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Study of Cytokeratin 17 Expression in Oral Squamous Cell Carcinoma

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ABSTRACT: -

Background -An significant category of neoplasia consists of head and neck tumors and its occurrence rises day by day. More than 95 per cent oral cancers aresquamous type. It is a major health problem and a chief cause of death as well. For detection of cancers, immunohistochemistry is generally used.Keratins are intermediate filaments thar are primarily expressed in epithelial cells. In mechanical support and Cytoskeleton formation it plays an important role that is crucial for cellular stability and integrity. The present study is designed with an aim to correlate Cytokeratin 17 expression by immunohistochemistry with histological grade of oral squamous cell carcinoma which predicts aggressiveness of the tumor and thus helps in early diagnosis and treatment.

Objectives - To evaluate Cytokeratin 17 expression and assess the correlation with the histological grade in oral squamous cell carcinoma.

Methods-This study is an observational, retrospective and cross-sectional type. The study will be carried for two years from October 2020-September 2022 in the Histopathology division and Immunohistochemistry section in the Pathology Department, Jawaharlal Nehru Medical College (JNMC) in coordination with Department of surgery, Department of Otorhinolaryngology, Oral and Maxillofacial surgery Department, Acharya Vinoba Bhave Rural Hospital (AVBRH), Sawangi(Meghe), Wardha, Maharashtra, India. To evaluate

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Cytokeratin 17 expression by immunohistochemistry and to correlate Cytokeratin 17 expression with histological grade of oral squamous cell carcinoma immunostaining for Cytokeratin 17 will be carried out.
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Results -The observations will be tabulated.
Conclusion - Conclusion will be drawn from the results obtained from the study.

Keywords- Cytokeratin 17, Expression, Oral Squamous cell carcinoma

INTRODUCTION:

Head and neck tumors constitutes a crucial part of neoplasia, incidence of which is increasing all over the world.[1] Worldwide oral cancer accounts for about 2-4% of the total oral carcinoma cases. Oral carcinoma accounts for a total of 45% prevalence in India. [2]
Oral carcinoma has an important component of neoplasms which affects oral cavity, pharynx and the salivary glands.[2] Somewhere above 95% of the oral cavity carcinomas are squamous type.[1] In developing countries they account for critical health issue thus being a chief cause of death. [2]
Oral squamous cell carcinoma (OSCC) has limited survival index as related withimprovement in diagnosis and management of other malignant tumor. Altogether incidence and mortality of oral squamous cell carcinoma and the altogether morbidity and mortality of 6.6/100,000 and 3.1/100,000 in males and 2.9/100,000 and 1.4/100,000 in females are growing.[1]

The main predisposing factors are alcohol and tobacco use. Tobacco smoking is linked to 75% overall cases of oral carcinoma. As compared to non-smokers tobacco smokers are 6 times more prone to develop oral cancer. Alsoalcoholic drinkers are6 times more prone as compared tonon-alcoholic drinkers to develop oral cancer.Together Tobacco and alcohol pose a 15 times more risk of cancer for users as compared tonon-users. [2]
Although alcohol and tobacco use are commonly the main predisposing factors but there are also other causes such as chewing of betel nut, in certain culturally biased people. Use of betel nut is common in Asian subcontinental population and therefore it is associated with increased risk of oral carcinoma. Other causes include areca nuts and narcotics.[2] In older males, low socioeconomic groups and certain culturally biased populations oral squamous cell carcinoma isoftenobserved.Other components that are also involved include decreased ability to fix muta gentdamaged DNA, decreased capacity to metabolize carcinogens, vitamin A, E or C or trace element deficiencies, defects of immune system. Theinefficient immunity may cause development of cancer. Patients infected with HIV and patients exposed to organ transplantation and the one underimmunosuppressive therapy are more prone to develop OSCC.[2]Therefore, thorough clinical examination and palpation of lymph node is an important diagnostic tool. Different diagnostic modalities include vital staining (Toluidine blue, Methylene blue, Rose Bengal), histopathology, immunohistochemistry, photospectometry, cytology includes FNAC and liquid based cytology, molecular studies are being implemented. Yet gold standard weapon used by pathologist while dealing with oral squamous cell carcinoma is Histopathological examination.[3]

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For obtaining precise morphological assessment of growth potential of Head and neck squamous cell carcinoma various grading system is used like TNM staging, Broders' system, Fisher, Lund , Willen , Crissman , Anneroth , Bryne's ITF ,Invasive Tumor Front Grading System.The Broders' histological classification for squamous cell carcinoma was proposed by Broders. Classification was set up in 1920 and was later on analysed in 1925which was formed on principle of differentiation of cells.Carcinomas, ranging from 1 to 4, are classified into 4 groups. Grade 1 carcinomas contain upto 25% of undifferentiated cells. Grade 2 has undifferentiated cells of 25-50 percent. Grade 3 has undifferentiated cells of 50-75 percent and grade 4 has undifferentiated cells of 75-100 percent.[4]

There is always a subjective difference to differentiate well , moderate and poorly differentiated carcinoma hence IHC marker are used.**Immunohistochemistry** is a technique which is used for identifying antigens by antigen- antibody interactions.In Histopathology it is used as a diagnostic procedure. Immunohistochemistry can be applied routinely. It is consistent with normal processesfor embedding and standard fixation. It can be performed retrospectively in archived material. It is sensitive and applicable to any molecule of immunological origin and in any morphological measure it is interpreted.[5] Immunohistochemistry uses antibodies to assess the tissue distribution of an antigen in health and disease. Immunohistochemistry is extensively used to detect cancers. It is used to classify number of proteins, enzymes and tissue structures since immunohistochemistry requires antigen antibody reactions.[6]

Cytokeratins are cytoskeleton intermediate filaments, candidates for OSCC diagnostic marker as they are more expressed in Oral squamous cell carcinoma than normal oral mucosa. While diagnostic and therapeutic advancements including surgery, radiotherapy and chemotherapy with advent of combination therapy the five year survival rate remains 70 to 80 percent due to late stage diagnosis and resistance to chemotherapy or radiotherapy. It is therefore essential to establish diagnostic markers of oral squamous cell carcinoma for the specific diagnosis and effective treatment of patients at an early stage. It is important that diagnostic markers for oral squamous cell carcinoma are identified. Biochemicalstudies and immunohistochemistry have shown that Oral squamous cell carcinoma expresses a wider range of Cytokeratins as normal epithelium cancerization can lead to variation in degree of differentiation.[7]

The low weight or acidic type 1 Cytokeratins and the high weight or basic or neutral type 2 Cytokeratins are two types of Cytokeratins.A variety of subtypes are comprised of high molecular weight cytokeratins or neutral Cyokeratins, namely CK1, CK2, CK3, CK4, CK5, CK6, CK7, CK8, CK9. Cytokeratins of low molecular weight or acidic comprise CK10, CK12, CK13, CK14, CK16, CK17, CK18, CK19 and CK20[8] CK 17 shou;ld be concentrated as a diagnostic marker of OSCC among CKs as in many studies it is seen that Cytokeratin 17 expresses in malignant tissues as compared with normal tissues in squamous cell carcinoma.

Cytokeratin 17 is a basal type cytokeratin. In normal tissues it is stained in basal cells of complex epithelia, breast myoepithelial cell, cervical and immature metaplastic cells, hair shaft epithelia, nail beds, sebaceous glands, urothelial metaplasia.[9]

In neoplastic tissues Cytokeratin 17 is positively stained in basal cells of skin, breast, cervical squamous intraepithelial lesion, cholangiocarcinoma, laryngeal premalignant changes or

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squamous cell carcinoma, pancreatobiliary, head and neck squamous cell carcinoma, thyroid, urothelial. Negative staining is seen in gastric adenocarcinoma.[9]

Cytokeratin 17 is positive in oral squamous cell carcinoma boundary surface cells, early invasion of

OSCC parabasal cells, and OSCC pearl nests. [10] The grading of Broders is dependent on cell differentiation. Cytokeratin 17 is associated with Broders' grading and Cytokeratin 17 is shown to be substantially expressed in grade 1 as compared to grade 2, 3 and grade 4. Thus as the tumor advances Cytokeratin 17 expression reduces or in other words higher is the grade of tumor lower is Cytokeratin 17 expression. The present study is designed with an aim to correlate Cytokeratin 17 expression by immunohistochemistry with histological grading of oral squamous cell carcinoma.

RESEARCH GAP:

The present research aims to close the gap of understanding between Cytokeratin 17 expression with the histological grade in oral squamous cell carcinoma. It will create a frame to predict the aggressiveness of the tumour which helps in early diagnosis and treatment.

RESEARCH QUESTION:

With understanding the effect of Cytokeratin 17 expression in Oral squamous cell carcinoma in correlation with histologic grade the following Research question is framed.

Does Cytokeratin 17 expression have significant association with the histological grade i.eBroders' histological gradingin oral squamous cell carcinoma.'

METHODOLOGY:

STUDY DESIGN - observational, retrospective and cross-sectional type

PLACE OF STUDY – Department of Pathology, JNMC, Sawangi(Meghe), Wardha, Maharashtra.

DURATION – 2020 to 2022 (2 years)

METHODS –

ROUTINE HEMATOXYLIN & EOSIN (H & E) STAINING[11]

Sections are deparaffinized in xylene: 3 changes; 10 minutes each.De-waxing of sections is done; sections are re-hydrated through graded alcohols to water.Sections are placed in running tap water for 1minutes.Removing of fixation stains if necessary, will be done.Sections are stained for 5-10 minutes with Harris's hematoxylin. For five min sections are once more placed in running tap water. The phenomenon called "bluing" takes place during this process.Differentiation is accomplished by putting the sections for 5-10 seconds in 1percent acid alcohol (1 percent HCL in 70 percent alcohol). Tap water is used again before bluing happens for 5 minutes.Differentiate sections in 70% acid ethanol containing 1 percent HCL for 5 sec. This will eliminate excess dye so that nuclear details will emerge.Wash thoroughly in tap water for 5 min before sections are again blue.Dehydration is carried out by 90 % ethyl alcohol and absolute alcohol.Sections are cleared by keeping them in xylene for 5minutes.Mount in DPX (DibutylphthalatePoolystyrene xylene) and examine under microscope.

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IMMUNOHISTOCHEMICAL STAINING

An appropriate paraffin block with adequate tumor mass and satisfactory amount of normal tissue is selected.3 micrometer sections are cut and mounted on PLL coated slides.De-paraffinization is achieved by placing the sections in xylene.Sections are rehydrated by subjecting them to descending concentrations of alcohol.Running tap water is used for washing.Sections are washed in distilled water for 1minute.Transferring the sections to Coplin's jar containing retrieval buffer is done.Antigen retrieval is carried out in a pressure cooker for 15-20 minutes. The solution used is comprised of 30 ml retrieval solution in 1500 ml of distilled water.The pressure cooker is left to cool to the room temperature.Sections are dipped once in distilled water.Tris buffer solution is used to wash the sections for at least 5 minutes at room temperature. This step is repeated three times.For peroxidase blocking which is performed for 30 minutes a combination of 3,5 hydrogen peroxide and methanol is used.Sections are washed in Tris buffer solution 3 times for 5minutes each.The antibody cytokeratin Brand- Dakocytomation diluted 1:60 is applied at room temperature for 1hour.Sections are again washed in Tris buffer solution 3 times for5 minutes each.Envision technique is carried out at room temperature by utilizing a labelled polymer for 30 minutes.Sections are washed again 3 times for 5 minutes each time in Tris buffer solution.The DAB (3, 3'- diaminobenzidine) substrate is applied for15-20 minutes. The working DAB solution is composed of 1 ml DAB buffer and 25 microliters of DAB concentrate.Washing of sections is done by Tris buffer, this time for5-10 minutes.Sections are washed in distilled water.Harris's Hematoxylin is applied as counterstain for 5minutes.Again, sections are washed in tap water.Sections are allowed to dry, mounted in DPX and examined under microscope.

SAMPLE SIZE –[12]

The resected specimens of clinically suspected cases of OSCC operated in department of oral surgery, AVBRH, Sawangi (Meghe) Wardha will be considered for this study-

Calculation: Sample size formula with desired error of margin.

$$n = (Z^{a/2})^2 x\ p\ x\ (1-p)/\ d^2$$

where, $Z^{a/2}$is the level of significance at 5% i.e. 95 % confidence interval

p= prevalence of oral squamous cell carcinoma. d= desired error of margin.

n=sample size

n = Approximately 40-50

Inclusion Criteria:

All cases diagnosed as squamous cell carcinoma of the oral cavity and all de novo cases of oral squamous cell carcinoma will be included in the study.

Exclusion Criteria:

1. A
ll cases of malignancy other than oral squamous cell carcinoma.
2. All cases of non - neoplastic lesions of oral cavity
3. Cases of recurrence.
4. Cases with previous history of chemotherapy and radiotherapy.

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Statistical Analysis:

Statistical analysis will be carried out using multi linear regression analysis. It will done to correlate Cytokeratin 17 and the histological grade of oral squamous cell carcinoma.

EXPECTED RESULTS:

The study will be conducted for a period of 2 years and all the observations will be depicted in a well-tabulated master chart.

DISCUSSION:

About 95% of head and neck cancers are oral squamous cell carcinoma, with the remainder c onsisting mainly of salivary gland adenocarcinoma. Squamous cell carcinoma pathogenesis is multifactorial . The main cause of

oropharynx is now considered to be infection with a highrisk papillomavirus. In India, the mo st common

predisposing variables are the chewing of betel nut and paan. Over the last two decades, HP Vassociated oropharyngealsquamous cell carcinoma has risen twofold. The fiveyear survival rate of early stage squamous cell carcinoma classic smoking and alcohol is approxi mately 80 percent, but the survival rate for late stage cancer decreases to 20 percent.[13]

A short review for the present work is entitled below, citing the studies from different centres establishing the Cytokeratin 17 expression in OSCC.

B.A Coelhoconducted a research on keratin 17 and keratin 19 expression in OSCC. In this study theyanalysed the expression of CK17 and CK 19 in oral squamous cell carcinoma patients via immunohistochemistry in tumoral and non-tumor tissues. From this study they concluded that CK17 and CK 19 are more highly expressed in oral squamous cell carcinoma tumor cells in comparison with non tumoral cells. [14]

Akiko Matsuhiracarried out a study on CK 13, CK 17, ki67 and p53 expression in upper layers of squamous cell carcinoma of tongue surrounding epithelial dysplasia. Total of 12 tongue SCC resection specimens were taken from 12 separate patients in the study. They concluded from this analysis that there was clear expression of CK13 in the normal epithelium, ki67 and p53 in the lower layer of normal epithelium, CK13 and CK17 in upper layer of epithelial dysplasia and cancer lesions[15]

SunakiNoguchi conducted a study on Cytokeratin 13 and Cytokeratin 17 expression in squamous cell carcinoma of tongue and intraepithelial neoplasia. In a review of this a total of eight patients with clinically diagnosed T1 and T2 squamous cell carcinoma of tongue were administered. Immunohistochemical staining from this study showed that CK 13 positive cells decreased starting with intraepithelial neoplasia and almost no CK 13 positive cancer cells and CK 17 positive cells increased starting with intraepithelial neoplasia and almost all cells were Cytokeratin 17 in cancer.[16]

K-J Wei et al conducted a study on Cytokeratin 17 protein overexpression in vitro and in vivo in squamous cell carcinoma of oral cavity. Six healthy individuals and 30 primary OSCC patients were admitted in total. The study showed increased expression of CK17 in OSCC patients cancer tissues as compared with non-malignant epithelia[17]

A number of different studies reported on Oral Squamous Cell carcinoma [18-21]. Handeet. al.

reported on immunohistochemical analysis of tumor-associated stroma in oral squamous cell carcinoma[22]. Gadbailet. al reported 4 studies on expression of Ki67, CD105, and α-SMAin oral squamous cell carcinoma[23-26]. Mohite et. al. reported about immunohistochemical evaluation of expression pattern of P53, P63, and P73 in epithelial dysplasia [27].

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