CHAPTER-2

REVIEW OF LITERATURE

2.1 Nutrition in HNSCC

2.1.A. Factors affecting nutrition

Malnutrition incancer is a multi-factorial process as discussed before, many factors put together result in increased energy and protein requirements, impairment of food intake, decreased anabolic stimuli (like physical activity), and alteration of metabolism in various organs and tissues. In 1989 Woodet al. noted that HNSCC patients have distinctive nutritional problems and this may have an affect on the response to treatment.[67] They suggested that the treating medical team must be x able to precisely evaluate the patient's nutritional status along with prescribing proper metabolic treatment.[67]

In cancer patients, malnutrition prevalence rate has been reported in various studies worldwide. A study from Spain reported 34% patients at hospital admission and 36% at discharge to be malnourished;[68] another study from Brazil reported malnutrition rate to be 71% overall, with 35% patients having moderate and 36% patients having severe malnutrition;[69] a French study found 39% patients were malnourished;[70] a study from Korea reported that 61% patients were malnourished depending on the type of cancer and disease stage.[71] A review article found 35% to 60% of all HNSCC patients were malnourished at diagnosis.[72] Another review article noted this to be 80% in HNSCC patients mostly due to their lifestyle choices and associated risk factors with HNSCC.[73] According to Ackerman et al. HNSCC patients face numerous nutritional problems before, during, and after treatment.[74] One reason being physical closeness of tumor to oral cavity, oropharynx, pharynx or larynx, which vital for normal eating; the second reason is that treatment related adverse effects are common, such as dynophagia, xerostomia, dysphagia, dysgeusia, thick saliva, mucositis, nausea, and vomiting. All the factors collude to promote impairment incapacity of the patientin maintaining sufficient nutritional intake. In HNSCC patients, unintentional weight loss and malnutrition, either during treatment or thereafter, were found to be associated with worse treatment outcome, worse morbidity/mortality and even poor QOL; this was also noted in overweight patients without low BMI.[74]

A prospective study followed up HNSCC patients from the beginning of cancer treatment to twelve months after completion of treatment. The impact factors associated with malnutrition (using GLIM criteria) were advanced tumor stage, CRT ± surgery, and severe mucositis.[75] The treatments for HNSCC are often a contributing factor for malnutrition; these include single modality like surgery, RT or chemotherapy alone, or multi-modality like combination CRT or surgery + RT or surgery + CRT;[76] the less commonly used treatments are immunotherapy, targeted therapy and hormonal therapy.[77] Kubraket al. also pointed out that treatment toxicities can compound the risk of malnutrition as the patient's intake is already limited by symptoms from the tumor.[78] In HNSCC patients, multi-modality treatment results in increased toxicity and adverse effects,[79] thus, patients suffer from significant weight loss and consequent poor nutritional status during the oncological treatment.[70] Linked to this are various unfavorable outcomes like poorer QOL, reduced physical functioning, also worsening of function of immune system, greater toxicity, treatment interruption, unplanned hospital admission, and even death.[80]

Crowderet et al. noted that as many as 90% HNSCC patients do experience symptoms impacting the oral intake resulting from the tumor or the treatment side effects.[81] In the literature, as mentioned above, the considerable challenges faced by HNSCC patients during the treatment are described. These adversely impact the patient's physical as well as emotional well being. However, limited research is done on the 'lived experience' and the altered meaning of 'food, eating, and the eating experience' during treatment in HNSCC patients. A systematic review aimed to study the affect of dysgeusia, swallowing disorders, xerostomia,and oral mucositis on factors like oral intake, nutritional status, and loss of weight in patients with HNSCC.[82] In this review, 25 studies were finally included. They found most studies looked at dysphagia, but it is known that the symptoms are interconnected, and usually affect one other. The review found limited data on oral mucositis, dysgeusia and xerostomia outcome HNSCC patients; concluding a lack of well-designed trials as well as multi-center prospective studies, and recommended that further research was required to establish which facet of these symptoms must be measured.

Another systematic literature review identified that HNSCC treatments lead to many inter-connected adverse effects that impact oral intake, prompting malnutrition.[83] The patients reported alteration in taste and a reduced desire totake food as a result of changes in appetite and pain. Xerostomia was reported in approximately 80% patients with HNSCC by Onseng et al.[84] The reduced saliva levels add to a reduced diet intake.[85] The treatments can also impact swallowing processes negatively due to the close approximation to anatomical structures (i.e., tongue, cranial nerves), leading to dysphagia.[86] Dysphagia in these patients is found to be directly associated with alteration in the usual diet intake.[87] Thus, loss of weight is recognized as an independent predictor and is usually associated with malnutrition in this patient population.

In patients with HNSCC, effects of tumor and treatment may also cause particular altered metabolic response, like alteration in resting energy expenditure.[88] The cancer treatment causes systemic inflammation and disrupts the metabolism of macronutrients; as a result there are changes in the energy expenditure requirements.[89] These alterations in the metabolism, along with reduction in food intake causes a negative energy-protein balance; this may result in cancer cachexia.[25] Jager-Wittenaar et al. found the prevalence of cachexia in HNSCC patients to be 42% and pre-cachexia to be 15%.[90]

In the months to years after CRT for HNSCC, >90% survivors may experience one or the other nutrition impact symptoms (NIS). Despite this high prevalence, the research on the long-term impact on nutrition and QOL in HNSCC survivors is limited. A systematic review evaluated the presence of NIS and the associated outcomes HNSCC survivors having received CRT.[91] The study included 849 HNSCC survivors with follow up of three months to >10 years after the CRT treatment. The functional deficits due to CRT were xerostomia, dysphagia, trismus, mucositis and pain. They concluded that NIS harmfully affect the HNSCC survivors much after the phase of ongoing active treatment and are connected to reduced nutrition and QOL. They recommended that dietary interventions are required beyond treatment completion to improve patient's eating challenge. If this is not done, then

in the long term, survivors may suffer as result of NIS leading to chronic malnutrition risk and would affect QOL.

A qualitative study in 2020 aimed to achieve a good perception of lived experience of the chronic NIS burden in HNSCC survivors.[92] They collected data by conducting semi-structured and face-to-face interviews with 31 HNSCC patients. The following 4 major categories were identified from the data— dietary preferences, symptom presence, addressing symptoms, and eating adjustments. The commonest symptoms were xerostomia, dysphagia, alterationin taste, and difficulty in chewing. The eating adjustments were increased time in consuming meals, taking less food, cutting food to smaller pieces, and taking more fluid. As a result of this, survivors noted dietary pattern changes post-treatment as compared to pre-treatment. All patients had experienced one or more chronic NIS and ~40% patients were not aware pre-treatment that NIS could persist chronically.

2.1.B. Assessment of nutrition in HNSCC patients

Assessment of nutrition status at the time of diagnosis can help identify the patients at greater risk of developing malnutrition, leading to institution of early interventions, thus reducing any possibility of malnutrition related morbidity.There are multiple assessment tools for assessment of malnutrition. Detailed history along with comprehensive physical examination are the most frequently utilized and finest means to evaluate the nutrition status. A good history must comprise of information on any unintentional recent loss of weight, appetite loss along with alteration in stamina. Physical examination should include presence of wasting in quadriceps deltoid and femoris muscles, stomatitis, cheilosis, along with dry scaling skin. Other anthropometric parameters can also be used for nutritional assessment. These may include triceps skin fold, and mid-upper arm diameter measurement. Nutrition assessment in cancer management must include a thorough diet history, disease stage, proposed plan of treatment with intent (curative/palliative), biochemical parameters when appropriate, and history of alcohol intake. The following factors should also be assessed—social setup (like social support, cooking facilities,

employment status), and patients perception of nutritional status, so as to ensure proper nutritional intervention.[93]

Body weight as the solitary indicator for nutrition status is insufficient, the usual anthropometric measures may also not reflect the sudden changes in nutritional status.[94] *Traditionally, malnutrition risk in oncology patients was determined by low BMI or body weight, and history of significant loss of weight. This approach anchored in weight only has been ineffective not only due to epidemic of obesity globally but also due to the novel concept of alterations in metabolism that occur before any recordable change in weight. Anorexia and appetite change are now accepted as early risk markers for malnutrition; these can arise regardless of the initial weight. Loss of weightis an important sign of increasing malnutrition, thus, this should be recognized early. Insufficient nutrition intake is defined by an inability to eat for 7 days, or an energy intake < 60% of requirement for 1 to 2 weeks.*[95]

Several biochemical markers are available to test for the nutritional status. Traditionally, nutritional depletion has been defined using albumin, prealbumin, and transferrin levels. Albumin is a poor reflection of acute nutritional change, as its half-life is 20 days. Nevertheless, albumin still is the crucial feature in starvation and finest marker for death in admitted patients.[96] *Transferrin is the main iron transport protein (half-life 8 days). As the iron metabolism affects its production by the liver, the reliability of serum transferrin level as a marker for the nutritional statusis limited.*[97] *Prealbumin is also produced by liver and its serum value range is usually 15 to 25 mg/dL; it transports proteinsand hormones (half-life 2-3 days). Prealbumin changes reflect nutritional depletion before any change in the albumin. Alteration in nutritional intake may be observed in 7 days by prealbumin levels, but very few studies have validated its efficacy over albumin levels, in predicting the outcomes.*[98] *As such, many medical conditions, like renal insufficiency, affect the plasma levels of prealbumin limiting its use in clinical practice.*

Other scales derived from above parameters are prognostic nutritional index, creatinine-height index, and SGA. These are used to identify nutritionally deficient patients. The SGA

being a validated scale that uses weight history, diet history, gastrointestinal symptoms, and physical assessment (muscle wasting, fat loss and edema) to assess overall nutrition status. SGA is validated for cancer patients, the patient generated SGA (PG-SGA) is also validated. SGA comprises of following parameters-

- *Weight in kilogram - usual, current, % weight loss*
- *Weight change in past 2 weeks*
- *Dietary intake-change, adequacy*
- *Gastrointestinal symptoms-nausea, vomiting, diarrhea, anorexia- with their frequency*
- *Functional capacity-change, grade*
- *Physical examination- subcutaneous fat, muscle wasting, oedema, ascitis*
- *Overall SGA rating*

Shirodkar et al. used SGA to evaluate the role of preoperative nutrition to predict post surgical in 266 cancer patients.[99] The cancer site distribution was as follows- 112 HNSCC, 53 gastrointestinal tract, 28 thoracic organs, and 73 patients other sites. The SGA score was A in 152 (57.2%), B in 98 (36.8%), and C in 16 (6%) patients; but, low BMI (< 18.5) in as many as 110 (41.8%) patients. SGA score B and C were associated with prolonged post-operative stay, greater antibiotic days (p=0.000) and higher rate of pre-defined adverse events (p=0.025;OR, 5.27; 95% CI, 1.35-20.51; p= 0.016).They found no such association with BMI groups with above mentioned outcome parameters. The authors remarked that SGA was an easy and cost-effective method to recognize malnutrition that was clinically important in Indian patients having surgery for cancer; also that BMI over estimated severe malnutrition in Indian patients and was not associated with adverse outcomes in their study.

PG-SGA is also a useful tool for assessment of the nutritional status.[100] PG-SGA score mayeven be utilized as an objective measurement to assess the outcome of any nutritional intervention. According to Isenring et al. it is precise in assessing well nourishedand malnourished patients.[101] Just like SGA scale, PG-SGA is divided into: A, B, and C grades,

with grade B and C indicating moderate and severe malnutrition, respectively. Nutritional status, measured using PG-SGA, before RT, has been used to predict response to RT; a score less than 9 was found associated with improved local disease control and reduced acute toxicity in HNSCC patients having treatment with RT.[102] In HNSCC patients with impaired speech, PG-SGA has been utilized for assessment of nutritional status in patients on enteral nutrition and gastrostomy.[103]

The Malnutrition Screening Tool compares satisfactorily with PG-SGA and is validated for nutritional assessment in cancer patients. Another important tool is Malnutrition Universal Screening Tool (MUST). MUST is not yet validated for cancer patients, but is being presently used in United Kingdom to screen for nutritional status in patients. It comprises of the following three parameters—loss of weight, BMI, and dietary state. Low, moderate, or high nutritional risk is indicated by score of 0, 1, or 2 respectively.

Peng et al. studied 3,232 patients to find out the relevance of the tool Nutritional Risk Screening 2002 (NRS2002) in nasopharyngeal cancer.[104] The NRS2002 comprises of three parameters—nutritional status, disease, and age score. Low nutritional risk is attributed to scores 0-2 and high nutritional risk to scores >3. The patients with a high nutritional risk need nutrition intervention. ESPEN recommended NRS2002 as a screening tool in 2002. Peng et al. also noted that MUST had highest concordance with NRS2002 and was a fine tool to classify patients at increased risk for prolonged hospitalization in cancer patients.[104]

Another tool, the Mini Nutrition Assessment (MNA), consists of three parameters—dietetic evaluation, anthropometric assessment, and global evaluation. The nutritional assessment is assigned as normal, nutritional risk, and malnutrition, for scores ≥24, 17-24 and <17 respectively. MNA was developed and established as nutrition assessment tool by Vellas et al. in the 1990s.[105]

Another Indian study aimed to study the role of various nutrition assessment tools in cancer patients undergoing nutritional intervention.[106] The outcome was assessed in this study using the following methods—laboratory, clinical, anthropomorphic assessment, Nutritional

Assessment Index and Prognostic Nutritional Index. They found that BMI, Hemoglobin percentage, the tricepsskin fold thickness (TST), and Mid Arm Circumference (MUAC) were dependable markers of malnutrition; and concluded that Nutritional Assessment Index was the best tool for malnutrition evaluation.

2.1.C. Literature on malnutrition affecting treatment outcomes in HNSCC

Cho et al. noted that existence of muscle wasting or sarcopenia was associated with malnutrition and treatment related toxicities.[107] In both under-weight and over-weight patients, sarcopenia was associated with increased rates of chemotherapy related adverse effects, lesser time to disease progression, worse surgical outcomes, and physical disabilities along with worse survival.[108-112] Trestiniet et al.studied the effect of nutrition on treatment outcome in HNSCC patients. They found that >10%weight loss was significantly associated with more mucositis (p=0.04) and dysphagia (p=0.0002) in patients receiving chemotherapy.[113] A review article noted that HNSCC patients were often malnourished and this may affect the prognosis in terms of morbidity and outcome of treatment.[114] They recommended that particular consideration ought to be given to nutritional requirements and interactions between diet and treatment in patients receiving CRT.

A recent pilot study looked at the nutritional status and HRQOL outcomes among the out-patients with HNSCC.[115] PG-SGA was used to evaluate the nutrition status, malnutrition being defined as a PG-SGA score B or C. HRQOL was assessed using European Organization for Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQ-C30). The main results of the study were—in comparison to malnourished patients, the well-nourished patients reported significantly less issues with fatigue, appetite loss, pain, sticky saliva, chewing, and coughing, along with social eating ($p<0.05$); a weak but statistically significant correlation between PG-SGA and HRQOL was found ($r =-0.37$, $p=0.012$); 70% patients were identified to have moderate or severe malnutrition pre-treatment through the PG-SGA score, but mean±SD of BMI was 29±5 kg/m^2 (overweight category); at the 1month follow up post-treatment, the patients reported increased severity of

swallowing, chewing, thick saliva, speech, dryness of mouth, difficulty in social eating and smell and taste sensations as compared to pre-treatment; xerostomia was seen to persist 3months after treatment (p=0.003); and as compared to well-nourished patients the malnourished had worse HRQOL symptoms. They recommended a regular assessment of nutrition and psychosocial parameters using the PG-SGA and EORTC QLQ-C30 scales to help in identifying patients requiring nutritional and psychosocial care.

Another parameter used in various cancer studies is Prognostic Nutritional Index. It was designed in 1984 by Onodera. It assesses the nutritional and inflammatory status. The formula for calculating PNI is as follows-

'Prognostic Nutritional Index = (10 x serum albumin [g/dL])+(0.005 x lymphocytes/μL)'

A higher value is considered better. ThePrognostic Nutritional Index was initially used to demonstrate association between post-operative complications and the prognosis in cancer of esophagus patients.[116] In HNSCC patients, a low index has been demonstrated as a predictor of worse survival[17] and also associated with higher grade RT-induced side effects.[18] It seems to be a reliable biomarker, with fair external and internal validity, and low variability due to the external factors. In addition to this, it is inexpensive, reproducible and available universally, and provides dependable assessment of patient's nutritional status.

Another marker used frequently for predicting treatment outcomes is pre-treatment and during treatment weight loss. Many studies suggest strongly that significant weight loss (>10%) is associated with adverse events like lesser response to RT and chemotherapy, higher morbidity, poor QOL, and even a higher mortality rate.[119]

2.1.D. Literature on treating malnutrition in HNSCC

According to ESPEN recommendations, in cancer patients, nutrition status should be evaluated frequently and even before the starting of anti-cancer treatment. Dietary interventions ought to be started before starting the cancer treatment and also when any nutritional deficiency is anticipated or observed. This will lead to optimal oncological and

QOL outcomes. Alshadwi et al. noted that a comprehensive nutrition assessment should be an integral component of treatment planning for HNSCC patients.[72]

Dietary counseling is the most commonly used nutritional intervention in management of cancer patients identified to be malnourished with a functional gastrointestinal tract. Interventions like oral supplements and dietary counseling, used in combination have resulted in better maintenance of weight, increased energyand protein intake, better QOL and improved tolerance to cancer treatment.[120] *Enteral feedingis considered in HNSCC patients if the patient is not able to maintain sufficient dietary intake (i.e., <60% of the energy expenditure) for a duration of>10 days; this could be due to dysphagia or severe toxicity like oral mucositis.*[121]

Baldwin noted that studies regarding use of oral nutritional supplements for management of weight loss in cancer patients are limited.[122] *The limitations of oral supplements may be due to a wide variety of patho-physiological changes that happen in cancer patients.These require a multifaceted and targeted approach, like adapting to the gastrointestinal insufficiency and modulation of the metabolic component during cachexia so as to allow nutritional interventions to beuseful.*[123] *A study evaluated nutrition counseling either with or without oral nutrition supplements and showed an improvement in nutritional outcomes (BMI, weight gain and improved scores on PG-SGA scale) with supplements.*[124]

It is important to note that, although nutrition interventions may assist the patients in consuming adequate diet as required, but nutrition interventions by themselves have not been seen to impact the development and maintenance of the lean body mass. Various studies demonstrated that reduction in the lean muscle mass duringthe cancer treatment may be contributary to weight loss, and was associated with reduced physical function as well as QOL. It was also linked with dose limiting response to treatment; this was seen to cause extended recovery periods.[125.126] *Nutrition support with nasogastric (NG) or percutaneous (PEG) tubes in HNSCC patients resulted in patients experiencing a reduced*

mean loss of weight during RT, but an increased frequency of nutrition related complications and hospital admissions.[121]

A study published in 2015, evaluated the impact of nutrition support with enteral versus parenteral nutrition in HNSCC patients undergoing CRT.[127]*In this study, 92.8% patients were given artificial nutrition during treatment, of which29.1% were on enteral nutrition (EN) via PEG, 41.8% on parenteral nutrition (PN), and 21.9% on oral support. EN was found to be better than PN formedian variations in all variables i.e., weight, serum levels of albumin, prealbumin and transferrin, fat free and fat body mass, and total body water.The incidence of Grade 3/4 oral mucositis was lower for EN (50% versus 66.7%), patient with EN had lesser duration of oral mucositis (14.7±19.1 days versus 22.5±22 days) as compared to PN. They concluded that enteral nutrition support during CRT for HNSCC results in better nutritional status, tolerance of treatment and progression free survival (PFS) as compared to parenteral nutrition.*

A single-center RCTevaluated the advantage of "oral nutritional supplements" (ONS) over and above nutritional counseling in patients with HNSCC undergoing RT.[128] *In the study, 159 HNSCC patients planned for RT were assigned randomly to nutrition counseling and ONS (N = 78) and no ONS (N = 81) groups, the intervention was carried out from beginning of RT and was continued for 3months post-treatment. The primary endpoint being changes in the body weight at the completion of RT; the secondary endpoints being changes in the muscle strength, protein/calorie intake, QOL and treatment tolerance. They found that in the counseling plus ONS group there was less loss of weight (mean difference in weight loss=1.6 kg (95%CI, 0.5-2.7; p=0.006), more protein, calorie intake, better QOL over time (p<0.001), reduced changes in scheduled of RT or CRT (HR=0.40; 95%CI,0.18-0.91; p=0.029) as compared to the counseling alone group. They concluded that use of nutritional counseling with ONSin HNSCC patients being treated with RT or CRT was associated with improved maintenance of body weight, protein/calorie intake, QOL, and treatment tolerance.*

A retrospective study assessed impact of the timing of PEG tube insertion on clinical outcomes in 111 HNSCC patients undergoing CRT.[129] The primary end points were weight loss during CRT, number of hospitalizations for nutritional deficit, and rate of complications and dependence of PEG. Early PEG tube placement had moderate correlation with reduced weight loss ($p<0.001$, $R=0.495$), mild correlation with hospitalization ($p=0.011$, $R=0.262$) and mild correlation with extent of continual loss of weight at 6 weeks treatment ($p=0.003$, $R=0.347$). No differences were found in complication and dependence rates associated with earlier PEG placement. Their conclusion was that patients requiring CRT for locally advanced HNSCC may benefit from early PEG placement due to better nutritional management in this group of patients with no added morbidity.

A prospective study assessed effect of early nutrition support (ENS) on nutrition markers and the treatment response in 102 HNSCC patients with >2points on MUSTscore before starting RT.[130] ENS consisted of nutrition counseling and oral/enteral nutrition supplements. In this study, 76% patients had stage IV disease; a slight decrease in BMI and increase in fat-free body mass ($p<0.001$) was seen post-treatment; biochemical nutritional parameters were stable in spite of a reduced oral intake. Interestingly, <40% patients had severe mucositis or skin reaction; 92% patients completed all planned RT; 22.8% patients had interruptions in RT sessions. They also found that the patients with malnutrition had a lower rate of RT completion thanthose without malnutrition ($p<0.001$); mortality was associated with poor performance status (PS), greater pre-treatment loss of weight and higher grade of oral mucositis or skin reactions ($p<0.05$). The authors concluded that ENS in HNSCC patients undergoing RT is an efficient strategy to prevent and limit malnutrition related morbidities.

An Australian RCT aimed to assess the efficacy of early nutrition intervention to improve outcomes in HNSCC patients undergoing prophylactic gastrostomy before starting curative treatment.[131] The randomization was performed to intervention group (70 patients) or standard care group (61 patients). Supplementary tube feeding commenced immediately in intervention group after the placement of tube. The primary outcome of this study was

percent weight loss after 3months post-treatment. On intention to treat analysis, no difference in weight loss in either groups (10.8±5.6% in intervention versus 10.9±6.6% in standard care, p=0.930, multivariate analysis p=0.624); no difference in QOL and clinical outcomes was found. They concluded that early nutritional intervention in this study had not improved outcomes.

An RCT aimed to assess improvement in nutritional status of patients with HNSCC during RT using a new training method for dietitians to carryout psychological techniques.[132] This trial assessed efficacy of "Eating As Treatment (EAT) program". The primary outcome in this study was nutrition status post-treatment and was measured using PG-SGA. They found that patients in the EAT intervention group had statistically significantly better PG-SGA scores (p=0.03), smaller % weight loss, fewer interruptions of treatment, lower depression score, higher QOL and less and shorter unplanned admission to hospital. This trial was the first to show the efficiency of psychological intervention for improvement in nutritional status in patients with HNSCC receiving RT.

2.2 Systemic immunity in HNSCC

2.2.A. Immunity and HNSCC

Even in the19th century, the perception was that cancer is associated with inflammation.The immune system has a vital role in HNSCC carcinogenesis as well as tumor prevention. Mantovani in an article published in Nature, stated that "Cancer related Inflammation" was an important element of tumors and may even represent the 7th hallmark of cancer.[133] Increasing evidence supports role of immune cells like neutrophils in inflammation, tumor promotion, and immune suppression associated with the tumors.[134]

Millrud et al. aimed to describe leukocyte activation pattern in blood in HNSCC patients and to study any association between the activation pattern and tumor progress or survival.[135] Leukocyte activation profilewas analyzed using flow cytometry in HNSCC patients and healthy persons as controls. They found raised total leukocyte, monocyte and neutrophil

counts, in addition to raised neutrophil/lymphocyte ratio (NLR) in HNSCC patients. HNSCC patients also had elevated % of NK cells and CD71^{+}, CD98^{+}, CD69^{+}T subsets. The pattern of changes correlated with tumor burden and nodal spread. In the HNSCC patients, a low neutrophil count, a high NLR, monocyte CD14^{high} CD16^{+}activation and a higher CD4 to CD8 ratio were found to be associated with worse survival. Where as, a high proportion of CD98^{+} Th cells was linked with better outcomes. Similar increase in number of monocytes and neutrophils has been noted by many reports other cancertypes.[136-138] Raised NLRmay be a reflection of amplified systemic inflammation, in addition to increased infiltration of the immature monocytes and neutrophils from the bone marrow due to raised turnover of leukocytes.

HNSCC tumors produce immune suppressive mediators affecting the immune function in the host patients via the following mechanisms—resistance to apoptosis; secretion of immune suppressive molecules like TGF-β, adenosine and prostaglandin (PG) E2 or cytokines like IL-6, IL-10; expression of the Fas ligand and that causes death of theTILs. Although many parts of immune system are responsible for the antitumor immune response, the T-cells are still believed to be the vital cells in the process of antitumor immunity. Multiple studies evaluated T cell apoptosis mechanisms in patients with HNSCC. One such mechanism involves Fas/Fas ligand signaling pathway.[139] Other pathway may beTNF-α induced Jurkat T cell apoptosis andTNF-related apoptosis inducing ligand (TRAIL).[140]

Locally advanced HNSCC has been identified to be a heterogeneous disease and there is need for better prognostic and predictive factors. Sparano et al. expressed that in patients of advanced HNSCC there was a reduced Th1 and higher Th2 immune response.[141] In addition to TNM stage, systemic inflammation and poor nutrition status were seen to impact survival negatively.[59] Lymph node density (LND) has recently been gaining increased importance recently for evaluation of degree of the node disease in many cancer types, like HNSCC. LND is the ratio of lymph nodes with metastasis to the total number of lymph nodes excised.[142] Many studies have assessed clinical application of LND in prognosis and the association with poo rresults in HNSCC patients.[143] A low LND has been found to have

strong correlation with better prognosis and reduced regional failure after treatment. Liao et al. pointed out that LND had a better prognostic value than the TNM system, as it simultaneously considers extent of nodal involvement and extent of nodal dissection.[143] Adel et al. noted that HNSCC patients who had an LND ≥0.06 were found to have significantly worse DSS and OS; LND ≥0.06 was the only factor associated with distant metastasis (HR=6.684; 95%CI,2.279–19.607; p=0.001).[144]

There have been suggestions that the immune system may ignore the primary tumors and an important immune response occurs in the regional lymph nodes only. Pretscher et al. evaluated the local distribution of T and B immune cells in the primary tumor tissue and in the nodal metastasis in HNSCC, and the prognostic effect of T and B cell distribution on disease free survival (DFS).[145] In the primary tumor they found an increased proportion of FoxP3+ Treg and cytotoxic T cells; in nodal metastasis they found an increased proportion of $CD20^+$B-cells in comparison to th eprimary tumors; a lower proportionof $CD8^+$ T cells was found in nodal metastasis incomparison to negative regional lymph nodes. This was suggestive of a local down modulation of the cellular immunity. Nodal metastasis in HNSCC patients is associated with clinically significant changes in systemic immunity as well.

Xu et al. evaluated the role of systemic immunity marker NLR in prognostication of 153 p16 negative HNSCC patients with unknown primary and nodal metastasis.[146] The primary endpoint was DSS. They found 5 year DSS to be 58%overall, 71% for patients with NLR1.4 to 3.7, 57% for patients with NLR 3.7 to 6.0, and 39% in patients with NLR 6.0 to 8.3 (p=0.001).Cox model analysis confirmed NLR to be an independent predictor of DSS.

2.2.B. Markers used for systemic immunity

The immune system has played a vital role inHNSCC carcinogenesis and even tumor prevention.[147] An endogenous immune response has been demostrated to be prognostic for HNSCC as well as for various other tumor types.[148] Systemic inflammatory markers like changes in acute phase proteins (hypoalbuminemia, raised C reactive protein,Glasgow Prognostic Score (GPS)); raised white cell counts (raised neutrophil count, reduced

lymphocyte count, and high NLR); have been used to predict adverse cancer treatment outcomes.[149,150]

The GPS has been promoted as an easily accessible and a strongly predictive marker for evaluation of systemic inflammation in cancer patients. It is calculated using serum levels of C reactive proteinand albumin. It is important to note that, GPS was systematically validated in clinical scenarios as predictor of prognosis and even mortality.[149,151]

The immune-modulating role of neutrophils and cancer has gained attention recently. Raised circulating neutrophils have been associated with worse prognosis in HNSCC. There has been ever increasing evidence to support the role of neutrophils in promotion of tumor growth, peri-tumoral and systemic inflammation, and immune suppression.[134] *Neutrophils are considered 'pro-tumorigenic' due to following two mechanisms—released pro angiogenic chemicals and the suppressed adaptive immune system. On the contrary, some studies have reported 'anti-tumorigenic' role for neutrophils.*[152] *Taking into consideration the newer perception of neutrophil biology and its association with cancer, their role in tumor development and prognosis is a matter of ongoing research. The role of intra-tumor neutrophils has also been evaluated in studies and their level independently affected the prognosisin terms of OS in HNSCC patients.*[153]

The importance of different TILT cell $CD4^+$ subtypes ($CD4^+FOXP3^+$, $CD4^+CD25^+$and $CD4^+CD69^+$) in prognosis has been evaluated in HNSCC patients. Studies found that elevated levels of $CD4^+CD69^+$ subtype was associated with improved prognosis, and the $CD4^+FOXP3^+$ subtype correlated with improved loco-regional disease control.[154] *Another marker noted was absence of/lower expression of ζchain of TILs in stage III/IV HNSCC patients. The low expression is predictive ofworse survival in comparison to patients with a normal ζ chain expression.*[155]

TILs with both $CD8^+$ and $CD4^+$ populations of T cells were established to be of prognostic importance in base of tongue and tonsillar squamous cell carcinomas(SCCs).[156,157] *Infiltration of the metastatic lymph node with $CD8^+$T cells or $CD20^+$ B cells were proven to be*

prognostic in the subsites hypopharyngeal and oropharyngeal SCCs, even though interestingly, TILs of the primary site were not of prognostic importance in this cohort.[158] *In oral cavity SCCs, peri-tumoral $CD8^+$subset of T cells were related to the tumor size, nodal metastases and clinical stage.*[159]

In the tumor microenvironment, cancer and tumor associated cells secrete or express many factors like IL-10, TGF-β, reactive oxygen species (ROS), and inducible nitric oxide synthase (iNOS) that cause T cell suppression. Tumor specific $CD8^+$ T cells were found to exhibit a reduction in the primary tumors and the metastases.[160] *On this basis these haematological markers linked with inflammation, like NLR*[161] *and the derived NLR (dNLR),*[162] *have been used for the prognostication of many solid tumors including HNSCC.*

Zahorec noted that the high NLR, seen in patients with HNSCC, indicated continuing systemic inflammation.[163] *Raised NLRwas related to reduced survival, suggesting that increased systemic inflammation was related to patient's life expectancy.*[136,163]

An elevated $CD4^+/CD8^+$ ratio has been linked topoor survival in patients with HNSCC in many studies, but its prognostic value has been doubtful as noted by some studies with contradictory results.[164-168]

2.3. Association between nutritional status and systemic immunity

2.3.A. In cancer

Various studies have demonstrated a steady association between symptoms of malnutrition and raised inflammatory markers as well as enhanced immune response.[149,169-172]

White blood cells (WBCs) are an integral part of all the phases of response to stress like injury, inflammation and infection; they have a fairly high metabolic need. Alshadwi et al. noted that absolute lymphocyte count <1,200 to 1,500/mm^3, without any reason for immune suppression, was a sign of malnutrition.[173] *They classified the absolute lymphocyte count*

range of 1,800 to 1,500/mm^3 as mild, 1,499 to 900/mm^3 as moderate and <900/mm^3 as severe malnutrition.

The interaction between malnutrition and infection is well established, and is probably due to alterations in immunological defense which is found in protein/energy malnutrition. This leads to impairment in immuno-competence, reduced Tcell proliferation and cell-mediated immunity (anergy).[174] Malnutrition also reduces production of the acute phase proteins thatare needed for survival at the times of infection, injury and stress.

Research relating to modulation of the immune function by using foods in patients ranging from healthy to immuno-compromised states have come to the general conclusion—foods can influence innate and even the acquired immunity. Immuno-nutrition is multifactorial and involves relationships between nutrition, infection, immunity, inflammation, and injury.[175] To give an example, immune-nutrition has a function in immune function and also in modulating gene expressions in the immune cells in neck and esophageal cancer patients who have receivedCRT. This improvement of the immune function, using immuno-nutrition, was also seen to allow the patients to adjust to oxidative and inflammation stress caused by the chemotherapy.[176] Curcumin which is an anti-oxidative and anti-inflammatory phytochemical, has been studied well for its potential as a natural anti-canceragent.[177]

2.3.B. In HNSCC

A study published in 2007, aimed to assess changes in the body composition and mass with respect to inflammatory state, physical function, and energy balance, before and after CRT in HNSCC patients.[178] The study included 17 HNSCC patients diagnosed with stage III or IVa cancer. The patients' body mass composition was assessed using dual energy X-ray absorptiometry, the assessment of resting energy expenditure (REE) was performed using indirect calorimetry, the assessment of physical and functional performancewas done pre- and post-CRT. Cytokines (IL-1β, IL-6, IL-8, and IL-10) and C-reactive protein levels were determined in the fasting venous blood samples. Energy intake was assessed by randomly placed telephone call for 24hour diet recalls. It was found in this study that the weight loss

started 1week after CRT; loss of lean body mass (LBM) was responsible for as much as 71.7 ± 21% (SD) loss of body mass; loss of LBM was significantly associated with reduced physical performance (r = 0.71, p=0.004) and increase in functional dependence (r=0.58, p=0.02); the intensity of total physical activity reduced significantly (p=0.003). They found the cytokine levels to be associated strongly with decline, both physical and functional. The conclusion of this study was that atypical alterations in the body composition, inflammatory state, and metabolism were found to be statistically linked to clinically significant impairment in physical and functional performance.

Luis et al. studied the effect of supplementation of enteral nutrition using 'arginine' on the markers of inflammation in HNSCC patients undergoing surgery.[179] The patients were allocated randomly to either of two groups at surgery i.e., patients who received supplementation of enteral nutrition with arginine (n=14, Group I), and patients who received iso-nitrogenous, iso-caloric enteral formula (n=15, Group II). The results were that IL-6 improved in both groups, whereas TNF-α and lymphocytes showed no change. They concluded that both the feeds improved CRPand IL-6 levels.

Marian et al. assessed the effect of peri-operative supplementation of nutritional support with 'arginine' on patient's nutritional, immune status, post-operative outcome, and survival in HNSCC patients undergoing surgery and had severe malnutrition (the authors defined severe malnutrition as >10% loss of weight).[180] They concluded that 9days of pre-operative feeding with tube, with or without the arginine supplementation, showed no significant improvement in either nutritional status or reduction in surgery related immune suppression or difference in post-operative clinical outcomes.

The association of systemic immunity and nutritional status in patients with HNSCC has also been studied. A study evaluated the prognostic importance of NLR in SCC of unknown primary (p16 negative) patients in head and neck. They found cancer cachexia in 10/153 (6.54%) patients with mean NLR 3.9 (range 1.4-8.3); thus, raised NLR was found to be linked significantly to cancer cachexia. Kano et al. established significant association

between raised NLR and hypopharyngeal or oropharyngeal subsite of HNSCC, T3/4 stage, N2b-N3 stage and clinical stages III/IV HNSCC.[181]

2.4. Prognostication in HNSCC

2.4.A. Use of novel markers

The endogenous immune response produced against HNSCC and the state of immunologic markers in a patient may be used to prognosticate and also help to guide the treatment strategies.[147] *Chronic inflammation encourages cancer development, progression, metastatic dissemination, even development of treatment resistance, and may be immuno suppressive.*[182]

Numerous molecular biomarkers predicting prognosis in HNSCC patients have been under research. Thecancer stem cells (CSCs) with CD133, CD44 and ALDH1 have been validated to be of prognostic significance. CD44 is implicated in inter-cellular interactions and also cell migration. Cancer cell in HNSCC with raised levels ofCD44 have been seen to be capable of self-renewal, and associated with distant metastasis and poor prognosis.[183,184] *Likewise, increased levels of CD133 were associated with invasiveness and metastasis inHNSCC.*[185] *Raised levels of ALDH1 were associated with invasion, self-renewal, metastasis in HNSCC, and may even have prognostic significance.*[184] *Epidermal growth factor receptor (EGFR), found to be over expressed in 80% to 90% HNSCC tumors, is associated with worse OS and PFS.*[186,187] *Raised levels of cytokine IL-6 and IL-6 receptors was also linked to worse prognosis in HNSCC.*[188,189] *Signal transducer and activator of transcription 3 (STAT3) signaling is hyperactivated in HNSCC tumors and is found to be linked toworse prognosis.*[7]

Bruixola et al. in a retrospective study on patients with HNSCC found that T4 stage (p=0.044), a PNI<45 (p=0.001) and N2b-N3 disease (p=0.025) were significantly associated with poor OS.[59] *In the training cohort, PNI (p=0.042) and dNLR (p=0.030) independently affected OS on multivariate analysis.*

Nguyen et al. studied the correlation of subtypes of TILs with the clinical variables and treatment outcome in 278 HNSCCpatients.[190] After controlling for other prognostic factors, raised $CD4^+$ T cells were predictive of better OS and DSS (p=0.003 and 0.004 respectively).

CRP and SCC antigen (SCC-Ag) have gained growing interest in cancerresearch. Several studies on HNSCC have found that SCC-Ag is linked with aggressive tumor, more recurrences and poor survival.[191] Adel et al. in their retrospective study of 277 HNSCC patients, studied the association between pre-operative serum markers (CRP and SCC-Ag) and post-operative marker of prognosis (LND).[144] LND, as mentioned previously, is defined as follows —the ratio of nodes with metastasis to number of nodes removed. The outcome measures were distant metastasis, OS and DFS. The prognostic value of pre-operative CRP and SCC-Ag levels was evaluated in comparison to LND. They found LND was associated significantly with distant metastasis, OS as well as DFS (all p<0.001). Pre-operatively, elevated levels ofCRP and SCC-Ag were significantly associated with LND (p=0.006), OS (p<0.001), and DFS (p<0.001). Patients with high LND were then classified into risk groups using the CRP and SCC-Ag levels (OS p=0.003, DFS p=0.010). They concluded that elevation in pre-operative SCC-Ag and CRP levels could predict a raised LND; SCC-Ag and CRP were found to be markers for further stratifying patients with increased risk LND into subgroups.

Many studies have paid attention to the clinical importance of LND in prognosis as well as the association with poor outcomes in patients with oral ororopharyngeal SCC.[143,192] Moreover, some authors have claimed LND as more prognostically accurate when compared toTNM staging in node positive HNSCC patients. Patel et al. in their multicenter international study, found that LND was better than the conventional node staging AJCC system in predicting outcomes in oral SCC.[193] The reason for this may be that LND considers not only number of nodal metastasis but also degree of lymph node dissected and the staging, surgically. Most studies have used 0.06 as the cut-off for LND. Liao et al. have used 0.16 as cut-off forneck dissection levels I to III and 0.048 for neck dissection stage I–V.[143]

2.4.B. Use of NLR as a prognostic marker

For a number of decades, raised leukocyte count was linked to poor prognosis in a variety of cancer types.[194,195] A rise in NLR points to a continuing inflammatory process and decreased regulatory pathways. The probable advantage of this inflammation based biomarker (NLR) is that first, it reflects core immune status and the host's inflammatory response; second, NLRcan be calculated easily using routine pre-treatment blood investigations at no added costs. These reasons could make NLR a promising biomarker of prognosis in low and middle income countries where HNSCC is a common clinical problem. But, there still is lack of agreement on the most favorable baseline NLR. An additional limitationis that NLR may be influenced by certain external factors like corticosteroids use or infections. Perisanidis et al. studied 97 patients with oral SCC, who had received pre-operative CRT. In this study, NLR>1.9 independently predicted shorter DSS in oral SCC patients.[196]

Studies have used various cut off points for NLR. These are usually calculated based on best specificity or sensitivity derived from an ROC curve for a particular outcome, like OS or PFS.Bruixola et al.found the bestspecificity and sensitivity for the prediction of poor OSto be NLR≥2.6.[59]A meta-analysisfoundaraisedpre-treatment NLR was linked to poorer prognosis in HNSCCpatients.[197] However, they noted that the data on the use of predictive models like NLR were scarce in patients with locally advanced HNSCC. Haddad et al. studied the role of NLR in patients treated for locally advanced HNSCC with CRT, and found that a pre-treatment NLR value of ≥5 was significantly associated with mortality.[198]

A meta-analysis published in 2018 studied the role of NLR in prognosticating patients being treated for HNSCC.[48] They analyzed 19 studies which included 3770 patients. The result of this analysis was that NLR > cutoff value in the respective study was significantly associated with poorer OS (HR 1.69; 95%CI 1.47-1.93; p<0.001) as well as DSS (HR 1.88; 95%CI 1.20-2.95; p=0.006), and concluded that raised NLR predicted poorer outcomes in HNSCC patients.

Asystemic review and meta-analysispublished in 2018, studied the effect of the pre-treatment NLR on OS in HNSCC patients; they also concluded that a raised NLR was predictive of worse OS in HNSCC patients.[199] Tham et al. in their meta-analysis and systematic review also studied the association between NLR and the prognosis in HNSCC, in which they included 15 studies with 5562 patients.They also demonstrated that araised NLR predicted significantly worse OS and DSS.[200]

The meta-analysis published in 2018, studied the association between pre-treatment OS and NLR in HNSCC patients.[199] This analysis included 24 studies in the analysis, including 6479 patients. They found an overall HR for OS in patients with a raised NLR (2.04 to 5) was 1.78 (95%CI 1.53-2.07; p<0.0001); subsite wise HR were- oral cavity 1.56 (95%CI1.23-1.98; p<0.001), larynx 1.55 (95%CI 1.26-1.92; p<0.001), nasopharynx 1.66 (95%CI 1.35-2.04; p<0.001) and hypopharynx 2.36 (95%CI 1.54-3.61; p<0.001). The HR was highest for subsite hypopharynx in this meta-analysis. They concluded that a raised NLR was predictive of worse OS in HNSCC patients. A meta-analysis published in 2018 also had similar findings and concluded that HNSCC patients with raised pre-treatment NLR in the peripheral blood hadworse prognosis, and were more prone to local recurrence and even distant metastasis.[197] Further literature on NLR is presented in the Discussion Chapter.

Data on Indian patients is limited in literature. An Indian study on HNSCC patients found that pre-treatment NLR and platelet/lymphocyte ratio (PLR) were inflammatory biomarkers clinically useful in cancer and were predictive of survival in non-metastatic HNSCC patients.[201] NLR values can be easily calculated from routine pre-treatment blood sample at no additional cost and could helpt he treating team in determining the prognosis in HNSCC patients.

2.4.C. Use of malnutrition as a prognostic marker

Many studies have attributed the cause of death in 10%-20% cancer patients to be malnutrition and not the cancer itself.[202-204] Thus, it would be appropriate to note that nutrition forms an important part of multi-modal cancer treatment. Van Bokhorst et al. noted that

malnutrition had significant harmful impact on morbidity, mortality, and quality of life.[205] *Martin et al. noted longer survival in cancer patients with stable weight and BMI ≥25.0 kg/m*^{2} *and shortest survival in patients with high percentage weight loss and low BMI.*[206] *In fact, loss of ≥5% weight during treatment was found to be linked to poorer survival in HNSCC.*[29,207]

There is literature on poor health related outcomes associated with malnutrition. Malnutrition was found associated with weight loss and muscle loss,[206] *impaired immune competence and higher infections,*[25,202,208] *psychosocial stress,*[209] *lower QOL,*[211] *treatment related toxicity,*[212] *and higher risk for mortality.*[202,206]

Mick et al. found pre-treatment loss of weight to be an independent predictor and strongly associated with survival in stage III/ IV HNSCC patients that were treated using multi modality treatment.[212] *Platek et al. in their study, concluded that pre-treatment weight was a very crude marker of nutritional status but may have a prognostic value in HNSC patients undergoing definitive CRT.*[213]

A retrospective study that was published in 2013 analyzed records of 194 stage III and IV HNSCC patients that were treated using CRT between years 2007 and 2009.[214] *They defined early mortality as death during CRT or within 60 days of completion of treatment. They found 14 (7.2%) patients had early mortality (78.6% were due to infection). On univariate analysis a significant correlation was found between early death and many pre-treatment variables, like Eastern Cooperative Oncology Group (ECOG) PS>1, albumin <3g/dL, hemoglobin <10g/dL, BMI <19kg/m*^{2} *and blood absolute lymphocyte count <700/µL. Multivariate analysis revealed that BMI <19kg/m*^{2}*, PS >1 and blood absolute lymphocyte count <700/µL were significantly associated with early mortality. They concluded that pre-treatment poor PS and malnutrition were independent predictors of early death in locally advanced in HNSCC patients receiving CRT.*