IMMUNOHISTOCHEMICAL EXPRESSION OF MUC 1 MUCIN IN DIFFERENT GRADES OF ORAL SQUAMOUS CELL CARCINOMA

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BRANCH –VI

ORAL PATHOLOGY AND MICROBIOLOGY

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MELMARUVATHUR- 603319

ORAL PATHOLOGY AND MICROBIOLOGY

CERTIFICATE

This is to certify that **Dr. P. HARIGANESH,** Post Graduate student(2017-2020) in the Department of Oral Pathology and Microbiology, Adhiparasakthi Dental College and Hospital, Melmaruvathur – 603319, has done this dissertation titled **"IMMUNOHISTOCHEMICAL EXPRESSION OF MUC 1 MUCIN IN DIFFERENT GRADES OF ORAL SQUAMOUS CELL CARCINOMA"** under our direct guidance and supervision in partial fulfilment of the regulations laid down by **THE TAMILNADU DR. M. G. R. MEDICAL UNIVERSITY**, Chennai– 600032 for **MASTER OF DENTAL SURGERY-(BRANCH-VI) ORAL PATHOLOGY AND MICROBIOLOGY** degree examination.

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DECLARATION

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DURATION OF THECOURSE	3 YEARS
NAME OF THE GUIDE	Dr. S.SHAMALA RAVIKUMAR, MDS.,
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I hereby declare that no part of the dissertation will be utilized for gaining financial assistance or any promotion without obtaining prior permission of the Principal, Adhiparasakthi Dental College and Hospital, Melmaruvathur – 603319. In addition, I declare that no part of this work will be published either in print or in electronic media without the guides who has been actively involved in dissertation. The author has the right to reserve for publishing the work solely with the permission of the Principal, Adhiparasakthi Dental College and Hospital, Melmaruvathur – 603319.

Co-Guide Guide & Head of the Department Signature of candidate

ABSTRACT

BACKGROUND:

AIM: To evaluate the expression of MUC1 Mucin in different grades of Oral Squamous Cell Carcinoma using ImunoHistoChemistry.

MATERIALS AND METHODS: A total of 40 samples were examined for the Immunohistochemical expression of MUC1 Mucin. The study group includes 40 cases of formalin fixed paraffin embedded tissue blocks of oral squamous cell carcinoma (10 cases of well differentiated OSCC,10 case of moderately differentiated OSCC, 10 cases of Poorly differentiated OSCC and 10 cases of Normal Oral Mucosa- control group). 3 µ thickness sections were made from each sample and stained with MUC1 Mucin antibody. The intensity and area of staining were assessed and scored. The data obtained was statistically analyzed using SPSS software.

RESULTS: There was a significant statistical difference in the intensity of staining of MUC 1 Mucin between normal oral mucosa and oral squamous cell carcinoma. There was also a significant statistical difference in the area of staining of MUC 1 Mucin between normal oral mucosa and oral squamous cell carcinoma. When comparing the staining intensity of MUC 1 Mucin between the different grades of oral squamous cell carcinoma, there was a significant difference statistically.

On comparison of area of staining of MUC 1 Mucin between the different grades of oral squamous cell carcinoma, there was again a

significant difference statistically. High immunopositivity and strong staining intensity for MUC 1 Mucin was observed in well differentiated squamous cell carcinoma when compared to moderately differentiated squamous cell carcinoma and poorly differentiated squamous cell carcinoma.

CONCLUSION: We conclude that MUC 1 Mucin biomarker can be used to detect higher grades of oral squamous cell carcinoma and hence, early detection in preventing invasion and metastasis.

KEY WORDS: Squamous Cell Carcinoma, Immunohistochemistry, MUC 1 Mucin

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ABBREVIATIONS

ACC - Adenoid Cystic Carcinoma

APC - Antigen presenting cell

ATP - Adenosine Triphosphate

CIC - Capicua Transcriptional Receptor

CT - Computed tomography

DPX - Dibutyl Phthalate Xylene

DAB - Diaminobenzidine

EDTA - Ethylene Diamine –Tetra – Acetic Acid

EGF - Epidermal Growth Factor

HRP - Horse Radish Peroxidase

HSP - Heat Shock Protein

HSPG - Heparan Sulfate Proteo Glycan

HNSCC	-	Head and Neck Squamous Cell Carcinoma
IHC	-	Immunohistochemistry
IQR	-	Inter Quartile Range
MAPK	-	Mitogen - Activated Protein Kinase
MDOSCC	-	Moderately Differentiated Oral Squamous Cell Carcinoma
MMD	-	Matrix Metalloproteinase
MRI	-	Magnetic Resonance Imaging
OSCC	-	Oral Squamous Cell Carcinoma
PBS	-	Phosphate buffered solution
PDGF	-	Platelet Derived Growth Factor
PDOSCC	-	Poorly Differentiated Oral Squamous Cell Carcinoma
RT-PCR	-	Reverse Transcriptase Polymerase Chain Reaction

SD - Standard Deviation

TBS - Tris Buffered solution

TCF - T- Cell Factor

TGF - Transforming Growth Factor

TNM - Tumor lymph Node Metastasis

WDOSCC - Well Differentiated Oral Squamous Cell Carcinoma

INTRODUCTION

Tumor markers are biochemical substances found in traces in body tissue and fluids which may get elevated in certain types of malignancies. They are released by the malignant cells and those other surrounding cells in response to the malignancy and are released into the blood and body fluids. Though they are useful in confirmation of diagnosis, staging and monitoring, to assess prognosis and also recurrence, their levels of specificity and sensitivity is of utmost importance. Hence quantitative analysis of these biomarkers have gained abundant attention to define alterations in normal to abnormal cells in malignant transformations[1].

Tumor markers should be highly specific for any tumor type and also should be highly sensitive to avoid false positive results. Though tumor markers are not regarded reliable for diagnosis as they are an adjunct to routine histopathology technique with H & E stains. There is an increasing number of tumor markers for oral squamous cell carcinoma every year. Recent studies have reported on MUC – 1 on changes from normal to oral squamous cell carcinoma and hence considered as useful indicator of the disease process. MUC – 1 overexpression in metastatic tumors has been reported and is correlated with aggressiveness, poor response to therapy and poor survival[1].

As reported earlier, around 95 - 98% of all oral malignancies seem to be oral squamous cell carcinoma and delayed diagnosis seem to be the reason for poor prognosis of the disease[2]. The high percentage of the disease are due to various etiological factors like chewing and smoking tobacco, alcohol intake and viral infections leading to development of premalignant lesions, invasive oral squamous cell carcinoma followed by metastasis. Though recent advances have evolved in diagnosing and treatment techniques of OSCC, yet the disease

presents with poor prognosis and decreased survival rate if detected in the later stages of the disease[3].

The development of oral squamous cell carcinoma is a multistep process, the transition from normal oral epithelium to oral dysplasia and cancer which results from accumulated multiple genetic and epigenetic alterations as well as by environmental influences including tobacco, alcohol, chronic inflammation and viral infections[4].

Mucins play a very important role in cell proliferation, differentiation and signaling. It has been reported that mucins are used by cancer cells for protection from adverse growth conditions and to control the local molecular microenvironment during invasion and metastasis[2].

MUC – 1 gene encodes secretion of MUC – 1 mucin which promotes tumor development, tumor survival and also secondary metastasis. Cancer cells express aberrant forms or amounts of mucin due to deregulation of mucin core proteins and the enzymes that modify them during the transformation of tumorcells[2].

Critical role of MUC – 1 in transcriptional regulation of genes associated with tumor invasion, metastasis, angiogenesis, proliferation, apoptosis, inflammation and immune regulation. MUC – 1 directly facilitates the cancer cell survival and growth by up regulating the glucose uptake and metabolism by tumorcells[5].

MUC – 1 mucin has been reported as an anti - adhesion molecule by many studies and overexpression on the cell surfaces reduces adhesion between cell to cell and extra cellular matrix indicating non interactions between the integrins and extra cellular matrix. Hence, in invasive and metastatic carcinomas there is an overexpression of MUC – 1 mucin has been reported. Immunoexpression of MUC – 1 expression has been reported to significantly

decrease from well differentiated to poorly differentiated OSCC which may be due to the lack of expression of mucins in the less differentiated squamous cells[2].

Within this background the present study has been undertaken, to study the immunohistochemical expression of MUC – 1 in different grades of OSCC.

AIM & OBJECTIVES

AIM

- To evaluate the expression of MUC 1 Mucin in different grades of Oral Squamous Cell Carcinoma using Immunohistochemistry.

OBJECTIVES

- To evaluate the expression of MUC1 Mucin in different grades of Oral Squamous Cell Carcinoma.
- To evaluate the expression of MUC1 Mucin in normal oral mucosa
- To compare the expression of MUC1 Mucin in different grades of Oral Squamous Cell Carcinoma
- To compare the expression of MUC1 Mucin in Normal Oral Mucosa

GENERAL REVIEW

MUCIN

Mucus is a biological lubricant that is present in over the wet epithelial surfaces of several organs in the body. The apical surface of epithelial cells in the lungs, stomach, intestines, eyes, salivary glands and oral cavity are lined by Mucins.[6] MUC1 gene in humans contain Polymorphic Epithelial Mucin and is associated with the cell surface . MUC1 is a glycoprotein with extensive o-linked glycosylation of its extracellular domain.[7]

SRUCTURE OF MUCIN:

Massive protein aggregates with a molecular mass ranging from 1-10 million Da are secreted as Mucins where the monomer linked aggregates has a non - covalent interaction.

Two distinct regions can be appreciated in case of mature mucins.

Cysteine rich amino and carboxy terminal regions are very lightly glycosylated. Mucin monomers are linked by disulfide linkages associated with cysteine residues. Multiple tandem repeats of 10 – 80 residue sequences form a large central region in which the amino acids like serine and threonine are predominant. N- linked oligosachharides are found on mucins but less than hundreds of O – linked oligosachharides.

Membrane bound mucins typically have a Sea urchin Sperm protein, Enterokinase and Agrin (SEA) that resides between glycosylated ectodomain and transmembrane domain.

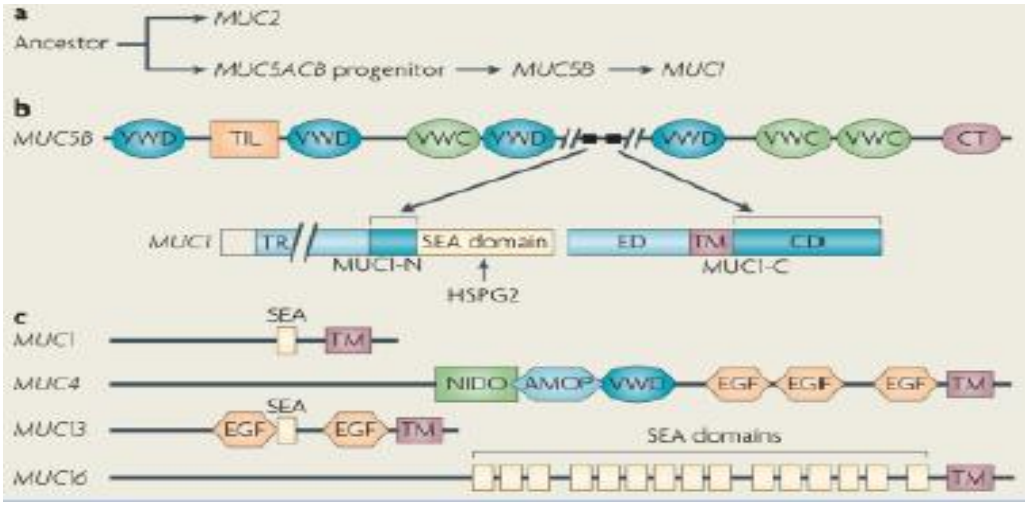


Figure No. 1. Domain structure of the transmembrane mucins[8]

The transmembrane mucins, protect the epithelial cell layer by functioning in cellular signaling pathways that promote growth and survival. Human cancers overexpress the transmembrane mucins, particularly MUC1, MUC4 and exploit the protective functions.

Human carcinomas and some hematologic malignancies overexpresses the transmembrane mucin (MUC 1) abberantly which is a heterodimer. Within the SEA domain, subunits MUC1 N – terminal (MUC1 – N) and C – terminal (MUC1 – C) are generated by autocleavage

The MUC1 cytoplasmic domain (MUC1 – CD) located downstream of the SEA domain is enough for creating anchorage – independent growth and tumorigenicity.[7]

MUC1 –CD (Cytoplasmic Domain) transforming function arose by diversification after evolution from MUC5B. HSPG2 is a tumor growth inducer. Heparin Sulphate ProteoGlycan 2 (HSPG2) gives rise to SEA domain structure of MUC1. MUC1 sequences upstream of its sea urchin Sperm protein, Enterokinase and Agrin (SEA) domain seem to have evolved from the MUC5B gene[8].

FUNCTIONS IN HUMAN:

Mucins limit the activation of inflammatory responses at the interface with the environment.[8] MUC1 protects and lubricates the mucous membranes of the human body. MUC1 is also involved in cell growth, differentiation and signaling.[9]

MUC1 regulates the transcription factor complex function which plays a major role in host immunity changes caused by tumor. Deregulation of mucin production acts as an important link between inflammation and cancer.

Mucus prevents the pathogen or infection from reaching cell surface as it binds to oligosaccharides in extracellular domain[10].

CLASSIFICATION:

21 mucin type glycoprotein belong to the MUC gene family. Mucin type glycoprotein are classified by the presence of tandem repeats structures containing a high proportion of Prolines, Threonines and Serines modified by O – glycosylstion (PTS domain)[10]

The human MUC family have been sub classified as **secreted** and **membrane bound** forms.

SECRETED MUCINS: Forms a physical gel barrier that protects epithelial cells lining ductal surface of specialized organs.

MEMBRANE BOUND MUCINS: Contribute to formation of protective mucous gel through ectodomains of O-glycosylated tandem repeats.

SECRETED MUCINMEMBRANE BOUND MUCIN

MUC 2	MUC 1
MUC 5AC	MUC 4
MUC 5B	MUC 3
MUC6	MUC12, 13, 16, 17[7]

EXPRESSION OF MUC1 IN NORMAL CELLS:

Mucin 1 protein is produced by means of the instructions given by MUC1 gene. This protein helps to produce mucus which helps in lubrication and protection of the airway, reproductive and digestive lining. It also plays a vital role in cell signalling. Mucin 1 contains nearly 20 - 100 repeated stretches of amino acids at a region called the mucin domain. Numerous chains of sugar molecules are attached to certain amino acids. These sugar molecules spread out preventing access for the foreign substances to enter the cell surface below. They also help in lubricating and hydrating the tissues by attracting the water molecules. The MUC1-CT (Cytoplasmic Tail) is the portion of mucin1 which transfers signals from the outside portion of the cell to inside. Thus Mucin 1 helps in cell proliferation, cell adhesion, motility and cell survival.

EXPRESSION OF MUC1 IN CANCER CELLS:

The heavy glycosylation in the extracellular domain of MUC1 inhibits the hydrophobic chemotherapeutic effect of the drugs to access the targets which usually reside within the cancer cells by means of creating a highly hydrophilic region. The interaction of the immune cells with the receptors on the cancer cell surface through steric hindrance is prevented by MUC1 causing inhibition of an anti-tumor immune response.[11]

Binding of MUC1 cytoplasmic tail (MUC1 – CT) to p53 is found to be associated with p53 response element of p21 gene promoter. Thus p21 is activated and it results in cell cycle arrest. MUC1 association with p53 inhibits p53 mediated apoptosis and causes promotion of p53 mediated cell cycle arrest.[12] Overexpression of MUC1 in fibroblasts increase the phosphorylation of AKT. Phosphorylation of Bcl 2 associates death promoter with Bcl-2 and Bcl-XL prevents release of Cytochrome C from mitochondria preventing apoptosis.[13] MUC 1-CT interacts with HSP-90 in mitochondria inducing phosphorylation of MUC1 by SRC gene. SRC activated by EGF receptor family ligand Neuregulin. Localization of MUC1 to mitochondria prevents activation of apoptotic mechanisms.[14]

REVIEW OF LITERATURE

Sandra J. Gendler et al (1991)[15] summarized about the structure and biology of a carcinoma associated mucin where he states that mucins play a role in natural killer cell resistance and in evasion of immune response by tumor cells and may act as suppressors of cell growth. MUC 1 is restricted to the apical cell surface by interaction with the microfilament network. The interactions between integral membrane proteins such as the mucin and cytoplasmic structural proteins may be involved in the maintenance of the polarity of the plasma membrane and stabilization of epithelial morphology. In undifferentiated malignancy, this interaction appears to be disturbed resulting in increased expression of the mucin in the cytoplasm of the cells by exposure of an epitope detected by the antibody SM-3. The epitope appears to be strongly associated with malignancy and may well be overexpressed on the cytoplasmic mucin component.

Michael A Mc Guckin *et al* (1995)[16]made a study to show the relationship between expression of MUC1 mucin a retrospective series of breast carcinomas and compares the prognostic significance of this expression with that of other prognostic factors, such as the presence of axillary node metastasis, histological type and grade, tumor size presence of coexistent carcinoma in situ, estrogen receptor status and menopausal status and concluded that mucin was mostly expressed by breast epithelial cells found adjacent to carcinomas. Expression was limited to the apical membrane of these cells.

Tetsuhiko Itoh *et al* (1996)[17] studied that in many human adenocarcinomas, Lewis X-related antigens and mucin antigens act as oncodevelopmentaltumor associated antigens and mucin core protein antigen expression is associated with MUC1 gene product (DF3 antigen, mammary type apomucin). They were associated with the earliest steps in the mucin

glycosylation in Normal Squamous Epithelium (NSE), Dysplastic Squamous Epithelium (DSE) and Squamous Cell Carcinoma (SCC) which were studied and found that DF3 is the effective marker for DSE and SCC in pharyngeal and laryngeal region and it is one of the MUC1 mucins and epithelial membrane antigen (EMA) is also a MUC1 mucin. Henceforth suggested that MUC1 mucin seems to be an effective tumor marker in Squamous Cell Carcinoma because of its high expression rates in SCC and negative expressions in NSE.

Mark J. H. Hudson *et al* (1996)[18] studied to identify the cytoskeletal alterations after MUC1 expression to analyze the effect of MUC1 on cell-ECM adhesion and to determine whether MUC1 expressing human pancreatic transfectant cell lines contributed to extracellular matrix remodeling by matrix contraction. The results indicate that cell surface expression of MUC1 mucin significantly reduces cell – ECM interaction with type I collagen.

Suguru Yonezawa *et al* (1997)[19]published a review in which he stated that the expression of MUC1 and MUC2 mucins was a useful indicator of the malignancy potential of tumors derived from other organs, like ampulla of Vater, stomach and breast. In another study by Yamamoto *et al* it has been mentioned that among the four isoforms of MUC1 Mucin antigens, sialylated- MUC1 mucin which was detected by monoclonal antibody, MY.1E12, was found to be expressed in all the invasive carcinomas and concluded saying that in eosophageal squamous cell carcinoma, MUC1 expression is an indicator of a poor prognosis.

Tetsuya Nitta *et al* (2000)[20]has conducted a study including Oral epithelial dysplasia, Carcinoma in situ and oral squamous cell carcinoma specimens which were stained

immunohistochemically by MUC1 mucin and were observed under both light and electron microscopy for clinicopathological findings and staining patterns in which he has suggested that the upregulation of MUC1 mucin expression in oral premalignant and malignant lesions show that mucin may play a role in the progression of oral carcinoma. Hence in oral malignant and potentially malignant lesions, expression of MUC1 Mucin can be considered as a diagnostic parameter.

Maria V CROCE *et al* (2000)[21]conducted a study on the expression of mucin in malignant laryngeal tumors. The study showed positive expression of MUC 1 Mucin and laryngeal carcinoma cells, therby stating the alteration of mucin during tumor development. Such change include differences in mucin associated antigenic expression between cancer cells and their normal counterparts. By immunohistochemistry, series of mAbs against epithelial mucins and carbohydrate related antigens have been evaluated which concludes that the expression may be related to an advanced malignant disease.

Joyce A Schroeder *et al* (2003)[22] conducted a study which indicate a potential mechanism for MUC1 promotion of invasive tumorigenesis in the breast through the modulation of β-catenin localization and subsequent cytoskeletal dynamics, where he suggests that the ability of β-catenin to interact with cytoskeleton modulating proteins promotes their redistribution and promotes cellular invasion. When MUC1 binds with β-catenin it promotes the novel interaction with invading cell margins by disrupting cell adhesion found at adherens junctions. This complex formation is not only the transition from hyperplasia to neoplasia in nonmetastatic diseases but also induce dynamic changes necessary for metastatic invasion.

Deepak Raina *et al* (2004)[13] studied and demonstrated that MUC1 induced transformation of 3Y1 fibroblasts is associated with increased levels of phospho-Akt and phospho-Bad activates the antiapoptotic PI3K/Akt and Bcl-x1 pathways and MUC1 also increases the expression of the antiapoptotic Bcl-x1 protein by a PI1K-independent mechanism and concluded that Overexpression of MUC1 in certain human tumors could confer resistance Bcl-x1 pathways and MUC1 activates other events that are responsible for induction of a malignant phenotype.

Xialong Wei *et al* (2005)[12] has studied to demonstrate that MUC1cytoplasmic domain associates with the p53 tumor suppressor and coactivates DNA damage-induced transcription of the p21gene and the overexpression of MUC1 by human tumors could be of importance to cell fate selection in the activation of p53 by genotoxic anticancer agents and hence MUC1 is a reliable marker for carcinomas.

Martin E Rabassa *et al* (2006)[23]conducted a study about anti muc1 immune response and expression of MUC1 statistically, according to different clinical and pathological parameters which may be useful to develop new anti head and neck squamous cell carcinoma therapeutic strategies where they have employed an anti MUC1 cytoplasmic tail Ab in HNSCC and it revealed a high cellular expression of MUC1. So they concluded that IgG capture the tumor cells which produce MUC1 mucin into circulation forming MUC1-IgG CIC and there is an inverse correlation of poorly differentiated oral squamous cell carcinoma with tumor and serum MUC1 detection

J Ren *et al* (2006)[14] studied to show that the MUC1 cytoplasmic domain binds directly to HSP70 in vitro is induced by c-Src- mediated phosphorylation of the MUC1 cytoplasmic domain. This study showed findings that indicate MUC1 is delivered to mitochondria by a mechanism involving activation of the ErbB receptor→c-Src pathway and transport by the molecular chaperone HSP70/HSP90 complex. MUC1 is aberrantly overexpressed in mitochondria. Overexpression of MUC1 is sufficient to induce anchorage-independent growth and tumorigenicity.

Maria V Croce *et al* (2008)[24] conducted a study stating that tumor markers may be helpful to evaluate prognosis accurately as well as to improve therapy selection and evaluated for MUC1, Tn, sTn and Lewis antigenic expression in primary head and neck squamous cell carcinoma lymph node metastasis and local recurrences and concluded determining that Lewis antigen were associated with membrane staining for MUC1CT which supports the previous reports that indicated a high expression of MUC1 in HNSCC

Farzana Mahomed (2011)[25]published a review about recent advances in mucin immunohistochemistry in salivary gland tumors and head and neck squamous cell carcinoma, in which he focusses on the immunohistochemical expression of members of the mucin family which belong to MUC type. In the distribution pattern of MUC1 in salivary gland tumors, MUC1 localizes to the apical cell membranes of normal salivary ducts, complete membranous and cytoplasmic expression of MUC1 has been found in varying proportions of neoplastic cells, particularly in malignant tumors. The identification of specific MUC1 type mucin expression profiles in different histopathologic types of salivary gland neoplasms may provide an important tool for improving diagnostic accuracy in salivary gland

tumorpathology. Targeting the expression of these mucins may indicate new directions for the development of anti-tumor strategies.

LD Roy *et al* (2011)[26] conducted a study which signifies the oncogenic role of MUC1 CT and is the first to identify a direct role of the MUC1 in initiating EMT during pancreatic cancer and its overexpression auguments metastasis and lack of tyrosines in the CT of MUC1 abrogates this process.

Tomofumi Hamada *et al* (2012)[27]conducted a study to evaluate the prognostic significance of DF3/MUC1 expression in patients with Oral Squamous Cell Carcinoma. It has been reported that the human DF3/MUC1 protein functions as an oncogene through its interaction with β – catenin and found that its expression is an independent risk factor for subsequent regional lymph node metastasis indicating poor prognosis in patients with OSCC. By the data from the study, the aberrant DF3/MUC1 expression is involved at an early stage of carcinogenesis and that it is activated during the process of squamous dysplastic transformation in patients with SCC. Thus DF3/MUC1 could be a marker of the carcinogenic risk of OSCC or could be used for the early detection of OSCC.

Sukhwinder Kaur *et al* (2014)[28] conducted a study to investigate the expression profile of MUC1 and MUC4 in the non-neoplastic bladder urothelium, in various malignant neoplasms of bladder and in bladder carcinoma cell lines and the results showed MUC1 expression on the apical surface or in umbrella cells of the normal non-neoplastic bladder urothelium and also in increasing pattern from normal urothelium to urothelial carcinoma. He concluded

saying that aberrant changes were observed both in expression and localization pattern with regard to MUC1 while there is a significant loss of MUC4 with progression of urothelial carcinoma.

Ping Li *et al* (2015)[29]studied to investigate the role of MUC1 in human oral squamous cell carcinoma progression where the hypothesis is that MUC1 regulate oral squamous cell carcinoma cells (scc-9) malignant biological behaviours and silencing MUC1 reduced scc-9 cellular colony forming ability, migration and invasion. MUC1 silencing markedly impaired the growth of ssc-9 cells in vivo and attenuate expressions of P13K, Akt and MMP-2/9 expression in tumor tissue. Since MUC1 is involved in growth and migration of oral squamous cell carcinoma cells, it acts as a main target for molecular targeting cancer therapy and indicate that MUC1 can be an important therapeutic target in oral squamous cell carcinoma as it plays a major role in scc-9 tumor migration and invasion.

M.Harishkumar *et al* (2017)[10]compared and correlated the expression of MUC1 mucin protein in Normal Oral Mucosa (NOM), Potentially Malignant Disorders (PMD) and Oral Squamous Cell Carcinoma (OSCC) by immunohistochemical method and suggested that determination of MUC1 expression may be a parameter in the diagnosis of malignant behavior of PMDs to OSCC. And also MUC1 expression may be a useful diagnostic marker for prediction of the invasive/metastatic potential of OSCC.

Arush Thakur *et al* (2018)[9] compared and correlated the immunoexpression of MUC1in Normal Oral Mucosa and Oral Squamous Cell Carcinoma using immunohistochemical

technique has concluded that upregulation of MUC1 immunoexpression was seen in OSCC as compared to NOM and MUC1 plays a vital role in the pathogenesis and progression of OSCC.

K.C.Shobhitha *et al* (2018)[2] conducted a study to evaluate, compare and correlate the expression of MUC1 mucin protein and its significance in normal oral mucosa (NOM) and OSCC by Immunohistochemical method and concluded that upregulation of MUC1 mucin expression in malignant lesions might play a vital role in the pathogenesis and its progression and will be a useful marker for prediction of the metastatic/invasive potential of OSCC.

MATERIALS AND METHODS

STUDY DESIGN AND CASE SELECTION

This Case Control study to analyse the Immunohistochemical expression of MUC1 Mucin was done on the archival retrieved, formalin fixed, paraffin embedded, histologically diagnosed case of different grades of oral squamous cell carcinoma tissues, obtained from the Department of Oral Pathology, Adhiparasakthi Dental college and Hospital and Department of General Pathology, Adhiparasakthi Institute of Medical Sciences and Research, Melmaruvathur.

SAMPLE SIZE

- Well Differentiated Squamous Cell Carcinoma – 10
- Moderately Differentiated Squamous Cell Carcinoma - 10
- Poorly Differentiated Squamous Cell Carcinoma - 10
- Normal buccal mucosa for control - 10
- Total No. of samples - 40

Control group included biopsies from normal oral mucosa (NOM) adjacent to the site of surgery during the surgical removal of third molar in 10 patients.

For positive control, archival retrieved, formalin fixed, paraffin embedded colon cancer was obtained from the department of General Pathology, Adhiparasakthi Institute of Medical Science and Research, Melmaruvathur.

ARMAMENTARIUM

- Microtome (Thermo scientific, MICROM HM340E)
- Paint brush
- Disposable microtome blades
- Hot plate
- Hot water bath
- PathnSitu positively charged slides
- Pressure cooker (5 Litres)
- Measuring jars
- Coplin jars
- Electronic timer
- Absorbent wipes
- Coverslip for slides
- Binocular Light Microscope (Olympus CX21i)
- Micropipette
- Rectangular steel trough
- Induction stove
- Incubator (Hitech Equipments)
- Liquid repellent slide marking pen
- Deparafinization stainless steel staining trough and rack
- pH meter (E1 digital pH meter)
- A DELTA PLAN2 AP 40 Trinocular Light Microscope with camera head

ANTIBODIES

1. **Primary antibody**

a) Anti-MUC1 Mucin [Rabbit monoclonal antibody] –

(PathnSitu Biotechnologies Private Limited)

2. **Secondary kit**

(PolyExcel HRP/DAB Detection System) –

(PathnSitu Biotechnologies Private Limited)

a) PolyExcel H_2O_2
b) PolyExcel Target Binder
c) PolyExcel Poly HRP
d) PolyExcel stun DAB – Chromogen
e) PolyExcel stun DAB – Buffer

REAGENTS

- Tris-EDTA Buffer – 50 X concentration

(PathnSitu Biotechnologies Private Limited)

- Immuno wash Buffer – 25 X concentration

(PathnSitu Biotechnologies Private Limited)

- Distilled water
- Xylene
- Absolute alcohol (Isopropyl Alcohol)
- Alcohol 90% (Isopropyl Alcohol)

- Alcohol 70% (Isopropyl Alcohol)
- Harris Hematoxylin
- Mountant (Dibutyl Phthalate Xylene)

IHC METHODOLOGY

- Formalin fixed paraffin embedded tissues were sectioned at 3µm and mounted on charged slides and kept for overnight incubation at 37^{o}C
- Prior to staining, slides were incubated at 60 – 70^{o}C for 1 hour
- Deparaffinized by 2 changes of xylene 10 minutes each
- Hydrated through descending grades of alcohols as follows:
    - Absolute alcohol – 1 change, 5 minutes
    - 90% alcohol – 5 minutes
    - 70% alcohol – 5 minutes
- Distilled water wash 2 changes each for 2 minutes
- Antigen retrieval done for 15- 20 minutes (upto 2 whistles in pressure cooker)
- Cooled for minimum of 30 minutes
- Distilled water wash 2 changes each for 2 minutes
- Washed in PBS / TBS for 2 minutes
- Circles were marked enclosing the section using liquid repellent pen
- Endogenous peroxidase blocking was done by adding PolyExcel H_2O_2 on the section, keep for 5 minutes
- Washed in wash buffer for 5 minutes, 3 changes
- Primary antibody was added and kept for 30 minutes for MUC 1 in a moist chamber

- Washed in wash buffer for 5 minutes, 3 changes
- PolyExcel Target Binder reagent was added and incubated for 12 minutes
- Washed in wash buffer for 5 minutes, 3 changes
- Polyexcel HRP was added and incubated for 12 minutes
- DAB solution was prepared (1 ml of DAB buffer + 1 drop DAB chromogen)
- Washed in wash buffer for 5 minutes, 3 changes
- Working DAB chromogen was added and kept for 2-5 minutes, then washed in distilled water.
- Counterstained with hematoxylin for 30 seconds
- 5 minute wash in running tap water
- Dehydrated through successive changes of alcohol and clear with xylene
- Dried and mounted with DPX

POSITIVE CONTROLS

Positive control section for MUC1 Mucin included colon cancer and was treated in the same manner as the test groups.

NEGATIVE CONTROLS

One section of test sample was selected and treated in the same manner as the test groups except that, the primary antibody was omitted for MUC1 Mucin.

ANALYSIS OF IMMUNOREACTIVITY OF MUC1 MUCIN

Immunoreactivity includes the assessment of Intensity of staining and Area of staining for MUC1 Mucin. The intensity of staining was evaluated by scanning 10 random fields under 40x magnification in both control group and study group

SCORES AND INFERENCE FOR INTENSITY OF STAINING

0 - No Stain

1 - Mild

2 - Moderate

3 – Intense

The Area of staining was analysed to determine the protein expression levels and its pattern. The entire surface of the epithelium was scanned for uptake of stain.

SCORES AND INFRENCE FOR AREA OF STAINING

0% - 5% of positive tumour cells – Score 0

6% - 25% of positive tumour cells – Score 1

26% - 50% of positive tumour cells – Score 2

51% - 75% of positive tumour cells – Score 3

76% - 100% of positive tumour cells – Score 4

FIGURE: 2

ARMAMENTARIUM

Fig 2 (a) Microtome

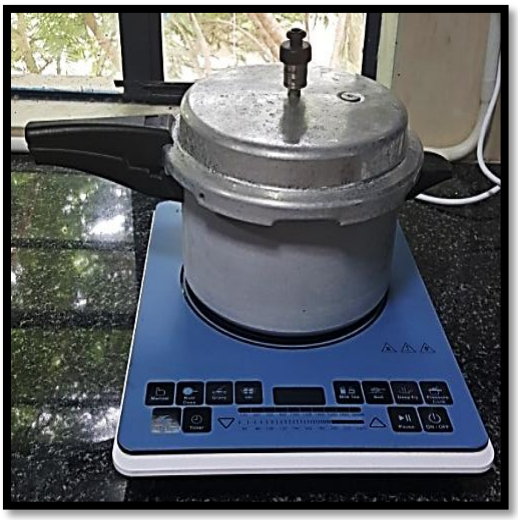


Fig 2 (b) Induction stove and Pressure cooker

Fig 2 (c) Electronic Timer

Fig 2 (d) Microscope

Fig 2 (e) Micropipette

Fig 2 (f) Incubator

Fig 2 (g) Reagent blocker

Fig 2 (h) Deparaffinization stainless steel staining trough and rack

Fig 2 (i) Tris – EDTA and Wash Buffer & pH paper

Fig 2 (j) Primary antibody Anti–MUC1 Mucin[Rabbit Monoclonal antibody]

Figure 2 (k) DAB Chromogen and DAB buffer[H2O2, Target Binder, Poly HRP]

Figure 2 (l) Secondary kit

Fig 2 (m) Hematoxylin

RESULTS

The present study was done to analyse the immunohistochemical expression of MUC1 Mucin in different grades of oral squamous cell carcinoma. Previously diagnosed 10 cases of well differentiated OSCC, 10 cases of moderately differentiated OSCC, 10 cases of poorly differentiated OSCC, and 10 cases of Normal Oral Mucosa (NOM) tissues were selected from the archives of the department of Oral and Maxillofacial Pathology.

Continuous sections of thickness measuring 3µ were made and stained for MUC1 Mucin using immunohistochemistry. 10 random fields under 40X magnification wereselected to determine the intensity of staining for MUC -1 Mucin

Scoring was done as follows:

0 - No Stain

1- Mild

2- Moderate

3-Intense

The percentage of immunopositive tumour cells were determined by examining the area of staining of MUC – 1 Mucin by viewing the entire section of the epithelium under 10x magnification for stain uptake.

Scoring was done as follows:

0%-5% of positive tumour cells – Score 0

6%-25% of positive tumour cells – Score 1

26%-50% of positive tumour cells – Score 2

51%-75% of positive tumour cells – Score 3

76%-100% of positive tumour cells – Score 4

Scoring for MUC1 Mucin staining was done for control group and study group. There were variable intensities of staining for MUC – 1 Mucin and scoring ranged from 0-3 for both control group (NOM) and study group (OSCC).The scores for area of staining ranged from 0-4 for control group (NOM) and study group.

Statistical analysis was done using SPSS.(VER 19, IBM. CHICAGO,U.S.A).

TABLE: 1

COMPARISON OF INTENSITY OF STAINING AND AREA OF STAINING BETWEEN HEALTHY CONTROLS AND OSCC GROUPS

Variables	HEALTHY CONTROLS				ORAL SQUAMOUS CELL CARCINOMA				p value
	Mean	Standard Deviation	Median	IQR	Mean	Standard Deviation	Median	IQR	
IOS	.3000	.48305	.0000	1.00000	1.4333	1.19434	1.0000	3.0000	***0.008**
AOS	.3000	.48305	.0000	1.00000	1.3000	1.26355	1.0000	2.0000	***0.02**

***Denotes statistically significant values using Mann-Whitney U Test**

IOS – Intensity of Staining

AOS – Area of Staining

IQR – Inter Quartile Range

INTENSITY OF STAINING

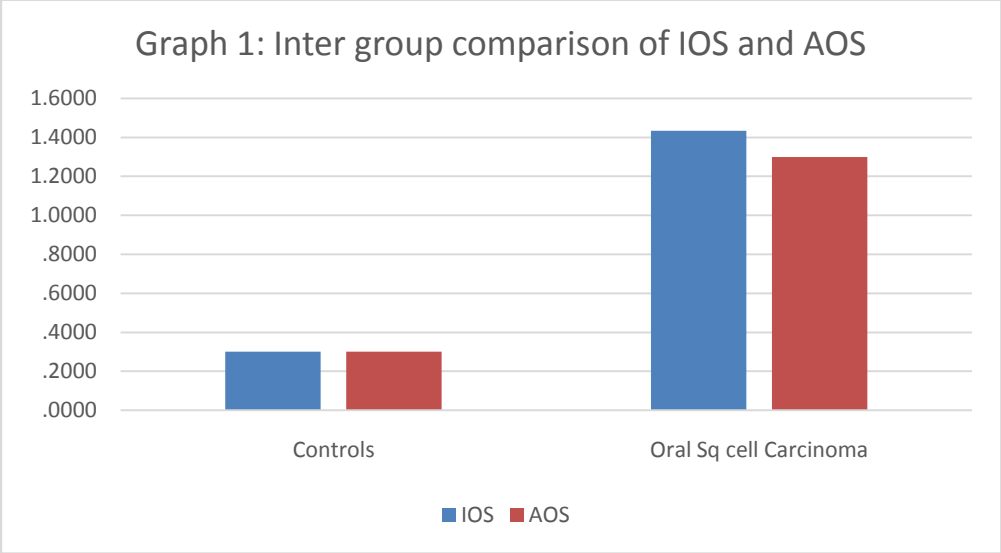
The above Table 1 depicts the scores for Intensity of Staining of Normal Oral Mucosa and Oral Squamous Cell Carcinoma was calculated to be 0.3 (SD – 0.48) and 1.43 (SD – 1.19) respectively. The median score for Intensity of Staining Normal Oral Mucosa and Oral Squamous Cell Carcinoma was calculated to be 0.0 (IQR – 1.0) and 1.0 (IQR – 3.0) respectively. Using Mann Whitney U Test, the difference between the scores was found to be statistically significant. (P value – 0.008).

AREA OF STAINING:

The table 1 depicts the score for Area of Staining for healthy controls and OSCC. The mean score for Area of Staining for MUC1 Mucin between Normal Oral Mucosa and Oral Squamous Cell Carcinoma was found to be 0.30(SD – 0.48) and 1.30(SD – 1.26) respectively. The median score for Area Of Staining for MUC1 Mucin between normal tissues and Oral Squamous Cell Carcinoma was 0.00(IQR – 1.00) and 1.0(IQR – 2.0) respectively. The difference between the scores was found to be statistically significant using Mann Whitney U Test (P Value – 0.002).

GRAPH: 1

INTERGROUP COMPARISON OF IOS AND AOS BETWEEN CONTROL GROUP AND STUDY GROUP



The above Graph1 shows comparison between control group and study group for Intensity Of Staining and Area Of Staining for MUC-1 Mucin

TABLE: 2

COMPARISON OF INTENSITY AND AREA OF STAINING BETWEEN HEALTHY CONTROLS AND OSCC GROUPS BASED ON PERCENTAGE

Variables	HEALTHY CONTROLS				ORAL SQUAMOUS CELL CARCINOMA				p-value
	Mean	Standard Deviation	Median	IQR	Mean	Standard Deviation	Median	IQR	
IOS%	58.0000	19.32184	70.0000	40.0000	40.0000	14.38390	40.0000	20.0000	***0.008**
AOS%	58.0000	19.32184	70.0000	40.0000	38.0000	20.23994	40.0000	40.0000	***0.002**

***Denotes statistically significant values using Mann-Whitney U Test**

INTENSITY OF STAINING BASED ON PERCENTAGE

The above table 2. depicts the mean score for Intensity of staining for healthy controls and study group using MUC1 Mucin based on percentage was found to be 58.0(SD – 19.32) and 40.0(SD -14.38) respectively.

The median score for area of staining for healthy controls and study group using MUC1 Mucin based on percentage was found to be 70.0(IQR – 40.0) and 40.0(IQR – 20) respectively. The difference between the scores were found to be statistically significant using Mann Whitney U Test (p Value – 0.02 which is less than 0.05).

AREA OF STAINING BASED ON PERCENTAGE

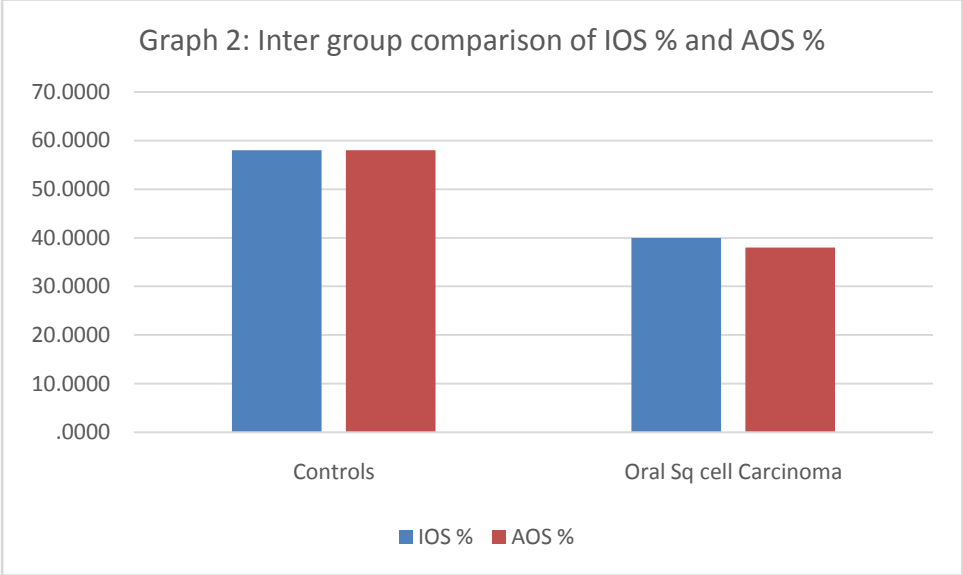
The table 2. Depicts the mean score for Area of staining on percentage basis for healthy controls and study group using MUC1 Mucin is 58.0(SD – 19.32184) and 38.0(SD - 20.23994) respectively.

The median score for Area of staining on percentage basis for healthy controls and study group using MUC1 Mucin is 70.00(IQR – 40.00) and 40.00(IQR – 40.00) respectively. The

difference between the scores were found to be statistically significant using Mann Whitney U Test (p value – 0.002 which is less than 0.05).

GRAPH: 2

INTERGROUP COMPARISON OF IOS AND AOS BETWEEN CONTROL GROUP AND STUDY GROUP BASED ON PERCENTAGE



The above graph 2. Shows comparison between control group and study group for intensity of staining and area of staining based on percantage for MUC-1 Mucin

TABLE 3

INTERGROUP COMPARISON OF INTENSITY OF STAINING AND AREA OF STAINING OF MUC1 MUCIN BETWEEN DIFFERENT GRADES OF ORAL SQUAMOUS CELL CARCINOMA

POST HOC ANALYSIS

	WDOSCC Vs MDOSCC	**WDOSCC Vs PDOSCC**	**MDOSCC Vs PDOSCC**	**p-value**
Intensity of Staining	0.1	0.001	0.04	*0.001
Area of staining	0.02	0.003	0.18	0.07

*Denotes statistically significant values using Kruskall Wallis ANOVA

Table 3. shows intergroup comparison of Intensity of staining among different grades of Oral Squamous Cell Carcinoma by MUC1 Mucin was scored as

0.1 (Between WDOSCC and MDOSCC)

0.001 (Between WDOSCC and PDOSCC)

0.04 (Between MDOSCC and PDOSCC)

And the p-value was found to be 0.001 (which is significant as it is less than 0.05) derived using Kruskall Wallis ANOVA test.

The intergroup comparison of Area of staining among different grades of Oral Squamous Cell Carcinoma by MUC1 Mucin was scored as

0.02 (Between WDOSCC and MDOSCC)

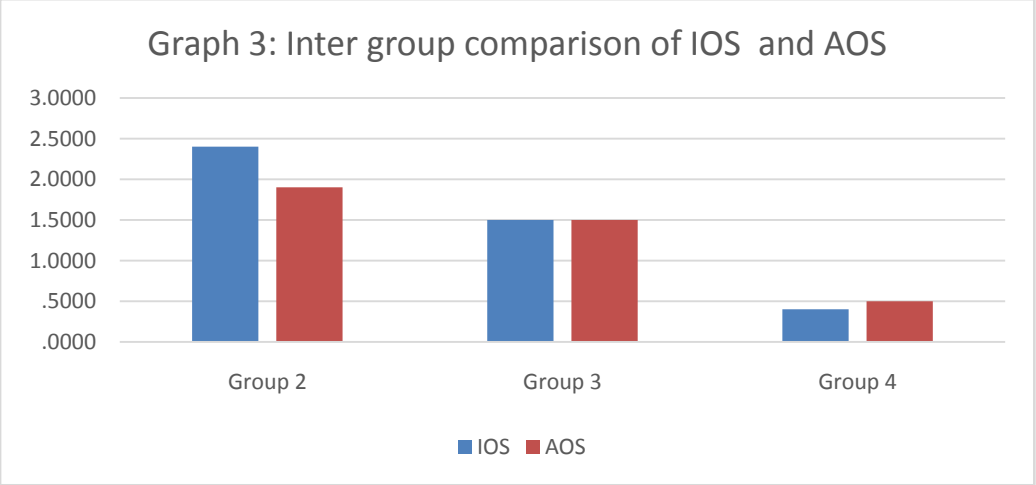
0.003 (Between WDOSCC and PDOSCC)

0.18 (Between MDOSCC and PDOSCC)

And the p-value is found to be 0.07 (which is not significant) derived using Kruskall Wallis ANOVA test.

GRAPH: 3

INTERGROUP COMPARISON OF INTENSITY OF STAINING AND AREA OF STAINING OF MUC1 MUCIN BETWEEN THE DIFFERENT GRADES OF ORAL SQUAMOUS CELL CARCINOMA



The above Graph 3. Shows intergroup comparison of intensity of staining and area of staining of MUC-1 Mucin between the different grades of Oral Squamous Cell carcinoma

INTENSITY OF STAINING:

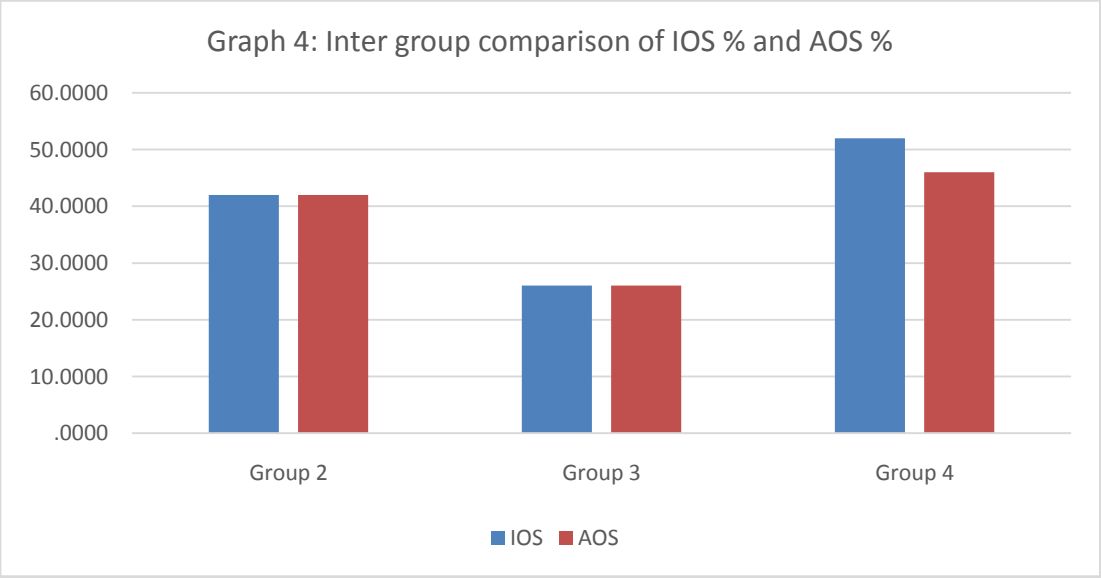
We compared the staining intensity of MUC1 Mucin between the different grades of Oral Squamous Cell Carcinoma using Kruskall Wallis ANOVA and P value obtained was 0.0010 (P Value<0.05) which was found to be statistically significant.

AREA OF STAINING:

We also compared the Area of Staining of MUC1 Mucin between well differentiated, Moderately Differentiated and Poorly Differentiated Squamous Cell Carcinoma using Kruskal Wallis ANOVA. The P Value was found to be 0.02 (P-value <0.05) which was also statistically significant.

GRAPH: 4

INTERGROUP COMPARISON OF INTENSITY OF STAINING AND AREA OF STAINING OF MUC1 MUCIN BETWEEN DIFFERENT GRADES OF ORAL SQUAMOUS CELL CARCINOMA BASED ON PERCENTAGE

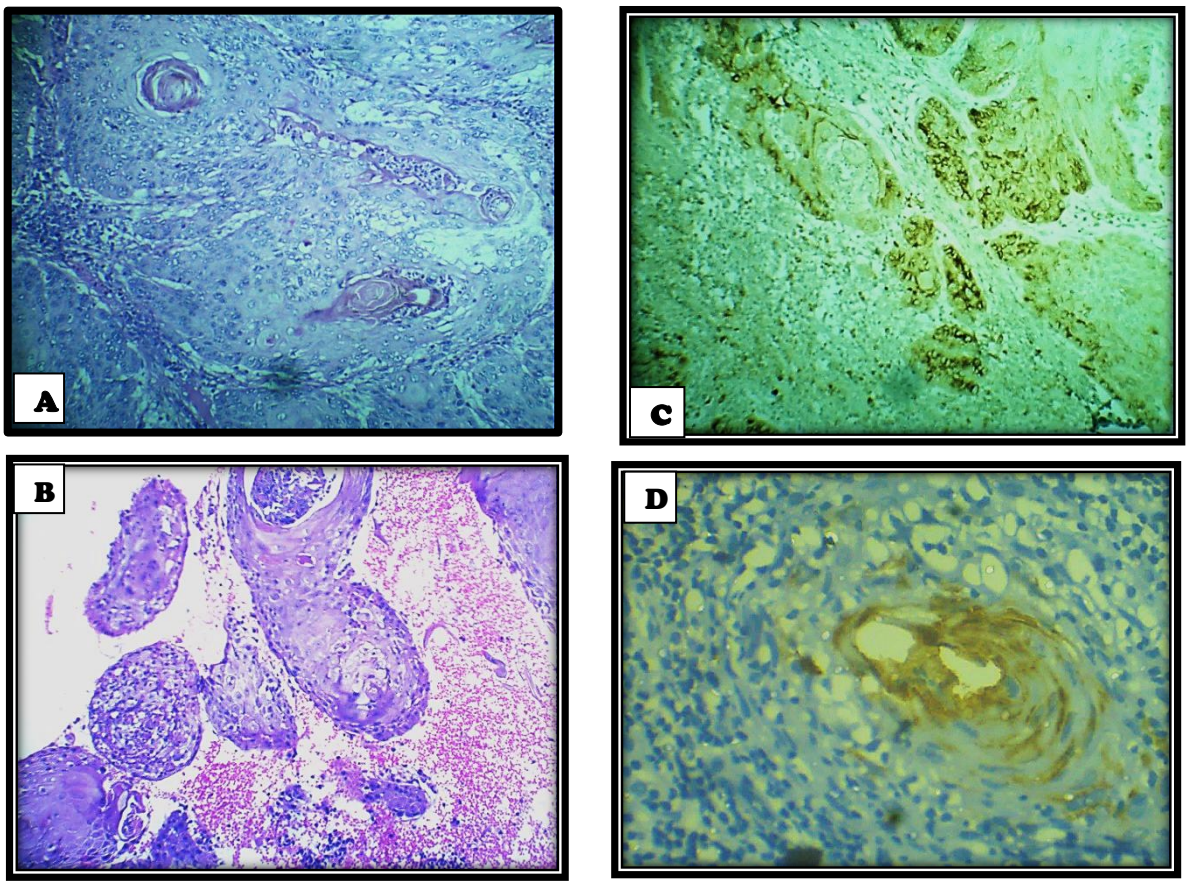


The above Graph 4. Shows intergroup comparison of intensity of staining and area of staining of MUC-1 Mucin between the different grades of Oral Squamous Cell carcinoma based on percentage.

SLIDE PICTURES

FIGURE: 3.WELL DIFFERENTIATED SQUAMOUS CELL CARCINOMA

HEMATOXYLIN AND EOSIN **IMMUNOHISTOCHEMISTRY**



A. 4x view of H&E showing invading dysplastic epithelial islands with keratin pearls

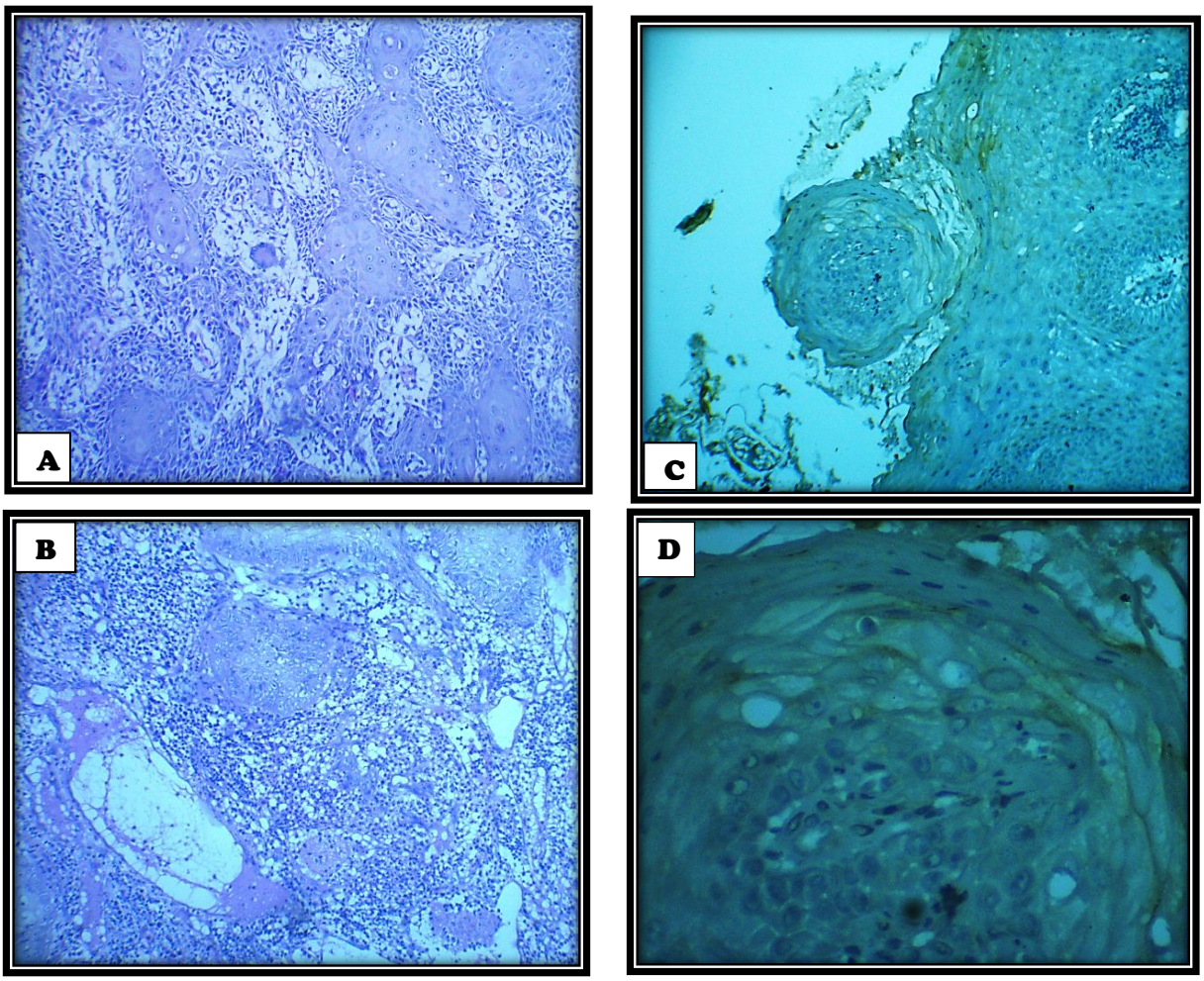
B. 10x view of H&E showing dysplastic epithelial islands and chronic inflammatory cells

C. 10x view showing strong positive expression for MUC1 in dysplastic epithelial islands

D. 40x view showing strong positive membrane expression for MUC1 in keratin pearl

FIGURE: 4.MODERATELY DIFFERENTIATED SQUAMOUS CELL CARCINOMA

HEMATOXYLIN AND EOSIN **IMMUNOHISTOCHEMISTRY**



A. 10x view of H&E showing invaded islands with chronic inflammatory cells

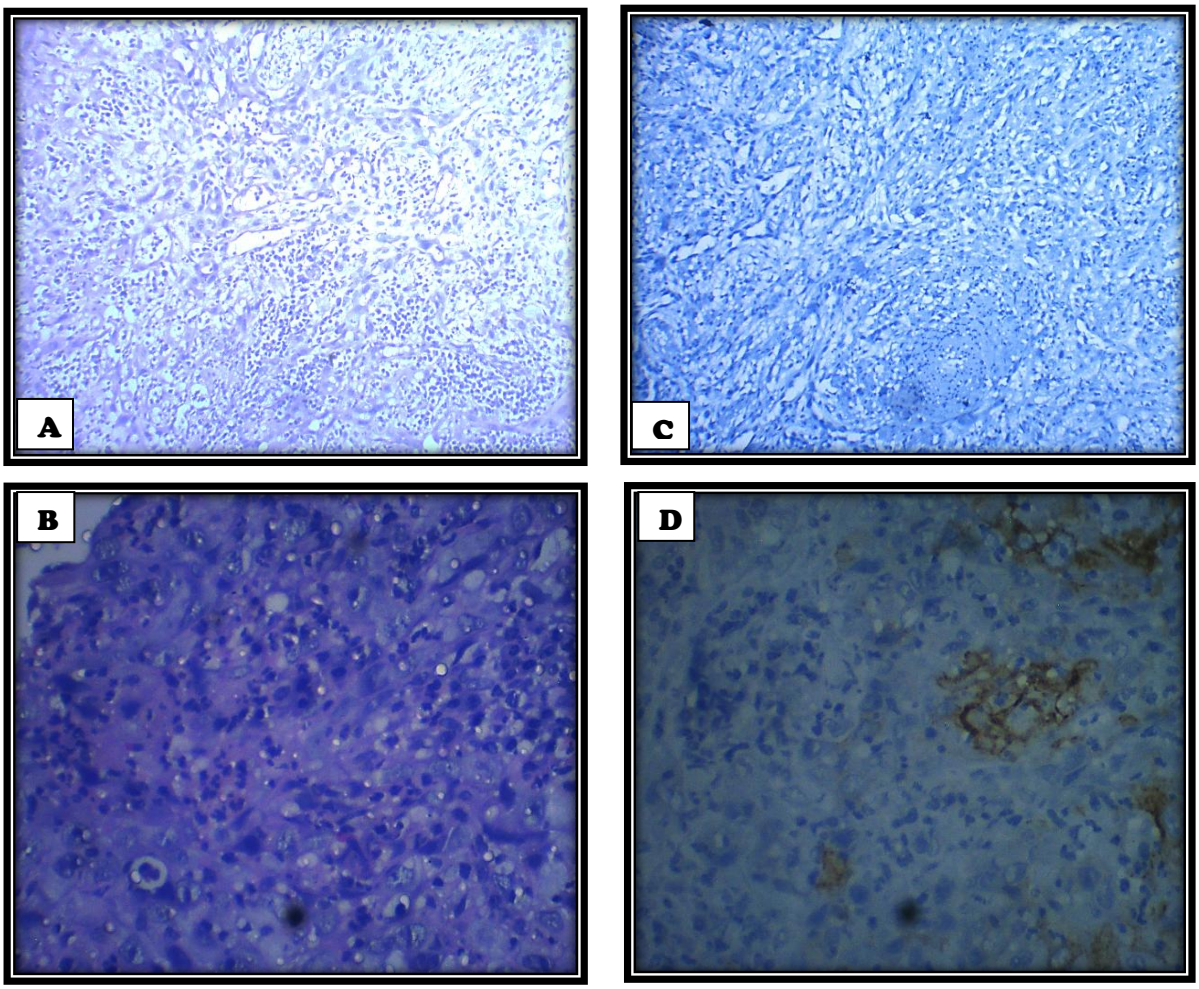
B. 10x view of H&E showing invaded islands with dysplastic cells

C. 10x view of MUC1 showing moderate membrane staining

D. 40x view of MUC1 showing moderate membrane staining seen in invading dysplastic cells

FIGURE: 5. POORLY DIFFERENTIATED SQUAMOUS CELLCARCINOMA

HEMATOXYLIN AND EOSIN **IMMUNOHISTOCHEMISTRY**



A. 10x view of H&E showing dysplastic cells with inflammatory cells

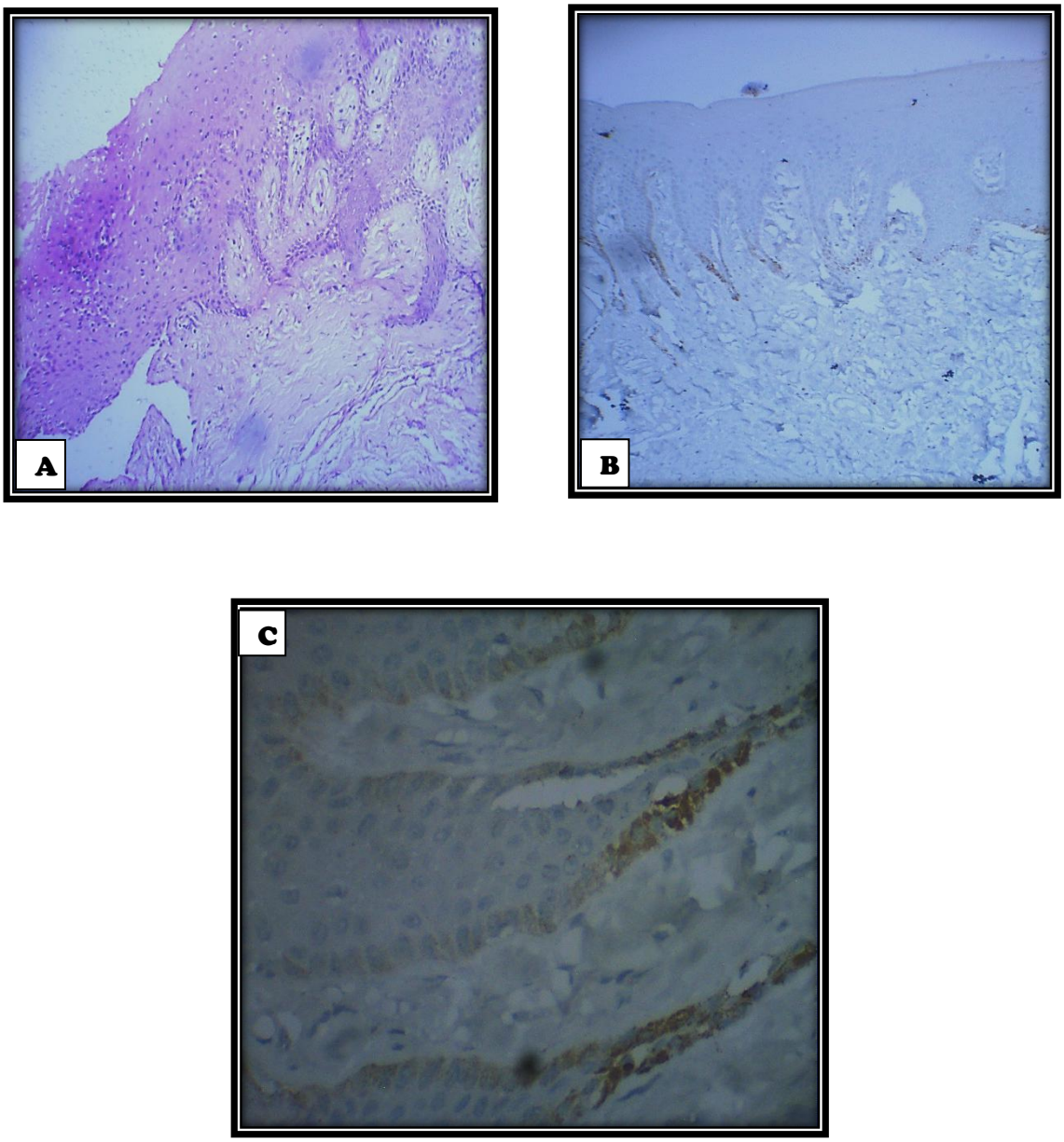
B. 40x view of H&E showing numerous dysplastic cells

C. 10x view showing absence of MUC1 expression

D. 40x view showing few MUC1 positive dysplastic cells

FIGURE 6: NORMAL ORAL MUCOSA

HEMATOXYLIN AND EOSIN **IMMUNOHISTOCHEMISTRY**



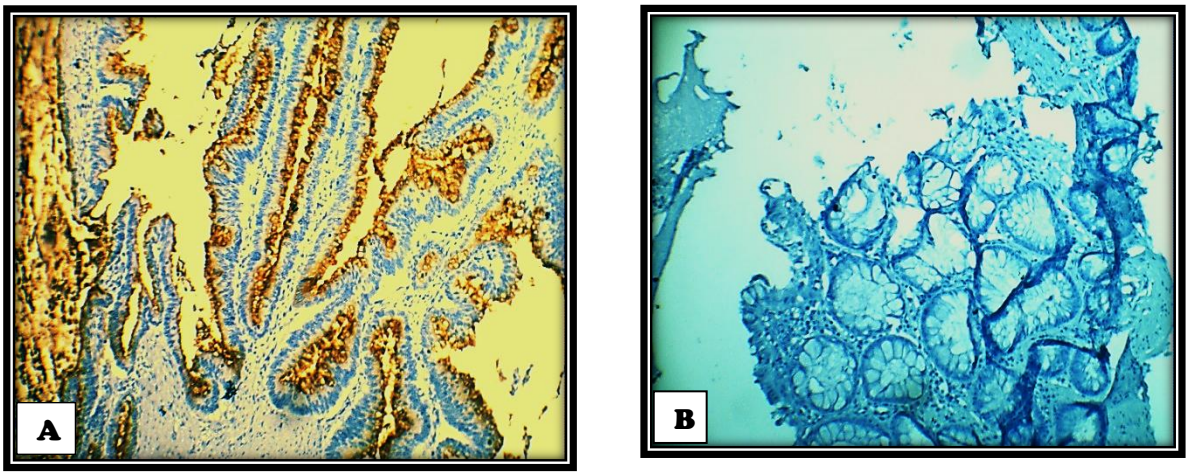
A. 10x view of H&E stained Normal Oral Mucosa

B. 4x view of Normal Oral Mucosa showing positive immuno-expression for MUC1 in basal and parabasal cells

C. 10x view of Normal Oral Mucosa showing positivity for immuno-expression for MUC1 in basal and parabasal cells

FIGURE: 7. CONTROLS

POSITIVE **NEGATIVE**



A. 10x view showing strong positive expression for MUC1 in colon cancer

B. 10x view showing negative expression for MUC1 in colon cancer

DISCUSSION

Of the top 10 cancers reported in the globe, Oral Squamous cell carcinoma is being highly prevalent and reported in all countries, though there may be geographical variations in incidence. Oral Squamous cell carcinoma is being frequently reported in Central and South Asian countries related to the use of tobacco products and alcohol and India has been reported with 50% of mortality as on 2018 due to being diagnosed at later stages.[30] Immunoexpression of Tumour markers has become a boon to early detection and hence preventing early invasion and metastasis.

Mucins play an important role in cell growth, cell differentiation and cell signalling[2].They belong to a family of high molecular weight glycoproteins. Most of the mucins are membrane bound due to the presence of a hydrophobic membrane that allows retention in the plasma membrane. Mucins are basically secreted as principle component of mucus .Sometimes, they are secreted as a component of saliva. Till now, about 20 different types of human mucin genes have been identified. Among them, MUC1 Mucin is a transmembrane mucin like glycoprotein encoded by the MUC1 gene and its expression has been identified in a variety of malignancies, including malignant oral lesions[20].

With this background we undertook the following study to evaluate the immunohistochemical expression of MUC1 Mucin in different grades of Oral Squamous Cell Carcinoma.

Our study included a total of 40 samples, which included formalin fixed archival retrieved paraffin embedded tissue blocks. Study group included 30 samples of clinically and histopathologically diagnosed Oral Squamous Cell Carcinoma comprised 10 samples of Well Differentiated Oral Squamous Cell Carcinoma (WDOSCC), 10 samples of Moderately Differentiated Oral Squamous Cell Carcinoma (MDOSCC), and 10 samples of Poorly

Differentiated Oral Squamous Cell Carcinoma (PDOSCC). Control group consisted of 10 samples of Normal Oral Mucosa (NOM).

Serial sections of 3µ thickness were made and were subjected to Immunohistochemical staining procedure using anti MUC1 Mucin (Rabbit Monoclonal Antibody – EP_{85}) (PathnSitu Biotechnologies Private Limited). The immunohistochemical staining pattern in colon carcinoma was used as positive control. The immunohistochemical reactivity was evaluated based on the presence or absence of brown coloured stain at the site of target antigen. All the IHC stained slides including study group and control group were evaluated based on the intensity of staining expression of MUC1 Mucin. The number of immunopositive tumor cells and their percentage was calculated based on the area of staining in all layers of the epithelium.

The intensity of staining of MUC1 Mucin was graded as 0- Negative, 1-Mild, 2-Moderate, and 3-Intense. All samples including control group and study group showed variable intensities of membranous and cytoplasmic staining for MUC1 Mucin. The area of staining was calculated first by examining the distribution of immunopositive tumor cells under 10X magnification, among which 5 random fields were selected under 40X magnification and the percentage of positive cells were scored as follows.

0%-5% of positive tumour cells – Score 0

6%-25% of positive tumour cells – Score 1

26%-50% of positive tumour cells – Score 2

51%-75% of positive tumour cells – Score 3

76%-100% of positive tumor cells – Score 4

In our study, control group included 10 cases of NOM. Out of 10 cases of NOM, 3 cases (30%) showed mild staining intensity for MUC1 Mucin and were graded as 1 and the remaining 7 cases (70%) showed no expression for MUC1 Mucin and were given score 0. These findings are similar to that of the results obtained by Harish Kumar *et al* in 2016. They also observed MUC1 Mucin immunoreactivity in 2 out of 20 cases of NOM. NOM showed weak staining as Mucins are involved in signalling pathways, cell differentiation, cell proliferation and apoptosis[2].

But, these findings are in contrast to that of the studies conducted by Nitta *et al* (1999) and Thakur *et al* (2018). Their studies did not reveal any immunoreactivity to MUC1 Mucin in control group samples that is normal oral mucosa.

Our study group comprised of 10 cases of WDOSCC. All 10 cases showed positive expression of staining intensity for MUC1- Mucin. Out of the 10 cases of WDOSCC, 5 cases (50%) showed intense staining for MUC1-Mucin and were scored as 3. 4 cases (40%) showed a moderate expression and were graded as 2.1 cases (10%) showed mild expression and was given a score of 1.

On evaluation, the area of staining in all 10 cases of WDOSCC, 2 cases (20%) showed 51% - 75% of positive tumour cells in the entire section of the epithelium examined. They were given a grade of 3. 6 cases(60%) showed 26% to 50% of immunopositiviy and were given a score of 2. 1 case showed (10%) a very mild immunopositivity with positive tumor cells less than 5% and so was given a score 0.

All 10 cases of WDOSCC in our study showed both membranous and cytoplasmic staining for MUC-1 Mucin. Keratin pearls also showed positive expression for MUC-1 Mucin. The distribution was either focal or diffuse. These findings are in accordance with the results obtained by Kumar *et al* (2016) and Shobitha *et al* (2018) and Nitta *et al* (1999). The

membranous and cytoplasmic staining of MUC-1 Mucin in the squamous epithelial cells can be correlated to its transmembrane and cytoplasmic subunits respectively[17, 31].

We examined a total of 10 cases of MDOSCC to evaluate the staining intensity and area of staining for MUC-1 Mucin. Out of the 10 cases of MDOSCC, 3 cases (30%) showed and intense staining expression for MUC-1 Mucin and were scored as 3. 2 cases (20%) showed a moderate staining expression and were given score 2. 2 cases (20%) showed a mild intensity of staining and were graded as 1. 3 cases (30%) did not show any staining for MUC-1 Mucin.

Out of the 10 cases of MDOSCC 2 cases (20%) showed immunopositive tumour cells ranging from 76% - 100% and were graded as 4. 1 case (10%) showed immunopositivity for MUC-1 Mucin in the range of 51% to 75% and so was given a score of 3. 1 case (10%) showed positive tumour cells ranging from 26% to 50% and was given grade 2. 2 cases (20%) showed immunopositivity for MUC-1 Mucin ranging from 6% - 25% and were given a score of 1. 4 cases (40%) showed very mild immunopositiviy which was less than 5% and so were scored as 0.

Out of 10 cases of PDOSCC, 6 cases (60%) did not show any expression for MUC-1 Mucin and so were scored as 0. 4 cases (40%) showed a mild staining intensity and so were given score 1. On evaluating the percentage of immunopositive tumour cells, 6 cases (60%) did not show any positive tumour cells and so were scored 0. 3 cases (30%) showed immunopositivity ranging from 6% to 25% and were scored as 1. 1 case (10%) showed immunopositive tumour cells in the range of 26% to 50%and so were given a grade of 2.

From our study we found that staining intensity of MUC-1 Mucin and also the area of staining of MUC-1 Mucin gradually decreased from WDOSCC to PDOSCC.

We are not able to compare these results with other studies, because a thorough search of literature did not reveal any studies done with equal samples in all the three grades of OSCC. More studies need to be done with increase in sample size to exactly substantiate the above findings obtained in our study.

We did a comparison between healthy controls (NOM) and study group (OSCC). The comparison was made for both intensity of staining and area of staining using Mann Whitney μ test. The P-Value obtained for intensity of staining was 0.008 which was statistically significant (P-Value < 0.05).

The P-Value obtained for intensity of staining based on percentage was also 0.008, which was again statistically significant (P-Value less than 0.05).

The P-Value obtained by comparing area of staining between healthy controls and OSCC was 0.02, which was found to be statistically significant. (P-Value less than 0.05). The P-Value obtained for area of staining based on percentage was 0.002, which was also statistically significant (P-Value<0.05)

Mentioned in [TABLE 1, TABLE 2, GRAPH1, GRAPH 2]

Our findings were in consistent with the results obtained by Nitta *et al* (1999), Kumar *et al* (2016), Thakur *et al* (2018) and Shobitha *et al* (2018) who also observed a gradual increase of positive expression of MUC-1 Mucin from NOM to OSCC. We also made a comparison between the study group, that is among the three grades of OSCC. The comparison was done for intensity of staining and area of staining for MUC-1 Mucin using Kruskall Wallis ANOVA test.

The P-Value obtained for intensity of staining between the three grades of OSCC was 0.010, which was statistically significant (P-Value <0.005). The P-Value for staining

intensity of MUC-1 Mucin based on percentage was less than 0.001 which was also statistically significant.

The P-Value obtained for area of staining of MUC-1 Mucin between the different grades of OSCC was 0.02 which was statistically significant (P-Value less than 0.05). The P-Value obtained for area of staining based on percentage was 0.07, which was not statistically significant (P-Value more than 0.05).

Mentioned in [TABLE 3, GRAPH 3, GRAPH 4]

The results obtained in the current study were similar to that of the results obtained by Shobitha *et al* (2018). They also made a comparison between the histological grades of OSCC and found a significant decrease in the immunoexpression of MUC1 Mucin from well differentiated OSCC to poorly differentiated OSCC through moderately differentiated OSCC.

The reason for this progressive decrease in the expression from WDOSCC to PDOSCC could be attributed to the inability of less differentiated squamous epithelial cells to express mucins when compared to well differentiated squamous epithelial cells. This phenomenon might be due to decelerated catabolism of certain inhibitory proteins for MUC1 mucin immunoexpression in well differentiated mature atypical squamous cells. Whereas in PDOSCC there might be an increased synthesis of certain intrinsic inhibitory proteins for MUC-1 for some unknown mechanism which might have altered maturation and de-differentiation of neoplastic cells.

From our study, we have observed that MUC-1 mucin is well expressed in the well keratinized areas, which are often seen in well differentiated OSCC.

From our study, we would like to conclude that upregulation of MUC-1 Mucin immuoexpression in neoplastic lesions may play a pivotal role in the pathogenesis and

progression of these lesions. MUC-1 Mucin may also serve as a useful marker for the prediction of metastatic potential, invasive potential and prognosis of OSCC. Hence, MUC-1 Mucin can be regarded as an important prognostic marker for OSCC.

SUMMARY & CONCLUSION

The aim of the study was to analyse the expression of MUC1 Mucin in different grades of Oral Squamous Cell Carcinoma using Immunohistochemistry. A total of 30 samples of squamous cell carcinoma and 10 samples of Normal Oral Mucosa (NOM) were taken from the archival blocks.

Immunohistochemical expressions of MUC1 Mucin were studied by analysis of staining intensity and area of staining.

From the present study, the following conclusions were drawn:

- Significant difference exists in the intensity of staining and area of staining of MUC1 Mucin between NOM and OSCC
- Significant difference exists in the intensity of staining and area of staining of MUC1 Mucin among well differentiated, moderately differentiated and poorly differentiated OSCC

We conclude that MUC1 Mucin is expressed in all grades of oral squamous cell carcinoma and weakly expressed in the proliferative layers (Basal Cell layer) in few cases of Normal Oral Mucosa. High immunopositivity and strong staining intensity for MUC1 Mucin were observed in well differentiated OSCC among the three grades of OSCC. A very mild immunopositivity and staining intensity was observed in poorly differentiated OSCC cases.

Hence, with our study we conclude that MUC-1 Mucin biomarker can be used to detect higher grades of Oral Squamous Cell Carcinoma and hence, early detection in preventing, invasion and metastasis.

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ANNEXURE

NORMAL ORAL MUCOSA		
S.No	**INTENSITY OF STAINING**	**AREA OF STAINING**
1	**1**	**1**
2	**0**	**0**
3	**0**	**0**
4	**1**	**1**
5	**0**	**0**
6	**0**	**0**
7	**1**	**1**
8	**0**	**0**
9	**0**	**0**
10	**0**	**0**

TABLE NO. 1: Scores of Intensity of staining and Area of staining on Normal Oral Mucosa tissues using MUC1 Mucin

WELL DIFFERENTIATED ORAL SQUAMOUS CELL CARCINOMA		
S.No	**INTENSITY OF STAINING**	**AREA OF STAINING**
11	**3**	**3**
12	**2**	**2**
13	**3**	**2**
14	**1**	**0**
15	**2**	**1**
16	**3**	**3**
17	**2**	**2**
18	**3**	**2**
19	**3**	**2**
20	**2**	**2**

TABLE NO. 2: Scores of Intensity of staining and Area of staining on Well Differentiated Oral Squamous cell Carcinoma tissues using MUC1 Mucin

MODERATELY DIFFERENTIATED ORAL SQUAMOUS CELL CARCINOMA		
S.No	**INTENSITY OF STAINING**	**AREA OF STAINING**
21	**2**	**2**
22	**3**	**3**
23	**3**	**4**
24	**2**	**0**
25	**0**	**0**
26	**3**	**4**
27	**0**	**0**
28	**0**	**0**
29	**1**	**1**
30	**2**	**1**

TABLE NO. 3: Scores of Intensity of staining and Area of staining on Moderately Differentiated Oral Squamous cell Carcinoma tissues using MUC1 Mucin

POORLY DIFFERENTIATED ORAL SQUAMOUS CELL CARCINOMA		
S.No	**INTENSITY OF STAINING**	**AREA OF STAINING**
31	**0**	**0**
32	**1**	**2**
33	**1**	**1**
34	**0**	**0**
35	**1**	**1**
36	**1**	**1**
37	**0**	**0**
38	**0**	**0**
39	**0**	**0**
40	**0**	**0**

TABLE NO. 4: Scores of Intensity of staining and Area of staining on Poorly Differentiated Oral Squamous cell Carcinoma tissues using MUC1 Mucin

Om Sakthi

ADHIPARASAKTHI
DENTAL COLLEGE & HOSPITAL
Recognised by Dental Council of India
Affiliated to The Tamilnadu Dr.M.G.R Medical University
A Unit of Adhiparasakthi Charitable, Medical, Educational & Cultural Trust

This Ethical Committee has reviewed the research Protocol submitted by Dr. P. Hariganesh, Post Graduate Student, Department of Oral Pathology and Microbiology, under the title "Immunohistochemical expression of MUC1 Mucin in different grades of Oral Squamous cell carcinoma:A Case Control Study" Ref no.: 2017-MDS-BrVI-Dev-13/APDCH under the guidance of Dr.S.Shamala Ravikumar for consideration of approval to proceed with the study.

This Committee has discussed about the Material being involved with the study, the Qualification of the investigator, the present norms and recommendations from the Clinical Research Scientific body and comes to a conclusion that this Research protocol fulfils the Specific requirements and the Committee authorizes the proposal.

URKUND

Urkund Analysis Result

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