“Prognostic significance of PD-L1 immunoexpression in Oral Potentially Malignant Disorder and Oral Squamous Cell Carcinoma” : A study Protocol

Abstract:

Background: “Oral squamous cell carcinoma” (OSCC) is a most common type of invasive disease. It involvs damage to the oral epithelium. It gives a bad prognosis. There are alterations in cells of the oral mucosa called “oral epithelial dysplasia” (OED) and are categarised as “Oral Potentially Malignant Disorders” (OPMD). As a result, prior alteration in premalignant lesions would encourage prior cancer treatment and could essentially diminish morbidity and mortality. “Programmed death ligand 1” (PD-L1) is functionally imputed ligand of the “co-inhibitory programmed death receptor 1 (PD-1)”. “(PD-L1)” is over-expressed on different cells including lymphocytes, tumor cells and different tissues in numerous malignancies. In this study, we will make effort to evaluate a new role of PD-L1 by correlating the immunoexpression of PD-L1 and clinicopathological characteristics and its prognostic significance in OSCC. We will evaluate and compare immunoexpression of PD-L1 in normal mucosa, OPMD and OSCC.

Methodology: Total 93 samples will be included in this study and they are divided into three groups, 31 in each group of the following: OPMD, OSCC and normal oral mucosa (control).Immunohistochemical staining will be performed and the integration of PD-L1 expression with various clinical features of OPMD and OSCC will be performed.

Expected Result: The present study will find immunohistochemical expression of “PD-L1” in OPMD and OSCC and its correlation with clinicopathological characteristics of OPMD and OSCC and 3 years disease-specific survivalof OSCC patients.

Conclusion: There is natable positive correlation of “PD-L1” appearance with OSCC . In case of OPMD, the progression of disease in terms of prognosis could be monitored. The unpredictable “PD-L1” appearance may be the main focus of integration therapy in OSCC. To provide personal immunotherapy to a variety of patients, the condition of PD-L1 should be considered.

Keywords- “Oral squamous cell carcinoma” (OSCC), “Programmed death ligand 1” (PD-L1), “Immunohistochemistry”, “Disease-specific survival rate”

Introduction:

"Oral squamous cell carcinoma "(OSCC) is a multifaceted neoplastic disorder. It emerges from the oral epithelium. It conveys bad predictions. Along with poor servival rates, treatment can lead to high morbidity, as the disease affects the aesthetic and there is a loss of function after treatment.

There is higher mortality rate because of delayed diagnosis and due to insufficient biomarkers for prediction of tumor development. So there is need to identify specific biomarkers that could help in making decision and earliest prognostication of OSCC.

There are alterations in cells of the oral mucosa called "oral epithelial dysplasia" (OED) and that are classified as "Oral Potentially Malignant Disorders" (OPMD). As a result, prior alteration in premalignant lesions would encourage prior cancer treatment and could essentially diminish morbidity and mortality [1].

"Programmed death ligand 1" (PD-L1) acts as "co-inhibitory programmed death receptor 1 (PD-1)". It contributes a very important role in the central and peripheral immunological forbearance [2]. "(PD-L1)" is a cell-surface protein that is up-regulated on different cells including lymphocytes, tumor cells and different tissues in numerous other malignancies.

Literature search reveled that larger tumor shows positive association with PD-L1 which was related with solid tumors, poor differentiation and node metastasis. The pertinent factors of PD-L1 surely forecast abnormal growth of tumor [3].

In this study, we will make a humble effort to evaluate a new role of PDL1 by correlating the immunoexpression of "PD-L1" and clinicopathological features and its prognostic significance in OSCC.

Objectives:

1. To assess immunoexpression of "PD-L1" in normal mucosa, OPMD and OSCC.
2. To compare immunoexpression of PD-L1 in OPMD with normal mucosa.
3. To compare immunoexpression of PD-L1 in OSCC with normal mucosa.
4. To compare the variation in immunoexpression of "PD-L1" in OSCC cases with OPMD cases.
5. To correlate immunoexpression of "PD-L1" with clinicopathological parameters and 3 years survival in OSCC patient.

Methods and Materials :

Inclusion and Exclusion Criteria -

For OPMD group, we will select 31 cases of hyperkeratosis, oral sub mucous fibrosis (OSMF). 31 cases of "normal oral mucosa" (NOM) will be taken from "gingival and vestibular mucosa" will be used as control group. The clinically and histopathological diagnosed cases of OSCC, who will be treated primarily with surgery, will be considered for inclusion in the eligibility criteria of the study.

Patients who gave past history of head and neck malignancies, recurrent diseases and radiotherapy, pre-operative chemotherapy, or surgery will be precluded from the study.

Information along with, detailed relevant habits history and their span, detailed clinical presentation, histopathological features will be noted. Also follow-up information will be noted for disease-free 3 years survival . All the Hematoxylin and Eosin stained tissue sections will be thoroughly screen at low power magnification (100X).

Sources of the Data -

The study comprises of surgical tissue specimens from patients, which will be perfom at the Department of "Oral Pathology and Microbiology", "Sharad Pawar Dental College and Hospital", "Datta Meghe Institute of Medical Sciences", "(Deemed to be University)", Sawangi (M), Wardha, Maharashtra, India, after acquiring acceptance from the "Institutional Ethical Committee". The surgically managed cases of 'OSCC' from year 2003 to 2019 in this institute will be retrieved from the archival of the department. Total 93 cases will be selected randomly with OPMD (31), OSCC (31)and normal oral mucosa (31). The histopathological grading of all OSCC cases will done using Broder's grading system. [4]

Immunohistochemistry –

For the detection of PD-L1 antigen the immunohistochemistry will be carried out. The method we will be using is "Universal Immuno-enzyme Polymer method". Deparaffinization of tissue section with xylene will be done and then hydration through alcohol with decreasing grade. Tissue sections will be heated in the micro-oven for ten minutes in 0.01M "sodium citrate buffer" (pH 6.0) for the retrieval of antigen for PD-L1 and then bench cooling will be done for 20 minutes. The same procedure will be again repeated. The section will be incubated with three percent hydrogen peroxide in methyl alcohol for thirty minutes to restrict the endogenous peroxidase activity after rinsing in PBS. The section then will be washed for 3 times with PBS for 5minutes. Non specific reactions should be prevented for that sections will be incubated with 10% serum for 10minutes.Then incubation with PD-L1 antibody in humidifying chamber for ninety minutes will be done at room temperature.

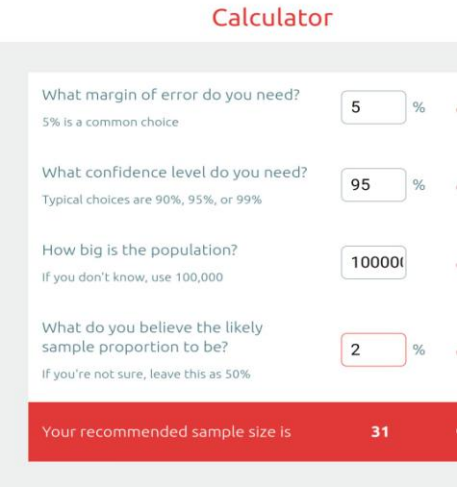
For positive control, pyogenic granuloma showing PD-L1 expression will be used. For negative, 1 section taken from each positive control and there is exclusion of the primary antibody. Then incubating it with serum. Then rinsing in PBS for 10 minutes three times after primary antigen- antibody reaction. The polymer anti-mouse (HRP-labeled) will be then incubated in humidifying chamber; at the room temperature for thirty minutes. The antigen-antibody reactions will be seen in the chromogen buffer solution of 3, 3' diaminobenzidine. Then mayer's Hematoxylin will be used for final counterstaining. PD-L1 immunostaining will be manually evaluated by three independent observers in blinded manner.

According to percentages stained of tumor cells there will be evaluation into five categories. (0= negative; 1=1-10%; 2= 11-25%; 3= 26-50%; 4= >50%). If there is more than 10% of PD-L1 expression the survival will be poorer. So 1% PD-L1 stained tumor cells will be considered as positive.

To determine the PDL1 cutoff, the combined positive score (CPS) of immunopositive tumor as well as immune cells will be analysed.

Study Design –

SAMPLE SIZE CALCULATION



Considering the prevalence of Oral Potentially Malignant Disorder as 2% in outpatient department of Oral Medicine and Radiology, using Single Proportion Formula, sample size is calculated by using the formula:-

$N = N*X / (X + N - 1)$

Where,

$X = Z_{\alpha/2}^2 *p*(1-p) / MOE^2$,

And $Z_{\alpha/2}$ is the critical value of the Normal distribution at a/2 (e.g. for a confidence level of 95%, a is 0.05 and the critical value is 1.96), MOE is the margin of error, p is the sample proportion, and N is the population size.

The sample size is calculated to be 31

There are total 3 groups

Hence, total 93 samples are required.

In this cross sectional study, a total of 93 samples divided into three groups: The groups are as follows:

Group I: 31 samples with Oral Potentially Premalignant Disorders

Group II: 31 samples with Oral Squamous Cell Carcinoma

Group III: 31 samples with Normal Oral Mucosa

To minimise the statistical bias a power analysis will be used to estimate the minimum sample size required for this project, which will give a desired significance level, effect size, and statistical power.

Expected Outcome :

This study emphasizes the importance of expression of PD-L1 in OPMD and OSCC and they will be correlated with the clinicopathological parameters like, TNM staging, histopathological grading and survival status of OSCC.

Discussion:

According to one study determination of the significance of "PD-L1" in the prognosis of

"OSCC" patients is given. PD-L1 immunoexpression was carried out by immunohistochemistry in three hundred five OSCC patients. It was observed that there was higher PD-L1 immunoexpression in OSCC patients who are females. High PD-L1 immunoexpression was related with distant metastasis in many patients. They revealed that, the high PD-L1 expression is a self determining risk factor in smokers. The conclusion of study was PDL-1 immunoexpression can be a self sufficient prognostic marker for OSCC patients having smoking habit.[5]

Some studies systematically reviewed surveying proof on algorithms and validation of test for immunohistochemistry tests. It was used to make out responsive patient for immunotherapy treatment with lung, gastric, ovarian or bladder cell cancer. Total 26 acceptable studies were evaluated using immunohistochemistry testing. The poorer concordance was reported. The majority of the studies of PD-L1 tests were carried out in lung cancer. [6]

Another author carried out immunohistochemistry for PD-L1 immunoexpression in OSCC of the tongue. The motive of the study was to find out the potential correlation of PD-L1 immunoexpression with clinicopathological factors of OSCC patient. It also found out the role of PD-L1in OSCC prognosis. They concluded that, 'PD-L1' may frequently overexpressed in tongue OSCC at the early stage, on the other hand, PD-L1 immunoexpression may not influence prognosis. [7]

One study determinedthe prognostic and clinicopathologicalsignificance of PD- L1 in OSCC. The poorer survival was associated with the cases whose PD-L1 immunoexpression was more than 10%. The conclusion of the study was PD-L1 immunoexpression was associated with lower "disease-specific survival" and tumor recurrence. He additionally discloses that neck node metastasis and tumor PD-L1 expression both were major independent factors for poor prognosis. They also concluded that if the PD-L1 immunoexpression is found in more than 10% of neoplastic cells then it would be a major factor which gives a poor prognosis in OSCC. [8]

A study find out the relation between the immunoexpression of "PD-L1", "p53"and "CK17" with "disease-specific survival" in OSCC and its clinicopathological features. They carried out IHC in 48 patients of OSCC to determine the relation between the expressions of PD-L1, CK17 and p53. The conclusion of the study was, there is positive correlation in the expression of p53 and PD-L in OSCC. In addition, a mark relation found between "p53" expression and "T stage" of OSCC. However there was not any significant relation between PD-L1 immunoexpression and "CK17" observed. [9] · Other studies on the similar aspects were reported[10-11]. Related studies by Gadbail et. al.[12-13], Hande et.al.[14], Sarode et. al. [15], and Tekade et. al.[16] were reviewed.

Conclusion:

According to the studies that have been carried out, the expression of PD-L1shows remarkable relation in OSCC. In case of OPMD, the progression of disease in terms of prognosis could be monitored. The associated appearance of "PD-L1" may be expected objective for combination therapeutic management in OSCC. To provide customized immunotherapy management to various patients, the PD-L1 immunoexpression status can be considered.

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Is there any ethical issue in this manuscript? - NO