CHAPTER-3

MATERIALS AND METHODS

3.1. Study design

Prospective Observational Cohort Study.

This study was performed at Cancer Research Institute (CRI), Swami Rama Himalayan University (SRHU),Dehradun.

3.2. Study population

Patients starting treatment for HNSCC at CRI, SRHU, Dehradun.

3.3. Inclusion and exclusion criteria

3.3.A. Inclusion criteria-

Patients with previously untreated and diagnosed HNSCC planned for treatment at this center.

3.3.B. Exclusion criteria-

1. Previously treated for HNSCC with surgery, RT, or CRT

2. Distant metastasis

3. Patient not willing to participate

4. Patients with skin, oesophagus or nasopharynx carcinoma or metastasis at any other primary site

5. Patients younger than 18 years

3.4. Recruitment

Ethics committee study permission number SRHU/ETHICS/2018/115. The patient once diagnosed with HNSCC was planned for elective (curative or palliative) treatment. The oncological treatment entailed surgery, RT, or CRT, i.e., single modality or multi-modality. The patients planned for oncological treatment were evaluated for inclusion in this study.

They were approached and informed written consent (Annexure-1) was taken before enrollment in the study. Once enrolled, the evaluation commenced. The patients' data was collected up tominimum 6 months following completion of the planned treatment to evaluate for disease outcome.

3.5. Evaluation

The patients were evaluated according to the study proforma (Annexure-2) at various points of time. Patients in the study were evaluated for nutritional status and systemic immunity before the commencement of treatment, during treatment, and at completion of planned treatment. The disease status and overall and progression free survival were assessed upto 6 months (at end of planned treatment, 6 weeks, 3months, and 6 months) of finishing the planned oncological treatment.

3.6. The data was collected for the following variables

a. Baseline data regarding patients' demographics, cancer disease details and planned treatments

b. ECOG (Eastern Cooperative Oncology Group) performance status

c. Nutritional status—pre-treatment weight loss, weight, BMI, SGA score, MUAC, hemoglobin, Bitot spots

d. Systemic immunity—peripheral TLC (Total Leukocyte Count), DLC (Differential Leukocyte Count), NLR (Neutrophil/Lymphocyte Ratio).

e. Treatment toxicity and complications, discontinuation and delay of treatment

f. Disease status and overall and disease specific survival at 6 months following completion of treatment.

3.7. The primary end points of the study

1. Nutritional status and systemic Immunity before and after treatment.

2. Disease status (progression free survival), overall survival and disease specific survival at 6 months of completion of treatment.

Two patient groups were created based on nodal metastasis -

a. N- patients without nodal metastasis at the time of presentation

b. N+ patients with nodal metastasis at the time of presentation

The data was documented in the proforma attached and anonymized.

3.8. Sampling technique

A cohort of consecutive patients diagnosed with HNCC starting treatment at CRI were screened for enrollment in this study till the sample size was reached.

3.9. Sample size calculation and sample size

Taking the correlation coefficient between nutritional status and systemic immunity as 0.5 (unknown), α at 0.05, β at 90%, design effect 1.5, the sample size was calculated as 55 in N- group and 55 in N+ group. Taking the loss to follow up rate as 20%, the final sample size was 66 in each group (**total 132 patients**).

3.10. Statistical Analysis

MS Excel 2010 was used for data entry, SPSS software version 22 was used to conduct the statistical analysis. The difference of data sets from normality was tested using one-sample Kolmogorov-Smirnov Test. Parametric tests were used for normally distributed data, non-parametric tests for non-normally distributed data and Chi square test for the categorical data using. Level of significance was taken as $p<0.05$.

Categorical variables were analyzed by the Pearson chi-square test. Paired-sample T test and unpaired Student t test was used to test for differences in mean. Non-parametric tests used were “Related-Samples Wilcoxon Signed-Rank Test, Related-samples Marginal Homogeneity Test, Independent-Samples Mann-Whitney U Test, and Independent-Samples Kruskal-Wallis Test”. Cochran's and Mantel-Haenszel Statistic was used to calculate the Risk Ratio (RR). Correlation was tested with Pearson's coefficient and Spearman’s coefficient. Multivariate analysis was performed using the Multi-nominal Logistic Regression model. ROCs were used to get cut-off values and their sensitivity and specificity to predict the outcomes under study.

Survival curves were generated using “Kaplan-Meier method and Cox-Regression model” and used to calculate the Hazard Ratio (HR) for outcomes i.e, Progression Free Survival (PFS) and Overall Survival (OS). Using the variable significantly associated with the outcome, RR and ROC cut-off values, novel risk stratification models were developed to predict outcomes such as failure to complete planned treatment, disease recurrence 6months, and death at 6 months.

3.11. Definitions, variables and methods

1. Head and Neck Squamous Cell carcinoma (HNSCC)-

HNSCC included primary tumor arising at the subsites-

a. the oral cavity,
b. the sinuses,
c. the oropharynx,
d. the hypopharynx, and
e. the larynx

2. Treatment for HNSCC-

Either single modality with surgery, radiation therapy or chemotherapy, or combined modality. The treatment may be with a curative intent or palliative intent.

3. Metastatic disease-

Presence of distant metastasis, regardless of the T and N stage was taken as metastatic disease.

4. TNM staging-

The American Joint Committee on Cancer, edition 8 schema wasused for each subsite (Annexure-3).

5. ECOG Performance status-

Table 3.1 The performance status of the patient was assessed using standard Eastern Cooperative Oncology Group grading system.

Grade	Activity
0	"Fully active, able to carry on all pre-disease performance without restriction"
1	"Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work"
2	"Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours"
3	"Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours"
4	"Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair"
5	Dead

6. Weight-

The weight of the patient was measured using Salter machine (unit 9069 PK3R-2914, d=0.1kg) in standing position.

7. SGA score-

The Subjective Global Assessment of nutritional status was performed using the standardized scale for malnutrition screening (Annexure-4).

8. Mid Upper Arm Circumference (MUAC)-

The MUAC is circumference of mid point (shoulder to tip of elbow) of left upper arm in centimeter (for muscle mass).

Table 3.2 Cut-off points for MUAC.[93]

	MUAC (in cm)	**Malnutrition**
Adults	17-21	Moderate
	18-21 along with recent loss of weight	Moderate
	<17	Severe
	<18 cm along with recent loss of weight	Severe

9. Body Mass Index (BMI)-

BMI formula: Weight in kilogram divided by the height in meter squared (kg/m^2) (for body fat mass).

Table 3.3 BMI cutoffs for nutritional status used.[93]

BMI(kg/m^2) cut-off	**Nutritional status**
>40	"Very obese"
30 to40	"Obese"
25 to 29.9	"Overweight"
18.5 to **24.9**	**"Normal"**
17 to 18.49	"Mild chronic energy deficiency"
16 to 16.9	"Moderate chronic energy deficiency"
<16.0	"Severe chronic energy deficiency"

10. Anemia-

Venous blood (10 mL) was obtained using standard phlebotomy and collected in an EDTA tube. LH 750 Coulter machine (Beckman Coulter) used volume conductivity and scatter method for obtaining the hemoglobin and hematocrit value.

Table 3.4 WHO hemoglobin levels to diagnose anemia (g/dL).

Group	No anemia	Mild	Moderate	Severe
Non pregnant female	>= 12	11- 11.9	8- 10.9	< 8
Male	>= 13	11- 12.9	8- 10.9	< 8

11. Peripheral total and differential counts-

Venous blood (10 mL) was obtained using standard phlebotomy and collected in EDTA tube. LH 750 Coulter machine (Beckman Coulter) used volume conductivity and scatter method for obtaining the TLC and the absolute DLC in addition to Leishman's stained peripheral blood smear method.

12. Treatment toxicity and complications-

All complications and toxicities related to treatment (surgery, radiation therapy, and chemotherapy) were looked for prospectively and documented in the case recording form.

13. Completion of planned treatment-

Completion of planned treatment wastaken as patient undergoing planned surgery and/or planned fractions of radiation therapy and/ or planned dose of chemotherapy without any delay or interruption.

14. Delay and interruption in completion of planned treatment-

Delay of >6 weeks between planned surgery and adjuvant RT, due to any cause, was taken as delay in completion of planned treatment. Delay in completion of planned RT once started was taken as interruption of planned treatment.

15. Discontinuation/default of planned treatment-

Unable to complete planned treatment, due to any cause, was taken as discontinuation in completion of planned treatment – default of treatment.

16. Disease progression-

Clinical or radiological progression of the primary tumor, regional nodes or appearance of distant metastasis as assessed from end of treatment upto 6 months after completion of treatment were taken as disease progression.

17. Disease free-

Clinical or radiological absence of disease at the primary tumor site, regional nodes and absence of distant metastasis as assessed from end of treatment upto 6 months after completion of treatment were taken as disease free status.

18. Stable disease-

Clinical or radiological absence of progression at the primary tumor, regional nodes and absence of distant metastasis as assessed from end of treatment upto 6 months after completion of treatment were taken as stable disease.

19. Overall / Disease specific survival-

The length of time from the completion of treatment, that patients are still alive. Any death due to disease was taken for disease specific survival. This was studied at 6 months of completion of treatment for each patient.