

## Research Article

# Status of Immunological Marker Interleukin 6 in Head and Neck Squamous Cell Carcinoma: Before and After Treatment

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## Abstract

Interleukin 6 (IL-6) has a potential to act as a biomarker for Head and Neck Squamous Cell Carcinoma (HNSCC). This study was conducted on 30 newly diagnosed histopathologically proven patients with HNSCC who were treated using standard chemoradiation and 30 age and sex matched healthy individuals. Serum levels of IL-6 were measured in the controls and in patients before and after 6 weeks of completion of treatment. All the patients presented in advanced stage disease (stage 3 and 4). The levels of IL-6 were found to be statistically significantly increased in patients of HNSCC before treatment as compared to controls ( $p < 0.0001$ ) and significantly decreased in patients after treatment ( $p = 0.001$ ) though the difference between stage 3 and 4 patients was not statistically significant ( $p = 0.298$ ). Thus, IL-6 might help in diagnosis and monitoring of patients with HNSCC and in better understanding of the etiopathogenesis of the disease.

**Keywords:** Head and neck squamous cell carcinoma; Interleukin 6; Chemo radiation; Biomarker

## Introduction

Non Communicable Diseases (NCDs) are the major challenge of the 21<sup>st</sup> century globally. Cancer is the leading cause of morbidity as well as mortality in general population. Out of all cancers, head and neck cancers rank as the sixth most common cancer worldwide and represent a serious challenge for the health community. The typical tumor is a Squamous Cell Carcinoma (SCC) with variable grade of differentiation (from well to undifferentiated) predominantly affecting males in their fifth to sixth decade of life [1,2].

Oncological treatment has advanced promisingly over the past decades. As per standard protocol, the patients are treated with surgery, Chemotherapy (CT), Radiotherapy (RT) or combination of these. Most suitable treatment modality for the patient is selected after the final diagnosis. The decision between RT and surgery may be taken on the factors including patient's performance status, the expected degree of functional impairment with surgery, and choice of the patient and the physician [3]. In view of the role played by host immune system in the development of cancer, immune biomarkers may be of immense help in management of patients with cancer.

Cytokines are small secreted proteins produced by cells having role in interactions and communications. It is a general name; other names are lymphokine, monokine, chemokine and interleukin [4]. Interleukin-6 (IL-6) is a multifunctional cytokine which plays an important role in a wide range of biologic activities in different types of cell including tumor cells. IL-6 is involved in the host immune defense mechanism as well as the modulation of growth and differentiation in various malignancies. These effects are mediated mainly by Signal Transducer and Transcription Activator 3

(STAT3) [5]. Clinical studies have revealed that increased serum IL-6 concentrations in patients are associated with advanced tumor stages of various cancers (multiple myeloma, non-small cell lung carcinoma, colorectal cancer, renal cell carcinoma, prostate cancer, breast cancer and ovarian cancer) and short survival in patients [6]. IL-6 is believed to promote growth of SCC by regulating a complex cytokine and protease network [5,6]. Therefore, this study was planned to evaluate the role of IL-6 in diagnosis, recurrence, prognosis and management of newly diagnosed histopathologically proven HNSCC patients by estimating the levels before and after treatment.

## Materials and Methods

The study was conducted in Department of Biochemistry in collaboration with Regional Cancer Institute. The study enrolled 30 newly diagnosed histopathologically proven patients with HNSCC and 30 age and sex matched healthy individuals. This study was approved by the Ethics Committee of our institution. Informed written consent was taken from all the participants. Diagnosis of HNSCC was established with the help of detailed history, clinical examination, radiological and histopathological examination. Staging was done according to American Joint Committee on Cancer 2014 criteria [7]. Written informed consent from the patients was collected to participate in the study and follow up nature of the study was also explained to the patients. Approval from Institutional Ethical committee was obtained for the research work. The pretreatment evaluation in all patients included complete history, general physical examination, and complete systemic examination. All the participants were subjected to anthropometric evaluation in the form of height and weight recording. Body Mass Index (BMI) was calculated using the formula weight/height and expressed as kg/m<sup>2</sup>. The assessment of general condition was done by using Karnofsky Performance Score

[8]. Hematological assessment was done by complete hemogram including hemoglobin. Radiological assessment included chest X-ray, X-ray of soft tissues of neck-lateral view and ultrasonography of abdomen and pelvis. Whenever clinically indicated, computed tomography scan of face and neck was done for accurate staging. All the patients were treated with the standard dose of radical external radiations (64 Gy/ 32 fractions for 6.2 weeks). Concomitant Carboplatin was also administered to late stage (III & IV) patients. Follow up of patients was done after 6 weeks of completion of treatment. Subjects with history of any chronic disease (renal, hepatic, endocrinal, malignancy) or who are on any medication/ supplements were excluded from the study. Pregnant and lactating females were also excluded.

Serum samples were analyzed for routine biochemical investigations (liver function tests, renal function tests, glucose and lactate dehydrogenase) and complete haemogram the same day and were stored at -200C for estimation of IL-6 in batches subsequently. Serum IL-6 levels were analyzed by Enzyme Linked Immunosorbent Assay (ELISA) method [9]. The data was compiled and analyzed using appropriate statistical methods using SPSS 25.0 statistical package. Quantitative data were expressed as mean  $\pm$  standard deviation. Student's paired t test was used to compare IL-6 levels before and after treatment.

## Results

The mean age of controls was 54.63 $\pm$ 8.07 years (43-72 years) and of cases 53.37 $\pm$ 9.12 years (36-74 years) with a p value of 0.57. Out of 30 cases and 30 controls, majority i.e. 29 subjects were males and 1 was female. BMI was 20.07 $\pm$ 3.36 Kg/m<sup>2</sup> (14.23- 25.15 Kg/m<sup>2</sup>) for controls and 19.13 $\pm$ 2.87 Kg/m<sup>2</sup> (13.02-28.46 Kg/m<sup>2</sup>) for cases (p=0.25). Among cases, 23 hailed from rural and 7 from urban background while among controls 20 belonged to rural and 10 from urban background. Site-wise and stage-wise distribution of HNSCC patients is given in (Tables 1 and 2) respectively.

Eight patients presented with difficulty in swallowing, 8 had hoarseness of voice, 12 complained of pain in throat region and 2 presented with swelling in the neck region. Personal habits posing as risk factors for HNSCC patients are presented in (Table 3).

Both alcoholic and smoker 15(50%) 15(50%)

Fifty percent of patients had Karnofsky Performance Score of 70 and 50% had score of 80. The levels of IL-6 in patients before (group A) and after treatment (group B) and in controls is presented in (Table 4). The difference in serum levels of IL-6 in stage 3 and stage 4 was statistically not significant (p=0.298).

## Discussion

In the present study, IL-6 levels were found to be statistically significantly increased in patients of HNSCC before treatment as compared to controls (p<0.0001) and significantly decreased in patients after treatment (p=0.001). As IL-6 is a pleiotropic inflammatory cytokine, its levels are observed to be raised, along with other immunological markers like IL-1, 8 and tumor necrosis factor-alpha, in malignant conditions of head and neck region in which inflammation also plays a part [10]. Increased expression of IL-6 has been reported in HNSCC and that an environment rich in

**Table 1:** Site-wise distribution of HNSCC in cases.

Location of cancer in cases	Number	Percentage
Ca Larynx	11	36.7%
Ca Oropharynx	10	33.3%
Ca base of tongue	6	0.20%
Ca Tonsil	3	0.10%

**Table 2:** Stage-wise distribution of HNSCC in cases.

Stage	Number	Percentage
Stage 4	11	36.7%
Stage 3	19	63.3%

**Table 3:** Risk factors (smoking, alcoholism and tobacco use) in HNSCC in cases and controls.

Risk factor	Cases	Controls
Smoker	28(93.3%)	25(83.2%)
Non Smoker	2(6.7%)	5(16.6%)
Alcoholic	20(66.7%)	15(50%)
Non Alcoholic	10(33.3%)	15(50%)
Tobacco User	25(83.3%)	10(33.3%)
Tobacco Non-user	5(16.6%)	20(66.7%)
Both Alcoholic and Smoker	15(50%)	15(50%)

**Table 4:** Serum IL-6 levels in HNSCC patients before treatment (Group A), after treatment (Group B) and in controls (Group C).

Parameter	Mean $\pm$ SD	Range
Group A	35.92 $\pm$ 6.08 pg/mL	8-152.83pg/mL
Group B	16.83 $\pm$ 2.81 pg/mL	2.83-62.17pg/mL
Group C	5.30 $\pm$ 0.27 pg/mL	2.34-8.81pg/mL

cytokines makes HNSCC cells more apt to invade and metastasize. IL-6 can also promote immune unresponsiveness and induce cachexia, both of which are observed in HNSCC patients who have a poor prognosis. Since patients with oral cavity carcinoma present at the later stages of the disease, it is expected that serum IL-6 levels increase with cancer stage. The increasing inflammation and lesion region would increase the serum IL-6 level [11]. Though, the IL-6 levels were not found to be statistically significantly different between stage 3 and 4 patients in the present study, which may be attributed to small sample size and lack of early stage patients for comparison. IL-6 can also trigger Signal Transduction and Activator of Transcription (STAT3) phosphorylation, which is associated with various human cancers and commonly suggests poor prognosis related to regulation of apoptosis and proliferative effects [12].

A very high percentage of patients were observed to be smokers, alcoholics and tobacco users in the present study. Smoked tobacco and alcohol are the major causative factors for head and neck cancers worldwide. The underlying carcinogenic mechanisms are not exactly clear although many have been proposed, the most important one being increased production of oxidative stress [13]. Ethanol is metabolized by epithelial cells and microflora into acetaldehyde, a known carcinogenic molecule [14]. High male preponderance of the disease may also be attributed to increased use of tobacco and alcohol in male population in Indian setting especially the rural background.

Our institute, being a tertiary care center, is the center of choice for the rural population due to lack of fully functional Radiotherapy department in other government hospitals of the state and services of private ones are not affordable for this population. This might be the reason behind majority of patients belonging to rural background rather than reflecting an increased incidence in this population. The lack of awareness and resources in this population might also have led to delayed diagnosis and presentation of patients in advanced stage of the disease.

The mechanism by which serum IL-6 contributes to or reflects cancer progression and biology is likely due to its dual effects on tumor initiation by paracrine or autocrine mechanisms and to its additional inhibitory effects on the immune response directed against the tumor. IL-6 inhibits dendritic cell differentiation, thus inducing immune tolerance of tumors and facilitating metastatic spread [15,16]. Though there are no patients in early stage (stage 1 and stage 2) to compare with advanced stage, role of IL-6 in detection of disease severity cannot be commented upon.

The limitations of the study mainly include small sample size, non-availability of early stage patients for comparison and non-inclusion of other immune biomarkers. As HNSCC patients, if get diagnosed early, have high cure rates. In spite of these limitations, the findings of the study suggest that IL-6 may act as a potential valuable immune biomarker for the disease.

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