Evaluation and Correlation of Preoperative Serum C-Reactive Protein, Neutrophil-lymphocyte Ratio and Platelet-lymphocyte Ratio for Cervical Metastasis in Patients of Primary Oral Squamous Cell Carcinoma

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Study Protocol

ABSTRACT

Background: The influence of cervical metastasis on prognosis in a person who is suffering from oral squamous cell carcinoma (OSCC) has been notorious. C- reactive protein (CRP) is a prior biomarker for inflammation. Recent literature provide evidence of systemic inflammatory markers playing important role in determining survival rate on patient with OSCC. Hence, in current study the "utility of Neutrophil-lymphocyte ratio (NLR) ,Platelet-lymphocyte ratio (PLR), and serum CRP are evaluated and correlated for cervical metastasis".
Objectives: This study intends to evaluate and correlate preoperative serum CRP, NLR, and PLR for cervical metastasis in patients of primary oral squamous cell carcinoma.
Methodology: The clinicopathological data, CRP and the preoperative complete blood investigation details were obtained from 40 primary OSCC patients who will undergo surgery. The optimal cut-off value of CRP, PLR and NLR was determined. The prognostic significance of markers will be determined by Univariate and multivariate analysis.
Expected Results: The elevation of CRP, NLR, and PLR levels will significantly evaluate and correlate with cervical metastasis.
Conclusion: Preoperative serum CRP, NLR, and PLR level will be a prognosticator in OSCC.

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1. INTRODUCTION

A commonly prevalent disease, Oral squamous cell carcinoma (OSCC) is the oral malignancy, characterizing up to 80-90% of all malignant neoplasms of the oral cavity [1]. They constitute one of the main causes for death worldwide. The rate of survival remains to be minute, when compared to the advancements in management of various malignant growth. Principal etiological and predisposing factors for OSCC include mostly tobacco, betel quid & areca nut, alcohol, viruses (e.g. HPV), nutritional deficiencies, a legacy of head & neck squamous cell carcinoma, and Candida infection [2,3]. In the year 2018, "oral cancer happened globally in about 3,55,000 people and resulted in about 1,77,000 deaths" [4]. OSCC has an overall 5 years survival rate. If diagnosed this varies from 'about 84% when localized compared to about 66% if it is spreading to the lymph nodes in the neck & 39% if it is also spreading to distant parts of the body' [5]. Survival rates have also been dependent on the 'location of the disease in the mouth' [6].

In OSCC, Cervical metastasis is the utmost crucial indicator which affects patient survival [7]. OSCC grows locally, it invades the adjacent surrounding tissue following the path of least resistance also spreads by lymphatic to metastasize to locoregional neck nodes, but it rarely develops distant metastasis. The cervical group of lymph node levels I, II and III often involved predictably in OSCC while level IV and V being only rarely involved [8].

Metastasis in the cervical region is the primary outway of metastasis, in different stages of OSCC, it is a significant factor to determine the prognosis of oral cancer. The prognosis of the patients is directly affected by "the size and the number of metastatic lymph nodes, whether or not capsular invasion. Also the extent of involvement of the neck region."[9]. Important elements impacting the cervical metastasis in the OSCC patients are the "degree of differentiation, growth pattern, depth of invasion, and NLR" [10]. 'The association between the growth pattern and lymph node metastasis is significant'. So, substantial risk of cervical metastasis for OSCC patients with an aggressive growth pattern. The grade of tumor differentiation is significantly related to cervical metastasis. The decrease in grade of tumor differentiation gradually increases the rate of cervical metastasis [11].

An annular protein present in extracellular fluid of blood whose concentration elevates in reaction to harmful stimuli known as "C- reactive protein(CRP)" [12]. It is of hepatic in nature, acute-phase protein that is controlled by "pro-inflammatory cytokines like interleukin-1(IL-1), interleukin-6(IL-6), and tumor necrosis factor(TNF)". Contrary to infection, it signifies its role in innate immunity as an early defence system [13,14]. The conditions such as "acute and chronic inflammation, malignancy, tissue injury and necrosis cause the unleash of IL-6 and other cytokines that trigger the synthesis of CRP and fibrinogen by the liver" [15]. In healthy adults, the average concentration of CRP varies between 0.8mg/L to 3.0mg/L. Nineteen hours in the plasma half-life of CRP.

In acute inflammation, the level can magnify 10,000 fold. Due to its relatively short half-life CRP levels fall quickly, hence inflammation subsides. In chronic inflammation CRP concentration between 2 and 10mg/l is metabolic inflammation [16,17]. Regulation of synthesis of CRP by "pro-inflammatory cytokines such as IL-1, IL-6, and TNF which are well known as growth factors" in various malignancies. These comprises of secretions of "t-lymphocytes, peripheral blood mononuclear cells, tumor cells, and by intra-tumor and peri-tumor resident normal cells".

The newly discovered tumor prognosis related indicators are "Neutrophil-lymphocyte ratio (NLR) and Platelet-lymphocyte ratio (PLR)", gaining most of the attention now a days. The increased value of NLR and PLR suggests systemic inflammatory conditions that may present as a decrease in lymphocyte range, a slight increase in platelet range, and an increase in neutrophil range [18,19]. Independent predictors of poor prognosis are NLR and PLR levels. It is proposed that NLR and PLR might be a prospective predictor of the survival of clinical response to chemotherapy and radiotherapy. Minimum values are 1.77 for NLR and 128.5 for PLR preoperatively.

So, the study is designed to evaluate preoperative 'serum CRP, NLR, and PLR and its

correlation with cervical metastasis in patients of primary OSCC.'

1.1 Objectives

1. To evaluate preoperative levels of serum CRP in patients with primary OSCC.
2. To evaluate preoperative NLR in patients with primary OSCC.
3. To evaluate preoperative PLR in patients with primary OSCC.
4. To correlate serum CRP with cervical metastasis.
5. To correlate NLR with cervical metastasis.
6. To correlate PLR with cervical metastasis.

2. METHODOLOGY

The present prospective observational study is scheduled to be conducted "Sharad Pawar Dental College and Hospital, Acharya Vinoba Bhave Rural Hospital, Sawangi (Meghe), Wardha, Department of Oral and Maxillofacial Surgery", for a duration of two years.

2.1 Sample Size

By using purposive sampling, 40 patients in each group will be selected during the period of October 2020 to MAY 2022. Patients fulfilling the following criteria will be recruited for the trial.

2.2 Criteria for Inclusion

- Patients with histopathologically confirmed case of oral squamous cell carcinoma and scheduled for surgical treatment with resection of primary lesion and appropriate neck dissection.
- Patients with no history of previous treatment for oral squamous cell carcinoma.

2.3 Criteria for Exclusion

- Medically compromised patient with ASA – III and IV category unfit to undergo surgery.
- Patient who have previously undergone neck dissection for cervical lymphadenopathy for oral squamous cell carcinoma.
- Patient who have received prior chemo/radiotherapy/both.

- Patients in which neck dissection were not performed.

2.3 Method

2.3.1 Pre-surgical work up

All the patients with a suspected malignant and oral cavity were subjected to biopsy for confirmation of OSCC histological into Well / Moderate / Poorly differentiated by Broder`s classification given in 1920. All patients underwent comprehensive pre-surgical assessment which concluded a medical history and complete physical examination, routine blood biochemistry complete blood count. Participant's socio-demographic history will be collected by questionnaire. A detailed clinical case history (Annexure-2) for all the patients will be recorded. Local clinical examination will be done for the site, size and type of growth. Neck evaluation will be done by manual palpation for any enlargement of neck nodes, size, shape, consistency, tenderness and fixity to overlying and underlying structures. On the basis of clinical findings the TNM staging will be done as per the 1997 American Joint Committee Cancer Research 8th edition.

3. MEASUREMENT OF CRP

Under all asepsis precaution and protocol, around "5 ml of Blood will be withdrawn from the ante-cubital vein into a sterile bulb. Then, the sample will be kept at room temperature to clot followed by centrifugation at 3,000 rpm for 10 min to separate the serum". The serum samples will be then collected. CRP levels will be measured by a "biochemistry analyzer-(Spectrophotometer EM 360; Erba Diagnostics, Mannheim, Germany) with cell holder and thermo-stable at 37oC, with a commercially available CRP assessment kit with it. CRP-Turbilatex (SPINREACT, S.A. Ctra. Santa Coloma, 7 E-17176 SANT ESTEVE DE BAS (GI) SPAIN) is a 'quantitative turbidimetric test' for the measurement of CRP in human serum".

Specific anti-human CRP will be coated with "latex particles which agglutinated when mixed with a sample containing CRP".

Absorbance change caused by agglutination, 'measured at 540 nm, dependent upon the CRP contents of the patient sample, that can be quantified by comparison from a calibrator of the known CRP concentration' (reference value:

normal value up to 6 mg/L). "Internationally adopted for inflammation, the minimum value for serum is 5.0 mg/L".

4. MEASUREMENT OF THE NLR AND THE PLR

The venous blood of around 3 mL will be obtained from patients. Then blood will be drained into an "EDTA-containing tube (BD Vacutainer, Plymouth, UK). It will be centrifuged at 3,000 rpm for 15 mins, and the plasma was frozen at −80°C until the analysis". The complete blood count will be obtained by using "Beckman Coulter LH-750 Hematology Analyzer (Beckman Coulter, Inc., Fullerton, California)". NLR and PLR would be calculated as a ratio of the circulating neutrophil, platelet count and lymphocyte counts. The normal ranges in our laboratory for the leukocyte are $1.2–3.4 \times 10^9$/L for lymphocyte count, $150\text{-}450 \times 10^9$/L for platelet count, $1.4–6.5 \times 10^9$/L for neutrophil counts. After obtaining a pre-anesthetic fitness, patient will undergo for the surgery, under General Anaesthesia

4.1 Surgery Includes

1. Excision of primary lesion with safe margin
2. Neck dissection – any one of the following on the basis of merit of cases "Selective neck dissection (SND) or Radical neck dissection (RND) or Modified radical neck dissection (MRND)"
3. Reconstruction using suitable flap

Resected specimen and resected levels of lymph nodes were assessed, grossed, labeled and after which the lymph node will processed for paraffin embedded sections, and sent for conventional Histopathological Examination.

The staining procedures were sequentially carried out on the section of the lymph nodes i.e. Histopathological examination using formalin fixed tissue: conventional H and E staining

5. DISCUSSION

Ravi Mehrotra, S Yadav (2006) review the recent advances as a causes, nature of history course and predictive value of genomic modification in oral squamous cell carcinoma. They concluded that global increase in incidence, fatality and poor diagnosis of OSCC, in current research efforts in the field of avoidance early detection of the disease.

Anastasios K. Markopoulos (2012) overview the important points of OSCC, stressing on its risk, genomic factors, the potential malignant disorders, and the medicinal approaches. It also emphasizes on the importance of advanced diagnosis.

Yonghong Li et al (2019) conducted a survey in 161 OSCC, "patients with clinical negative lymph nodes and cited them for therapeutic management, to see the hazardous factors present in cervical lymph node metastasis in OSCC".

Mohamad Adel et al. (2016) look into the relation between "preoperative serum markers squamous cell carcinoma antigen (SCC Ag), CRP and postoperative prognostic marker i.e. Lymph node dissection (LND)" in this study. They had done retrospective study in 277 OSCC patients who underwent primary resection and neck dissection with or without adjuvant therapy. They concluded that increased preoperative SCC Ag and CRP levels are predictors for LND. As well, SCC Ag and CRP are markers for classifying high risk LND patients with OSCC into subgroups.

Sunil D. Khandavilli, Et al. (2009) studied about worse overall survival, and tumor size and stage which was associated with a raised preoperative CRP. CRP levels increases predictive power of the indicator.

S. Acharya, J. Kale, K. Hallikeri, V. Anehosur, D. Arnold (2017) conducted a study intended "to evaluate the preoperative serum CRP levels in OSCC patients and to investigate its relation with clinical and pathological features. CRP values for 30 healthy controls and 60 OSCC patients are evaluated and CRP was to a higher side in OSCC than in control group. 70% of OSCC patient had raised CRP which was associated with clinical nodal status and lymph node metastasis".

Swetha Acharya Et al. (2015) assessed the NLR and PLR before whole surgical staging and that provided evidence on lymph node metastasis in OSCC patients. They concluded that nodal involvement status of OSCC is directly related to the preoperative PLR. Hence, PLR is exceptional to NLR to predict lymph node metastasis in OSCC.

A number of studies on related aspects of oral squamous cell carcinoma and lymph node metastasis were reported [20-23]. Agrawal et. al.

reported on Touch Imprint Cytology [24]. Debta et. al. conducted evaluation of myeloid cells infiltration in different grades of oral squamous cell carcinoma [25]. Related studies by Agrawal et. al. [26] and Jaiswal et. al.[27] were reviewed [28].

6. CONCLUSION

Preoperative serum CRP, NLR, and PLR level will be a prognosticator in OSCC.

CONSENT AND ETHICAL APPROVAL

As per international standard or university standard guideline Patient's consent and ethical approval will be collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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