CHAPTER-1

INTRODUCTION

1.1 Epidemiology of HNSCC

Head and neck squamous cellc arcinoma (HNSCC) arise from the mucosal epithelium of oral cavity, larynx and the pharynx. Worldwide, HNSCC was the 6th commonest malignancy; there were 890,000 newly diagnosed cases and 450,000 mortalities in 2018 reported in literature.[1,2] It is the commonest cancer in the developing countries, particularly in Southeast Asian countries.[3-5] HNSCCis an important problem in India, constituting nearly 1/3rd cancer cases ascompared to only about 4–5% in developed countries. The age standardized rates for HNSCC globally are shown in Figure 1.1. In India, HNSCC accounts for 1/4thmale cancers but only 1/10th female cancers. The high rates of HNSCC in India are largely due to widespread use of oral tobacco, areca nut tobacco smoking and alcohol.[6] As noted by Indian Council of Medical Research (ICMR) Atlas, about 0.2 to 0.25 million new HNSCC patients are diagnosed in India every year.[5] As of date, none of the screening strategies for HNSCC have proven helpful in India and only carefully performed physical examination seems to be the primary strategy for early detection. Also, there is a lack of adequate awareness among people with regards to high risk lifestyle choices and early symptoms of HNSCC. Due to all these reasons majority patients present in locally advanced Stage III/IV HNSCCin India.

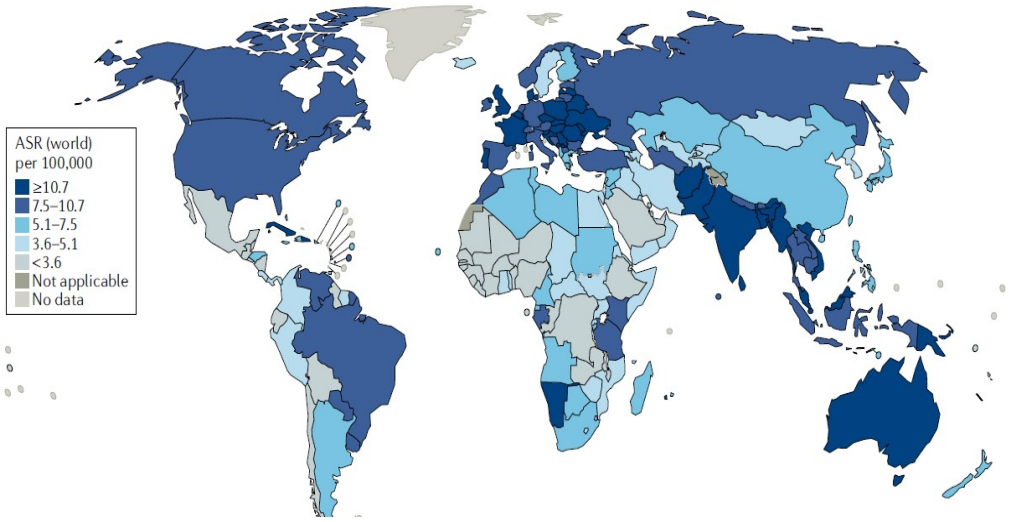

Figure1.1 World map depicting the Global incidence of Head and Neck Squamous Cell Cancer andestimated age standardized rates (ASRs).(GLOBOCAN 2018[1])

There are many risk factors for HNSCC, these are tobacco consumption (oral or smoking), alcohol use, contact with environmental pollutants and viral infections (high-riskhuman papillomavirus (HPV) and Ebstein-Barr Virus (EBV). It is noteworthy that many risk factors show cultural, geographical and habitual prevalence. Alcohol and tobacco use are thefactors occurring most extensively geographically and are generally associated with cancer of oral cavity and larynx. In contrast, pharyngeal cancer is more and more attributed to high-risk HPV infection, mainly HPV-16.[7] It has been noted that heavy usage of tobacco and alcohol both has more than 35 fold greater risk for HNSCC.[8] The consumption of products like areca nut, betel quid or oral tobacco are found to be associated with a high prevalence of oral cavity carcinoma in South-Asian countries like India, Taiwan and some areas in China.[9] Additional risk factors are advancing age, poor oral and dental hygiene and also dietdeficient invegetables.[10,11] Slaughter et al. introduced the 'field cancerization' model in HNSCC; it suggests the carcinogens damage large anatomical fields that are exposed to it.[12] In tobacco-related HNSCC, this may increase with age of the patient.[13,14] A model of systematic histological evolutionin HNSCC is depicted in Figure 1.2.

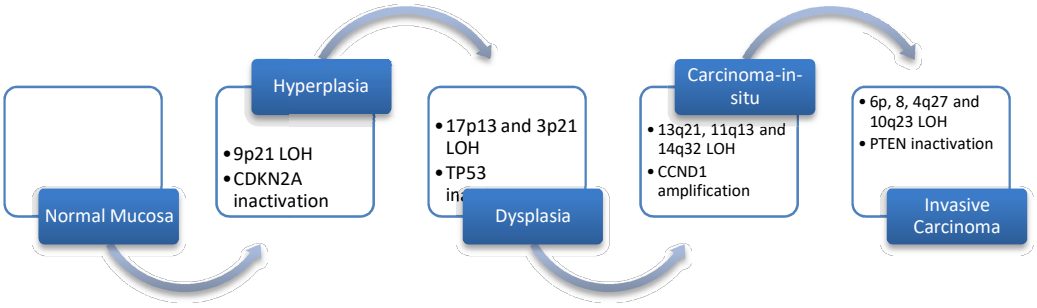

Figure 1.2Genetic events in HNSCC. (This figure depicts the evolution of HNSCC and associated Key Genetic events. Normal mucosa undergoes epithelial cell hyperplasia to dysplasia to carcinoma in situ to invasive carcinoma. Definite genetic events augmented at each phase of evolution are shown.[15])

1.2 Overview of treatment in HNSCC

For HNSCC, multimodal treatment consisting of surgery, radiotherapy (RT) or chemo-radiotherapy (CRT) is advised, depending of the stage and poor prognostic factors. Cancer of the subsite 'oral cavity' usually requires surgery (with or without induction chemotherapy) which is followed by adjuvant RT/CRT depending on the tumor (T) stage, presence of nodal metastasis, resection margins, depth of invasion (DOI) of primary tumor, presence of lymphovascular invasion (LVI), peri-neural invasion (PNI) or extracapsular nodal extension (ENE). For cancer of pharynx or larynx, the primary treatment approach has been CRT. Exempting early oral cavity cancer (single modality treatment-surgery alone) or early larynx cancers (surgery/RTonly), a majority of HNSCC patients' treatment entails a multimodality approach. Lee et al. have noted cure rates of >80% in HNSCC patients with small primary and no or single nodal metastasis with single modality approach (surgery or RT).[16] For patients having advanced primary tumor or higher nodal stage, post-operative RT or CRT,as guided by poor risk factors mentioned above, reduce risk forloco-regional recurrence and also seen to improve the survival.[17,18] But this approach of three-modality therapy (i.e., surgery + CRT) comes at the cost of increased rates of late toxicities such as poor quality of

life (QOL), chronic xerostomia, chewing problems, dysphagia and micro-aspiration; this may raise non-cancer mortality in HNSCC survivors.[19]

1.3 Malnutrition in HNSCC

First, let us look at some definitions of malnutrition. In recent years, several international organizations have made efforts to define malnutrition. These resulted in the Global Leadership Initiative on Malnutrition (GLIM). They used the following factors to diagnose malnutrition – "phenotypic criteria such a sinvoluntary weight loss, low body mass index (BMI), reduced muscle mass; and etiologic criteria such as reduced food intake, disease burden, and inflammation."[20] In this consensus, diagnosis of malnutrition is made by combination of-

- Minimum one phenotypic criterion,with
- One of the etiologic criterion

The European Society for Parenteral Nutrition (ESPEN) Guidelines-19 defined "severe nutritional risk" as follows-

• Loss of weight >10-15% within past six months

• BMI <18.5 kg/m^2

• Subjective Global Assessment (SGA) Scale GradeC

•Serum albumin <30g/L

•Failure to maintain nutrition intake >60% of the recommendation for a period of >10 days

Malnutrition in patients with HNSCC is multi-factorial. HNSCC may cause significant symptoms like pain, burning sensation, bleeding, halitosis, trismus, dysphagia, odynophagia and aspiration; bulky tumors can obstruct upper aerodigestive system. Use of excessive alcohol and tobacco often exacerbates the problem. In addition, treatment options for HNSCC like surgery, chemotherapy and RT have side-effects further contributing to

malnutrition. Surgery may alter anatomy leading to temporary or permanent dysfunction of chewing and swallowing.[21] Common adverse effects of RT and CRT are anosmia, mucositis, dysgeusia, trismus, xerostomia, and nausea/vomiting. HNSCC patients usually have a poor dentition contributing to increased trouble with mastication. In addition to above factors,the systemic effect of tumor may lead to an increase in nutrient demand.[22] The tumor nutrient demand then 'competes' with the host patient for nutrients, thus, resulting in disturbances in host metabolism. This leads to increased basal metabolic rate, anorexia and abnormal nutrient metabolism. Tumor necrosis factor alpha (TNF-α) and other cytokines like interleukin 6 (IL-6) and interferon-gamma (IFN-ɣ) have been found to be linked to cachexia in certain animal models; in part this may be regulated by Nuclear Factor (NF)-kappaB.[23] A combination of released cytokines from host and tumor give rise to multiple abnormalities in metabolism of substrates like lipid, carbohydrate and protein. In patients with malnutrition there is muscle wasting in all types-skeletal, cardiac, and smooth muscle, leading to generalized weakness, reduced respiratory function or decreased cardiac function.

'Disease-related malnutrition' may be defined as resulting from commencement of the systemic inflammation due to an underlying pathology like the cancer. The tissue breakdown and anorexia that caused he inflammatory response to tumor, consecutively, leads to significant weight loss, changes in composition of body and waning physical function.The 'cachexia' is a multi-factorial wasting syndrome that is distinguished by an involuntary weight loss and continuing loss of skeletal muscle mass in addition to loss of fat mass. This wasting cannot be corrected using the usual nutritional care, thus leading to significant impairment of functional.[24] In 'pre-cachexia', widespread involuntary weight loss and muscle wasting is preceded by early metabolic and clinical signs. Risk factors for development and worsening of cachexia are type and stage of cancer, response to the anti-cancer treatment and degree of systemic inflammation. 'Sarcopenia' may be described as-reduced lean body mass (i.e., muscle), with fatigue being commonly present associated with lessened strength and limited physical function.[25,26] A review article published in 2009 noted,cancer cachexia involved loss of muscle and fat and is caused by catabolism induced by unusual host response to tumor

and tumor factors. In addition to cachexia, difficulty in functions like smell, taste, swallowing, saliva flow, etc. add to long-term nutritional problems and poor outcome.[27]

Malnutrition in HNSCC patients is a risk factor linked to worsening in QOL, poor prognosis, and unfavorable recovery from treatment. Wasting of muscle, associated with malnutrition, influences muscle function and loss of strength, thus increasing fatigue and decreased QOL. Health-related QOL(HRQOL) in patients with HNSCC is influenced by limitation of function, as a consequence of tumor and its treatment options. During treatment, depending on the tumor subsite, patients with HNSCC may be predisposed to varying degrees of oral dysfunction, dysphagia, and speech related problems that usually improve by 6 months after completion of treatment.[28] RT and chemotherapy have several side effects associated and toxicities resulting in oral mucositis, xerostomia, and other gastrointestinal problems. A high incidence of these add to reduced nutrition intake and consequently worsening of patient's nutritional status.[29] Long-term (at 10 years) QOL in HNSCC patients I scommonly reduced.[30] HNSCC survivors, having received RT, have impairments of speech and swallowing in around half of the patients, and such impairments are usually seen for a long duration.[31] In a study, Kraaijenga et al. noted that 68% HNSCC survivors were found to have voice problems even at 10 years after RT.[32] Furthermore, Hutcheson et al. reported the dysphagia prevalence at 2 years was 45% in HNSCC survivors and also that dyphagia was 4-8 times more likely to happen in these HNSCC survivors as compared to the general population.[33]

1.4 Immunity in HNSCC

Now, the causal link connecting inflammation, innate immunity and cancer is extensively accepted; even so, a lot of the molecular/cellular mechanisms modulating this association stay unresolved. Recently, efforts are underway to better understand the tumor-elicited inflammation that is detected in nearly every type of solid malignancies. It has been noted that tumor cells may interfere with key mechanisms of interface between inflammation and cancers in order to promote tumor colonization of host. Tumor cells release numerous cytokines and chemokines which attract leukocytes.[34] The tumor microenvironment (TME)

has an inflammatory component; this may include a varied leukocyte population like lymphocytes, neutrophils, macrophages, eosinophils, dendritic cells, and mast cells. All these leukocytes are proficient at producing a varied range of the following chemicals—cytotoxic mediators (like reactive oxygen species), cytokines, serine and cysteine proteases, matrix metalloproteinases (MMPs), membrane-perforating agents, and soluble cytotoxic mediators (TNF-α, ILs and IFNs).[35]

The components which are important to understand immunity and HNSCC are TME and immune invasion. In HNSCC, the TME is complex and has a heterogeneous combination of the tumor and various types of stromal cells. The stromal cells include immune cells, cancer associated fibroblasts (CAFs) and endothelial cells. CAFs secrete various growth factors, cytokines (like IL-6), and chemokines. These have a role in promoting cell growth in tumors, in angiogenesis, and in recruiting immunosuppressive immune cells.[36] As noted, immune element of TME in head and neck cancer comprises of tumor infiltrating lymphocytes (TILs like T cells, B cells, and the naturalkiller (NK) cells), in addition to those of myeloid lineage (such as neutrophils, dendritic cells, macrophages,and the myeloid derived suppressor cells (MDSCs)). HNSCC tumors usually have vast infiltration of these immune cells, but the composition and grade of infiltration is different according to the anatomical subsites and etiology (smoking or HPV).[37,38] In HNSCC, there is better outcome with high levels of TILs, although this dependson the equilibrium of various cells that exert an antitumor effect (effector T cells) and cells with immunosuppressive effect (regulatory T cells(Tregs)).[39,40] Many studies have demonstrated highly immunosuppressive nature of TME of majority HNSCC tumors. Anti-tumor immunity of TME is mainly by NK cellsand effector T cells. The immuno-suppression and growth of the tumor is related to the activity of Treg cells, macrophages and MDSCs. Raised levels of NK and CD8+ effector T cells inTME were found to be associated with better survival.[41] Pro-inflammatory cytokines secreted by tumors may provoke a systemic inflammation.[42]

Tumor-associated antigens(TAAs) and tumor-specific antigens (neo-antigens) expression is a result of genetic alterations in cancer cells, abnormal quality-control mechanisms,and

reprogramming of epigenetics.[43] During 'immune surveillance', antitumor immunity is activated by these antigens, and sometimes, may also lead to rejection of early cancers.[43,44] HNSCC tumors may evade immune surveillance by various mechanisms. TheTME has abundant immunosuppressive cytokines and growth factors, IL-6, IL-10, transforming growth factor beta (TGFβ) and vascular endothelial growth factor (VEGF).These promote activity of Treg cells, MDSCs and macrophages. They also inhibit anti-tumor activity of Teff and NK cells.[45] Advanced stage HNSCC tumors, exhibit upregulation of programmed death ligand-1 (PDL1), which reduce cytolytic activity of the T cells.[46] Other mechanisms to evade immune-surveillance are antigen loss variants, including immune-editing, generation of immunogenic tolerance, and major histocompatibility complex (MHC) downregulation.[44]

The response of systemic inflammation is important for invasion of tumor cells through promotion of tumor metastasis, microvascular regeneration, and proliferation of tumor cells. The neutrophils play considerable role in progression of cancer. Neutrophil to lymphocyte ratio (NLR) is promoted for its reliability and accuracy in predicting the systemic inflammation. NLR was associated with prognosis of solid tumors.[47] A meta-analysis by Takenaka et al. (2018) evaluated the role of NLR in prognostication of patients with HNSCC.[48] The analysis included 19 studies with 3770 HNSCC patients, and they found that NLR more that cutoff value (as used in various studies) was significantly associated with poor overall survival (OS) (Hazard Ratio(HR) 1.69; 95% Confidence Interval(CI) 1.47-1.93; p=0.001) and disease specific survival (DSS) (HR 1.88; 95% CI 1.20-2.95; p=0.006).

1.5 Association between Nutrition and Immunity

The complex interaction between metabolism of the nutrients and immune system happens at numerous levels, like direct sensing of the nutrients by the immune cells and signaling via the endocrine system. Leptin may be reviewed as a case study of the multifaceted inter-relationships. Leptin modulates appetite along with being a pleiotropic cytokine, thus sustaining thymic cellularity along with output, modulating dominance of Th1 over Th2 cells,[49,50] and inhibiting number of Tregs.[51] Low leptin levels may lead tolower cellular

immunity and is associated with nutrient deprivation.[50] Leptin may have various effects on the innate immune cells, ranging from activation and migration of neutrophils, to monocytes and macrophages activation.[49]

There may be aggravation of the systemic inflammation by overflow of tumor derived pro-inflammatory cytokines that consecutively interfere with body metabolism of nutrients like carbohydrates, fats and proteins.[42,52,53] There is good evidence supporting the signaling by tumor-derived cytokines likeTNF-a and IL-1, 6.[54,55] Neuro-endocrine control of appetite can be affected by cytokines, leading to anorexia.[25] Also, these cytokines give rise to wasting of muscle, which results in higher fatigue levels and also impairment in physical activity.[42] Cytokine regulates adipose tissue loss via defectivelipogenesis, higher lipolysis and depletion of the energy reserves fat deposits.[56] The cytokinesin the circulation also modify liver synthesis of the acute phase proteins, suppressing pathways for drug clearance; this may bring about increased risk of adverse effects from anti-cancer drugs and treatments.[53]

In malnourished patients, there is certain degree of immune-compromise, specially decreased cell mediated immunity. It has been noted that the extent of poor nutrition correlated with the burden of tumor, and affected the outcome. It was also found that, in malnourished patients, the immune suppression observed was associated with unhindered tumor growth.[57]

As summarized in Figure 1.3, the tumor secretes various chemicals including inflammatory factors that have an affecton function of organs like brain, liver, muscle and fat. The alteration in the appetite signals eminating from the brain result in anorexia, ensuing reduction in intake of calories; imbalances in anabolism and catabolism result in wasting of muscle, reducing muscle strength and even fatigue; production of acute-phase proteins is stimulated in the liver, leading to reduced drug clearance and higher treatment toxicity; there is depletion of fat deposits due to cytokine stimulated lipolysis along with faulty lipogenesis, leading to wasteful, maladaptive response to small intakes of food.

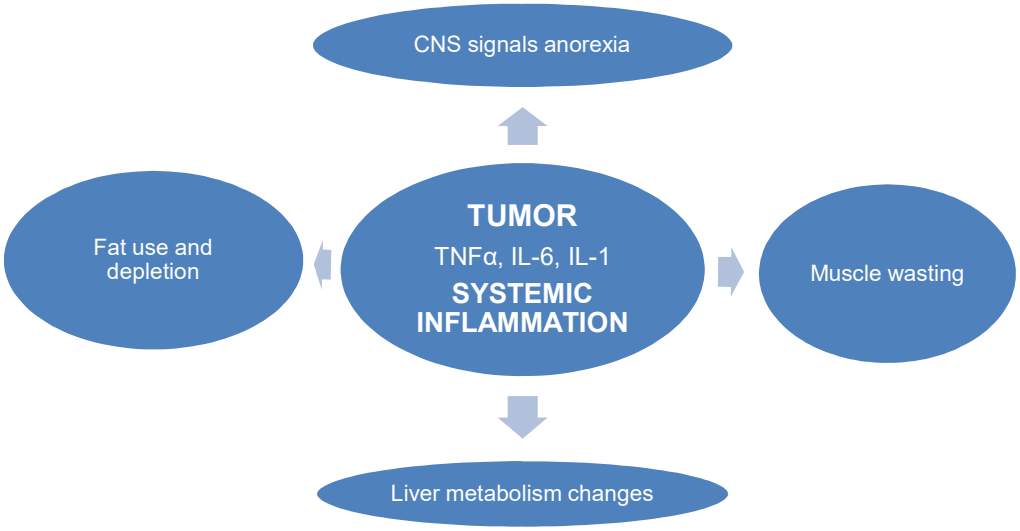

Figure1.3 Metabolism in the presence of tumor and systemic inflammation. (TNF, Tumor necrosis factor-alpha; IL, Interleukin)

1.6 Prognostic factors in treatment of HNSCC

The evaluation, staging, prognosis and treatment of HNSCC have evolved over years. For the conventional HNSCC staging, tumor, node and metastasis system was given by 2017 Union for International Cancer Control (UICC). The American Joint Commission on Cancer (AJCC) staging system also integrates certain additional information for patients with HPV positive disease. In the 8th edition of "Cancer Staging Manual" (released in 2017 byAJCC/UICC) there were 3 main changes that aimed to improve outcome predictability in Head and Neck SCC. These were addition of –

- depth of Invasion (DOI) for T stage in oral cancer
- extracapsular nodal extension in non-HPV HNSCC,and
- a new staging system based on HPV positivity in oropharyngeal SCC.[58]

In HNSCC patients, risk factors like T stage, node status, DOI, degree of differentiation, LVI, PNI, and extra-capsular spread have been used to predict the survival and guided the adjuvant treatment. Still, it has been widely noted that patients with similar risk factors and similar treatment have different OS and DSS. As of now, the major progress in building prognostic models for HNSCC has been the novel classification of oropharyngeal cancer subsite according to the p16 status, as mentioned above, which was implemented in January 2018.[58] It is a paradigm changing classification, as it recognizes the p16-immuno positive oropharyngeal cancersas having a totally different biological and molecular behavior. However, Bruixola et al. reported this subgroup to be only 25-30% of the whole HNSCC population.[59] In the past 3 decades, survival of HNSCC patients has improved modestly; the 5-year survival was 55% during 1992–1996, and 66% during 2002–2006, across all age groups and subsites in the Surveillance, Epidemiology, and End Results (SEER) registry.[60] Martin et al. reported reduction in survival by half of the HNSCC patients in the presence of node metastasis.[61] Duray et al. elucidated that, the poorer prognosis in node positive patients may be linked to the strong effects of HNSCC on host immune system.[62] Revelations in molecular genetics landscape of HNSCC in the last decade have also hinted at new prospects in future therapeutic interventions. Continuing endeavours must aim to understand the biology of HNSCC, immune-biology, and nutritional factors in order to identify novel prognostic biomarkers. This will help the physician to deliver most successful therapies with least toxicity.

1.7 Need for a low cost model

In the developed world, a reduction in incidence of tobacco-related HNSCC has been observed due to the declining use of tobacco over last few decades.[63] In the West, there has been a parallel increase in HPV-positive oropharynx, due to the reduction in tobacco related HNSCC. In India, the government has made attempts to reduce use of tobacco (oral and smoking), but, given the scale of the problem, there is a long way ahead to notably reduce

the incidence of tobacco related HNSCC in the country. This increase in the incidence of HNSCC related to HPV is not yet seen in India, regardless of literature suggesting up to 40% coexisting HPV prevalence in Indian HNSCC.[64] It is noteworthy, that tobacco or alcohol associated HNSCC have more adverse prognosis as compared to HPV related HNSCC, and nearly 50% patients may develop recurrence or metastatic disease.[65]

Many HNSCC patientsin the West may present in advanced stage. In the United States, more than 2/3rd HNSCC patients were seen to have nodal dissease or metastasis at distant site at diagnosis, and more than 1/2 patients required salvage surgery or RT for tumor recurrence as early as within 2 years of cancer treatment.[66] To improve the survival in clinical practice, simple and low cost indices predicting a raised risk for poor clinical outcome in terms of recurrent disease, distant metastasis, or mortality in HNSCC patients arerequired. In India, majority cancers present in advanced Stages III/IV, associated with elevated degree of malnutrition and immune response.

The World Bank classified India as a lower-middle-income group country. As per Indian Council for Medical Research (ICMR) as many as 90% patients with oral SCC were from rural areas and belonged to lower or lower-middle socio-economic class, and 3.6% were below poverty line.[6] In developing countries, HNSCC differs from that in the Western world in terms of age at presentation, subsite of disease, etiology (more tobacco and alcohol related), higher malnutrition, and molecular biology. Certain special challenges faced by developing countries like illiteracy, poverty, lack of awareness, advanced stage of cancer, limited access to specialized health care, and limited treatment infrastructure create difficulties in management of cancer patients. There is need for a low cost model to predict prognosis in Indian HNSCC patients for better management protocols, specially tailored to the needs of a lower-middle-income country.

1.8 Aims and objectives of the study

1. To study the nutritional profile and systemic immunity in patients with Head and Neck Squamous Cell Carcinoma (HNSCC).

2. To study the correlation between the nutritional status and systemic immunity with outcome of treatment in patients being treated for HNSCC.

3. To develop a low cost model for early prognosis using nutritional status and systemic immunity marker in patients being treated for HNSCC.

1.9 Hypothesis

H_0 - There is no correlation between nutritional status and systemic immunity in patients with Head and Neck Squamous Cell Carcinoma.

H_1 - there is a positive correlation between nutritional status and systemic immunity in patients with Head and Neck Squamous Cell Carcinoma.