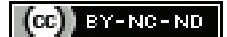# Comparative Evaluation of Serum Interleukin-6 Expression with Neutrophil-lymphocyte Ratio and Platelet-lymphocyte Ratio in Pre and Post Surgery Oral Squamous Cell Carcinoma Patients: A Research Protocol

Dentistry Section

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ABSTRACT

Introduction: Oral Squamous Cell Carcinoma (OSCC) is the 3rd most common malignancy. In Indian scenario, oral submucous fibrosis is the most common condition associated with OSCC. Pre and postsurgical evaluation of serum Interleukin-6 (IL-6), Neutrophil Lymphocyte Ratio (NLR) and Platelet Lymphocyte Ratio (PLR) will help in predicting the prognosis, along with planning of postsurgical treatment protocol. The NLR and PLR are easily available and cost-effective biomarkers from peripheral smear.

Need of Study: The IL-6 and NLR are convenient and inexpensive prognostic markers, they could be widely available in routine clinical practice, which might serve as a valuable marker for predicting the prognosis of OSCC and assessing the magnitude of systemic inflammation.

Aim: To compare the serum IL-6 with NLR and PLR in pre and postsurgical OSCC patients.

Materials and Methods: A prospective analytical study, for duration of two years from December 2020 to October 2022 will be conducted at Department of Oral Medicine and Radiology, Sharad Pawar Dental College and Hospital, Sawangi (Meghe), Wardha, Maharashtra, India, in which total 40 patients from lower and middle socio-economic status and histological confirmed cases of OSCC will be included, after obtaining an informed written consent. A detailed clinical, radiological and histological evaluation of all cases will be recorded in the structured format. Presurgical and postsurgical 5 mL venous blood will be collected for evaluation of IL-6, NLR, Platelet–Lymphocyte ratio. Data analysis will be done using unpaired 't' test. For categorical data, Chi-square test will be used to compare the proportions.

Conclusion: The present study results are expected to provide evidence for the role of serum IL-6 alongwith NLR and PLR as adjuvant biomarkers for OSCC.

INTRODUCTION

Oral squamous cell carcinoma accounts for 2-3% of all cancers and is the 11th most common cancer worldwide. It remains a major cause of morbidity and mortality in patients with head and neck cancer [1]. Recently, several studies have reported that biomarkers involved in inflammation and the immune system are useful for understanding the biological behaviour of OSCC [2,3].

The IL-6 is a multifunctional cytokine that regulates inflammatory responses [1]. It plays an important role in many tumour functions, including development, migration, invasion, growth, proliferation, apoptosis, progression, angiogenesis and differentiation of tumour cells [1]. Although neutrophils have anti-tumour effects, increasing NLR is less efficacious but lymphocytes are more responsible for controlling cancer progression [1]. Several clinicopathologic factors, such as age, primary tumour size, regional lymph node metastasis and surgical margin involvement have been shown to contribute to the poor prognosis in patients with oral cancer [4]. Therefore, it is necessary to search for a potential prognostic indicator that would be available before surgery. Therefore, NLR elevation has been suggested to be associated with poor prognosis [1].

Recent studies have shown an increase of circulating IL-6 after surgery, this relationship was evaluated before and after tumour resection [1,3]. Elevated NLR and PLR have been reported to be associated with a prognosis of survival in various cancers, including oesophageal, nasopharyngeal, gastric, colorectal and endometrial carcinomas [4], but in the previous study only pretreatment evaluation

was conducted for IL-6 [3] and NLR, PLR [4,5]. Hence, present study will be undertaken after ethical approval from Institutional Ethics Committee for evaluation of the serum IL-6 with NLR and PLR in pre and postsurgical OSCC patients.

The objective of the study would be estimate and compare pre and postsurgical serum levels of IL-6, PLR and NLR in OSCC patients. The null hypothesis considered for the study was preoperative and postoperative changes in serum levels of NLR, PLR and IL-6 may not be present with cases of OSCC. Though, there is an alternative hypothesis that states that preoperative and postoperative elevated serum levels of NLR, PLR and IL-6 may be present with cases of OSCC. Hence, the present study results would provide a comparative evaluation of these markers in OSCC patients.

MATERIALS AND METHODS

The present prospective analytical study will be conducted in Department of Oral Medicine and Radiology, Sharad Pawar Dental College and Hospital, Sawangi (Meghe), Wardha, Maharashtra, India, from December 2020 to October 2022. Study will be commenced after the approval of Institutional Ethics Committee [Ref.No.-DMIMS(DU)/IEC/2020-21/9427].

Inclusion criteria: Patients aged between 20-60 years with middle and low socio-economic status, clinical and histopathological confirmed diagnosis will be included in the study.

Exclusion criteria: Patients with history of other body benign/malignant tumour in the past and who underwent preoperative chemotherapy

or radiation therapy will be excluded. Patient with diagnosed cases of Cardiovascular System (CVS)/ Respiratory System (RS)/Gastrointestinal Tract (GIT)/endocrinal diseases will also be excluded.

Sample size calculation: Using single proportion formula, for the sample size n:

n=N*X/(X+N-1),

Where,

$X = Z\alpha/22 * p * (1-p)/MOE^2$,

And $Z\alpha/2$-is the critical value of the normal distribution at $\alpha/2$.

(e. g. for confidence level of 95%, α is 0.05 and the critical value is 1.96)

MOE-is the Margin Of Error

P=2.65 which is the sample proportion based on the number of patients reporting at Sharad Pawar Dental College and Hospital (SPDC) having OSCC.

N=population size.

The total sample size was calculated to be 40.

Study Procedure

Patients visiting Department of Oral Medicine and Radiology with clinical and histopathological confirmed diagnosis of OSCC will be included in the study. Clinical Tumour Node and Metastasis (TNM) classification and histopathological grading will be followed to categorise the OSCC cases [6]. Imaging evaluation for lymph node status will also be done.

Parameters assessed: Patients peripheral smear will be used to evaluate NLR and PLR. Preoperative samples will be collected after biopsy confirmation and postoperative samples will be collected on 7th day. The NLR, PLR and IL-6 will be assessed and compared pre and postoperatively. The correlation between NLR, PLR, IL-6 with clinical size of the tumour, lymph node status will also be assessed.

Preoperative NLR and PLR with IL-6 will be correlated with the prognosis as it is found that a high overall tumour stage and lymph node involvement are linked to poor prognosis [3,4]. Increased levels of both preoperative and postoperative NLR, PLR (cutoff value of 2.7 and 135, respectively) and IL-6 (cutoff value serum values ≥5 pg/mL) [4] may indicate grave outcome of the disease.

Estimation of serum IL-6: In the present study human IL-6 Enzyme Linked Immunosorbent Assay (ELISA) kit (Diaclone, France), batch: 1006-120, Cat.No: 950.030.096 will be used according to company's guidelines for estimation of IL-6.

STATISTICAL ANALYSIS

Data will be entered into Microsoft excel worksheet (Microsoft, USA). Data analysis will be done using IBM Statistical Package for Social Sciences (Statistics for Windows, version 21.0. Armonk, NY: IBM Corp). Categorical data will be described in terms of frequencies and percentages. Continuous data will be presented by mean and Standard Deviation (SD). Comparison of means will be done using unpaired 't' test. For categorical data, Chi-square test will be used to compare the proportions. Spearman correlation coefficient will be used to analyse the correlation between NLR, PLR and IL-6 with clnical size of the tumour, lymph node status. For analysis, p-value less than 0.05 will be considered statistically significant.

DISCUSSION

Interleukin-6 is a multifunctional cytokine that regulates immune responses [1]. IL-6 activates the Janus Kinases (JAK)/Signal Transducer and Activator of Transcription (STAT), Phosphoinositide 3-kinase (PI3K), and the Mitogen Activated Protein Kinase (MAPK) pathways [1,5]. This signalling influences cell migration, malignant tumour growth and invasion, anti-apoptotic signalling, angiogenesis, and bone remodelling. In this study, we will examine the association

between IL-6 expression and clinicopathological factors in patients with OSCC [1,6]. The IL-6 expression is associated with Primary Ovarian Insufficiency (POI), vascular invasion, and pathological nodal status in OSCC. In particular, vascular invasion strongly correlated with IL-6 expression, suggesting that IL-6 is involved in lymphangiogenesis in OSCC [7,8]. Additionally, some studies have reported that IL-6 over expression plays transcriptional and regulatory roles in invasion and metastasis, leading to poor prognoses for patients with head and neck cancers [7,9,10]. The IL-6 production is increased in response to various stimuli, such as infection and inflammation [1,11].

Leukocyte count is usually increased in response to infection, inflammation, allergic reaction, and malignancy [12]. The NLR elevation has been suggested to be associated with poor prognosis [4,13-14]. Platelets can promote tumour growth by increasing angiogenesis, increasing microvessel permeability and extravasation of cancer cells [15,16]. Platelets could interact with tumour cells through receptors or ligands and increase tumour cell growth or invasion platelets have a negative effect in host immune attack against tumours [4,6]. Researchers observed that the numbers of neutrophils and lymphocytes were inversely correlated and that NLR was an indicator of poor prognosis [4,17]. Based on these findings it is conceivable that the white blood cell differential in Head and Neck Squamous Cell Carcinoma (HNSCC) is preferentially skewed towards either a myeloid or a lymphoid lineage, with the lymphoid preponderance being associated with better disease outcome [12]. It is important to point that the roles of different cellular, subsets in dictating cancer behaviour [14,16,18].

Limitation(s)

The main limitation of this study includes areca nut, tobacco and alcohol habit which are responsible for metabolic syndrome, coronary artery disease, chronic obstructic pulmonary disease and hepatic disease which can change the NLR, PLR and IL-6 values, and in turn influences the final outcome of the results in the present study.

CONCLUSION(S)

The NLR and PLR can be an adjuvant biomarker for the OSCC in predicting prognosis and are easily available investigation from the peripheral smear of the patient but IL-6 is a proinflammatory biomarker even if it is not easily available in routine practice, it helps in binding the correlation with NLR and PLR.

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