DOI: 10.7860/JCDR/2022/58414.17238

Research Protocol

Dentistry Section

Immunoexpression of Podoplanin in Leukoplakia and Oral Squamous Cell Carcinoma and its Correlation with Survival: A Research Protocol

SAMIHA JAMEEL AHMED KHAN[1], MADHURI GAWANDE[2], ALKA HANDE[3], SWATI PATIL[4], ARCHANA SONONE[5]

ABSTRACT

Introduction: Podoplanin (PDPN) is a well-conserved mucin-type transmembrane glycoprotein. According to various studies, podoplanin expression is seen in various human cancers and it also encourages the progression of the tumour. A high PDPN expression, specifically in oral cancers, shows a significant relation to the metastasis of lymph nodes and poor patient survival, suggesting its substantial role in identifying the malignant transformation of a lesion by its expression in initial oral tumourigenesis. Only few studies have mentioned the use of podoplanin marker in detection of malignant transformation of Oral Potentially Malignant Disorders (OPMDs). Most malignant transformations are seen in cases of oral leukoplakia. The present study will help in early diagnosis of malignant transformation of leukoplakia by showing an increased expression of podoplanin, thereby, resulting in better treatment and prognosis of the disease.

Aim: To evaluate immunoexpression of podoplanin in leukoplakia and oral squamous cell carcinoma and also to correlate it with the clinicopathological characteristics of leukoplakia and Oral Squamous Cell Carcinoma (OSCC) and the survival of OSCC patients.

Materials and Methods: This retrospective study will be conducted in the Department of Oral Pathology at Sharad Pawar Dental College, Sawangi (Meghe), Wardha, Maharashtra, India. Surgically operated OSCC cases from year 2005-2019 in this Institute will be retrieved from the archival of the department. Ninety samples in total will be taken for the study, which will be further divided into three groups, consisting of 30 samples in each group as follows: leukoplakia, OSCC and normal oral mucosa (control). Immunohistochemical staining will be carried out, and podoplanin (PDPN) immunoexpression with different clinical characteristics of leukoplakia and oral squamous cell carcinoma will be assessed. Broder's grading system will be used for histopathological grading of all cases of OSCC. The Chi-square test, Kaplan-Meier method, and the log-rank test will be used to statistically analyse the data.

Keywords: Immunohistochemistry, Oral neoplasms, Prognosis, Transmembrane glycoprotein

INTRODUCTION

Oral cancer is considered to be the most common cause of the death by cancer. The most widely known cancer of the oral cavity is Oral Squamous Cell Carcinoma (OSCC). The OSCC is usually led by Oral Potentially Malignant Diseases (OPMD). These OPMDs can be identified on a clinical basis owing to their characteristic presentation [1]. These include: leukoplakia, Oral Submucous Fibrosis (OSMF), erythroplakia and lichenoid dysplastic lesions [2,3]. Most malignant transformations are seen in cases of oral leukoplakia. Oral leukoplakia is a non scrapable, white lesion of oral mucosa that cannot be characterised as any other lesion [4]. The paramount modalities for managing OPMDs and OSCC are prevention and early detection [1].

Patients' quality of life is deteriorated by premalignant lesions of the oral cavity and their pathological sequelae, as managing the disease is unaffordable to the common people [5]. Many researchers have found that OPMDs showing epithelial dysplasia are at more risk of malignancy than OPMDs showing no dysplastic features [6,7]. Histopathologic assessment is considered a "gold standard" for examining lesions with the possibility of transforming into malignancy. In histopathological examination, one of the important criteria is histopathological grading, which helps to determine the clinical and biological course of OSCC [8].

Local invasion/expansion, recurrence, the potential for metastasis, and disease-free survival of the tumour are predicted by assessing cell proliferation in histopathology through immunohistochemical evaluation [9]. Researchers have found objective molecular markers capable of identifying lesions with a high potential of turning into malignancy [10]. These markers help to erase the differences that arise due to inter-observer variability while diagnosing different grades of OSCC [11].

Podoplanin is a well-conserved mucin-type transmembrane glycoprotein marker that is primarily used for lymphatic endothelial cells. PDPN expression is seen in various human cancers and encourages tumour progression [12]. The PDPN expression is seen only on lymphatic endothelium and is not expressed on blood vessel endothelium. PDPN helps in the prevention of cellular adhesion [12]. A high PDPN expression, seen in head and neck cancers, specifically in oral cancers, showed a significant relation to the metastasis of lymph nodes and poor survival of the patient. Some dysplastic and hyperplastic lesions, which were close to primary oral cancers, also showed expression of PDPN. This indicated that PDPN plays a notable role in identifying the malignant transformation of a lesion by its expression in initial oral tumourigenesis [13].

Previous studies conducted by Deepa AG et al., and Patil A et al., have mentioned the role of podoplanin in diagnosis of various OPMDs [14,15]. But, the present study will focus mainly on evaluating the podoplanin expression in leukoplakia, which is the most capable of malignant transformation. Also, leukoplakia is more common in the population of this western region of India, where this study will be conducted. Evaluation of PDPN in leukoplakia will help in preventing further progression of disease by detecting its risk of malignant transformation at an early stage. The various clinicopathological features of leukoplakia will be compared and correlated with OSCC. A three-year survival analysis of OSCC patients will also be done in present study to see the prognosis of the disease.

The aim of the research protocol is to evaluate immunoexpression of Podoplanin (PDPN) in Leukoplakia (LP) and oral squamous cell carcinoma and its correlation with the survival of OSCC patients. The null hyposthesis is that podoplanin immunoexpression will remain the same in normal oral mucosa, oral leukoplakia and OSCC. Alternative hypothesis is that, podoplanin immunoexpression will increase through normal oral mucosa to leukoplakia to OSCC.

MATERIALS AND METHODS

This retrospective study will be conducted in the Oral Pathology Department at Sharad Pawar Dental College, Sawangi (Meghe), Wardha, Maharashtra, India, after obtaining the approval of the Institutional Ethical Committee {DMIMS(DU)/IEC/2022/760} and informed consent of the patients.

Inclusion criteria: Total 30 OSCC cases and 30 LP cases that will be clinically and histopathologically diagnosed and who will be treated surgically for primary treatment, will be included. Normal oral mucosa will be used as control to compare the podoplanin expression in normal and abnormal conditions.

Exclusion criteria: The cases with head neck cancer history in the past, recurrent or distant disease, and patients who have undergone preoperative treatments, except biopsy will be excluded from the the study.

Sample size calculation: Sample size was estimated using Cochran's formula [16] for the present study

$$N=Z^2pq/e^2$$

Where;

Z is the level of significance at 5%

i.e. 95% Confidence interval=1.96

p=Samples showed positive podoplanin expression focally

in small group of cells in the basal layer of epithelium=35%=0.35

q=(1-p)

e=Error of margin=10%=0.10

N=1.962×0.35×(1-0.35)/0.102

N=90, hence, 90 patients needed in the present and 35% prevalence was taken from previous study by Deepa AG et al., [14].

Study Procedure

Ninety samples in total will be taken for this study which will be further divided into three groups, consisting of 30 samples in each group as follows:

- Leukoplakia (n=30): Random cases of leukoplakia will be selected for OPMDs group from the archival of department.
- Oral squamous cell carcinoma (n=30): Surgically operated OSCC cases from year 2005-2019 in this Institute, will be retrieved from the archival of the department.
- Normal oral mucosa (control) (n=30): Normal oral mucosa will be taken from surgically extracted third molar cases after patient's verbal consent.

The histopathological grading of all OSCC cases will be done using Broder's grading system [17].

The patient's details like age, gender, habits and site of the lesion, clinical history, duration of disease, and microscopic features of the lesion will be recorded. A three-year retrospective follow-up will be done for the patients selected for OSCC for survival analysis since the time of surgery. Under low power view (100X), all the Haematoxylin and Eosin (H&E) stained tissue sections will be screened. Immunohistochemical staining will be carried out, and Podoplanin (PDPN) immunoexpression with different clinical characteristics of leukoplakia and oral squamous cell carcinoma will be assessed.

Immunohistochemistry

Tissue sections (4 µm thickness) will be retrieved from paraffin-embedded tissue blocks (fixed with formalin). They will be transferred on 3-Amino Propyl Tri-ethoxy Silane (APES) glass slides. Deparaffinisation will be done using xylene, and the slides will be rehydrated with lower ethanol concentrations. Antigen retrieval will be done by heating the slides in tris-Ethylenediamine Tetraacetic Acid (EDTA) in a pressure cooker for 5 mins. Blocking of endogenous peroxidase activity will be done with 3% H_2O_2 (Hydrogen Peroxide) for 10 mins. Ultraviolet block reagent will be used to treat the slides for five minutes. Incubation of slides will be done for one hour at room temperature with mouse monoclonal anti-human podoplanin primary antibody (D2-40). Later, secondary antibodies will be followed using horse radish peroxidase for 30 minutes. A substrate chromogen (3'-Diaminobenzidine Tetrahydrochloride) will be used for staining, and counter staining will be done using Harris haematoxylin. Dehydration of sections will be done, followed by clearing and mounting, and podoplanin expression will be observed according to the grades of podoplanin mentioned above. These findings will be observed under a light microscope [14].

Expected outcome: The podoplanin immunoexpression is expected to increase through normal oral mucosa to leukoplakia to oral squamous cell carcinoma, suggesting its use in assessing the potential for malignancy of leukoplakia and aggressive behaviour of OSCC. Immunoexpression of podoplanin will help predict the transformation of leukoplakia into malignancy and the prognosis of OSCC patients.

STATISTICAL ANALYSIS

The association between podoplanin expression status and clinicopathologic parameters will be analysed using the Chi- square test. Also, the survival of OSCC patients will be assessed using Kaplan-Meier method, and the log-rank test will be used to test for significant differences.

DISCUSSION

Deepa AG et al., conducted a study in which podoplanin immunoexpression was compared in twenty cases each of OL, OSMF, and OSCC to normal oral (buccal) mucosa using IHC. For this, D2-40 (monoclonal antibody) was used. When OSCC, OSMF, and OL were compared to normal oral mucosa, a remarkable upregulation in the grades of PDPN immunoexpression was seen. This increase in podoplanin expression was seen with decreasing grades of differentiation. PDPN expression was seen increasing in oral submucous fibrosis compared to oral leukoplakia. The conclusion of this study was that PDPN expression in epithelial cells of dysplastic lesions could significantly predict the risk of malignancy [14].

A study performed by Aiswarya A et al., evaluated podoplanin expression in 25 samples of leukoplakia and 30 samples of OSCC as a molecular marker to predict the risk of malignancy in leukoplakia cases. Podoplanin expression was seen to be increasing from normal oral mucosa to oral leukoplakia to OSCC. The podoplanin staining score was seen to be remarkably increasing from mild dysplasia to carcinoma in-situ in cases of oral leukoplakia. Well-differentiated OSCC group displayed the highest expression of podoplanin. Their study concluded that the progressive increase in podoplanin expression in case of oral leukoplakia through the increasing grades of dysplasia suggests a high risk for malignancy [18].

Kawaguchi H et al., evaluated the expression of podoplanin to see the progression of oral cancer in 150 cases of leukoplakia using IHC along with long-term follow-up. The follow-up was done to assess the correlation of demographic features with PDPN expression. Oral leukoplakia cases which were positive for podoplanin showed a significantly greater risk for oral cancer compared to those with negative PDPN. They stated that, histology and PDPN expression help further stratification of oral cancer development. This study

concluded that PDPN expression is most frequent in leukoplakia cases. PDPN, along with histology, can be a vital marker for predicting potential for oral cancer development in leukoplakia cases [19].

Patil A et al., conducted a study where they assessed the significance of PDPN as a biomarker for cancer probability in leukoplakia. They also correlated PDPN expression to different grades of OSCC. The investigation of PDPN expression was done in 40 cases each of leukoplakia and OSCC using IHC. A remarkable increase in PDPN expression was seen from mild dysplasia to severe dysplasia and from well-differentiated OSCC to poorly differentiated OSCC in this study. This study came to a conclusion that, PDPN could be used as a biomarker for the identification of initial oral tumourigenesis [15].

Karunagaran M et al., conducted a study in which comparison and analysis of the expression of podoplanin and its pattern of distribution in normal oral mucosa and OSMF were made. This was compared with the increase in grades of dysplasia from mild to moderate to severe dysplasia using IHC. Forty cases were taken, and four groups were formed comprising- normal oral mucosa (group A), OSMF-mild dysplasia (group B), OSMF- moderate dysplasia (group C), and OSMF- severe dysplasia (group D). They observed that podoplanin expression increased with an increase in scores of dysplasia. This study indicated that expression of PDPN increases as the grades of dysplasia increase, implying its part in the potential of the disease for malignant transformation [20].

Limitation(s)

The only limitation of the present study is the small sample size. Further studies with greater sample size and follow-up are recommended, to demonstrate the exact role of podoplanin in OL and OSCC.

CONCLUSION(S)

Podoplanin plays a significant role in identifying malignant transformation of a lesion by its expression in initial oral tumourigenesis. Podoplanin, along with histology, can be a valuable marker for predicting the potential for oral cancer development in leukoplakia cases.

REFERENCES

[1] Hande A, Sonone A, Gadbail A, Gawande M, Patil S, Sharma P. Modalities to restrain the progression of oral potentially malignant diseases and oral squamous cell carcinoma in COVID-19 pandemic. J Oral Oncol. 2021;114:105072.

[2] Van der Waal I. Potentially malignant disorders of the oral and oropharyngeal mucosa; terminology, classification and present concepts of management. Oral Oncol. 2009;45(4-5):317-23.

[3] Gupta B, Ariyawardana A, Johnson NW. Oral cancer in India continues in epidemic proportions: evidence base and policy initiatives. Int. Dent J . 2013;63(1):12-25.

[4] Dhole P, Lohe VK, Sayyad A, Kadu RP, Thakare G, Sune RV. A case report on capillary hemangioma and Leukoplakia on Tongue. Med. Science. 2020;24(106):4211-16.

[5] Deolia SG, Khare MV, Arora RP, Chikhale RN, Korde RD, Reche AM. Assessment of the oral health seeking behavior of patients with premalignant lesions. J. Fam Med. Prim Care. 2020;9(1):141-46.

[6] Reibel J. Prognosis of oral pre-malignant lesions: Significance of clinical, histopathological, and molecular biological characteristics. Crit Rev. Oral Biol Med. 2003;14(1):47-62.

[7] Jr SS, Gorsky M, Ms FL. Oral leukoplakia and malignant transformation. A follow-up study of 257 patients. Cancer. 1984;53(3):563-68.

[8] Sonone A, Patil S, Hande A, Gawande M. Correlation of p53 immunoexpression with depth of tumor in microinvasive oral squamous cell carcinoma. Journal of Pharmaceutical Research International. 2021;33(37B):98-03.

[9] Gadbail AR, Sarode SC, Chaudhary MS, Gondivkar SM, Tekade SA, Yuwanati M. Ki67 labelling index predicts clinical outcome and survival in oral squamous cell carcinoma. J Appl Oral Sci. 2021;29:e20200751.

[10] Condurache Hritcu OM, Botez AE, Olinici DT, Onofrei P, Stoica L, Grecu VB, et al. Molecular markers associated with potentially malignant oral lesions. Exp Ther Med. 2021;22(2):1-5.

[11] Jagtap MM, Shukla S, Acharya S, Tamhane A, Bhake A. Utility of histochemical and immunohistochemical profile in grading of squamous cell carcinoma of the oral cavity. J Clin Diag Res. 2020;14(1):EC01-EC05.

[12] Rudzińska M, Gaweł D, Sikorska J, Karpińska KM, Kiedrowski M, Stępień T, et al. The role of podoplanin in the biology of differentiated thyroid cancers. PLoS One. 2014;9(5):e96541.

[13] Yuan P, Temam S, El-Naggar A, Zhou X, Liu DD, Lee JJ, et al. Overexpression of podoplanin in oral cancer and its association with poor clinical outcome. Cancer. 2006;107(3):563-69.

[14] Deepa AG, Janardanan-Nair B, Varun BR. Podoplanin expression in oral potentially malignant disorders and oral squamous cell carcinoma. J Clin Exp Dentistry. 2017;9(12):e1418.

[15] Patil A, Patil K, Tupsakhare S, Gabhane M, Sonune S, Kandalgaonkar S. Evaluation of podoplanin in oral leukoplakia and oral squamous cell carcinoma. Scientifica. 2015;2015:135298.

[16] Cochran WG. Sampling Techniques. jjohn Wiley and Sons. Inc., New York. 1977.

[17] Broders AC. Squamous-cell epithelioma of the lip: A study of five hundred and thirty-seven cases. J Am Med Association. 1920;74(10):656-64.

[18] Aiswarya A, Suresh R, Janardhanan M, Savithri V, Aravind T, Mathew L. An immunohistochemical evaluation of podoplanin expression in oral leukoplakia and oral squamous cell carcinoma to explore its potential to be used as a predictor for malignant transformation. J Oral Maxillofac Pathol. 2019;23(1):159.

[19] Kawaguchi H, El-Naggar AK, Papadimitrakopoulou V, Ren H, Fan YH, Feng Let al. Podoplanin: A novel marker for oral cancer risk in patients with oral premalignancy. J Clin Oncol. 2008;26(3):354-60.

[20] Karunagaran M, Murali P, Palaniappan V, Sivapathasundharam B. Expression and distribution pattern of podoplanin in oral submucous fibrosis with varying degrees of dysplasia–an immunohistochemical study. J Histotechnol. 2019;42(2):80-86.

PARTICULARS OF CONTRIBUTORS:

1. Postgraduate Student, Department of Oral Pathology, Sharad Pawar Dental College, Datta Meghe Institute of Medical Sciences, Wardha, Maharashtra, India.
2. Professor and Head, Department of Oral Pathology, Sharad Pawar Dental College, Datta Meghe Institute of Medical Sciences, Wardha, Maharashtra, India.
3. Professor, Department of Oral Pathology, Sharad Pawar Dental College, Datta Meghe Institute of Medical Sciences, Wardha, Maharashtra, India.
4. Associate Professor, Department of Oral Pathology, Sharad Pawar Dental College, Datta Meghe Institute of Medical Sciences, Wardha, Maharashtra, India.
5. Assistant Professor, Department of Oral Pathology, Sharad Pawar Dental College, Datta Meghe Institute of Medical Sciences, Wardha, Maharashtra, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Samiha Jameel Ahmed Khan,
Postgraduate Student, Department of Oral Pathology, Sharad Pawar Dental College,
Datta Meghe Institute of Medical Sciences, Sawangi (Meghe),
Wardha, Maharashtra, India.
E-mail: samiha.khan26@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]

- Plagiarism X-checker: Jun 21, 2022
- Manual Googling: Sep 22, 2022
- iThenticate Software: Nov 11, 2022 (3%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:

- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Jun 13, 2022**
Date of Peer Review: **Aug 10, 2022**
Date of Acceptance: **Sep 23, 2022**
Date of Publishing: **Dec 01, 2022**