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Original Research Article

Assessment of programmed cell death ligand- 1 (PD-L1) expression in oral potentially malignant disorders and oral squamous cell carcinoma - An immunohistochemical study

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ABSTRACT

Introduction: OSCC is multifactorial and is a multistep process. It may arise de-novo or may be preceded by oral potentially malignant disorders(OPMD). During malignant transformation certain molecular changes are evident at cellular level even in the absence of phenotypic changes in the tissue. With the recent advances in molecular understanding of cancers, immune checkpoint inhibitors have been recognized as perpetrators or supporters in the pathophysiology of various cancers. One of the main immunosuppressive pathways is the programmed death 1 (PD-1)/programmed death-ligand 1 (PD-L1) in which there is an interaction between T-cell PD-1 receptor and PD-L1 on cancer cells. Upregulation of PD-L1 is associated with disease progression.
Aims and Objectives: To assess and compare the PD-L1 expression in OPMD and OSCC.
Materials and Methods: 64 Paraffin embedded tissue sections of histopathologically diagnosed cases of 32 OSCC and 32 OPMD were immunohistochemically stained with PD-L1 & its membranous expression was evaluated. Descriptive statistical analysis was applied.
Results: Thus the study showed 100 % PD-L1 positive expression in OSCC and PD-L1 expression increased with increase in histopathological. In case of OPMD 93.74 % showed positive PD-L1 expression. We found PDL1 expression is significantly higher in OSCC (Mean= 4.59 ± 1.965) compared to OPMD (Mean= 2.03 ± 1.204).
Conclusion: Assessment of PD-L1 expression in OPMD patients will help us to screen the subjects with or without risk of malignant transformation as increase in PD-L1 expression signifies the increased risk of malignant change.

1. Introduction

OSCC is a problem with major concern not only because of consequential mortality but also the treatment rendered results in facial disfigurement and loss of function. Despite of catastrophic progress in recent therapeutic approaches, the prognosis of OSCC is penurious with a 5-year survival rate of 35-50%.[1] This possible finding is mainly because patients present with terminal advanced stages of OSCC at the time of diagnosis, suggesting that early detection of the disease is needed to improve the treatment outcome and reduce the rising burden of OSCC in today's global population.[2] A typical feature of OSCC, is its association with certain precursor lesions that often give warning signals to the clinician; aiding in early diagnosis.

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Aetiology of OSCC is multifactorial and is a multistep process. It may arise de-novo or may be preceded by oral potentially malignant disorders(OPMD), including Oral leukoplakia (OLK), erythroplakia, Oral submucous fibrosis (OSMF) and Oral lichen planus (OLP).[3]

The malignant transformation rates of OL, erythroplakia, Proliferative verrucous leukoplakia (PVL), OSMF and OLP are 0.13-34% (Warnakulsurya et al.),[4] 14-50% (Peter A Riechart et al.),[5] 68-100% (Speight et al).[6] 7-30% (Bari S et al)[7] and 1.09%(Sarah G et al)[8]respectively. Therefore, there is an urgent need to identify specific biomarkers for both OPMD and OSCC that would help in clinical decision making, early diagnosis, treatment strategies and prediction of prognosis in OPMD and OSCC.[9]

During malignant transformation certain molecular changes are evident at cellular level even in the absence of phenotypic changes in the tissue. Identification of such molecular changes may help in early diagnosis, in understanding tumor behavior and in precise stratification of patients into different risk categories. Therefore, molecular pathology using the potential biomarkers for diagnosis, prognosis and therapeutics can be applied for better patient outcome.[2]

With the recent advances in molecular understanding of cancers, immune checkpoint inhibitors have been recognized as perpetrators or supporters in the pathophysiology of various cancers; and hence considered as a promising treatment options for various cancers and hence considered as a promising treatment options for various cancers. However, their role in OSCC and OPMD in unknown. One of the main immunosuppressive pathways is the programmed death 1 (PD-1)/programmed death-ligand 1 (PD-L1) in which there is an interaction between T-cell PD-1 receptor and PD-L1 on cancer cells.[10]

This upregulation of PD-L1 is associated with disease progression. Therefore, present study is planned to asses and compare the PD-L1 expression in OPMD and OSCC.

2. Material and Methods

The present study was Cross sectional descriptive study. This study was executed in the Department of Oral Pathology and Microbiology of the Institute with valued co-operation from Cancer Hospital, with the prior sanction of Institutional Ethical Committee This study included clinically and histopathologically diagnosed cases of OPMD and OSCC reported to the institute. Total sample size comprises of 64 patients. Study population is categorized into two groups.

Group 1 - 32 Patients with OPMD (OLK/ OSMF/ OLP)

Group 2 - 32 Patients with OSCC

Tumors were staged according to the seventh edition of American Joint Committee on Cancer (AJCC) Cancer Staging Manual. Clinical data were obtained; these included gender, age, location, smoking, alcohol consumption, betel quid chewing, tumor stage, T, N, and M stages.

Formalin-fixed, paraffin-embedded tissue sections were sectioned at a thickness of 4 μm, and they were routinely deparaffinized in xylene and rehydrated in graded alcohol solutions. Sections were then submerged into EZ Retriever buffer (1 mm, pH=8.0), and antigen retrieval was performed using pressure cooker. To attenuate non-specific protein binding, they were incubated in 5% BSA buffer for 1 hr. Endogenous peroxidase activity was eliminated by treating with 3% H2O2 for 20 min at 37 °C. The sections were then treated for 1 hr in a moist chamber with primary antibodies for PD-L1. After treating with an HRP polymer-conjugated anti-mouse secondary antibody for 1 h at 37 °C, the slides were visualized with diaminobenzidine (DAB) solution and counterstained with hematoxylin. For negative controls, the primary Ab was replaced with a rabbit IgG isotype. Human placental tissues were used as positive controls for PD-L1.

The positive staining rate of immunohistochemical staining was evaluated and blindly scored by two pathologists. The proportion of PDL1- expressing cells was scored as follows: 0, no staining; 1, ≤25%; 2, 25–50%; and 3, > 50%%. Cells were considered PD-L1-positive if the staining was notable on cell membrane, regardless of the intensity. Intensity will be scored as 0, negative; 1 – weak ; 2- moderate and 3- strong intensity. The scores for the intensity of staining and the percentage of stained cells will be multiplied to yield a integrated score for each tissue section.

2.1. Statistical analysis

Data obtained was compiled on a MS Office Excel Sheet (v 2010, Microsoft Redmond Campus, Redmond, Washington, United States). It was subjected to statistical analysis using Statistical package for social sciences (SPSS v 21.0, IBM). Descriptive statistics like frequencies and percentage for categorical data, Mean & SD for numerical data has been depicted.

1. Inter group comparison (2 groups) was done using t test.
2. Comparison of frequencies of categories of variables with groups was done using Fisher's exact test.

For all the statistical tests, $p<0.05$ was considered to be statistically significant, keeping α error at 5% and β error at 20%, thus giving a power to the study as 80%.

3. Results

In OPMD cases age of the patients ranged from 11-70 years with a mean value of 37.5±11.78 years. Peak incidence was seen in 3rd decade In OSCC cases, the age of patients ranged from 21-70 years with mean value of 48.43±11.19 years. Peak incidence was seen in 5th decade. A strong male predominance was observed in the current study in

both the study groups with M:F ratio 31:1 for OPMD group and 4.3:1 for OSCC cases. In OPMD groups the site commonly affected was buccal mucosa (43.75%) while alveolar mucosa was the most common site (37.5%) to be involved in OSCC patients. In present study chewing tobacco was found to be most commonly associated in OPMD (62.5%) as well as OSCC (71.87%) cases.

In the present study PDL1 expression was located primarily on the cell membrane. The normal mucosa was negative for PD-L1 staining. Our results demonstrate that PDL1 expression is significantly higher in OSCC (Mean= 4.59 ± 1.965) patients compared to OPMD (Mean= 2.03 ± 1.204) patients

In present study out of 32 OPMD cases 46.87% (n=15) belonged to OSMF, 40.65% (n=13) OLK and 12.5% (n=4) were OLP. When we compared scoring group for immunoexpression of PD-L1 6.25% (n=2) belonged to scoring group 0 (no expression), 71.87% (n=23) belonged to scoring group 1 and 21.87% (n=7) belonged to scoring group 2. Minimum PD-L1 expression was present in OLP (Figures 3 and 8). This may be because of association of PD-L1 expresssion with presence of epithelial dysplasia which is more in OLK than OSMF and minimum in OLP Amongst all cases of OPMD maximum IIS was found in leukoplakia. Out of 13 OLK cases 46.15% (n=6) showed IIS=4, 30.76% (n=4) showed IIS=1 while 23.07% (n=3) showed IIS=2 (Figures 1 and 8). This indicates that among OPMD, leukoplakia shows more PDL-1 expression than OSMF and OLP this may be because of presence of more degree of epithelial dysplasia in leukoplakia than OSMF and OLP. Out of 15 OSMF cases 86.67% (n=13) belonged to scoring group 1 and 6.67% (n=1) each belonged to both scoring group 0 and 2. From this 60% (n=9) showed integraged IHC score (IIS) - 2, 26.6% (n=4) showed IIS-1 and 6.66% (n=1) showed IIS-0 and IIS-4 each (Figures 2 and 8). Uptil now, PD-L1 expression was not studied in only OSMF. In present study total 12.5% (n=4) oral lichen planus cases were present out of which only 75% (n=3) showed IIS=1-2 and 25% (n=1) showed IIS=0. When we compared mean IIS in OSCC and OPMD, statistically significant difference was found for IIS between OPMD (2.03 ±1.204) and OSCC (4.59 ±1.965) with p<0.001*(Figure 12).

In the present study among OPMD maximum number of cases have shown integrated score from 0 to 4 while in group 2 it was from 2 to 9. Which implies that PDL1 expression increases as lesion progress from OPMD to OSCC.

Our study reports higher prevalence of stage III & IV tumours which is in line with local studies that have reported a higher prevalence of advanced stage of oral cancers (104). In our study we found that Mean integrated IHC score for clinical stage 1 was 4.0 ± 0.0, for stage II was 6.0±2.1, for stage III was 4.3 ± 2.2 and for stage IV was 4.0 ± 1.5 (Figure 9). According to this result no correlation was found within the PDL1expression and clinical stages of

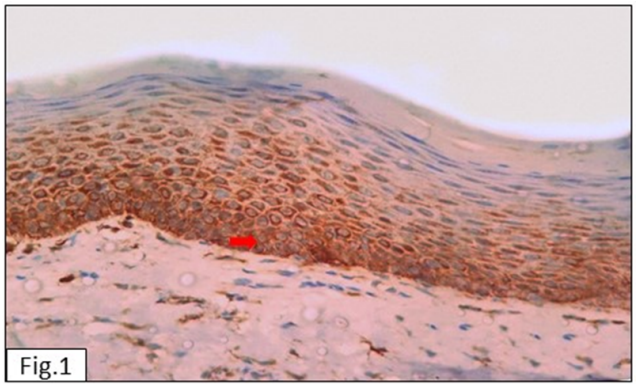

Fig. 1: Oral leukoplakia- membranous PD-L1 expression (IHC, PD-L1, 400x magnification

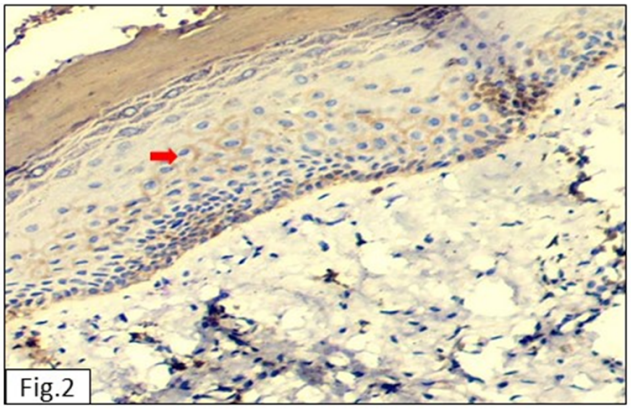

Fig. 2: OSMF- membranous PD-L1 expression (IHC, PD-L1, 400x magnification)

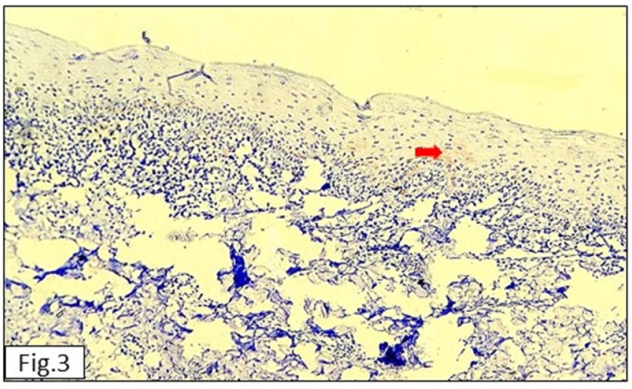

Fig. 3: Oral lichen planus- membranous PD-L1 expression (IHC, PD-L1, X400 magnification)

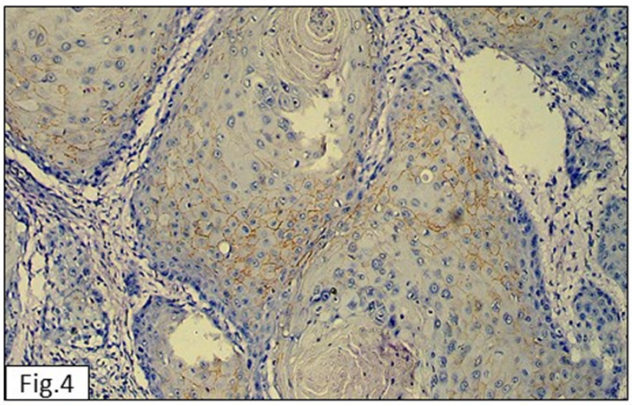

Fig. 4: OSCC Grade 1 (membranous expression (IHC, PD-L1. X100 magnification)

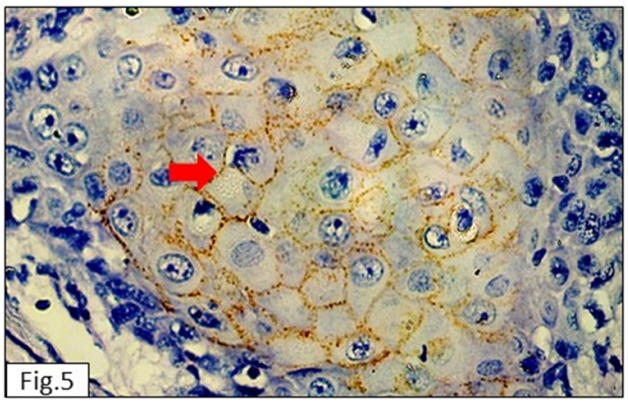

Fig. 5: OSCC Grade I (membranous expression) (IHC, PD-L, X400 magnification)

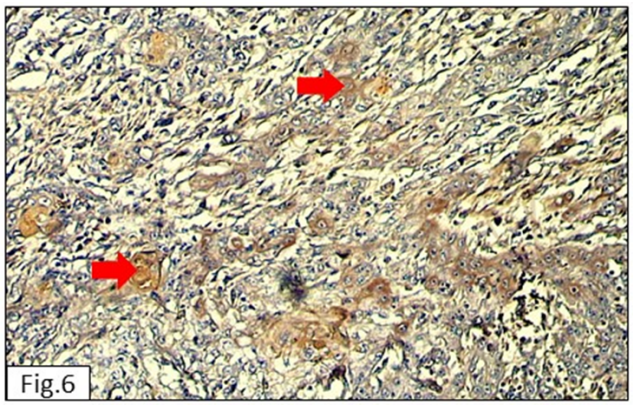

Fig. 6: OSCC Grade II (membranous expression) (IHC, PD-L1, X400 magnification)

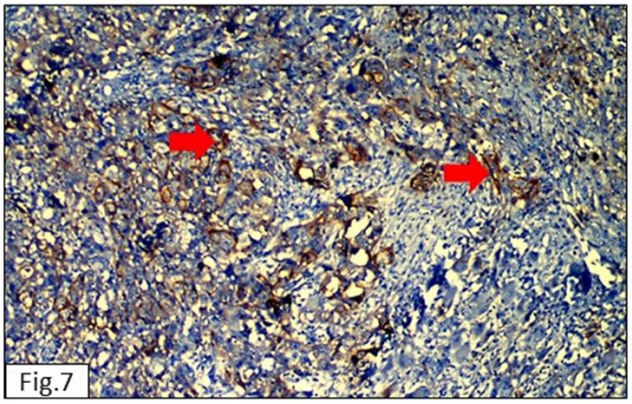

Fig. 7: OSCC Grade III (membranous expression) (IHC, PD-L1, X400 magnification)

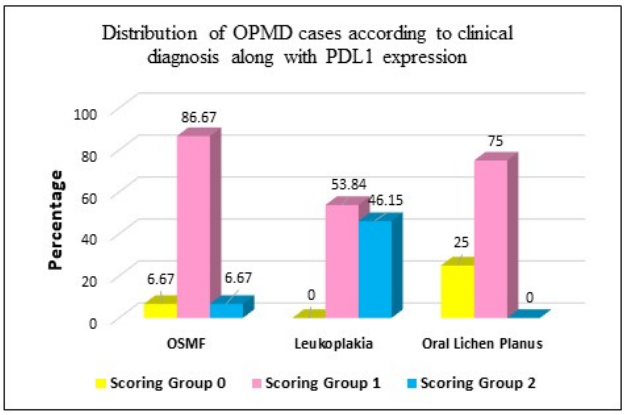

Fig. 8: Distribution of OPMD cases according to clinical diagnosis along with PDL1 expression

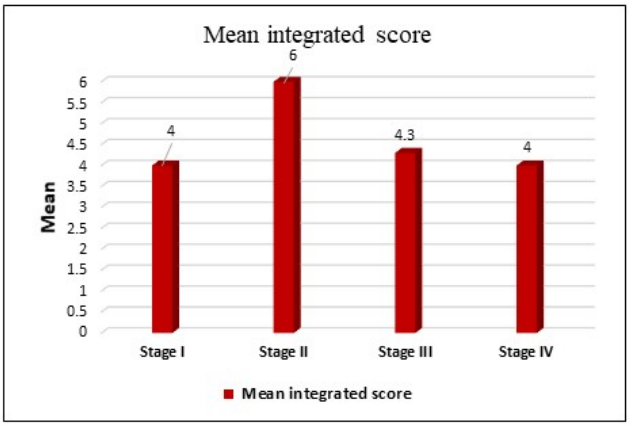

Fig. 9: Clinical stage wise distribution and comparison of mean integrated score of PDL1 expression among OSCC cases

OSCC. In present study we found direct correlation among the degree of differentiation and PD-L1 expression based on histopathologic grades. Mean IIS of histopathological grade I was 4.0 ± 1.6, for grade II was 4.3 ± 1.2 and for grade III was 9.0 ± 00. As the histopathological grade increased PDL1 expression also increased (Figure 10). There were statistically significant association with histopathological grade and score with p=0.001*.

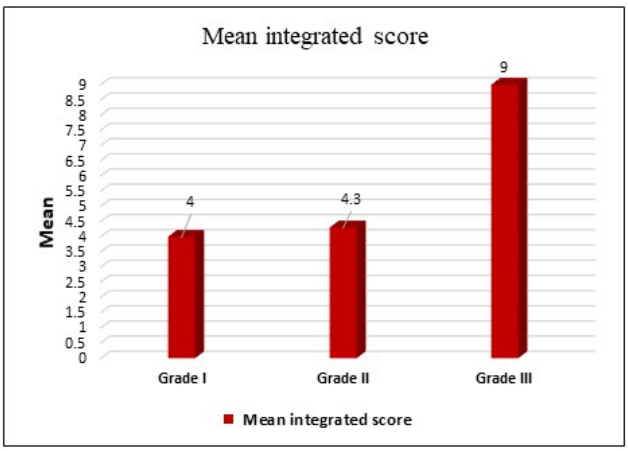

Fig. 10: Histopathological grade wise distribution and comparison of mean integrated score of PDL1 expression among OSCC cases

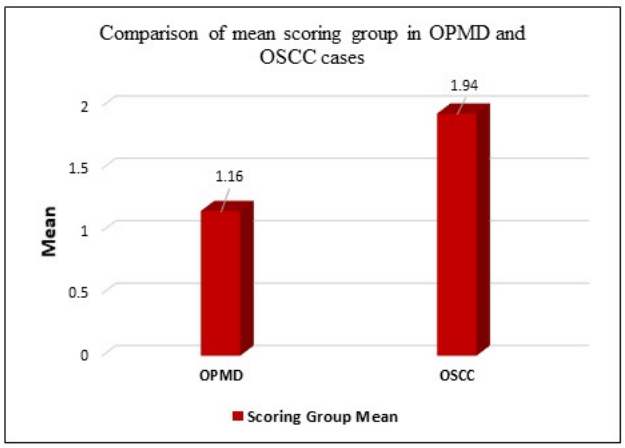

Fig. 11: Comparison of mean scoring group in OPMD and OSCC cases

Thus, the study showed 100% PD-L1 positive expression in OSCC and PD-L1 expression increased with increase in histopathological. In case of OPMD 93.74 % showed positive PD-L1 expression. We found PDL1 expression is significantly higher in OSCC (Mean= 4.59 ± 1.965) compared to OPMD (Mean= 2.03 ± 1.204) (Figure 12)

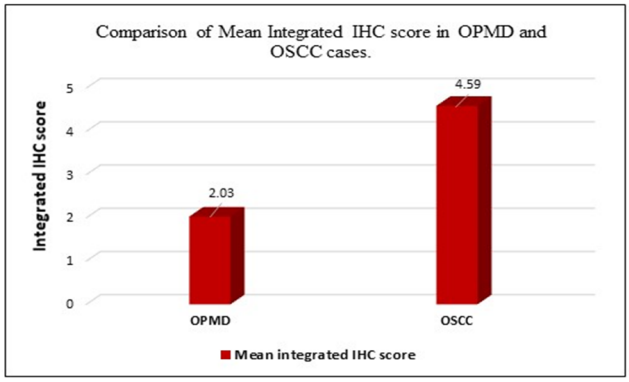

Fig. 12: Comparison of mean integrated IHC score in OPMD and OSCC cases

4. Discussion

With more and more extensive demographic studies on Oral cancers reflecting the grave nature of the disease, the research arena is rife with finding ways to combat this threat. Amidst the facts and figures impressing upon the widespread prevalence of cancers, the need for more precise diagnosis and management still persists.

OPMD is the latest WHO recommended term for a group of disorders that carry an unpredictable risk of malignant transformation.

Prevention and early detection of such Oral potentially malignant disorder (OPMD) have the potential of not only decreasing the incidence but also in improving the survival of those who develop oral cancer In recent years, several biomarkers have been identified that can provide useful diagnostic and prognostic information in progression of OPMD into malignancies. Immune escape is a key mechanism of cancer progression and metastatic dissemination and creates a serious obstacle for successful cancer treatment. Identifying the molecular changes within cancer cells like expression of programmed cell death ligand -1 which inhibits lysis of cancer cells by immune system will provide diagnostic information.[11] The immunosuppressive network between cancer cells and host immune cells may contribute to the ability of tumors to evade immune attacks. Many investigators have mentioned that lymphocytes infiltrating and adjacent to tumor cells play an important role in immune responses that limit and correlate with tumor invasion, and these are considered to be associated with clinical outcomes of tumors including OSCC.[12]

PD-L1 is usually expressed by macrophages, some activated T cells and B cells, DCs and some epithelial cells, particularly under inflammatory conditions.[13] In addition, PD-L1 is expressed by tumor cells as an "adaptive immune mechanism" to escape anti-tumor responses[14] Patients with high PD-L1 expression had poor clinical

outcome and might require PD-L1-targeted immunotherapy to improve their prognosis. According to Chen et al (2019) PD-L1 Expression is higher in OSSC than in OPMDs. Therefore, PD-L1 expression has been implicated in malignant transformation of OPMDs to OSCC.[15]

The present study was an effort towards understanding the alterations that occurred in potentially malignant disorder and malignant state (OSCC) in order to study the process of transformation. This was attempted to achieve by studying the immunohistochemical expression of PDL1 in OPMD and OSCC cases.

4.1. Immunohistochemical expression of PDL1 among the study groups

An increasing number of studies have revealed that the level of PD-L1 expression is increased in several human malignancies, such as non-small cell lung carcinoma, breast cancer, small cell neuroendocrine carcinomas, and esophageal squamous cell carcinoma.

These malignancies can generate an immunosuppressive tumor microenvironment by expressing high-aggregate PD-L1 to avoid cytolysis by activated T cells. It may explain why overexpression of high-aggregate PD-L1 in tumors leads to poor prognosis in cancer patients.[16] Most studies reveal a worse outcome correlation whereas favorable outcome has been observed in PD-L1 positive cancers in melanoma and colon cancer. These conflicting results led us to investigate the role of PD-L1 in our OSCC population.[8] Many times this OSCC is preceded by OPMDs which may be attributed to development of some molecular and genetic changes within OPMDs. Identifying such molecular changes may help in early diagnosis. According to study of Saraggi et al.(2017).[8] PD-L1 expression is seen in dysplastic lesions than in normal and is further increased in cancer. So, in present study we assess and compare the PD-L1 expression in OPMD and OSCC.

In the present study PDL1 expression was located primarily on the cell membrane. The normal mucosa was negative for PD-L1 staining. This finding is in accordance with the findings of Wang et al(2019), Gonçalves AS (2017).[17,18] In normal tissues, PD-1 signaling in T cells regulates immune responses to decrease damage to adjacent tissue, and counteracts the development of autoimmunity by promoting tolerance to self-antigens. PD-L1 receptor is also expressed on the surface of CD4+Foxp3+ regulatory T cells (Tregs), a subset of CD4+ T cells that play a critical role in maintaining immune tolerance and weakening immune responses, to promote the development, maintenance, and immunosuppressive function of Tregs through inhibiting mTOR and AKT phosphorylation.[19]

Our results demonstrate that PDL1 expression is significantly higher in OSCC (Mean= 4.59 ± 1.965) patients compared to OPMD (Mean= 2.03 ± 1.204) patients.

4.2. Expression of PDL1 in OPMD and OSCC

Cancer immunosurveillance is an essential protective response that prevents the development of malignant tumors through the early elimination of transformed cells134. Early elimination can also contribute to the selection of tumor cells that can evade antitumor responses based on the concept of immunoediting.[20] The immune checkpoint system is one of the mechanisms through which tumors can escape antitumor responses evading eradication by the host immune system by attenuating T-cell mediated responses.[21] Immunomodulatory monoclonal antibodies, which target the PD-1/PD-L1 pathway, have shown promising results in clinical trials of several cancers. However, most of our understanding of PD-1/ PD-L1 role in cancer is based on models in which tumors have already escaped immunosurveillance, and very few studies have investigated their roles in pre-cancerous lesions.

In this context, oral premalignant lesions represent an excellent model for understanding the expression of PD-1/PD-L1 and how this immune checkpoint pathway is involved in malignant transformation for the following two reasons: 1-OED can precede malignant transformation but already shows various genetic abnormalities, including mutations in the P53 gene and differential expression of several genes involved in the regulation of immune responses.[22] 2. OED is commonly associated with an increase in the inflammatory infiltrate, particularly TCD4 cells and neutrophils, which can be seen in direct contact with the epithelial cells.[23] These interactions are occurring primarily at the basal epithelial layer, but invasion is still not present.

To date, the prognostic value of PD-L1 has been studied in several cervical premalignant lesions.[24,25] According to the immunohistochemical assessment of Saraggi et al.(2017),[26] PD-L1 protein was significantly overexpressed in ampullary dysplastic lesions compared to the normal samples.[24] Consistently, PD-L1 protein has been found to be constitutively expressed in premalignant trophoblast subtypes of gestational trophoblastic diseases, independently from FIGO (International Federation of Gynecology Obstetrics) prognostic score, chemoresistance, or fatal outcomes.[27]

In present study out of 32 OPMD cases 46.87% (n=15) belonged to OSMF, 40.65% (n=13) OLK and 12.5% (n=4) were OLP. When we compared scoring group for immunoexpression of PD-L1 6.25% (n=2) belonged to scoring group 0 (no expression), 71.87% (n=23) belonged to scoring group 1 and 21.87% (n=7) belonged to scoring group 2. The cases of scoring group 0 were of OLP and OSMF. At present no any study was found which compares PD-L1 expression among OPMDs. In our study out of 32 OPMD cases all OLK cases showed PD-L1 expression. Whereas only 1 case each from OLP and OSMF showed negative expression. Minimum PD-L1 expression

was present in OLP. This may be because of association of PD-L1 expresssion with presence of epithelial dysplasia which is more in OLK than OSMF and minimum in OLP.[22]

Amongst all cases of OPMD maximum IIS was found in leukoplakia. Out of 13 OLK cases 46.15% (n=6) showed IIS=4, 30.76% (n=4) showed IIS=1 while 23.07% (n=3) showed IIS=2. This indicates that among OPMD, leukoplakia shows more PDL-1 expression than OSMF and OLP this may be because of presence of more degree of epithelial dysplasia in leukoplakia than OSMF and OLP. This finding is in accordance with Chen XJ et al (2019)[15] who found density of PD-L1 in OLK was significantly higher than the control. Moreover, the percentage of PD-L1-positive OLK tissue samples was 61.9% compared to 0% in the control tissues, suggesting that PD-L1 has potential as a biomarker for OLK. At present, the only research available has revealed that the expression of PD-L1 protein was higher in OLK than in control ($P < 0.05$).[15,18]

Another OPMD which shows malignant transformation in OSCC is OSMF. In present study we found out of 15 OSMF cases 86.67% (n=13) belonged to scoring group 1 and 6.67% (n=1) each belonged to both scoring group 0 and 2. From this 60% (n=9) showed integraged IHC score (IIS) - 2, 26.6% (n=4) showed IIS- 1 and 6.66% (n=1) showed IIS-0 and IIS-4 each. Uptil now, PD-L1 expression was not studied in only OSMF. Hongzhi Q et al (2020)[28] investigated the expression of PD-L1/ PD-1 between OSCC with or without OSMF and found that level of PD-L1 expression was significantly higher in OSCC associated with OSMF than in OSCC without OSMF ($p = 0.006$). They concluded that PD-L1/PD-1 signaling might play an important role in the malignant transformation of OSMF, and targeting PD-L1/PD-1 signaling may be a new therapeutic strategy for OSCC, especially in OSCC patients with OSMF.[28]

In present study total 12.5% (n=4) oral lichen planus cases were present out of which only 75% (n=3) showed IIS=1-2 and 25% (n=1) showed IIS=0. This findings are in consistent with Costa et al (2020)[29] who showed most OLP samples were considered negative for PD-L1 (n=22, 66.6%).[29] In line with this, Zhou et al. (2012), by evaluating PD-1, PD-L1, lymphocyte, and inflammatory cytokines in peripheral blood and using other assays in OLP, observed that the PD-1/PD-L1 pathway negatively modulates the immune response mediated by lymphocytes.

Although data are sparse and divergent about the possible participation of the PD-1/PD-L1/ PD-L2 pathway in the immunopathogenesis of OLP, the hypothesis of the Lenouvel et al., (2019)[30] study was that a reduction of PD-L1 and PD-L2 expression associated with a high cytotoxic immune response, especially in clinically more severe OLPs, may occur in the microenvironment of this autoimmune disease. They hypothesized that the PD-L1/PD-1 immunoinhibitory pathway may be compromised in OLP.[31] This provides a explanation for low PD-L11 expression in Lichen planus.[30]

When we compared mean IIS in OSCC and OPMD, statistically significant difference was found for IIS between OPMD (2.03 ±1.204) and OSCC (4.59 ±1.965) with $p<0.001$*. In the present study among OPMD maximum number of cases have shown integrated score from 0 to 4 while in group 2 it was from 2 to 9. Which implies that PDL1 expression increases as lesion progress from OPMD to OSCC. This is in accordance with Kanan et al (2020)[32] who reported that increase in the number of PD-L1 positive cells in oral dysplastic lesions and OSCCs. however, it is well known that not all dysplastic lesions will transform to OSCC. The significant increase in PD-L1 expression in basal epithelial cells and inflammatory cells in lesions that progress to cancer suggests that the activation of mechanisms that suppress the elimination of transformed cells precede cellular invasion which is the hallmark of cancer. Therefore, results of Kanan et al(2020)[32] study highlights the importance of the immune responses in early transformation as a potential tool to predict and monitor malignant transformation.

Takahiro Yagyu et al(2017)[33] elucidated the role of programmed death ligand 1(PD-L1), tumor microenvironment and tumor escape mechanisms that allow malignant transformation of oral precancerous lesions. They indicated that PD-L1 expressing dysplastic epithelial and recruited subepithelial cells in oral precancerous lesions may evade the host immune system and that the inhibition of PD-1/PD-L1 pathway may potentially prevent malignant transformation of oral precancerous lesions. Our findings also showed significant increased expression of PDL1 from OPMD cases to OSCC cases. Similar findings are also observed in Saraggi et al (2017)[26] and Chen XJ et al (2019).[15]

4.3. Expression of PDL1 among various clinical stages of OSCC

Our study reports higher prevalence of stage III & IV tumours which is in line with local studies that have reported a higher prevalence of advanced stage of oral cancers (104). In our study we found that Mean integrated IHC score for clinical stage 1 was 4.0 ± 0.0, for stage II was 6.0±2.1, for stage III was 4.3 ± 2.2 and for stage IV was 4.0 ± 1.5. According to this result no correlation was found within the PDL1expression and clinical stages of OSCC. This was in accordance with Chen XJ et al (2019)[15] who reported that PD-L1 immunoreactivity was independent of tumor size (T1–T4), lymph node status (N–, N+), local recurrence, and distant metastasis ($P > 0.05$) in OSCC.[34,35] But according to Khan et al study (2020).[36] They found a significant p value for the association of PD-L1 with stage II (p=0.029) and stage IV (p=0.001) tumors, This may be because of large sample size i.e 140 OSCC cases that were included by khan

et al in their study.

4.4. Expression of PDL1 among various histological grades of OSCC

In present study we found direct correlation among the degree of differentiation and PD-L1 expression based on histopathologic grades. Mean IIS of histopathological grade I was 4.0 ± 1.6, for grade II was 4.3 ± 1.2 and for grade III was 9.0 ± 00. As the histopathological grade increased PDL1 expression also increased. There were statistically significant association with histopathological grade and score with p=0.001*. These findings are in accordance with Kouketsu et al. (2017)[12] and Chen XJ et al. (2019)[15] according to Chen XJ et al. (2019) more PD-L1 expression was found in grade III OSCC than grade I and II with p value= 0.001.

Thus, the study showed 100% PD-L1 positive expression in OSCC and PD-L1 expression increased with increase in histopathological. In case of OPMD 93.74% showed positive PD-L1 expression. We found PDL1 expression is significantly higher in OSCC (Mean= 4.59 ± 1.965) compared to OPMD (Mean= 2.03 ± 1.204).

5. Limitations and Clinical Significance of Study

Although this study was satisfactory in terms for realizing its aim and objectives, there were certain restraints associated with it. These restraints were realized as variances among observations when compared to different studies which could have resulted from the differences in the sample size, lack of uniformity in selection of OPMD and OSCC cases, difference in the exposure of tissues to chemicals such as formalin etc. which may have an adverse effect on epitopes etc.

Also, the present study had a relatively small sample size and was unicentric. For clinical application, prospective studies involving larger number of patients are needed to further evaluate the clinical utility of PDL1 as a biomarker for malignant transformation of OPMD providing additional value beyond the clinical and histological parameters.

On establishment of role of PDL1 in malignant transformation of OPMD, it may serve as an effective diagnostic and prognostic biomarker. Increased expression of PDL1 in OPMD cases may help in identifying them to have a greater risk of cancer progression. Early management of such cases may arrest cancer progression and their subsequent transformation into malignancy.

Overexpression of PDL1 in OSCC also contributes to a more aggressive course and also suggested that PDL1 can be clinically used as therapeutic target for cancer treatment. Futher research is required to prove its role as a potent therapeutic target.

6. Conclusion

According to studies PD-L1 expression in OPMD is increased as compared to control tissue depending upon the degree of dysplasia present in tissue and is further increased in OSCC cases. In the present study, a total of 64 subjects including OPMD and OSCC cases were assessed for PDL1 expression. In OSCC cases PD-L1 expression was compared amongst the different clinical and histological grades. Amongst all OPMD cases 100% PD-L1 expression was seen in OLK, 93.33% in OSMF cases and 75% in OLP cases. PD-L1 expression among OSCC cases increased with advancing histopathological grades.

It could thus be concluded from the present study that assessment of PD-L1 expression in OPMD patients will help us to screen the subjects with or without risk of malignant transformation as increase in PD-L1 expression signifies the increased risk of malignant change. These high risk subjects could be referred to specialized centers for further evaluation. Hence, this could be used as a screening marker which may help further in diagnostic information and disease monitoring.

7. Source of Funding

None.

8. Conflict of Interest

None.

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