

Influence of Circadian Rhythm in Radiation Induced Mucositis in Head and Neck Malignancies

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ABSTRACT

BACKGROUND: In India, more than 60% of the head and neck malignancies present as locally advanced disease. Chemoradiation has been the main stay of treatment for the past three decades. In spite of the multimodality treatment, normal tissue toxicity in the form of mucositis has been a limiting factor.

MATERIALS AND METHODS: We investigated the plausible impact of circadian rhythm of normal mucosa to the development and severity of mucositis, based on the timing of radiation delivery in patients receiving radiation treatment in head and neck malignancies. We analysed the data of 142 patients of histologically proven squamous cell carcinoma of the head and neck sites (stage II to IVA), treated from January 2014 who underwent radical external beam radiation therapy (EBRT) to a dose of 60 – 66 Gy in 30 – 33 fractions. All the patients were treated in a cobalt teletherapy unit, with lateral parallel pair (60 -66 Gy/33 #) and low anterior neck (50 Gy /25 #) radiation fields. Stage III and IVA patients with normal renal parameters and good performance status received concurrent weekly Cisplatin 40mg/m². Patients were stratified based on whether they received EBRT in the morning (8am-11am) or in the evening (5pm-8pm). The clinicopathological characteristics were relatively well balanced in both the groups.

RESULTS: Of the analysed 142 patients, 42.47% versus 60.9% developed RTOG Grade 3 or 4 mucositis [severe] after morning versus evening EBRT, respectively (p = 0.028). The median time to development of severe mucositis was prolonged in the morning group (6 weeks vs 5 weeks). Morning EBRT was also associated with significantly lesser no of patients for whom treatment had to be interrupted due to severe mucositis 17.8% vs 42.0% (p = 0.002).

DISCUSSION: The potential of chronomodulation at improving therapeutic efficacy has been highlighted through our study and may be utilized more in the clinical setting

KEYWORDS: *Circadian rhythm, Chronotherapy, morning versus evening RT, head and neck cancers*

I. INTRODUCTION

In India more than one third of the newly diagnosed cancer cases are head and neck malignancies. The use of tobacco in various forms either smoking or chewing still predominates as the etiological factor for the development of head and neck malignancies[1]. Of these more than 60% of the cases are locally advanced and present with involved regional cervical lymph nodes. Concurrent chemoradiation with Cisplatin as the radiosensitizer agent has been documented in metanalysis to improve locoregional control(LRC) and overall survival(OS) when compared to radiation therapy alone[2]. But this gain in LRC and OS has come at the expense of increase in the toxicity rates, with acute Grade 3 or 4 toxicities like skin toxicity, mucositis, dysphagia, drop in hematological parameters, all increased to upto around 43% when compared to RT alone.[3-8]

Acute mucositis has been and continues to be one of the most worrisome toxicities in head and neck irradiation. Nearly all the patients who receive head and neck radiation do develop some grade of mucositis. But as the dose increases the grade of toxicity increases as well and at radical doses of around 66-70 Gy as conventional fractionation the chances for severe mucositis dramatically increases. This can result in decreased oral intake, poor quality of intake, increased number of hospitalizations resulting in increased financial burden and mostly importantly interruptions in treatment culminating in decrease in expected locoregional control.[9-15] Pharmacological methods to alleviate this discerning side effect has met with only very limited success with agents like palifermin and benzydamine and researches into this area has more or less reached the dead end. [16-18]

The mechanisms involved in the pathogenesis of mucositis appear to be much more complex than a direct damage to epithelium theory, albeit radiation induced and chemotherapy induced mucositis considered to be of similar pathogenesis. This includes the complex interactions between cytokines, innate microbial and local immunological effects, resulting in both direct and indirect tissue damage.[18-20]

It is a well studied and documented concept that almost all human biological functions run based on a circadian rhythm (day/night rhythm). These circadian rhythms are controlled by the suprachiasmatic nucleus of the hypothalamus, with numerous biological processes are dictated by this clock including sleep, emotions, cellular proliferation, release of hormones etc . Preclinical data suggests the existence of an interaction between clock genes like PER1,PER2,CLOCK,BMAL1 etc and the cellular proliferation in the cell cycle. [21-25]

Employing this concept of an existing circadian rhythm , treatment strategies have been developed to identify the best time of the day to treat , to maximize the effect of treatment on tumour cells and minimize the same on normal cells. This form of therapy utilizing the diurnal rhythm is known as chronotherapy.

Preclinical experimental data suggests the existence of a difference in the diurnal rhythm of tumour cells and normal cells with regards to DNA synthesis and cellular proliferation. well [26,27]. Henceforth the ideal time of the day to treat a tumour , would be in the morning hours , to maximize tumour kill as the cells would be more or less in the radiosensitive G2/M phase and the normal cells would be minimally affected as they would be cycling in the radioresistant G1 phase.[28,29]

In our study we investigated the notion of chronotherapy by retrospectively analyzing whether the degree of development of radiation induced mucositis had any relation to the timing of delivery of radiation .

II. MATERIALS AND METHODS

208 patients with histopathologically proven head and neck squamous cell carcinoma patients were registered in the Department of Radiotherapy and Oncology of our institution during the period from January 2014 to June 2014. The subsites that were included in the study were oral cavity, oropharynx and nasopharynx. All patients included in the study were Stage II, III or IVA. We excluded 66 patients from the final analysis who underwent radiation treatment between 11 am to 4 pm, who had treatment interruptions due to personal reasons, hypopharynx and larynx subsites, and those who did not receive the radical radiation dose of 60-66 Gy in 30 – 33 fractions. The data of 142 patients who received RT either during the morning shift (8 am – 11 am) or during the evening shift (5pm – 8pm) were available for the final analysis. The study design and the protocol were approved by the institutional review board and the regional ethical committee.

All the patients included for the final analysis had been treated to a dose of 60-66Gy in 30-33 fractions, 2 Gy per fraction, one fraction a day, five fractions a week, with a lateral parallel pair/unilateral field and a low anterior neck field intended to irradiate the head and neck primary site and the regional lymph node stations. As per the departmental protocol, all head and neck patients on RT are reviewed once a week. The toxicities are graded based on the Radiation Therapy Oncology Group (RTOG) toxicity criteria and documented in the master file of each patient. The data on mucositis grade on each review, the highest grade of mucositis, time to development of the highest grade of toxicity and any treatment interruptions due to the toxicity were utilized for the study. All relevant statistical analysis was done using the SPSS software version 18.0 using descriptive analysis method. Comparison of acute mucositis between the groups was done using independent t-test or Chi square test whichever was applicable. p-value of <0.05 was considered significant.

III. RESULTS:

A total of 142 patients were available for the final analysis. The patient characteristics, tumor and treatment characteristics were balanced between the two arms. 51.4% (n=73) of the patients received RT in the morning arm and 48.6% (n=69) received RT in the evening arm. Mean age of the morning group was 56.57 years and 55.61 years in the evening group. More than 90 % of the patients were males (n=130) which was higher than the general demographical pattern of head and neck cancers in India. Oropharyngeal malignancies were the predominant subset (52.8%, n=75) with Stage III disease forming the majority of cases (57%,n=81). 76 out of the 142 patients (53.5%) received concurrent chemoradiation as a part of the treatment protocol. 47 percent patients in the final analysis reportedly used tobacco even during the period of radiation (n=67). (TABLE 1)

TABLE 1: PATIENT CHARACTERISTICS

VARIABLES	MORNING (n=73)	AFTERNOON (n=69)	TOTAL (n= 142)
MEAN AGE (years)	56.57	55.61	56.11
SEX (Male)	69(94.5 %)	61(87.1 %)	130(91.6%)
(Female)	04(05.5 %)	08(12.9 %)	12(8.4%)
SUBSITE			
ORAL CAVITY	28(38.3%)	29(42.0%)	57(40.1%)
OROPHARYNX	38(52.1%)	37(53.6%)	75(52.8%)
NASOPHARYNX	07(09.6%)	03(04.4%)	10(07.1%)
STAGE			
II	28(38.3%)	17(24.6%)	45(31.7%)
III	39(53.4%)	42(60.8%)	81(57.0%)
IV A	06(08.2%)	10(14.4%)	16(11.3%)
PRESENT SMOKERS	29	38	67(47.1%)
NASOGASTRIC TUBE	36	30	66(46.5%)
ECOG PS			
1	47(64.3%)	46(66.6%)	93(65.5%)
2	26(35.7%)	23(33.4%)	49(34.5%)
CONCURRENT CHEMOTHERAPY	33(41.1%)	43(52.2%)	76(53.5%)

51.4 % of patients (n=73) in the entire study population developed severe mucositis i.e Grade 3 or 4 with 42.47 % of the patients in the morning arm (n= 31) and 60.9% (n=42) in the evening arm developing the same. This difference was statistically significant ($p=0.028$). Thus the patients treated in the evening arm developed a higher percentage of severe mucositis compared to the morning arm. Mean time to develop the Grade3 or 4 mucositis was lesser in the evening arm 5 weeks to 6 weeks in the morning arm. (TABLE 2)

TABLE 2: ACUTE MUCOSITIS GRADING

VARIABLES	MORNING RT (8am – 11am) (n=73)		EVENING RT (5pm – 8pm) (n=69)	
GRADE 1	N=07	09.6%	N=05	07.2%
GRADE 2	N=35	48.0%	N=22	31.9%
GRADE 3	N=29	39.7%	N=32	46.4%
GRADE 4	N=03	02.7%	N=10	16.5%
GRADE 3 & 4 COMBINED	N=31	42.4%	N=42	60.9%
MEAN TIME TO DEVELOPMENT OF GRADE 3/4 OF TOXICITY (IN WEEKS)	6 WEEKS		5 WEEKS	

On multivariate analysis, use of concurrent chemotherapy and current smokers were independent predictors for the development of severe mucositis. 62.7 % of the patients who smoked (n =67) during RT, developed severe mucositis compared to 41.3 % who didn't smoke ($p = 0.011$). Of the 76 patients, who received concurrent

chemotherapy, 68.4 % developed severe mucositis, whereas only 31.8 % of patients who didn't receive developed severe mucositis. (**p= 0.0001**)

The evening arm RT was associated with a statistically significant increase in the percentage of patients who had treatment interruptions and had to stop concurrent chemotherapy due to the higher incidence of mucosal toxicity and decrease in the general condition. 17.8 % versus 42.0 in favour of the morning arm (**p=0.002**) with regards to the treatment interruptions and 21.2% versus 41.9% favouring the morning RT arm with respect to percentage of patients who had to be withheld from weekly chemotherapy. (**p=0.028**) (TABLE 3)

TABLE 3: TREATMENT INTERRUPTIONS

VARIABLES	MORNING RT (8am – 11am)		EVENING RT (5pm – 8pm)	
	INTERRUPTIONS IN RT	N=13	17.8%	N=29
STOP CONCURRENT CHEMO	N=7/33	21.2%	N=18/43	41.9%

IV. DISCUSSION

In our study we assessed whether the timing of delivery of radiation in head and neck malignancies had any relation to the severity of development of mucositis. The incidence of Grade 3 or 4 mucositis in general was 51.4% which is in similar range to the percentage of severe mucositis that has already been published in literature.[30]The analysis of our study revealed that the incidence of severe mucositis was significantly less when radiation was delivered in the morning hours (8am-11am) compared to the when delivered during the evening hours (5pm-8pm).

Our results reverberate the published work of Bjarnson et al, who conducted a randomized trial to corroborate the advantage of morning radiotherapy versus afternoon radiotherapy with respect to normal tissue toxicity. The results of his prospective randomized trial demonstrated a significant improvement in weight loss and reduced incidence of oral mucositis among male patients (49.4% versus 64.1%), for the morning group when compared to patients receiving radiotherapy in the afternoon. [30]

Bashir et al, similarly compared in a nonrandomized prospective manner the difference in normal tissue toxicity when radiation to head and neck was delivered using Intensity Modulated Radiation treatment (IMRT) technique. The results were similar with 28.6% patients in the morning group compared to 43.7% patients in the evening group developing Grade 3 mucositis and the severe mucositis free interval (SMFI) was 33 days compared to 22 days in favour of the morning arm.[31]Goyal M et al similarly in a randomized fashion proved that development of higher grade mucositis were higher in the evening irradiated group than in the morning irradiated group 38% versus 26%.[32]There are clinical data in other subsites demonstrating the effect of chronotherapy on normal tissue toxicity and as well as the effect of chemotherapy in a diurnal rhythm.

Preclinical and clinical evidence suggest a difference in drug induced toxicities existing on a circadian rhythm. Cisplatin was demonstrated to be less toxic when administrated in the evening hours as the susceptible tissue such as marrow elements and renal tissue are less susceptible to Cisplatin induced damage. This was clinically substantiated by Focan et al[39] in Carcinoma Esophagus patients and Verma et al in head and neck malignancy patients[38]In a single institution retrospective analysis on the incidence of acute skin toxicity developing in chest wall irradiation of breast patients, a similar result with the incidence of higher grade skin toxicity being more in the evening arm compared to the morning arm was demonstrated.[33]

Shukla et al had clinically substantiated a decrease in incidence of diarrhea in cervical cancer patients for whom radiation was delivered in the morning, confirming the presence of a diurnal rhythm to the cell cycle of the intestinal mucosal cells.[37]All these results circle to the fact that there is a circadian rhythm existing in the oral mucosa cells resulting in a time bound variation in the response to radiation with the hypothesis that majority of cells during the morning hours are present in the relatively radioresistant G1 phase as compared to evening hours when they are in the radiosensitive G2 phase of the cell cycle. The experimental data to substantiate the findings come from the Bjarnason et al where they studied the nuclear expression of cell cycle proteins by immunohistochemistry as a function of the time of day in oral mucosa biopsies from healthy male human volunteers. An evident circadian rhythm was found for all studied proteins, with the high point of expression for p27 at 6:00 am (early G1-phase marker), p53 at 10:50 am (late G1-phase marker), cyclin-E at 2:50 am (S-phase marker), cyclin-A at 4:00 pm (G2-phase marker), and cyclin-B1 at 9:10 pm (M-phase marker). [34]

Concurrent administration of chemotherapy and smoking have previously been documented to be risk factors for developing mucositis and this effect was reiterated in the analysis of our present study showing a significant increase in the incidence of severe mucositis .[3]There were a statistically significant higher percentage of treatment interruptions in the evening arm 42.0% versus 17.8% when compared to the morning arm. The percentage of patients who had to be taken off the concurrent chemotherapy was also much higher in the

evening arm. Randomised data suggests that treatment interruptions have a very detrimental effect on the locoregional control as well as on the overall survival in head and neck malignancies.[35,36] Thus in our study we were able to demonstrate that evening hours of RT delivery would result in a more severe mucosal toxicity and cause treatment interruptions, negatively impacting the treatment outcome.

V. CONCLUSION

The results of our study substantiate the fact the radiation treatment delivered in the morning hours is associated with lesser incidence of Grade 3 or 4 mucosal toxicities and lesser treatment interruptions. Though large randomized trials are lacking, there is sufficient clinical evidence signifying the importance of utilizing chronotherapy as an important treatment strategy. Since there is no active interventional or pharmacological measure to minimize normal tissue toxicity, chronomodulated radiochemotherapy would be a feasible and cost effective treatment strategy that can be put into clinical practice.

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