



## **Correlation of p53 Immunoexpression with Depth of Tumor in Microinvasive Oral Squamous Cell Carcinoma**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Author AS designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author SP managed the analyses of the study. Authors AH and MG managed the literature searches. All authors read and approved the final manuscript.*

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## **ABSTRACT**

**Introduction:** "Oral squamous cell carcinoma (OSCC)" is a major health issue in India, the incidence of OSCC is 3-7 times more in developing countries than developed countries. OSCC is the '3<sup>rd</sup> most common cancer' in India followed by "cervical and breast cancer". One side of OSCC that has not much explore is the 'microinvasive squamous cell carcinoma' which is an early stage neoplasm without infiltration in the deeper tissues. There is no particular definition of "microinvasive oral squamous cell carcinoma (MIO SCC)" There are no specific guideline are present to categories the "microinvasive squamous cell carcinoma (MIO SCC)". Most of the time the infiltrating neoplastic cells are masked under the background of the inflammatory cell infiltrate present connective tissue stroma. So this study is humble attempt to recognized and measured depth of invasion of infiltrative neoplastic cells to categories MIO SCC and to find better management protocol for it

**Aim:** This study aims to: Measure p53 immunoexpression in "microinvasive oral squamous cell carcinoma, evaluate the depth of invasion in MIO SCC in H & E stained section, and correlate the p53 immunoexpression with the depth of tumor in it.

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**Methodology:** The 25 cases of “microinvasive oral squamous cell carcinoma” will be selected and 10 cases of “normal oral mucosa (NOM)” will be obtained from “gingiva and vestibular mucosa” as controls after extraction of impacted teeth. “The depth of tumor” will be measured from the “basement membrane or in areas of basement membrane loss, from an imaginary line reconstructing the basement membrane from the adjacent epithelium to the deepest point of invasion in connective tissue” by Leica DMLB2 research microscope with Leica Q-win standard software (Switzerland).

**Results:** The results show that the depth of invasion in MIO SCC, will be categorized the lesion and give the better guidelines for histological grading and treatment protocol for MIO SCC

**Conclusion:** There are no definite guidelines for histological grading and final treatment protocol for MIO SCC. The assessments of depth of tumor through p53 immunoexpression may be one of the criteria for grading in MIO SCC. Thus the correlation of p53 immunoexpression with the depth of tumor in MIO SCC helps to determine the treatment modalities of MIO SCC.

*Keywords: Microinvasive squamous cell carcinoma; P53 markers; depth of tumor.*

## 1. INTRODUCTION

“Oral squamous cell carcinoma (OSCC)” is a major health problem and considered as the main reason for the mortality from oral diseases in most of the countries. It is the 6<sup>th</sup> most common cancer worldwide [1]. In India the incidence of OSCC is 3-7 times more than developed countries. OSCC is the 3<sup>rd</sup> most common cancer in India followed by cervical and breast cancer [2].

Lot of research and technologies in modern era, but the survival rate of the “oral squamous cell carcinoma remains unchanged, The five year survival rate is estimated to be about 50%. [3] The early diagnosis and its managements is the best approach for better prognosis.

The final diagnosis of any clinical malignant lesion of OSCC depends on the histopathological examination of the tissue. Histopathological diagnosis considered as the gold standard for the final diagnosis of the malignancy. In histopathological examination one of the important criteria is histopathological grading which helps to determine the clinical and biological course of OSCC. The first histological grading system was given by Broder which was established on the degree of differentiation and keratinization of tumor cells. It has four grades, if 75- 100% cells are differentiated then it considered as grade I and known as well differentiated squamous cell carcinoma, in grade II 50-75% cells are differentiated known moderately differentiated squamous cell carcinoma. In grade III 25-50% cells are differentiated and it considered as poorly differentiated squamous cell carcinoma and in

last grade IV where only 0-25% cells are differentiated it is known as anaplastic tumor [4].

One aspect of the OSCC was least discuss in the literature that is microinvasive squamous cell carcinoma which is an early stage tumor without invasion of deep tissues.[5,6]. As such no specific guideline are available to categories the microinvasive squamous cell carcinoma(MIO SCC). Most of the time the infiltrating neoplastic cells are masked under the background of the inflammatory cell infiltrate present connective tissue stroma.

So this study is humble attempt to recognized and measured depth of invasion of infiltrative neoplastic cells to categories MIO SCC and find suitable treatment plan for it.

## 2. MATERIALS AND METHODS

The present study will be carried out at the Department of Oral and Maxillofacial Pathology and Microbiology”, “Sharad Pawar Dental College and Hospital, Datta Meghe Institute of Medical Sciences, Deemed to be University, Sawangi (M), Wardha, Maharashtra, India”. The surgically treated cases of OSCC from year 2009 to 2020 in this institute were retrieved from the archival of the department.

### 2.1 Inclusion and Exclusion Criteria

The histopathologically diagnosed cases of MIO SCC, will be considered for inclusion in the eligibility criteria of the study. The staging of the patients will be done according to the American Joint Committee of Cancer staging system. Patients with history of previous head neck cancer, pre-operative, radiotherapy,

chemotherapy or surgery (other than a biopsy) and recurrent or distant disease were excluded from the study. Clinical data collected included age, sex, site, the clinical appearance of the lesions, tobacco and alcohol habits (smoking or smokeless tobacco or both, the type of tobacco usage, the presence or absence of alcohol consumption) and lymph node status.

## 2.2 Study Design

In this “cross sectional, retrospective cohort” study a total 25 samples will be selected.

## 2.3 Statistical Data

“Sample size is calculated using the formula:  $n = \frac{Z^2 \cdot P \cdot (1 - P)}{e^2}$ ”

where:

“Z = value from standard normal distribution”

“Corresponding to desired confidence level (Z= 1.96 for 95% CI)”

“P= is expected true proportion”

“e = is desired precision (half desired CI width)” values

Estimated proportion – 0.5

Desired precision of estimate – 0.2

Confidence level – 0.95

Population size – N/A

Results - Sample size required for specified inputs = 25

## 2.4 Sample Selection

The 25 cases of microinvasive oral squamous cell carcinoma will be and 10 cases of “normal oral mucosa (NOM)” was obtained from “vestibular mucosa and gingiva as controls after extraction of impacted teeth. The histopathological diagnosed cases of “MIO SCC” will be considered for inclusion in the eligibility criteria of the study. All the Hematoxylin and Eosin stained tissue sections will be thoroughly screened at low power magnification (10X).

## 2.5 Depth of Invasion

“The depth of invasion will be measured from the basement membrane or in areas of basement membrane loss and from an imaginary line reconstructing the basement membrane from the adjoining epithelium to the deepest point of invasion in connective tissue by Leica DMLB2 research microscope with Leica Q-win standard software (Switzerland)”. The point of deepest

infiltration of the tumor epithelial cells was recognized in a “four  $\mu$ m thick section” of the H & E stained and IHC stained slide. The depth of tumor will be measured and recorded.

## 2.6 Immunohistochemistry

For the detection of P53 antigen the immunohistochemistry will be carried out. The method of immunohistochemistry was “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 P53 and then bench cooling was done for 20 minutes. The same procedure will again be 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 washed for 3 times with PBS for 5 minutes. Non specific reactions should be prevented for that sections will be incubated with 10% serum for 10 minutes. Then incubation with P53 antibody in humidifying chamber for ninety minutes will be at room temperature. Then rinsing in PBS for 10 minutes three times after primary antigen- antibody reaction. The polymer anti-mouse (HRP-labeled) then incubated in humidifying chamber; at the room temperature for thirty minutes. The chromogen solution of 3,3’ diaminobenzidine in buffer will be taken to see the antigen-antibody reactions. Then Mayer’s Hematoxylin will be used for final counterstaining. P53 immunostaining were manually evaluated by three independent observers in blinded manner.

“P53 antibody stain the ‘neoplastic’ epithelial cells. This antibody stains which are highly expressed in the basal and supra-basal cells, helps to differentiate the neoplastic positively stained epithelial cells from mesenchymal cells and further aid in measurement of depth of invasion”.

## 3. DISCUSSION

“The microinvasive squamous cell carcinoma which is an early stage tumor without invasion of deep tissues.”[5,6] In WHO classification ( 2005) , there is no clarity on defining MIO SCC although classification systems is present for similar lesions occurring elsewhere, for example microinvasion in cervical cancer. “Microinvasive

OSCC is an early stage relatively thin tumor confined to the papillary lamina propria as defined by the depth of the rete process". For MICSCC in the other site such as larynx, breast, cervix, they have the specific histopathological criteria such as in MISCC in larynx ,microinvasive cancer includes the presence of scattered malignant cells within the submucosa just below the basement membrane or within 1-2 mm of basement membrane.[7] "Barnes (2001) defined microinvasive carcinoma of larynx as invasive SCC that extends into the stroma by  $\leq$  0.5mm, as measured from adjacent epithelial basement membrane [8]" Microinvasive carcinoma of the cervix is described based on "the sub-division of stage I cancers as a lesion which invades the cervical stroma to the depth of 3 mm below the base of the epithelium and not more than 7 mm width" [9] " Microinvasive carcinoma of breast is a term used to describe a borderline difference between completely contained ductal carcinoma in situ and a minimally invasive ductal carcinoma. A very small amount of malignant cells are found beyond the duct lining and into the surrounding stromal tissue." [10]

In case of MIOSSC, as there are no specific guidelines given, pathologist uses their judgments and experience, so there is intraobserver variability present in the pathological reports, which affect the treatment modalities and there is continual debate on treatment plan of MIOSSC with no nodal involvement, this could be due to unexplored literature data about MIOSSC and no specific well-defined grading system present to categories OSCC. The specific criteria are required to such lesions because some of them having minimal risk of "lymph node metastasis" and "recurrence" and thus can be treated conservatively and unnecessary elective neck dissection, and post treatment morbidity can be avoided. For an oral pathologist difficulty in diagnosing this condition is due to very small the invasive component, which may remain undetected. Sometimes the dense inflammation itself can mask the basement membrane integrity so analytical studies are necessary to understand the definite clinical presentations, measures of diagnosis and understand the prognosis of these lesions

In new classification of American Joint Committee of Cancer<sup>1</sup> staging system (AJCC) two parameters are included one is depth of tumor. (DOI) and extranodal spread [11]. DOI,

also "known as reconstructed tumor thickness, is different from clinical tumor thickness, particularly in exophytic and ulcerated lesions." DOI was originally envisaged as distance from a theoretical reconstructed normal mucosal surface line to the deepest extent of growth [12]. "Proximity to blood vessels and lymphatics determines the risk of developing nodal metastases. Therefore, it may be more accurate to consider the actual mass that is present beneath the theoretical reconstruction of a basement membrane (DOI), rather than the thickness of the whole tumor (TT)." (6) "Microinvasive carcinoma is a biologically malignant lesion potentially capable of gaining access to lymphatics and vascular channels. Even though majority of lesions with microinvasion have a better prognosis, it is prudent to assess the depth of invasion as propinquity to blood vessels and lymphatics increases the risk of nodal metastasis" [13]

There are very few studies present in the literature which measures the depth of invasion in MISCC one study showed the range from 0.53 to 1.16 mm, deepest point is 1.16 upto the lamina propria .(6) According to Sheeba et al the range was from the 0.02 to 0.07 and it was upto the papillary part of the lamina propria. [14]

As in the MIOSSC invasive component being very small, which may remain undetected and the dense inflammation itself can mask the basement membrane integrity, as well as it is difficult to identify the neoplastic cells in dense inflammation background. So it is required to use the marker to identify these cells. P53 is a proliferative marker , in routine histopathological section. It is used for staining of neoplastic cells, it stains the nucleus of the neoplastic cells, so it will be easily possible to recognize the infiltration of neoplastic epithelial cells in the lamina propria in presence of dense inflammatory cell infiltrate background.

P53 is a product of TP53 gene, it is also known as "the guardian of the genome" because of number of roles, particularly allowing the DNA stability by regulating the G1/S cell cycle. If DNA damage is present then p53 activates the DNA repair proteins and it also activates apoptosis if the damage is beyond repair. There are so many studies are present in literature which shows the strong correlation between TP53 gene overexpression and neoplastic development and progression. So TP53 controls the cycle cycle and maintain the genomic stability

by preventing the accumulation of genetic damage. Mutation of TP53 have been observed in “early cancerous development in most of OSCC”. [15]

A number of studies on p53 immunoexpression in different cancers were reported[16-18]. Different studies on oral squamous cell carcinoma were reported by Agrawal et al.[19] , Alvi et al.[20], Anmol et. al.[21], Bagri et. al.[22] and Gadbaill et. al.[23].

There are various classifications for the OSCC but it does not reveal the extension of neoplastic epithelial cells into the connective tissue , that’s why there is a need of hour to evaluate the depth of invasion of neoplastic epithelial cells into deeper tissue by molecular marker thus diagnosing the early MIO SCC to predict better treatment plan and better prognosis. On this basis, the objective of the study is to correlate the p53 immunoexpression with the depth of tumor in MIO SCC.

#### 4. CONCLUSION

There are no definite guidelines for histological grading and final treatment protocol for MIO SCC. The assessments of depth of tumor through p53 immunoexpression may be one of the criteria for grading in MIO SCC. Thus the correlation of p53 immunoexpression with the depth of tumor in MIO SCC helps to determine the treatment modalities of MIO SCC.

#### CONSENT

Written Informed Consent obtained from the patient regarding use of their data and archival tissue sample

#### ETHICAL APPROVAL

Institutional Ethical Clearance is taken. IEC NO. – DMIMS (DU) /IEC/2020-21/42

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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