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Research Article

COMPARISON OF BIODOSIMETRY IN HEAD AND NECK CANCER PATIENTS TREATED WITH CONCURRENT CHEMO RADIOTHERAPY VERSUS ACCELERATED FRACTIONATION RADIOTHERAPY BY DICENTRIC CHROMOSOMAL ABERRATION ASSAY

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Abstract

Aim: To compare biodosimetry in head and neck cancer patients undergoing concurrent chemoradiotherapy versus accelerated fractionation radiotherapy using the dicentric chromosomal aberration assay. **Methods**: The study was conducted at Maulana Azad Medical College and Lok Nayak Hospital, New Delhi, spanning 12 months, focusing on histopathologically confirmed cases of head and neck cancers. Patients were randomly assigned in a 1:1 ratio to either the CTRT or AFRT treatment groups. Cytogenetic analysis was performed on all 32 patients. Dicentric chromosome yield was measured in blood samples collected before treatment initiation and at various points during radiotherapy (baseline, 7th, and 13th fractions), with assessments conducted one hour post-radiotherapy each time. **Results**: In the chemoradiotherapy group, the mean dicentric chromosome yield per cm² exceeded that of the accelerated radiotherapy group by 7.52% and 16.20% on days 7 and 13 of treatment, respectively. Additionally, the CTRT arm showed a significantly higher overall response rate (p=0.024). **Conclusion**: Our data confirm that there is a benefit of adding chemotherapy with radiotherapy when compared with accelerated radiotherapy in terms of the number of dicentric chromosomal aberrations.

Keywords: Radiation, DNA damage, Cytogenetic analysis, Lymphocyte, Dicentric chromosome.

INTRODUCTION

Head and Neck Cancer (HNC) affects over 650,000 people annually, resulting in 330,000 deaths worldwide. According to GLOBOCAN 2018, it ranks seventh in global cancer incidence at 4.9%.[1] Common risk factors include smoking, alcohol consumption, Human Papillomavirus (HPV) infection (particularly for oropharyngeal cancers), and Epstein-Barr virus (EBV) infection (especially for nasopharyngeal cancers in Asia). [2] Optimal disease control often requires combined approaches involving surgery, radiotherapy (RT), and/or chemotherapy. Treatment strategies may include primary surgery followed by postoperative RT or concurrent chemoradiotherapy, induction chemotherapy, concurrent chemoradiotherapy, or sequential therapy. Locoregionally advanced (stage III/IV) squamous cell carcinomas are particularly associated with a high risk of both local recurrence and distant metastases.[3] Decisions regarding the sequencing and selection of surgery, radiotherapy (RT), and/or chemotherapy require input from multiple disciplines. Factors such as tumor size and extent, patient characteristics (age, comorbidities, treatment preferences), potential functional outcomes and morbidity, organ preservation, and quality of life improvement must all be considered. Among these modalities, RT plays a significant role, exerting its effects by inducing DNA damage, including single and double-strand breaks, DNA-protein crosslinks, and oxidative damage [3].

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In locally advanced non-distant metastatic head and neck cancers most randomized trials show the superiority of concurrent chemoradiotherapy (CTRT) to conventional radiotherapy alone. CTRT represents the most commonly used strategy and is a more attractive approach. The rationale for combining radiotherapy with chemotherapy stems from the concept that the cell killing resulting from the combination is greater than the sum of the two separately and the interaction is described as “synergistic.” One such drug is Cisplatin which acts by the formation of DNA adducts that blocks DNA replication and transcription. These crosslinks represent about 90% of the total DNA damage induced, and it significantly enhances the cell-killing effects of radiation. It is the most commonly used radiosensitizer in the current scenario. According to the meta-analysis of chemotherapy in head and neck cancer (MACH-NC)[4], CTRT has an absolute survival benefit of 6.5% in HNC patients. Similarly, another strategy to intensify radiation treatment is altered fractionation with the same efficacy. The meta-analysis of radiotherapy in carcinomas of the head and neck (MARCH) shows the superiority of altered fractionation over conventional fractionation. Altered fractionation is associated with an absolute benefit of 6.4% in locoregional control and 3.4% in overall survival.[5] Two large randomized trials have shown improved outcomes of accelerated fractionation of 6 fractions per week over conventional fractionation of 5 fractions per week (DAHANCA6/7)[6] and (IAEA)[7]. The rationale behind accelerated fractionation radiotherapy (AFRT) is that by reducing the overall treatment duration, there's less time for tumor cell regeneration during treatment, thereby enhancing

the likelihood of tumor control with a given total dose.[8] Here, in this study, we intend to directly compare both these approaches of treatment intensification in terms of biodosimetry. Radiation biodosimetry involves quantifying absorbed doses using biological material from exposed individuals.[9] Dose estimation relies on cytogenetic analysis, which correlates chromosome aberration frequency with absorbed dose. Ionizing radiation acts by damaging the DNA, causing single and double-strand breaks, DNA-protein crosslinks, and oxidative damage. Although radiation induces many types of chromosomal changes in addition to dicentric chromosomes, dicentric aberrations are considered the most sensitive and specific for assessing radiation dose.[10] Blood lymphocytes are the preferred sample for analyzing aberration frequency, as they exhibit various types of chromosome aberrations, including dicentric chromosomes (DC), centric rings, acentric fragments, and translocations. Among these, dicentric aberrations are the most sensitive and specific for assessing radiation dose, formed through exchange between centromeric pieces of two broken chromosomes, often accompanied by acentric fragments. Multicentric chromosomes may also appear at high doses, while centric rings are less frequently observed. There is a paucity of worldwide data regarding cytogenetic changes induced by radiation in HNC patients receiving concurrent chemo-radiation or accelerated fractionation radiotherapy. In this study, our aim is to directly compare both of these treatment intensification approaches in relation to biodosimetry.

MATERIALS AND METHODS

This comparative observational study was conducted in the Department of Radiation Oncology, Genetic Lab (Department of Pediatrics), and Department of Otorhinolaryngology at Maulana Azad Medical College and Lok Nayak Hospital, New Delhi, spanning 12 months. It involved patients with histopathologically confirmed cases of head and neck cancers.

Inclusion Criteria:

1. Head and neck squamous cell carcinoma confirmed via histopathological examination.
2. Age group-18 to 70 years.
3. Eastern Co-operative Oncology Group (ECOG) ≤2.
4. Complete blood count: absolute neutrophil count>1500/dl, white blood cell count>4000/dl, platelets >100000/dl, hemoglobin>10 mg/dl.
5. Kidney function test: serum creatinine≤1.2, glomerular filtration >60ml/min.
6. Liver function test: serum bilirubin<1.0.
7. Ejection fraction >60% for fitness of treatment.

Exclusion Criteria

1. History of previous malignancy
2. Previous irradiation
3. Distant metastasis
4. Presence of immunodeficiency syndromes
5. Uncontrolled hypertension, diabetes mellitus, chronic kidney disease, hypothyroidism or any other chronic disease despite medication
6. Pregnancy

Sample size: Since it is a pilot study, hence sample size as per convenience (due to laboratory and time constraints) is being taken. A total of 32 patients were analyzed.

Randomization: Patients were randomly allocated in a 1:1 ratio to either the CTRT or AFRT groups. Group assignments were determined using a computer-generated number sequence and concealed in sequentially numbered opaque envelopes to maintain blinding.

METHODOLOGY

All subjects provided written informed consent before enrollment, and a comprehensive proforma capturing relevant demographic and medical history was completed. Cytogenetic analysis was conducted on all 32 patients, measuring dicentric chromosome yield in blood samples collected before treatment initiation and at various points during radiotherapy (baseline, 7th fraction, and 13th fraction), each time one-hour post-radiotherapy. We examined the correlation between dicentric chromosome yield in lymphocytes and individual radiotherapy doses, comparing it with the absorbed radiation doses. Given the absence of similar prior studies, we deemed a ≥ 10% increase in dicentric chromosomes significant. A total blood volume of 20 ml was withdrawn from each patient over the study period. Radiotherapy planning followed a standard protocol using two parallel opposed fields. Delivery was via TELECOBALT Co-60 (THERATRON 780E). The CTRT arm received a daily dose of 200 cGy for 5 days per week, while the AFRT arm received 200 cGy for 6 days per week, totaling 70 Gy. Patients undergoing concurrent chemoradiotherapy received weekly cisplatin chemotherapy at 40mg/m2 before radiotherapy.

Cytogenetic Analysis

0.5 mL of heparinized whole blood was cultured in RPMI 1640 medium in duplicate and then incubated at 37 ºC for 48 hours. Demecolcine (Sigma) was added at a concentration of 0.11µg/mL, 3 hours before cell harvest to arrest cells at the metaphase stage. Cells were collected by centrifugation and subjected to hypotonic treatment (KCl 0.075 mol/L) at 37 °C for 10 minutes. Following this, cells were fixed twice with ice-cold methanol: acetic acid (3:1, v/v) for 5 minutes at 454xg, dropped onto coded slides, and stained with 4% Giemsa (Merck) in phosphate buffer (PBS) (pH 6.8, Merck) for 10 minutes. Stained slides were covered with coverslips and mounted with Entellan®. Chromosomal aberrations were analyzed using a Nikon Eclipse E200 light microscope, with one hundred metaphases examined for each individual at magnifications of 500x and 1250x. Dicentric chromosomes were identified and recorded per hundred metaphases scored, with analyses performed blindly by the same reader.[11]

Statistical analysis

Collected data underwent statistical analysis with SPSS software version 24. Mean group comparisons utilized the Mann-Whitney U Test, while categorical data was compared using the chi-square test. A p-value < 0.05 indicates statistical significance.

RESULTS

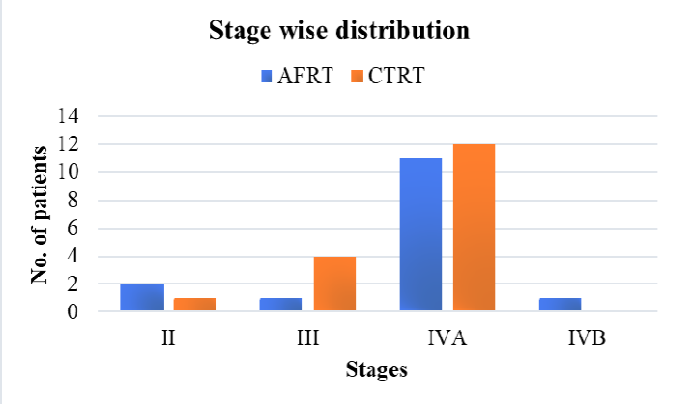
Among the 32 patients, 30 were males. The mean age in the AFRT group was 55.5 years, while in the CTRT group, it was 50.2 years, with no significant difference between the two (p = 0.082). Tobacco use (smoking/chewing) was reported in 22

patients, with no significant difference observed between the two groups in terms of tobacco smoking/chewing distribution (p = 0.197) as depicted in Table 1.

Table 1. Smoking and alcohol distribution among the study groups

	AFRT		CTRT		Total			
Smoking	N	%	N	%	N	%	χ2	p-value
Present	12	37.55	10	31.25	22	68.8%	1.663	0.197
Absent	3	9.4%	7	21.9%	10	31.2%		
Alcohol Consumption								
Present	4	12.5%	4	12.5%	8	25%	0.042	0.838
Absent	11	34.4%	13	40.6%	24	75%		

The most common pathology was a moderately differentiated squamous cell carcinoma (present in 87.5% of the patients). The majority of cases of head and neck cancer were from the oropharynx in both groups 21.9% in each followed by the oral cavity and larynx. The majority of cases in both groups belonged to stage IVA. There was a similar distribution according to the stage in both groups (Graph 1).



Graph 1. Stage-wise distribution

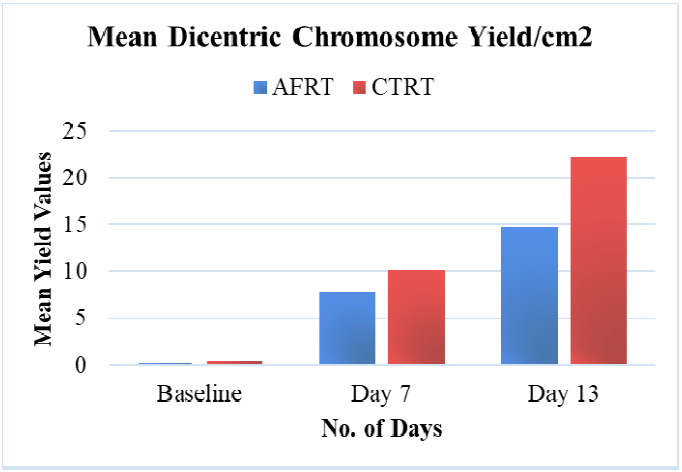
Significant differences were observed between the two groups regarding the mean dicentric chromosome yield per cm² on day 7 (p < 0.001) and day 13 (p < 0.001) of treatment. The mean dicentric chromosome yield per cm² was 7.52% higher in the chemoradiotherapy group compared to the accelerated radiotherapy group on day 7 and 16.20% higher on day 13 of treatment (see Table 2).

Table 2. Comparison of CTRT and AFRT arms in terms of mean dicentric chromosome yield/cm²

Mean dicentric chromosome yield/cm²	AFRT		CTRT		Difference (%)	Mann Whitney U Test	
	Mean	SD	Mean	SD		U-Value	p-value
Pre Treatment	0.26	0.593	0.47	0.62	1%	102.5	0.350
Day 7 of RT	7.86	1.187	10.17	2.743	7.52%	22.00	0.000*
Day 13 of RT	14.66	1.234	22.17	5.886	16.20%	15.00	0.000*

*: statistically significant

At the first follow-up (after six weeks of completion of treatment), 6 patients (20.7%) had a complete response (CR) of local disease in the CTRT arm whereas 1(3.4%) patient had CR in the AFRT arm. Stable disease (SD) was seen in 1 patient (3.4%) in the CTRT arm and 6 patients (20.7%) in the AFRT arm. Partial response was seen in 5 patients (17.2%) in the CTRT arm and 6 patients (20.7%) in the AFRT arm.



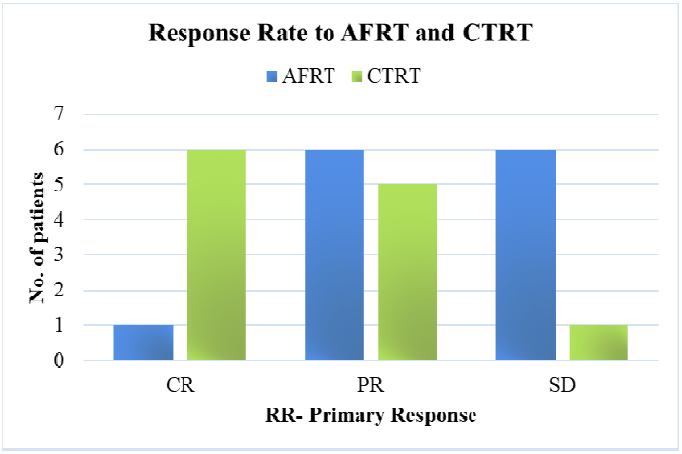
Graph 2. Mean DC yield / cm2 on the Y-axis and days of treatment on the X-axis

The overall response rate was significantly higher in the CTRT arm (p=0.024) as shown in Table 3.

Table 3. RR/primary response to chemoradiotherapy or accelerated radiotherapy by RECIST 1.1

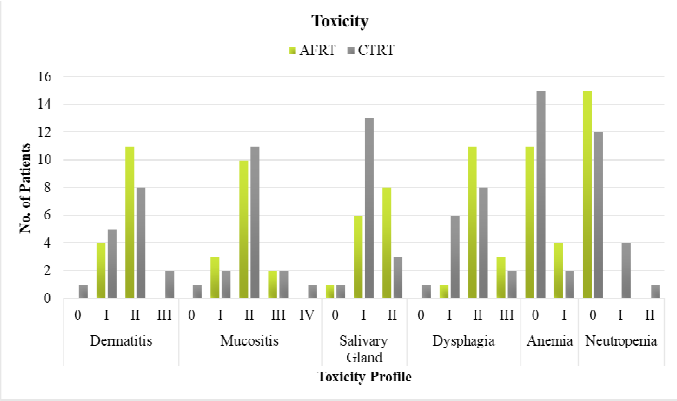
Season	AFRT	CTRT	Chi-square	p-value	S/NS
CR	1(3.4%)	6(20.7%)			
PR	6(20.7%)	5 (17.2%)	11.213	0.024	S
SD	6 (20.7%)	1 (3.4%)			
Progressive	1(3.4%)	3(10.2%)			

S: Significant



Graph 3. RR as primary response to chemoradiotherapy or accelerated radiotherapy by RECIST 1.1

Most of the patients suffered from grade II skin toxicity, which was comparable in both arms. Grade II mucositis was seen in the majority of the patients in both arms and grade IV mucositis was seen in one patient of the CTRT arm. It was statistically not significant. The majority of the patients suffered from grade I salivary gland toxicity. The frequency of the grade II salivary gland was higher in the AFRT arm (53.3%) as compared to the CTRT arm (17.6%). Grade II dysphagia was more commonly seen in the AFRT arm (73.3%) as compared to the CTRT arm (47%) but statistically not significant (p=0.162). Grade I anemia was seen more in the AFRT arm (26.7%) as compared to the CTRT arm (11.8%). Grade I and II neutropenia was seen in the CTRT arm, but statistically not significant (p=0.073) as shown in Graph 4.



Graph 4. Showing acute toxicity between concurrent chemoradiation and accelerated fractionation radiotherapy

Discussion

This study aimed to compare the in vivo dose-response relationship of dicentric chromosome aberration formation in peripheral blood lymphocytes among head and neck cancer patients undergoing either chemoradiotherapy or accelerated radiotherapy. The mean age at presentation was 50.2 years for the chemoradiotherapy group and 55.5 years for the accelerated radiotherapy group, with no significant difference between the two ($p = 0.082$). The most common primary site was the oropharynx (21.9% in each group), followed by the oral cavity (12.5% in the accelerated radiotherapy group and 3.1% in the chemoradiotherapy group) and the larynx (6.2% in each group). Most patients were at stage IVA (37.5%). Alcohol consumption history was reported in 25% of patients, and tobacco use (smoking/chewing) was present in 22 patients. In our study, the mean dicentric chromosomal aberrations on day 7 and day 13 of the AFRT arm were 7.86 and 14.66, and the CTRT arm were 10.17 and 22.17 respectively. So, it has been seen that there is an increase in dicentric chromosomal aberrations formation with an increase in radiation dose. Similar observations were noted in various dosimetric investigations. Tichy et al.[12] observed an increase in the number of dicentric chromosome aberrations in all patients with head and neck tumors following successive radiotherapy fractions. In another study by Roch-Lefevre *et al.* [13], significant post-irradiation elevation in cytogenetic markers was observed in eight patients treated for head and neck cancer. Additionally, Kadam *et al.* [14] reported a dose-dependent rise in cytogenetic damage observed in non-target cells, specifically lymphocytes.

Our study revealed a significant disparity between the two groups regarding the mean dicentric chromosome yield per cm^2 at day 7 of RT ($p < 0.001$) and day 13 of RT ($p < 0.001$). The mean dicentric chromosome yield per cm^2 in the chemoradiotherapy group exceeded that of the accelerated radiotherapy group by 7.52% and 16.20% on days 7 and 13 of RT, respectively. This indicates a substantial impact of concurrent chemoradiotherapy on dicentric yield compared to accelerated fractionation radiotherapy alone. In our study, the locoregional response in the CTRT arm of our study is better than the AFRT arm whereas in a similar trial by Gupta *et al.* [15], who compared CTRT 66Gy/33#/5.5 weeks with concurrent weekly Cisplatin 30 mg/m^2 vs AFRT 66Gy/33#/5.5 weeks (6#/week) and found comparable response rates (82.1% vs 78.8% for local response rates at 6 weeks). This can be explained due to the small number of patients in our study and the majority of the patients belonged to stage IVA/IV B (75% vs 58% in Gupta et al's study).[15] The correlation of dicentric chromosome aberration formation in days 0, 7, and 13 to the overall response rates in both arms were not statistically significant. No similar study has been done previously. However, this should be tested in a trial with a larger sample size to confirm the finding. We observed that the toxicities were comparable in both arms. In the majority of the patients, the onset of acute toxicities occurred 2-3 weeks from the start of the radiation treatment. Most of the patients suffered from grade II skin toxicity, which was comparable in both arms. Grade II mucositis was seen in the majority of the patients in both arms and grade IV mucositis was seen in 1 patient in the CTRT arm. The patients in our study tolerated the treatment well with acceptable toxicity. In the trial by Gupta et al.[15], most of the patients had grade III dermatitis which was comparable in both arms. In the CTRT arm, Grade IV dermatitis incidence was notably higher at 32.8% compared to 12.1% in the other arm ($p=0.02$). Most patients experienced Grade II/III mucositis, with significantly higher Grade III mucositis observed in the CTRT arm (62.7% vs. 40.9%, $p=0.015$).

Our study directly compares concurrent chemoradiotherapy with accelerated fractionation radiotherapy in terms of dicentric chromosomal aberrations with biodosimetry. This is the first study of this kind. Even studies with the head-on clinical comparison of these arms are limited in the literature. We have tried to find a correlation between dicentric chromosomal aberrations formation and response to treatment in these arms. Our study is conducted with robust methodology and the results have been described in terms of the dicentric assay. Since one investigator conducted this study so interviewer bias is minimal. Also, strict inclusion and exclusion criteria were followed to minimize selection bias.
The major limitation of the study was a smaller sample size and the limited duration of the study and follow-up. The radiotherapy was delivered with 2D conventional techniques. A further larger study with a larger sample size, longer follow up and use of modern radiotherapy techniques will provide more insights into biodosimetry and its application in clinics.

Conclusion

From our study, we conclude that

- There is a dose-dependent increase in the yield of dicentric chromosomes in human lymphocytes of head and neck cancer patients receiving concurrent chemoradiotherapy or accelerated radiotherapy.
- Chemotherapy significantly increases dicentric chromosome yield per cm^2 compared to radiotherapy alone.
- There was no statistically significant correlation found between the formation of dicentric chromosomal aberrations and response rates in either group.
- The overall response rate was significantly higher in the CTRT arm.
- The dicentric chromosomal aberration assay is a recognized biomarker for assessing chromosomal damage in cytogenetic biodosimetry.

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