Original Article

Relationship between the nutritional status and antimicrobial protein levels with the periodontal condition in untreated head and neck cancer patients

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Abstract

Background: Chronic periodontitis might be associated with oral premalignant lesions, tongue cancers, and other oral neoplasms, which is a foremost public health problem throughout the world. The nutritional status of the patient with cancer becomes very important for tolerating the treatment course as most of the newly diagnosed patients with head and neck cancer are malnourished before treatment begins. Antimicrobial proteins are also essential contributors to maintaining the balance between health and disease in this complex environment. Therefore the aim of this study was to determine whether an association exists between the measures of nutritional status and the levels of antimicrobial proteins with the periodontal condition in newly diagnosed, untreated head and neck cancer patients. **Materials and Methods:** A total of 50 patients newly diagnosed with head and neck carcinoma were included. Saliva and plasma samples were collected, together with clinical periodontal recordings. Nutritional status parameters consisted of body mass index, serum albumin, hemoglobin, and total lymphocyte count. Cystatin C and lysozyme were the antimicrobial proteins. **Results:** A logistic regression model showed that periodontal parameters were inversely related to their nutritional status; however, antimicrobial protein levels showed to be directly related to periodontal condition. **Conclusion:** This study suggests an association between periodontal disease, nutritional status parameters, and antimicrobial protein levels.

Keywords: Antimicrobial proteins, chronic periodontitis, nutrition, squamous cell carcinoma

Introduction

The dental biofilm that harbors gram-negative anaerobic bacteria has known to be associated with periodontitis, a chronic inflammatory disease that eventually leads to irreversible destruction of tissues supporting teeth, detectable as periodontal pockets and alveolar bone loss, during which there is continuous release of bacterial and inflammatory markers into gingival crevicular fluid (GCF), saliva and, to a certain degree, into blood and which adversely affect systemic health. Therefore, aiming at treating periodontal infections has shown to prevent and reverse systemic adverse effects.[1] Cancer as of today is a major public health problem and by 2020, the World Health Organization (WHO) has estimated that, globally, >15 million people will experience cancer and 10 million will die from it each year.[2] The consumption of alcohol and the usage of tobacco are considered to be the predominant and established etiologic factors for head and neck cancer (HNC). Other risk factors

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include periodontal disease, poor oral hygiene and dental status, and human papilloma virus infections.[3] A positive association between periodontal disease and cancer risk have also been suggested by several epidemiologic studies.[4,5] Periodontitis is known to contribute to constant low-grade systemic inflammation with elevated levels of circulating inflammatory markers and various identified inflammatory markers, and include proinflammatory plasma cytokines, peripheral white blood cells, prostanoids, and proteases including matrix metalloproteinases and acute-phase proteins. Periodontal pathogen-induced chronic inflammation may also result in the breakdown of normal cell growth control and potential carcinogenesis; in addition to all this, the immune system in an individual with chronic periodontal disease may be deficient at clearing infection, and subsequently inadequate at surveillance for tumor growth.[6] Summing up all the above-mentioned reasons, periodontitis is considered as a marker that has potential influences on tumor growth and progression.[3] These reported observations might be explained by another plausible mechanism, which is the increased production of carcinogenic nitrosamines. The endogenous carcinogenic nitrosamines that get formed in the oral cavity are elevated by poor oral hygiene, periodontal disease, tobacco smoking, and certain dietary factors. Oral microorganisms also affect nitrosamine production. Acetaldehyde production from alcohol by oral microbiota is another possible reason as to why consumption of alcohol might be a risk factor of HNC.[4] Studies have thus concluded that the mechanisms associated with the systemic inflammatory burden and increased levels of carcinogenic compounds generated in response to periodontal pathogens and as a sequela of inflammatory mechanisms could be a contributing factor to the development of cancer.[5] HNCs include those of the upper aerodigestive tract, including the oral cavity, nasopharynx, oropharynx, hypopharynx and larynx, paranasal sinuses, and salivary glands. Nearly 40% of newly diagnosed patients with HNC are malnourished even before treatment begins. Among those patients with advanced-stage III and IV disease, the prevalence of malnutrition is even greater (56%).[6,7] It is important to identify malnourished patients in need of restorative medical nutritional therapy because malnutrition is associated with adverse outcomes in terms of cancer prognosis (response rate and survival), poor wound healing, impaired immune function as evidenced by susceptibility to infectious agents, prolonged hospitalizations, and overall cost of care.[8,9] Pragmatic marker of general health status is the level of serum albumin. Inflammation and malnutrition cause a decrease in its rate of synthesis, thereby a reduction in albumin concentration. The presence of hypoalbuminemia has been thought to be the result of nutritional depletion auxiliary to the tumor and to be coupled with tumor size and site of disease in patients with advanced cancer.[8]

Oxygen in the body is carried by hemoglobin (Hb) and the measure of Hb will affect the oxygen content in the body and tumor.[9] A fall in the Hb level will eventually result in tumor hypoxia, increased hypoxic cells, angiogenesis, and resistance to chemotherapy and radiotherapy in cancer patients.[10] In the last few decades, numerous studies have been performed to study the relationship between cancer and cellular immunity and immunologic changes in advanced malignancy is the reduction in the total lymphocyte in blood.[11] HNC patient often experience dysphagia, odynophagia, passage difficulties, and pain in the mouth which in turn may result in changes in taste or appetite, eventually resulting in difficulties with nutritional intake leading to critical weight loss.[12] This weight loss in turn increases morbidity and mortality and decreases treatment tolerance and overall quality of life.[13] The microbial ecology of the healthy mucosa is always kept in check and they maintain the microbial invaders with the help of antimicrobial peptides which are part of the innate immune system.[14] Studies have shown that cystatins are involved in inflammatory disease, bone resorption, tumor progression, infection, and in the pathogenesis of the periodontal disease.[15,16] Cystatin C is generally downregulated in tumors, the lower levels of cystatin C could allow a surplus of harmful tumor-associated proteolytic activity.[16,17] Both the primary and secondary granules of neutrophils and macrophages are the major source of lysozyme, which not only exhibits antibacterial effect but also may function in a regulatory capacity in the inflammatory or immune response.[18,19] Lysozyme because of its ability to inhibit bacterial aggregation may participate in the regulation of the oral microflora, however, lysozyme also inhibit PMN chemotaxis and depress superoxide generation of PMNs. Lysozyme is considered to be an important protective enzyme in periodontal diseases.[20]

To date, the interplay between these parameters remains unclear in those patients with HNC. It was hypothesized by us that patients with newly diagnosed, untreated HNC will have a poor oral health status indicating a heightened risk for malnutrition-related complications. Hence, the purpose of this study was to investigate the nutritional status and antimicrobial protein levels and correlate them with the periodontal condition in newly diagnosed untreated HNC patients.

Materials and Methods

A total of 50, newly diagnosed, untreated HNC patients aged 28–80 years were recruited from the in-patient department of Otorhinolaryngology, Sri Devaraj Urs Medical College and Hospital, Kolar, Karnataka, India. Subjects were explained about the study and on the basis of their approval were asked to read carefully and sign the consent form. The design of the study and procedures for obtaining informed consent was approved by the Ethical Committee of AECS Maaruti College of Dental Sciences and Research Centre, Bangalore (301/2012–2013) and Sri Devaraj Urs Medical College and Hospital, Kolar, Karnataka, India, (2598/2012–2013) and was performed in accordance with the Code of Ethics of the World Medical Association according to the Declaration of Helsinki of 1975 as revised in 2000.

Individuals included in the study were initially diagnosed with squamous cell carcinoma of oral cavity, oropharynx, or larynx within 45 days and who had not undergone any treatment. The diagnosis was done according to International Classification of

Diseases for Oncology, third edition revision 1.[21] Tumor stage was assigned according to the American Joint Committee on Cancer staging criteria, seventh edition.[22]

The inclusion criteria were patients aged 18 years and older with the presence of at least 6 natural teeth. Individuals previously treated for malignant disease or with the history of cancer, immunocompromised subjects, patients with gastrointestinal disorders, patients who have undergone periodontal therapy in the last 6 months, and patients lacking data variables required for the scoring analysis were excluded from the study.

General measurements were performed before cancer therapy. Medical charts were reviewed to obtain information on cancer site and cancer stage. A personal interview was conducted to obtain information on drinking habits (Yes/No), tobacco chewing habits, and smoking habits (pack-years, i.e., number of packs smoked per day multiplied by the number of years of smoking). The evaluation of nutritional status parameters of study group members consisted of reviewing their medical records for anthropometric, biochemical, and hematological data obtained during the first 48 hours of their hospital admission.

Abstracted data consisted of:

1. BMI: Body mass index (BMI) which was calculated by dividing the body weight in kilograms by the height in square meters and was classified as malnutrition if ≤18.5 kg/m^2 (WHO, 2004; MUST, 2013)[23,24]
2. Serum albumin: Serum albumin <2.7 g/dL was considered consistent with malnutrition (ESPEN guidelines, 2003).[25] It was measured by the bromocresol green albumin method
3. Hb: Hb levels ≤11.9 g/dL was considered consistent with malnutrition (WHO, 2011).[26] It was measured using XS-800i SYSMEX
4. Total lymphocyte count: Total lymphocyte count (TLC) was calculated as the number of white cells per microliter multiplied by the percentage of lymphocytes (ESPEN guidelines, 2003).[25] It was considered consistent with malnutrition at values ≤1499/μL. It was measured using XS-800i SYSMEX.

A 10 mL blood sample was drawn and was then centrifuged at 2000 ×*g* for 10 min, and plasma was separated and stored at –20°C until analysis. Whole saliva samples were obtained by expectorating into polypropylene tubes. Patients were advised to rinse their mouth several times with water and then to relax for 5 minutes and then asked to lean their head forward over the polypropylene tube. The samples were centrifuged for 20 minutes at 1000 ×*g*. The samples were stored in aliquot at –20°C until the sample collection period and were thawed immediately before assays. A physiologist masked to the oral status of patients measured the salivary levels of cystatin C and lysozyme in each patient by ELISA.

Subsequent to saliva collection, all participants underwent a clinical periodontal examination. Periodontal clinical examination was performed using a UNC 15 pressure-sensitive periodontal probe (AXE, China). Number of teeth present, number of mobile teeth, number of decayed teeth, probing pocket depth (PPD), clinical attachment level (CAL), Plaque Index (PI),[27] and percentage of sites with bleeding on probing[28] (BOP) were recorded. Third molars were excluded from the measurement. In addition, the number of occluding tooth pairs (functional dental units) with intact crowns were measured. Pontics attached to fixed prostheses were counted as if they were natural teeth. Removable dentures were not counted because they were deemed too mobile to be effective masticatory elements, and residual roots were not counted because they were not involved in occlusion.

Statistical analysis

Statistical software namely SPSS 16.0, SAS 9.2 were used for data analysis and data processing. Descriptive and inferential statistics have been carried out in the present study. A P value < 0.05 was considered as statistically significant. Pearson's correlation coefficient was used to show the linear relationship between the 2 sets of data. A sample size of 50 from a population of 100 has a 95% confidence interval with probability of 0.05 and sum of square of means equal to 7.6 when the standard deviation for the sample is 0.42. Therefore, the power of the study was calculated by using the above values and was estimated to be 0.84. This inferred that a sample size of 50 was adequate to get significant values.

Results

A total of 50 patients were enrolled, 30% of the subjects were between 55 and 64 years of age group. The male/female ratio was 25/25. Subjects were distributed according to their cancer stage and site, 58% of subjects fell in the IV stage of cancer and 56% of the subjects had cancer at buccal site. In accordance with their habits, 40% of the subjects were alcohol consumers; on the basis of smoking habits in pack-years, 16% of the subjects smoked 20 packs in a year. According to their consumption of the number of tobacco packets, 20% of the subjects chewed 6 packs per day. The mean albumin and Hb levels were 3.23 ± 0.61 and 11.96 ± 0.85 g/dL and the TLC was 2.17 ± 0.97 × 10^3 μL, respectively. The mean levels of lysozyme and cystatin C were 16.62 ± 4.79 μg/mL and 14.66 ± 2.84 ng/mL, respectively.

The dental and oral health status was assessed using the following variables: number of teeth present, mobile teeth, decayed teeth, anterior occluding teeth, posterior occluding teeth, PPD, CAL, bleeding on probing, and PI have been outlined in Table 1. The relationship between nutritional status and antimicrobial protein parameters with periodontal variables was analyzed by using Pearson's correlation coefficient. Table 2 showed that PPD increased with a decrease in serum albumin; there was an increase in PPD, CAL, and PI with a decrease in Hb levels. Similarly, PPD and PI increased with an increase in TLC; however, PPD, CAL, PI, and BOP% increased with an increase in cystatin C levels, however, BMI and salivary lysozyme levels decreased with a decrease in BOP%.

Depicts the prevalence of malnutrition according to the different nutritional parameters assessed in our study.

Discussion

Although the original hypotheses linking inflammation and cancer appeared more than a century ago, the relationship between the 2 has been well documented in the recent literature. Periodontal pathogens induce chronic inflammation to already initiated cells, leading to break down of normal cell growth control and potential carcinogenic transformations. Increased production of carcinogenic nitrosamines is another plausible mechanism. Poor oral hygiene and periodontal disease by tobacco use and certain dietary factors promote the formation of endogenous nitrosamines in the oral cavity by nitrate-reducing bacteria. Tooth loss resulting from poor oral hygiene may also be a contributing factor to greater nitrosamine production.[5] Saliva is of paramount importance for maintaining oral health and contains proteins, and peptides involved in human salivary defense have been identified and characterized.[17,29] Cysteine proteinases are known to play an important role in an inflammatory disease like periodontitis, psoriasis, multiple organ failure, bronchiectasis, and malignancy. This could happen either directly by tissue degradation or indirectly by the activation of proinflammatory mediators and other proteolytic enzymes.[30] The antimicrobial activity of lysozyme is generally associated with its lytic action on bacteria by catalyzing the hydrolysis of cell wall polysaccharides.[24] It has been observed that there is a close correlation between the lysozyme concentrations and biological features of tumors. And there have been minimal rises in lysozyme activity in tumors of lower immunogenicity and high metastatic capacity.[31]

Malnutrition-related complications are seen more among untreated patients with malignant tumors of HNC region. The BMI decreases in proportion to inadequate caloric intake or added need in debilitating illnesses like cancer, and similarly, Hb concentrations decrease in response to deficiencies of iron, proteins, vitamins A, B1, B2, B6, B12, E, folate, and copper. Lymphocyte numbers are known to decrease in association with deficiencies in proteins, calories, vitamins, mineral, and trace elements.[32] Serum albumin is known to be an independent predictor of survival in lung, pancreatic, gastric, colorectal, and breast cancers.[33] Medow *et al*[34] reported that the patients with HNC with high preoperative serum albumin concentrations had a better prognosis than those with lower serum albumin concentrations.

In this present study, a total sample of 50 newly diagnosed, untreated HNC patients were recruited from the in-patient department of Otorhinolaryngology, Sri Devaraj Urs Medical College and Hospital, Tamaka, Kolar, Karnataka. The average age range in our study was between 25 and 84 years. Maximum incidence of carcinoma was found in the sixth decade of life (38%). Our study also found that the prognosis of patients with HNC is poor when the age of onset ≥55 years old. This could be attributed to the deterioration of physical or nutritional status that tends to make older patients more vulnerable. Buccal mucosa was the most common site for oral cancer followed by oropharynx. Sixty percent of our subjects were in TNM stage IV, which could be explained by a high prevalence of betel quid chewing.

Multiple systemic factors, including smoking and excessive alcohol ingestion, may also impair the nutritional well-being

Table 1: Dental and oral health status in patients with head and neck cancer

Parameters	Mean	SD
Teeth present	22.92	4.457
Mobile teeth	4.55	2.204
Probing pocket depth	5.67	1.07
Clinical attachment level	6.49	0.80
Bleeding on probing percentage	19.28	6.37
Plaque Index	1.63	0.64
Decayed teeth	2.36	1.20
Anterior occluding teeth	3.0	2.1
Posterior occluding teeth	4.6	3.2

Table 2: Relationship between periodontal parameters (PPD, CAL, PI, and BOP%) with nutritional status and antimicrobial protein parameters in patients with head and neck cancer

	Body mass index (kg/m²)	Serum albumin (g/dL)	Hemoglobin (g/dL)	Total lymphocyte count (µL)	Lysozyme (µg/mL)	Cystatin C (ng/mL)
Probing pocket depth						
r	-0.029	-0.326	-0.271	0.293	0.128	0.274*
P	0.839	0.021*	0.035*	0.039*	0.377	0.054*
Clinical attachment level						
r	-0.065	0.042	-0.247	-0.055	-0.118	0.292
P	0.655	0.773	0.027*	0.703	0.414	0.04*
Plaque Index						
r	-0.118	-0.013	-0.399	0.325	-0.051	0.259
P	0.414	0.93	0.004*	0.021*	0.727	0.05*
Bleeding on probing percentage						
r	0.261	0.087	0.039	0.164	0.384	0.257
P	0.047*	0.546	0.786	0.255	0.006*	0.05*

*There is a significant correlation at $P<0.05$. r: Regression analysis

of individuals with HNC. Cigarette smoking is associated with leanness and is often seen in patients newly diagnosed with HNC. This may occur because nicotine modulates the appetite-regulating areas of the hypothalamus, leading to an anorexic effect and a decrease in food intake.[32] In the present study on the basis of smoking habits in pack-years, 16% of the subject consumed 20 packs in a year. Our study developed a statistically significant association between smoking and the HNC stages. Heavy users of alcohol are often malnourished, this issue is exacerbated because alcohol also has a direct effect on the gastrointestinal tract and liver. The metabolism of alcohol in the liver by both dehydrogenase and microsomal ethanol-oxidizing system generates toxic products such as acetaldehyde, which can interfere with the metabolism of lipids and nutrients. Ethanol, the main component of alcoholic beverages, determines the risk of cancer.[32] In our study, 40% of the subjects were alcohol consumers. Our study developed a statistically significant association between alcohol consumption with the HNC stages.

The type of tobacco, duration, and patterns of use are likely to be important modifiers.[1] According to their consumption of tobacco, 70% of the subjects were tobacco chewers. On the basis of the number of tobacco packets, 20% of the subjects consumed 6 packs per day, and according to the duration of usage greater part of the subjects, that is, 16% of the subjects chewed tobacco since 30 years and 4% of the subjects chewed tobacco since 50 years. Our study developed a statistically significant association between the duration and number of packets used with the HNC stages. In our study among 50 patients with HNC, 16 had a BMI of ≤18.5, 23 had a Hb of ≤11.9 g/dL, 14 had serum albumin levels of ≤2.7 g/dL, and 14 had TLC of ≤1499/μL.

According to BMI, malnutrition was found in 32% of the subjects (n = 16), TLC demonstrated immunologic depletion in 28% of the subjects (n = 14), low levels of serum albumin was found in 28% of the subjects (n = 14), and low levels of Hb was found in (46%) of the subjects (n = 23). The association between periodontal disease (as possibly indicated by poor oral hygiene, tooth loss, and bleeding gums) and HNC is biologically plausible, as periodontal infection can lead to release of inflammatory mediators. The host response to bacterial inflammation is known to play a role in the development of cancer. Poor dentition has also been associated with oral flora that can produce carcinogenic by-products to increase the risk of cancer.[35] To study the oral and dental health status, we took the mean of the number of teeth present (22.92 ± 4.457), mobile teeth (4.55 ± 2.204), decayed teeth (2.36 ± 1.20), anterior occluding teeth (3.0 ± 2.1), and posterior occluding teeth (4.6 ± 3.2). Missing teeth could be a risk factor because it is an indicator of periodontal disease. Abnet *et al.*[36] have suggested that individuals with missing teeth may have a greater burden of an oral flora that is more effective in the reduction of nitrate to nitrite or in the production of acetaldehyde, a metabolite of alcohol that has proved to be carcinogenic. The number of anterior and posterior opposing tooth pairs were found to have a U-shaped distribution. For anterior teeth, 2 (4%) were edentulous, 17 (34%) had all their anterior pairs, and 31 (62%) subjects had values between these extremes. For posterior teeth, 4 (8%) were edentulous and 2 (4%) had all their posterior pairs. Andrade *et al.*[37] showed that the number of posterior occluding pairs was the strongest predictor of a higher risk of malnutrition. Our results are at some variance from Zheng *et al.*[38] who noted excessive numbers of missing teeth in patients with HNC and hypothesized that this leads to dietary alterations and a malnourished state. This discrepancy in results probably arose because in our study we gauged masticatory function by defining occluding pairs of teeth on the basis of the number and type of teeth present which is more discriminatory and descriptive of masticatory potential than the mere number of teeth. A significant percentage of weight loss is observed in patients with HNC, which correlates with the prognosis of the patient.[39] The mean BMI of subjects in our study was of 19.37 ± 2.71. An inverse association between BMI and HNC risk has been observed in previous studies by Gaudet *et al.*[40] wherein they showed that low BMI indicated a heightened risk for malnutrition-related complications encountered significantly in patients with malignant tumors of head and neck region. Our study hypothesized that BMI was positively correlated with BOP% which infers that decreased BMI has been associated with decreased gingival bleeding. This may be because BMI has a positive relationship with proinflammatory markers and inverse relationship with antioxidants. BMI alters periodontal microflora, increases susceptibility to various infections, and exaggerates host immune response. Serum albumin level is a practical marker of general health as it demonstrates the severity of an underlying disease and mortality in the elderly. Inflammation and malnutrition both reduces albumin concentration by decreasing the rate of synthesis.[41] In our study, the mean serum albumin concentration for all patients was 3.23 ± 0.61 g/dL. In our study, we defined a cutoff value for serum albumin as 2.7 g/dL, which was considered consistent with malnutrition. Several studies[42,43] have demonstrated the predictive value of hypoalbuminemia for morbidity, mortality, and increase of length of stay and costs. Therefore, hypoalbuminemia is often considered as an indicator of the illness severity rather than the nutritional status. However, there is an indirect relationship between inflammations with nutritional status. Inflammation contributes to an increase in net protein loss caused by catabolism, and also induces anorexia, reducing the probability that a patient will get an adequate intake to his requirements, thus accelerating the undernutrition process.[44] The possible mechanism behind decreased sera levels of albumin could be related to the consumption of a major fraction of serum albumin in the quenching of free radicals that are supposed to be increased.[45] Free radical production is ubiquitous in all respiring organisms and is enhanced in many disease states and in carcinogen exposure.[46] In this study, Pearsons correlation analysis shows a significant association between serum albumin concentration and PPD. In patients with HNC, it is important to maintain adequate serum albumin concentrations because the serum albumin concentration is one of the determinants of survival rate. Although additional studies are necessary, the periodontal condition may contribute to

increased survival rate in patients with HNC. In the present study, our results showed an inverse independent relationship with serum albumin concentration and PPD which was in agreement with the study conducted by Majon *et al*[47] in institutionalized older adults with vertical tooth mobility and periodontal pockets >6 mm which showed significantly lower albumin concentrations. Although the precise mechanism underlying the serum albumin–periodontal relationship is not well understood, this relationship might be explained by the following 2 conceivable possibilities, namely the influence of nutritional aspect or chronic disease aspect.[48] Majon *et al*.[47] reported that a compromised oral functional status has little influence on the nutritional status of semidependent elders, whereas those in poorer health might be more susceptible to poor oral function. In addition, the rate of albumin synthesis in elderly subjects might not be sensitive to changes in protein intake. Our results suggested that not only the nutritional aspect but also the inflammatory reactants might be influenced by both serum albumin concentration and periodontal disease condition. In addition, hypoalbuminemia can be associated with cancer, may occur after radiation or chemotherapy, and has been attributed to many causes, including hypermetabolism of the tumor, inadequate nutrient intake, and/or malabsorption. Low albumin levels have been reported to accompany or follow acute infections or inflammation.[49] Anemia is well known to be prevalent in the general population and is even more common among cancer patients. It is widely accepted that anemia causes radiation resistance because the Hb level presumably mediates tumor responses to radiation through the delivery of oxygen to the tumor.[50] Hb level has been reported to be a prognostic factor in number of malignancies treated with radiation therapy, particularly carcinoma. Chua *et al*.[51] reported low pretreatment Hb levels to be a prognostic factor for tumor control and/or patient survival. The mechanism by which anemia worsens outcome is a multifactorial process, and it may be in part explained by low Hb levels resulting in the regions of low oxygen tension within the tumor, which leads to hypoxia-induced radioresistance in the tumor cell population.[52] Nordsmark *et al*[53] thought that Hb was an important prognostic factor affecting the locoregional recurrence-free survival rate in HNC patients treated by radiotherapy. The reason may be that anemia promotes tumor angiogenesis and causes tumor cells resisting apoptosis signal, thus affecting the efficacy of cancer treatment. In addition, anemia leads to an increase of hypoxic cells, which are resistant to radiotherapy and are considered as the main reason for local uncontrolled disease or recurrence after radiotherapy. Twenty-three of our subjects displayed value of ≤11.9 g/dL. The results of this longitudinal study showed that a reduction in Hb was associated with the progression of periodontal disease. Hb shows a statistically significant negative correlation with PPD and CAL, and PI which infers that with increase in PI, PPD, CAL, and Hb decrease. Studies have tried to evaluate the relationship between periodontitis and Hb levels. Hutter *et al*[54] have suggested that periodontitis may cause lower numbers of erythrocytes and consequently lower Hb levels. This pathogenesis was reported to be similar as for rheumatoid arthritis, that is, depressed erythropoiesis by circulating proinflammatory cytokines process. Cytokines such as interleukin (IL)-1, IL-6, tumor necrosis factor (TNF)-α, and interferons are hypothesized to be involved in the maintenance of red blood cell production or stability. Undernutrition induces immunologic changes, such as drop in TLC, which increase frequency and severity of infection. This accounts for much of the morbidity and mortality associated with malnutrition. TLC has been suggested as a useful indicator of nutritional status and outcomes.[47] A 4-fold increase in mortality has been reported with a TLC of <1500/μL.[55] In our study, 14 of our 50 subjects had TLC ≤1499/μL which was considered consistent with malnutrition. It has been suggested that malnutrition leads to suppression of cellular immunity. The immune system has long been thought to be important in the prevention and control of cancers. HNSCC is an immunogenic tumor, as shown by a variable amount of infiltrating lymphocytes and other immune cells.[56] The reduction of total lymphocytes in the blood is the main immunologic change in advanced malignancy.[11] Apart from the cytokine levels, periodontal disease is known to affect the cellular response in systemic circulation. An increase in the white blood cell levels and the presence of hyper-responsive monocytes has been demonstrated in systemic circulation of patients with periodontal disease.[57] Our assessment of TLC in HNC patients showed a mean of $2.17 \pm 0.97 \times 10^3$ μL. TLC was found to be comparatively on the higher side in patients with HNC. Malnutrition is a factor that affects the TLC, thereby compromising immunologic status. However, the lymphocyte count can increase in the presence of bacterial infection. Lee[58] has reported that patients with higher peripheral blood CD8 count experienced increased survival. Better clinical outcomes associated with high lymphocyte count have been reported in gastric and HNC. Our results showed TLC to be positively correlated with PPD and PI. Normal humoral immunity requires adequate function of B lymphocytes under proper regulation of T-helper lymphocytes. Researchers have proved that protein-energy malnutrition affects both B-lymphocytes differentiation and T-lymphocyte regulation by causing a defect in humoral immunity and antibody synthesis. This defect was readily restored by nutritional repletion, as shown by Law *et al*.[59] ILs are a complex group of proteins and glycoproteins that are produced by macrophages and T lymphocyte. They exert a pleotrophic effect on the number of different target cells but primarily affect the T-lymphocyte function.

Saliva is a defender of the oral cavity. Periodontal disease has been associated with cysteine proteinases in gingival tissues and GCF. The origin of these cathepsins during inflammatory reactions most probably are monocytes or macrophages. Cystatins are cysteine proteinases inhibitors present in a variety of tissues and body fluids, including saliva. Because cysteine proteinases are suspected to play a role in the pathogenesis of periodontal disease, cystatins in saliva have been studied as possible protective proteins in the processes of tissue and bone destruction.[17] The main physiological role of cystatin C is believed to be regulation of cysteine proteinases secreted from the cells or leaked from the lysosomes during necrotic or

apoptotic processes, which links cystatin C with the etiology of various diseases, including cancer. The present study reported a mean of cystatin C levels as 14.66 ± 2.84 ng/mL. Cystatin C is increased in subjects with malignant diseases, is associated with impaired renal function, and seems to be a better marker than creatine.[60] A large body of literature has been accumulated to suggest that cystatin C participated not only in the transformation of cells to a malignant state but also in tumor growth, invasion, and metastasis. Among the subjects involved in our study, 32% of the subjects were smokers and 72% of the subjects chewed tobacco; our study was similar to Warfel *et al.*[61] where he reported that alveolar macrophages from smokers secreted more cystatin C. This could be because of tobacco byproducts present within the smoke that served as an irritant, increasing constitutive secretion of cystatin C elaborated by alveolar macrophages. Cystatin C levels were positively correlated with mean PPD, CAL, PI, and BOP%.

Lysozyme is a major secretory product of macrophages and is found in both primary and secondary granules of neutrophils. Lysozymes antibacterial role has generally been attributed to its muramidase property in the hydrolysis of bacterial cell wall peptidoglycan. However, it has been suggested that lysozyme may participate in the regulation of oral microflora through its ability to inhibit bacterial growth and mediate bacterial aggregation.[19] This enzyme exhibits antibacterial effects and has the regulatory capacity in inflammatory or immune response, because of which this enzyme has been studied as a protective enzyme. Eccles and Alexander[62] have already shown that the macrophage content of tumors is a reflection of a host immunologic response to the tumor and that there is an inverse relationship between macrophage content and the development of metastases.

Our results revealed that HNC subjects had markedly lower levels of salivary antibacterial protein lysozyme (16.62 ± 4.79 μg/mL). A localized primary tumor is under some form of host restraint associated with large numbers of macrophages resident within the tumor mass and its draining regional lymph nodes. This macrophages response could account for the elevated serum lysozyme levels. The development of metastatic disease because of the failure of host resistance is associated with sparse macrophage infiltration of the tumor mass and therefore with lower levels of lysozyme which explains lower levels of lysozyme in cancer patients.[63] In our study, we observed a negative correlation with higher stages of cancer. Lysozyme may be an important protective enzyme in periodontal diseases. Decreased concentration of lysozyme is also found in the saliva of patients with periodontitis, and increased levels in the crevicular fluid have been noted.[29] This could be attributed to the fact that a large amount of leukocytes concentrated in gingival sulci over a short period of time can cause a decrease in the amount of leukocytes in nonstimulated saliva, which was also reported in saliva of patients with periodontitis in our study, which was in agreement with Ito *et al.*[17] and Syrjanen *et al.*[64] We also found that lysozyme was positively correlated with BOP%.

Conclusions

To the best of our knowledge, this is the first study to assess the relationship between nutritional status parameters and antimicrobial protein levels and the oral health status in patients with untreated HNC. The present investigation revealed statistically that PPD increased with a decrease in serum albumin; there was an increase in PPD, CAL, and PI with a decrease in Hb levels. Similarly, PPD and PI increased with an increase in TLC; however, PPD, CAL, PI, and BOP% increased with an increase in cystatin C levels, whereas BMI and salivary lysozyme levels decreased with a decrease in BOP%. Some nutritional status parameters (low Hb) indicated a heightened risk of malnutrition-related complications in HNC patients. Other nutritional status parameters (BMI, serum albumin, and TLC) could be encountered as more significant in untreated HNC.

Oral cancer control is altering, with an increasing emphasis on prevention, early diagnosis, and patient experience during and after treatment. At the identical time, primary care is progressively encouraged by government body and health funders international. Therefore, it is well timed to consider how primary care can influence cancer control and periodontal disease, which is one of the dominated ones by highly technical interferences centered on treatment, and in which, the role of primary care has been largely perceived as marginal. Thus, commission, expert opinion from primary care, public health professionals along with oral health professionals with academic and clinical cancer expertise, such as epidemiologists, and oral oncologists is essential to prevent oral cancer and periodontal disease by promoting awareness and preventive programs in primary-care and community-care settings with minimal cost-effective strategies, which also enhances interdisciplinary collaborations among health care provider to improve the quality of life of their patients.

In the future, we aim to gather more detailed nutritional status information using questionnaires and the assessment of muscle mass depletion and percentage of weight loss with time. Longitudinal studies are necessary to examine if a causal relationship exists between nutritional status and antimicrobial protein levels.

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Conflicts of interest

There are no conflicts of interest.

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