two thirds of patients it was WHO grade III or IV (1) although, one study by Archibald et al. states the addition of chemotherapy to the treatment regimen did not increase the incidence of complications when compared with historical controls receiving radiotherapy alone (Archibald *et al.,* 1986).

Conclusion

The oral neutrophil has long been utilized as a marker of severity of periodontal disease condition. In the event of new knowledge that unplanned treatment breaks or reduction in dose intensity during chemo-radiotherapy can lead to decreased loco-regional control, poorer quality of life, and shortened overall survival (Rosenthal, 2007), the assessment aids in mucositis can become valuable in future.

ACKNOWLEDGEMENT

I would like to sincerely acknowledge the guidance of Prof. Dr. Lal P. Madathil; M.D.S, Department of Oral Pathology & Microbiology, A.B. Shetty Memorial Institute of Dental Sciences, Mangalore, Karnataka, India. Dr. Suresh Rao; M.D.,Head of Department,Father Muller Institute of Oncology,Fr. Muller Road, Mangalore. Dr. Pratima S. Rao;M.D.S., Department of Oral Pathology & Microbiology, A.B. Shetty Memorial Institute of Dental Sciences, Mangalore, Karnataka, India and especially the management of Father Muller Medical College, Mangalore, India

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