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Original Research Article

EVALUATION OF TOXICITY PROFILE AND PROGNOSIS OF TREATMENT OF HPV ASSOCIATED HEAD AND NECK SQUAMOUS CELL CARCINOMA (HNSCC), WITH CHEMORADIOTHERAPY.

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Abstract:
Head and Neck cancer is generally referred as squamous cell carcinoma of Head and Neck and are located at the oral or nasal cavity. It has been one of the leading cause of death around the globe and has significantly increased epidemiological growth and rate of mortality over the last three decades. A significant subset of the 600,000 annual cases of HNSCC includes approximately 85,000 HPV associated (oropharyngeal) tumors, which means that the head and neck region is the second most common HPV+ tumor site. In 90% of the HPV associated tumors, HPV16 detection can be seen. The objective of the proposed study is to evaluate the effects of chemoradiotherapy and its toxicity profile for the treatment of HPV negative and HPV positive carcinomas. In a case-control study, 100 patients were enrolled and grouped into two groups with HPV negative and HPV positive carcinogenic tumor in the ratio of 50:50. All the patients are exposed to the treatment with concurrent chemoradiotherapy and radiotherapy. From the study, it can be concluded that HPV- positive tumors have less cumulative exposure to multiple risk factors (tobacco chewing, alcohol, smoking). The association of tumor HPV status with treatment response observed in our study showed a trend of better treatment outcome, consistent in the design and analysis of current and future clinical trials of treatments for head and neck cancer patients.

Keywords: Head and Neck Carcinoma, chemoradiotherapy, Human Papilloma Virus (HPV), Smoking, alcohol.

Introduction:

Cancer of head and neck includes all cancers arising from the upper aerodigestive tract and typically referred to squamous cell carcinoma of head and neck. Head and neck cancers comprise a group of cancers that are anatomically located in the oral cavity, the oropharynx, the nasal cavity, paranasal sinuses, the nasopharynx, the hypopharynx and the larynx[1].The incidence of Head and

Neck Squamous Cell Carcinoma (HNSCC) has been gradually increasing over the last 3 decades. It is 7th leading cause of cancer by incidence and 6th leading cause of cancer mortality in the world. It is likely that approximately 600,000 new cases worldwide will arise this year, and that only 40-50% survive for 5 year[2]. Treatment has always remained a big challenge for this deadly disease, hence "Prevention is better than cure" holds true for this disease[3].

Males are affected significantly more than females with a ratio ranging from 2:1 to 4:1. The incidence rate in males exceeds 20 per 100,000 in regions of France, Hong Kong, the Indian subcontinent, central and eastern Europe, Spain, Italy, Brazil, and among African Americans in the United States. Oral cavity and tongue cancers are more common in the Indian subcontinent; nasopharyngeal cancer is more common in Hong Kong, and pharyngeal and/or laryngeal cancers are more common in other populations; these factors contribute disproportionately to the overall cancer burden in this Asian countries[4].

HNSCC develops mostly via one of the two primary carcinogenic routes, namely the chemical carcinogenesis through exposure to tobacco and alcohol abuse, which are known to be synergistic, and high-risk human papillomavirus (HPV) induced carcinogenesis.[5] Besides the exogenous risk factors, certain inherited disorders such as Fanconi anemia show more susceptibility to HNSCC. Interestingly, epidemiological studies demonstrated a decrease or stabilization of laryngeal, hypopharyngeal and oral cavity cancers. This decrease is ascribed to the gradual decrease of the use of primary exogenous risk factors (smoking and alcohol).

The most common high-risk HPV types are HPV16, HPV18, HPV31, HPV33, and HPV35. These types are estimated to cause about 5% of the cancer burden worldwide, which includes 99% of cervical cancers, 25%–60% of head and neck cancers, 70% of vaginal cancers, 88% of anal cancers, 43% vulvar and 50% of penile cancer. A significant subset of the 600,000 annual cases of HNSCC includes approximately 85,000 HPV associated (oropharyngeal) tumors, which means that the head and neck region is the second most common HPV+ tumor site[6].

HPV-positive tumors are characterized by high expression of p16INK4A. Moreover, because transcription of the E7 oncogene is required for p16INK4A upregulation, it has been suggested that carcinomas overexpressing p16INK4A represent those tumors in which HPV has been involved in the carcinogenic process[7]. Thus there is good evidence that p16INK4A positivity may be regarded as a biomarker for tumours harbouring clinically and ontogenetically relevant HPV infections.[8]

Objective:

The aim of the present study to know the prognostic significance of HPV Infection in Head and Neck Carcinoma patient undergoing Chemoradiation. Toxicity Profile of HPV Positive vs HPV Negative Head and Neck Cancer Patients undergoing chemoradiotherapy (CT-RT)

Material and Method:

Statistical Analysis:

The actuarial values of the endpoints were evaluated by the Kaplan-Meier analysis and compared using the log-rank test

A multivariate Cox proportional hazard analysis was used to evaluate prognostic parameters and treatment with respect to the risk of locoregional. The data were analyzed using the software GraphPad Prism (7.03). The results were expressed as Mean ± Standard Deviation (SD). Data were analyzed using chi-square test, unpaired t-test, $P<0.05$ was considered as significant.

Selection of subjects:

A case-control study was conducted at King George's Medical University, KGMU), Lucknow, India. Cases suffering from Head and Neck cancer and visiting the OPD of Radiotherapy Department of KGMU from July 2016 to July 2017 were included in this study. The cases had squamous cell carcinoma of the Head and Neck, which was confirmed by histopathological examinations and were advised for Immunohistochemistry for P16+(Positivity)/P16-(Negativity) and put on combination treatment of chemoradiotherapy (CT-RT). Informed consent of the cases was obtained before inclusion in the study. All study subjects completed a questionnaire covering medical, residential and occupational history.

Inclusion Critera[9]:

- Previously untreated histologically proven squamous cell carcinoma of Head and Neck.
- Karnofsky Performance Status (KPS) 70 and above.
- Adequate bone marrow reserve WBC >4000/cu. mm, platelet count > 1 lac/ cu. mm.
- Age 20-70 year.
- All Patients should belong to a same ethnic group of North India.
- Normal liver, renal, cardiac, lung function.
- Carcinoma cases of Oral Cavity, Oropharynx, Hypopharynx, and Larynx.
- Consenting Patient.
- History of exposure to Tobacco in the form of chewing or Smoking and Alcohol.

Exclusion Criteria:-

- Patients having any concurrent illness with Head and Neck Cancer or history of prior malignancy.
- Prior treatment of Chemotherapy, Radiotherapy or Chemoradiation.
- KPS less than 70.
- Patients who defaulted during the treatment.
- Patients whose expected survival was not more than 6 months
- Metastasis.
- Post-operative patient.
- Nonconsenting Patient.

Treatment

- The presenting history & chief complaints were taken with a special inquiry about predisposing factors.
- General & systemic examination.
- Local examination including oral cavity proper, D/L copy & neck examination.
- Routine investigations like Hemogram, LFT, KFT, cardiac evaluation, chest X-ray.
- Special investigations like CT scan.
- TNM staging (according to AJCC 2010)[10].
- Treatment consisted of concurrent chemoradiotherapy (Total dose of 70Gy in 35#, in 7 weeks with concurrent cisplatin 35 mg/m2 weekly)
- The response was categorized as complete response, partial response, stable disease or progressive disease based on RECIST assessment criteria.
- Toxicity will be graded according to RTOG Toxicity criteria.
- Radiotherapy treatment was given by Co60 (Bhabhatron BARC Mumbai/ Theratron 780E, Ottawa, Canada).
- Planning of patient will be done on x-ray simulix evolution simulator, Siemens.

Identification of P16+(Positivity) by IHC of Histopathology Sample[11]-

Patients and Tissues-

All Patients in this group received primary conventionally fractionated radiotherapy (70Gy in 35 fractions, 5 fractions/wk) as the only treatment. Routine paraffin-embedded, formalin-fixed pretreatment tumor tissues were sent to the pathology for histopathology and IHC marker for P16 positivity. The patients gave

written informed consent. Both the main study and the tumor tissue analyses were approved by the ethical committee of the institution.

Evaluation of p16INK4A Expression[12]-

Immunohistochemistry for p16INK4A expression was performed on a BenchMark XT autostainer (Ventana Medical Systems, Illkirch, France) according to the manufacturer's recommendations. Briefly, paraffin sections were cut at 5 micrometers on Superfrost plus charged glass slides (Menzel-Glaser, Braunschweig, Germany), heated at 60°Cfor 1 hour, and deparaffinized in the instrument with EZ prep solution (Ventana Medical Systems). Heat-induced antigen retrieval was carried out using Cell Conditioning 1 solution (CC1, VentanaMedical Systems). p16INK4A was detected by incubating sections with antibody clone JC8 diluted 1:25 for 32minutes. This is a mouse monoclonal immunoglobulin G2a antibody raised against full-length human p16, particularly suitable for use on formalin-fixed, paraffin-embedded sections. Its specificity has been confirmed by Western blotting.

Specific reactions were detected using ultraView Universal DAB Detection Kit (Ventana Medical Systems), and the slides were counterstained with hematoxylin. Sections of p16INK4A-positive Tonsillar carcinoma were used as positive controls. p16INK4A expression was associated with distinct diffuse nuclear and cytoplasmic staining of epithelial cells. Tumors were classified dichotomously as either p16INK4A-positive (strong, diffuse nuclear and cytoplasmic staining in 10% of carcinoma cells) or negative.

Detection of HPV Infection-

To demonstrate the correlation between HPV16 infection and tumor cell expression of p16INK4A, a 5-micrometer formalin-fixed, paraffin-embedded tumor sections were deparaffinized and treated with Dako Target Retrieval Solution (Dako, Glostrup, Denmark). Sections were digested with proteinase K and rinsed in deionized water. Biotinylated HPV16-type-specific DNA probe (Dako, code Y1407) in hybridization mixture was used according to the manufacturer's recommendations. Denaturation and hybridization were carried out using a Hybridizer Instrument (Dako). Sections were denatured for 5 minutes at 92°C and hybridized overnight at 37°C. After stringent washing, the hybridized probe was detected using the tyramide-based GenPoint Catalyzed Signal Amplification System (Dako, code K0620), with the consecutive application of primary peroxidase-conjugated streptavidin, biotinyl tyramide, and secondary peroxidase-conjugated streptavidin. Signals were visualized with the chromogen 3,3-diaminobenzidine. Positive and negative control sections were included.

Treatment and treatment response[13]:-

Patients were subjected to concurrent chemotherapy with radiotherapy (CT-RT). Chemotherapy included administration of 35mg/m2 of cisplatin once every week for 7 weeks along with 70 Gray of radiation @ 200 cGy/Fraction; 5 Fractions/week.

For studying the treatment response, specific monitoring of the patients was done by thorough serial inspection of the head and neck region - looking for disease recurrence as well as second primary tumors. Investigations in the form of CT scan or D/L copy were done as and when required.

On the basis of RECIST criteria, the treatment outcome was divided into the following three categories:

Response Evaluation Criteria in Solid Tumors (RECIST):-

- **Complete Response (CR):** Disappearance of all target lesions.

- **Partial Response (PR):** At least a 30% decrease in the sum of the LD of the target lesion, taking as reference the baseline sum LD.
- **Stable Disease (SD):** Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started.
- **Progressive Disease (PD):** At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions.

Those exhibiting CR & PR were categorized as responders while patients exhibiting SD & PD were classified as non-responders.

Result and Discussion:

Detection of HPV16 Carcinoma by tumor cell expression of p16INK4A

Carcinomas were classified as HPV16 positive when a discrete signal was seen localized to the nuclei of tumor cells. Signals were categorized as either punctuate and/or diffuse, representing integrated and episomal virus, respectively. There is a strong correlation between HPV16 DNA detection and p16INK4A expression is established by this study. p16 may not be a specific marker of HPV infection, it can provide important prognostic information and future therapies aimed at targeting this pathway may be effective in treating p16-positive, HPV-negative squamous cell carcinoma (SCC)

Subjects:

100 cases were registered. This formed the study group made of the equal number of Group I (HPV+) and Group II(HPV-). The cases had squamous cell carcinoma of the oral cavity, oropharynx, larynx, and hypopharynx.

Table-1: Age-wise distribution of patients in the groups

Age in years		Group I (n=50)		Group II (n=50)	
		No.	%	No.	%
<40		17	34.0	4	8.0
41-50		20	44.0	19	38.0
51-60		11	22.0	16	32.0
>60		2	4.0	11	22.0
Total	Male	35	70.0	42	84.0
	Female	15	30.0	8	16.0

Table-2: Site-wise distribution of patients in the groups

Site of a tumour	Group I (n=50)		Group II (n=50)	
	No.	%	No.	%
Oral cavity	5	10.0	17	34.0
Oropharynx	43	86.0	21	42.0
Hypopharynx	2	4.0	9	18.0
Nasal cavity+ Nasopharynx	0	-	3	6.0

Table-3: Association of risk factors between the groups

Risk factors	Group I (n=50)		Group II (n=50)	
	No.	%	No.	%
Tobacco				
Yes	17	34.0	23	46.0
No	33	66.0	27	54.0
Smoking				
Yes	25	50.0	34	68.0
No	25	50.0	16	32.0
Alcohol				
Yes	19	38.0	18	36.0
No	31	62.0	32	64.0
Smoking+Tobacco				
Yes	10	20.0	15	30.0
No	40	80.0	35	70.0
Smoking+Alcohol				
Yes	13	26.0	15	30.0
No	37	74.0	35	70.0
Tobacco+Alcohol				
Yes	5	10.0	7	14.0
No	45	90.0	43	86.0
All				
Yes	5	10.0	6	12.0
No	45	90.0	44	88.0
None				
Yes	12	24.0	6	12.0
No	38	76.0	44	88.0

HPV positive tumors were more likely than HPV negative tumors to arise from the oropharynx, to be poorly differentiated, and to have basaloid features. Additionally, patients with HPV-positive tumors have less cumulative exposure to multiple risk factors (tobacco chewing, alcohol, smoking).

Table-4: Distribution of study population according to TNM staging between the groups

TNM staging	Group I (n=50)		Group II (n=50)	
	No.	%	No.	%
T stage				
T2	3	6.0	2	4.0
T3	14	28.0	13	26.0
T4A	28	56.0	30	60.0
T4B	5	10.0	5	10.0
N stage				
N0	2	4.0	4	8.0
N1	19	38.0	18	36.0
N2A	7	14.0	5	10.0
N2B	9	18.0	8	16.0
N2C	12	24.0	12	24.0
N3	1	2.0	3	6.0
M stage				
M0	0	0.0	0	0.0

Table 5: Distribution of study population according to group staging between the groups

Group staging	Group I (n=50)		Group II (n=50)	
	No.	%	No.	%
Stage III	12	24.0	9	18.0
Stage IVA	24	48.0	30	60.0
Stage IVB	14	28.0	11	22.0

Table-6: Distribution of study population according to neck node level between the groups

Neck node level	Group I (n=45)		Group II (n=41)	
	No.	%	No.	%
Level IB	10	20.8	9	19.6
Level II	27	56.2	25	54.3
Level III	11	22.9	12	26.1

Table-7: Distribution of response between the groups

Response	Group I (n=45)		Group II (n=41)		p-value
	No.	%	No.	%	
CR	34	68.0	27	54.0	0.04
PR	7	14.0	11	22.0	
SD	6	12.0	6	12.0	
PD	3	6.0	6	12.0	

Table-8: Comparison of the study population according to Toxicity profile between the groups

Toxicity profile	Group I (n=50)		Group II (n=50)		p-value
	No.	%	No.	%	
Skin reactions					
Grade I	21	42.0	21	42.0	0.06
Grade II	21	42.0	20	80.0	
Grade III	7	14.0	8	16.0	
Grade IV	1	2.0	1	2.0	
Mucosal					
Grade I	20	40.0	20	40.0	0.11
Grade II	22	44.0	21	42.0	
Grade III	6	12.0	8	16.0	
Grade IV	2	4.0	1	2.0	
Xerostomia					
Grade I	26	52.0	23	46.0	0.14
Grade II	9	18.0	10	20.0	
Grade III	15	30.0	17	34.0	
Dysphagia					
Grade I	20	40.0	17	34.00	0.09
Grade II	10	20.0	9	18.0	
Grade III	15	30.0	17	34.0	
Grade IV	5	10.0	7	14.0	
Systematic					
Hematological	36	72.0	24	68.0	0.13
Normal	5	10.	6	12.0	
Grade I	6	12.0	7	14.0	
Grade II	2	4.0	2	4.0	
Grade III	1	2.0	1	2.0	
Grade IV	0	0.0	0	0.0	
Vomiting	13	26.0	14	28.0	0.82
Myelosupression	3	6.0	4	29.0	0.001*

*Significant

From the study, it is evident that there is not much difference in the toxicity profiles between the study population of Group-I (HPV Positive) and Group-II (HPV negative). Only Group-II showed higher incidences of decreased bone marrow activity. The study showed a trend that if multiple risk factors are present, treatment outcome may be grave (particularly in oropharyngeal tumor) even if HPV status is positive.

Table-9: Comparison of treatment time between the groups

Groups	Treatment time in days
Group I	53.68±5.58
Group II	55.04±5.63
p-value	0.26*

Data are expressed as Mean ± SD

There is significant difference found between the time of treatment of both the groups.

Table-10: Distribution of disease status at follow-ups between the groups

Follow-up	Group I		Group II		p-value
	No.	%	No.	%	
One month	**(n=49)**		**(n=50)**		
Present	12	24.5	25	50.0	0.009*
Absent	37	75.5	25	50.0	
Three month	**(n=47)**		**(n=46)**		
Present	11	23.4	18	39.1	0.10
Absent	36	76.6	28	60.9	

the study also showed a trend towards improved treatment outcome for patients with HPV positive tumors.

Table-11: Comparison of treatment time with a response

Response	Group I	Group II	p-value
CR	51.67±3.59	51.88±2.72	0.89
PR	59.00±7.18	59.40±6.02	0.92
SD	61.50±4.35	60.66±4.08	0.56
PD	-	65.00±0.00	-

In the analysis of association of tumor p16INK4A expression with therapeutic response in a prospectively collected cohort of patients with HNSCC treated with radiotherapy alone, showed that tumor cell expression of p16INK4A in this group of patients is associated with markedly improved treatment response and survival and that p16INK4A expression status is an independent prognostic factor for both outcomes[14].

Conclusion:

From this study, it can be concluded that the current treatment options are still suboptimal for both groups of HNSCC patients due to high resistance and recurrence (HPV´) and high toxicity (HPV´ and HPV+) issues[11]. It should be kept in mind that the slopes of clinical dose-response curves indicate that enhancement of dose of RT by just 10% will increase tumor control rates by 5%–30% depending on tumor sites and current control rates. Since it is not possible to increase the total radiation dose to the entire tumor due to high levels of normal tissue toxicity, novel therapeutic approaches are needed.

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