

**"EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL
EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH
PRESENCE OF MAST CELLS: A RETROSPECTIVE
COMPARATIVE STUDY"**

DISSERTATION

Submitted to

BABU BANARASI DAS UNIVERSITY, LUCKNOW, UTTAR PRADESH

In the partial fulfilment of the requirements for the degree

of

MASTER OF DENTAL SURGERY

In

ORAL & MAXILLOFACIAL PATHOLOGY & ORAL MICROBIOLOGY

By

Dr. DEEP SHARMA

Under the guidance of

Dr. JIJ GEORGE

Professor & Head

Department of Oral & Maxillofacial Pathology & Oral Microbiology

BABU BANARASI DAS COLLEGE OF DENTAL SCIENCES, LUCKNOW

(Faculty of Babu Banarasi Das University)

YEAR OF SUBMISSION: 2025

BATCH: 2022-2025

Enrollment No.: 11200004044

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled “**EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY**” is a bonafide and genuine research work carried out by me under the guidance of **Dr. JIJ GEORGE**, Professor & Head and **Dr. ANKITA SINGH**, Reader as Co-Guide in Department of Oral & Maxillofacial Pathology & Microbiology, Babu Banarasi Das College of Dental Sciences, Babu Banarasi Das University, Lucknow, Uttar Pradesh.

Date: 21-04-25.

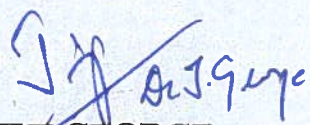
Place: Lucknow

Deep Sharma
Dr. DEEP SHARMA

CERTIFICATE BY THE GUIDE

This is to certify that the dissertation entitled **“EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY”** is a bonafide work done by **Dr. DEEP SHARMA**, under my direct supervision and guidance in partial fulfillment of the requirement for the degree of MDS in Department of Oral & Maxillofacial Pathology & Oral Microbiology, Babu Banarasi Das College Of Dental Sciences, Babu Banarasi Das University, Lucknow, Uttar Pradesh.

Date: 21/4/25


Dr. JIJI GEORGE

Professor & Head

Department of Oral & Maxillofacial

Pathology & Oral Microbiology

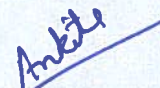
BBDCODS, BBD University

Lucknow

CERTIFICATE BY THE CO-GUIDE

This is to certify that the dissertation entitled “**EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY**” is a bonafide work done by **Dr. DEEP SHARMA**, under the supervision of **Dr. Ankita Singh** as Co – Guide, Reader, Department of Oral & Maxillofacial Pathology & Oral Microbiology, Babu Banarasi Das College Of Dental Sciences, Babu Banarasi Das University, Lucknow, Uttar Pradesh.

Date : 21.4.25



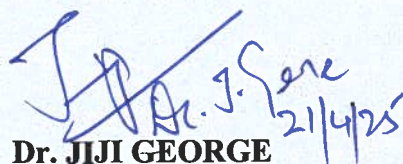
Dr. ANKITA SINGH

Reader

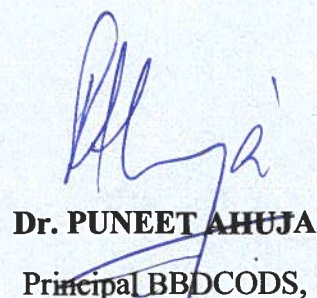
Department of Oral
& Maxillofacial Pathology &
Oral Microbiology
BBDCODS, BBD University
Lucknow

ENDORSEMENT BY THE HOD / HEAD OF THE INSTITUTION

This is to certify that the dissertation entitled “**EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY**” is a bonafide work done by **Dr. DEEP SHARMA** under the supervision of **Dr. JIJ GEORGE**, Professor & Head, Department of Oral & Maxillofacial Pathology & Oral Microbiology, Babu Banarasi Das College of Dental Sciences, Babu Banarasi Das University, Lucknow, Uttar Pradesh.


Dr. JIJ GEORGE

Professor & Head Department of
Oral & Maxillofacial Pathology
& Oral Microbiology
BBDCODS, BBD University
Lucknow


Dr. PUNEET AHUJA
Principal BBDCODS,

BBD University
Lucknow
PRINCIPAL
Babu Banarasi Das College of Dental Sciences
Babu Banarasi Das University
Ayodhya Road, Lucknow-226028

COPYRIGHT

DECLARATION BY THE CANDIDATE

I hereby declare that the **Babu Banarasi Das University** shall have the rights to preserve, use and disseminate this dissertation in print or electronic format for academic / research purpose.

Date: 21-04-25

Place: Lucknow

Deep Sharma
Dr. DEEP SHARMA



*“Dedicated Solely
To My Parents”*





ACKNOWLEDGEMENT



ACKNOWLEDGEMENT

**DEDICATED TO MY BELOVED PARENTS - MR. PRABHAT CHANDRA
SHARMA AND MRS. BIDYA BOTI SHARMA**

"Your love, values, and sacrifices have been the foundation of everything I have achieved.

This journey would not have been possible without your constant support and blessings."

"When you look back at your journey, remember who walked beside you."

With utmost gratitude, I begin by thanking the ALMIGHTY for bestowing His blessings, strength, and wisdom upon me, guiding me through every step of this academic pursuit.

I express my deepest respect and sincere thanks to **Dr. Jiji George**, M.D.S., Professor and Head, Department of Oral Pathology and Microbiology. Her remarkable mentorship, keen academic insight, and unwavering encouragement have played a crucial role in shaping this work. I am privileged to be her student.

I owe heartfelt thanks to **Dr. Ankita Singh**, M.D.S., Reader, my co-guide, for her consistent support, guidance, and patience. Her timely advice and motivating presence helped me overcome many challenges during the course of this study.

My sincere appreciation goes to **Dr. Puneet Ahuja**, M.D.S., Principal, Babu Banarasi Das College of Dental Sciences, Lucknow, for his leadership, encouragement, and constant motivation that inspired me to strive for excellence.

I am also grateful to **Dr. Devangi Dwivedi**, M.D.S., Senior Lecturer, and **Dr. Sandhya Sahetya**, M.D.S., Senior Lecturer, for their invaluable support, suggestions, and encouragement throughout my postgraduate journey.

To my loving wife **Mrs. Luna S. Sharma**, for her unconditional love, patience, and strength. She has been my unwavering support system and has stood by me in every moment. Her belief in me made all the difference.

To my precious Son, **Arohan Sharma**, his smile gave me light even on the most exhausting days. He is my greatest inspiration and joy.

A special word of thanks to my Co-PG, **Dr. Anuradha Ojha Giri**, for her companionship, support, and collaboration throughout this academic journey. Her presence made this experience meaningful and memorable. A word of gratitude for my senior **Dr. Debo Das** and juniors **Dr. Nikhil** and **Dr. Priyanshu** for their support and help.

Lastly, I extend my heartfelt gratitude to **Vedprakash**, for lab support & to everyone who has supported, encouraged, or believed in me during this endeavor. This thesis is not only the culmination of academic effort but a reflection of every hand that lifted me up along the way.

Every journey has milestones; not endings. This is just the beginning of a new chapter.

Dr. Deep Sharma

TABLE OF CONTENTS

S. NO.	PARTICULARS	PAGE NO.
1.	ABSTRACT	1-2
2.	INTRODUCTION	3
3.	AIM & OBJECTIVES	4
4.	REVIEW OF LITERATURE	5-29
5.	MATERIALS & METHOD	30-34
6.	LEGENDS	35-42
7.	OBSERVATIONS AND RESULTS	43-51
8.	DISCUSSION	52-56
8.	CONCLUSION	57
9.	BIBLIOGRAPHY	58-70
10.	APPENDICES	71-81

LIST OF TABLES

TABLE 1	Criteria used for dysplasia
TABLE 2	Endoglin (CD105) expression in normal human tissues
TABLE 3	Descriptives of Mast cell density with respect to different grades of dysplasia
TABLE 4	Intergroup comparison of mast cell density
TABLE 5	Post hoc pairwise comparisons of mean Mast cell density using Tukey's test
TABLE 6	Descriptives of Microvessel density with respect to different grades of dysplasia.
TABLE 7	Intergroup comparison of Microvessel density using one way ANOVA test
TABLE 8	Post hoc pairwise comparisons of mean Microvessel density using Tukey's test
TABLE 9	Correlation between Mast cell density & Microvessel density among dysplasia cases

LIST OF GRAPHS

GRAPH 1	Mean Mast cell density with respect to different grades of dysplasia
GRAPH 2	Mean Microvessel density with respect to different grades of dysplasia
GRAPH 3	Scatter plot showing the correlation of Mast cell density & Microvessel density among mild dysplasia cases
GRAPH 4	Scatter plot showing the correlation of Mast cell density & Microvessel density among moderate dysplasia specimens
GRAPH 5	Scatter plot showing the correlation of Mast cell density & Microvessel density among severe dysplasia cases
GRAPH 6	Scatter plot showing the overall correlation of Mast cell density & Microvessel density among all dysplasia cases

LIST OF FIGURES

FIGURE 1	Semi-Automatic Rotary Microtome (Yorco YSI 062)
FIGURE 2	Decloaking chamber
FIGURE 3	Reagents used for Hematoxylin & Eosin staining
FIGURE 4	CD 105 antibody (BioGenex) used for Immunohistochemical staining
FIGURE 5	Other reagents used for Immunohistochemical staining
FIGURE 6	Tris buffer
FIGURE 7	Ultra Vision HRP DAB
FIGURE 8	Penta head microscope (Olympus Magnus MLXi Plus)
FIGURE 9	Control Section for CD105
FIGURE 10	Photomicrograph showing mast cells in Mild dysplasia (Toluidine Blue stain) (x400)
FIGURE 11	Photomicrograph showing CD 105 positive endothelial cells in Mild dysplasia (x400)
FIGURE 12	Photomicrograph showing mast cells in Moderate dysplasia (Toluidine Blue stain) (x400)
FIGURE 13	Photomicrograph showing CD 105 positive endothelial cells in Moderate dysplasia (x400)
FIGURE 14	Photomicrograph showing H & E stained section of Severe dysplasia (x400)
FIGURE 15	Photomicrograph showing mast cells in Severe dysplasia (Toluidine Blue stain) (x400)
FIGURE 16	Photomicrograph showing CD 105 positive endothelial cells in Severe dysplasia (x400)

LIST OF APPENDICES

ANNEXURE – I	INSTITUTIONAL RESEARCH COMMITTEE APPROVAL
ANNEXURE – II	INSTITUTIONAL ETHIC COMMITTEE APPROVAL
ANNEXURE – III	OBSERVATIONS
ANNEXURE – IV	FORMULA USED FOR THE ANALYSIS
ANNEXURE – V	PLAGIARISM REPORT

LIST OF ABBREVIATIONS

OED	Oral Epithelial Dysplasia
OSCC	Oral Squamous Cell Carcinoma
OPMDs	Oral Potentially Malignant Disorders
OSMF	Oral Submucous Fibrosis
VEGF	Vascular Endothelial Growth Factor
CD105	Cluster of Differentiation105 (Endoglin)
CD32	Cluster of Differentiation32
CD34	Cluster of Differentiation34
MVD	Mean Vascular Density / Microvessel Density
MCD	Mast Cell Density
MC	Mast Cell
TGF-β	Transforming Growth Factor Beta
ECM	Extracellular Matrix
PG	Pyogenic Granuloma
HRP IHC	Horseradish Peroxidase Immunohistochemistry
DAB	Diaminobenzidine
RGD	Arg-Gly-Asp (Tripeptide)
hTERT	Human Telomerase Reverse Transcriptase
CK	Cytokeratin
EGFR	Epidermal Growth Factor Receptor
bFGF	Basic Fibroblast Growth Factor
MMP-9	Matrix Metalloproteinase 9
COX	Cyclooxygenase
PAI-1	Plasminogen Activator Inhibitor-1
uPA	Urokinase-Type Plasminogen Activator
TSP-1	Thrombospondin-1
ALK1	Activin receptor-like kinase 1
TbetaR-II	TGF- β Receptor II
Smad	Smad Proteins, Involved in TGF- β Signaling
HHT1	Hereditary Hemorrhagic Telangiectasia Type 1
TNF	Tumor Necrosis Factor



ABSTRACT



EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY

ABSTRACT

Background and Objectives:

Among the oral diseases, oral cancer is a critical health issue due to its life-threatening potential.

Lesions with dysplastic features have been categorized under oral potentially malignant disorders (OPMDs), such as Oral leukoplakia, Erythroplakia, Oral Submucous Fibrosis (OSMF) etc. Morphologically altered tissues are highly susceptible to malignant transformation; for example, in leukoplakia, malignancy may arise elsewhere in clinically normal mucosa. Angiogenesis, is one of the essential factor for tumour growth and metastasis. Many studies suggest that mast cells are immune cells which play an active role in angiogenesis. Hence, this study was conducted, to assess the role of angiogenesis by immunohistochemical expression of endothelial cell markers like Endoglin (CD105) and mast cell density using toluidine blue stain. This can help in identifying the biological behaviour of Oral epithelial dysplasia and aid in developing angiogenesis as a novel target for anticancer therapy.

Materials and Methods:

10 tissue samples each of different grades of Oral epithelial dysplasia and pyogenic granuloma as control, were immunohistochemically analysed for expression of Endoglin (CD 105) and mast cell density using Toluidine Blue stain.

Result:

We observed increased micro vascular density which corresponded to an increase in angiogenesis with progressing grades of epithelial dysplasia. Also, an increase in Mast cell density was observed with increasing grades of oral epithelial dysplasia.

EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY

Conclusion:

Angiogenesis plays an important role in the progression of oral epithelial dysplasia which can be quantified by assessing micro vascularity. This can be used to develop strategies to improve the treatment outcome.



INTRODUCTION



EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY

INTRODUCTION

Oral Potentially Malignant Disorders are a group of disorders which possess an increased propensity to transform into a malignancy. These were earlier grouped as precancerous lesions and conditions. Oral leukoplakia, is the most commonly encountered OPMD. Epithelial dysplasia is descriptive of its histological changes. The transition of dysplastic oral epithelium to oral squamous cell carcinoma involves an 'angiogenic switch', marked by enhanced neo-vascularization in the sub- epithelial lamina propria. This increase in blood vessel formation is considered a key indicator of malignant transformation. Mast cells contributes significantly to this process, as they are rich source of various angiogenic factors. In addition, they also release proteolytic enzymes that break down the extracellular matrix, facilitating the formation of new blood vessels. Quantification of angiogenesis can be done by evaluating mean vascular density using Endoglin (CD105) expression and mast cell density using toluidine blue stain. Hence, this retrospective comparative study was conducted to assess the expression of Endoglin in different grades of oral epithelial dysplasia, and to compare the expression of mast cells using toluidine blue stain.



*AIM AND
OBJECTIVES*



**EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL
EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF
MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY**

AIM & OBJECTIVES

Aim: To determine the Mean Vascular Density using Endoglin (CD105) and assess mast cell density using Toluidine Blue in Oral epithelial dysplasia and evaluating their relationship with the biologic behaviour of these lesions.

Objectives:

1. To determine and compare the Mean Vascular Density in various grades of Oral Epithelial Dysplasia using CD105 (Endoglin).
2. To determine and compare Mast Cell Density in various grades of Oral Epithelial Dysplasia using Toluidine Blue Stain.
3. To compare the Mean Vascular Density and Mast cell density in different grades of Oral Epithelial Dysplasia using the Endoglin and Toluidine blue.



*REVIEW
OF
LITERATURE*



EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY

REVIEW OF LITERATURE

ORAL POTENTIALLY MALIGNANT DISORDERS

Oral cancer is the 11th most common cancer in the world, with an estimated 300,000 new cases and 145,000 deaths¹. In India, 20/100,000 population are affected by oral cancer, which accounts for around 30% of all types of cancer². Most cases of oral cancer are associated with habits (tobacco/areca nut) and are preceded by asymptomatic clinical lesions collectively referred to as oral potentially malignant disorder (OPMD)³.

Precancerous lesion was defined as 'a morphologically altered tissue in which oral cancer is more likely to occur than in its apparently normal counterpart'; examples of which include leukoplakia, erythroplakia and palatal changes in reverse smoking. Precancerous conditions were defined as 'a generalized state associated with a significantly increased risk of cancer' and included- Oral submucous fibrosis, Erosive oral lichen, lupus erythematosus etc.

Pre-cancerous lesions and precancerous conditions as they were earlier known (WHO 1978)⁴, are now obsolete; and have been collectively replaced by the term 'Oral potentially malignant disorders'. The change in terminologies were due to the fact that not all precancerous lesions/ conditions gave rise to cancer despite having the potential to do so; also a malignancy could arise at a site distant to the premalignant lesion, showing a field change. The term OPMD was introduced in 2007 following an expert group meeting coordinated by the WHO Collaborating Centre for Oral Cancer⁵.

Introduction of the term OPMD's where all these diseases are grouped under a single term connotes their capability to transform into a malignancy but also means the

EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY

malignant change always may not necessarily happen. Oral potentially malignant disorders are recognised and defined clinically and dysplasia may or may not be present in them. Even when dysplasia is present, it still only indicates a risk of malignant transformation; most dysplastic lesions never transform in the life of the patient and the time to transformation in those that do is highly variable⁶. OPMDs include leukoplakia, erythroplakia, reverse smoker's palate, erosive lichen planus, oral submucous fibrosis, lupus erythematosus and actinic keratosis⁷.

Oral leukoplakia, the most commonly encountered OPMD is defined as "A predominantly white plaque of questionable risk having excluded (other) known diseases or disorders that carry no increased risk for cancer"⁵. The term 'leukoplakia' is a clinical diagnosis of exclusion⁸. Clinically white lesions can histologically range from frictional keratoses, mild, moderate, severe dysplasia and carcinoma in situ to varying grades of squamous cell carcinoma⁹.

Epithelial dysplasia is descriptive of histological changes only and has no clinic-morphological parameter. Dysplasia is the disorderly growth which includes cytological and architectural features¹⁰.

EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY

There are criteria used for diagnosing oral epithelial dysplasia, which are listed as cytological and architectural features (Table 1):¹¹

Table 1: Criteria used for dysplasia

Architectural/Tissue Changes	Cytological Changes
Irregular epithelial stratification	Abnormal variation in nuclear size (anisokaryosis)
Loss of polarity of basal cells	Abnormal variation in nuclear shape (nuclear pleomorphism)
Basal cell hyperplasia	Abnormal variation in cell size (anisocytosis)
Drop-shaped rete ridges	Abnormal variation in cell shape (cellular pleomorphism)
Increased number of mitotic figures	Increased nuclear-cytoplasmic ratio
Abnormally superficial mitoses	Increased nuclear size
Premature keratinization in single cells (dyskeratosis)	Atypical mitotic figures
Keratin pearls within rete ridges	Increased number and size of nucleoli, Hyperchromasia

GRADING OF DYSPLASIA

Neville et al¹² graded dysplasia as:

- i. Mild: Hyperchromatic and slightly pleomorphic nuclei are noted in the basal and suprabasal cell layers of stratified squamous epithelium.
- ii. Moderate: Dysplastic changes extend from the basal layer to the midportion of the spinous layer and are characterized by nuclear hyperchromatism, pleomorphism and cellular crowding. Hyperkeratosis on the epithelial cell layer with prominent granular cell layer.
- iii. Severe: Cellular crowding and disordered arrangement throughout most of the epithelial thickness, although slight maturation and flattening of the cells appears to be present at the epithelial surface. Epithelial cells are seen

EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY

to mature very little as they progress toward the hyperparakeratotic surface.

- iv. Carcinoma in situ: When the entire thickness of the epithelium is involved, the term carcinoma in situ is used. Dysplastic cells extend from the basal layer to the surface of the mucosa (top to bottom change) with no invasion into the underlying connective tissue.

Ljubljana Grading System¹³

This classification was developed to cater for the special clinical and histological problems of laryngeal abnormalities; and this classification was proposed for grading of epithelial hyperplastic lesions of the larynx, to hyperplastic epithelial lesions arising in the oral cavity.

- i. Simple hyperplasia: A benign hyperplastic process with retention of the normal pattern of the epithelium which is thickened because of an increase prickle cell layer. The cellular components of the basal and parabasal region remain unchanged. There is no cellular atypia.
- ii. Abnormal hyperplasia: A benign augmentation of basal and parabasal layers. This is seen upto one and a half of the total epithelial thickness. Stratification is fully retained. Nuclei in the cells of the basal and parabasal layers may be moderately enlarged but still maintain a uniform distribution of nuclear chromatin. Small numbers of epithelial cells, less than 5% are dyskeratotic.
- iii. Atypical hyperplasia or 'risky' epithelium: It demonstrates a recognizable alteration of epithelial cells toward malignancy, but not to such a degree as

EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY

seen in carcinomatous cells. Stratification is still preserved in the general epithelial structure. The nuclei are enlarged and nuclear contour may be irregular with marked variations in staining intensity. The nuclear cytoplasmic ratio is increased. Mitotic figures are increased and are found within two-third of the epithelium. Civatte bodies (apoptotic cells) may be present.

- iv. Carcinoma in situ: It shows features of carcinoma without invasion. There is loss of stratification throughout the epithelium although 3 to 5 layers of compressed cells may be present on the surface. Marked atypia and mitotic abnormalities are characteristic. Mitotic figures present throughout the epithelium, including its upper one-third and abnormal mitoses are frequently found.

WHO System (2005)¹⁴

Grading On the basis of architecture and cytology

- i. Hyperplasia: Describes increased cell numbers. This may be in the spinous layer leading to hyperplasia or acanthosis in the basal/parabasal cell layers (progenitor compartment), termed basal cell hyperplasia. Architecture shows regular stratification and there is no cellular atypia.
- ii. Mild dysplasia: Slight nuclear abnormalities, most marked in the basal third of the epithelial thickness. Cells show normal maturation and stratification. A few, but no abnormal mitoses may be present in the parabasal layers.
- iii. Moderate dysplasia: More marked nuclear abnormalities are seen in the basal two-third of the epithelium. Cell maturation and stratification are

EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY

evident in the upper layers. Mitoses are present in the parabasal and intermediate layers, but none is abnormal.

- iv. Severe dysplasia: Marked nuclear abnormalities involve more than two-thirds of the epithelium. Mitoses, some of which are abnormal, may be present in the upper layers. Maturation and stratification was still seen in most superficial layers.
- v. Carcinoma in situ: It is defined as 'a lesion in which the full thickness, or almost the full thickness, of squamous epithelium shows the cellular features of carcinoma without stromal invasion.' Requires top-to bottom change with undifferentiated, primitive cells from the basal layer to the topmost layer.

Severity of dysplasia is directly correlated with the likelihood of malignant progression, although non-dysplastic lesions can also transform into malignancy¹³.

Previous studies had shown that vascularity increased in a stepwise fashion from normal mucosa to dysplasia to carcinoma and also demonstrated a close association between vascularity and tumor progression in oral mucosa. There are multiple steps that are associated with tumor progression; one of them is neo-angiogenesis, which is essential for tumor growth and metastasis^{15,16}.

Angiogenic switch that occurs in the early stages of malignant transformation, emphasize the role of mast cells in the progression from normal to dysplastic tissues, leading to the development of OSCC¹⁷.

Yokoyama M¹⁸ in their study found an increased microvessel density, inflammation and neovascularization in moderate dysplasia and suggested that, it is at that stage where the switch to malignant transformation occurs. Therefore, it is imperative to

EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY

assess the microvessel density and neo-vascularisation in different grades of oral epithelial dysplasia. The presence of mast cells in the tumor margins may be attributed to higher vascular density. Chymase secreted by the mast cells has been found to be associated with matrix degeneration, angiogenesis, and tumor growth¹⁹.

It has been suggested that use of molecular markers along with clinical and histological grading can better predict the disease progression. Many genes and signaling pathways have shown to be involved in the development of oral squamous cell (OSCC). Molecular markers that correlate oral epithelial dysplasia with malignant transformation include: Overexpression of EGFR, c-Jun, Ki67/Mcm2, Cyclins D and E, p53, p63, MMP-9, TGF alpha, COX-1 and-2, amplification of Cyclin D1, loss of c-erbB2, pRB, Upregulation of telomerase (human telomerase reverse transcriptase; hTERT, aneuploidy, loss of heterozygosity (Chromosome loci 3p, 8p, 9p, 4q, 11q, 13q, 17p), cytokeratins (CK 1, CK 8 and CK 18), high-risk Human papillomavirus, p16²⁰.

ANGIOGENESIS

Angiogenesis, defined as the growth of new blood vessels (neovascularization) from pre-existing ones²¹.

It is a complex process, needed for the continued growth and survival of solid neoplasia. The process of angiogenesis is in itself a multi-step process involving extensive interplay between cells, soluble factors, and extracellular matrix (ECM) components. Sequential steps in angiogenesis include: degradation of basement membrane by proteases; migration of endothelial cells into the interstitial space and sprouting; endothelial cell proliferation at the migrating tip; lumen formation, generation of new basement membrane with the recruitment of pericyte, formation of

EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY

anastomoses and finally the blood flow. Angiogenesis can be determined histologically by the immunohistochemical expression of various endothelial markers like CD31, CD34, VEGF-A, Endoglin etc²².

Under normal circumstances, angiogenesis is a highly ordered process under tight regulation, and it requires inducing quiescent endothelial cells in a monolayer to divide and spread the vascular network only to the extent demanded by demands of growing tissues. Many positively and negatively acting factors that influence angiogenesis; are soluble polypeptides, cell-cell and cell-matrix interactions, and hemodynamic effects. Some of the soluble growth factors, membrane-bound molecules, and mechanical forces that mediate these signals are VEGF, TGF- beta, EGF, Ang1, Ang2, TNF-alpha, Angiogenin, Angiotropin etc. Amongst these, most well-characterized angiogenic factor is vascular endothelial growth factor (VEGF), which, increases endothelial cell permeability, stimulates endothelial cell uPA/PAI-1 production, stimulates endothelial cell proliferation, inhibits endothelial cell apoptosis, enhances endothelial cell migration and stimulates in vivo angiogenesis²³.

In a process very similar to normal angiogenesis, a tumor may elicit formation of blood vessels from pre-existing capillaries. Tumor cells are able to grow around an existing vessel and at least initially, do not need to induce angiogenesis for adequate vascularization²⁴.

Tumor angiogenesis, formation of peritumor and intratumor new blood vessels, is necessary to growth and metastasis of the solid tumors, and is regarded as one of the most important events occurring in the neoplastic process²⁵.

Neovascularization allows tumor growth by supplying nutrient and oxygen, disposing metabolites and releasing growth factors that promote proliferation of the tumor cell²⁶.

EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY

Neoplasms promote angiogenesis by secreting growth factors- vascular endothelial growth factor (VEGF), hepatocytes growth factor and platelet- derived growth factor that stimulate endothelial migration and proliferation. Angiogenesis contributes to the metastatic process by providing large numbers of leaking blood vessels for vascular invasion²⁷.

Proliferating capillaries have fragmented basement membranes and are leaky, making them more accessible to tumor cells than mature vessels. Immunohistochemical expression of various other endothelial markers like CD31, CD34, and Endoglin etc., can also determine angiogenesis²⁸.

Endoglin is highly specific and potent pleiotropic angiogenic factor for endothelial cells of capillaries, veins and arteries. It is used as a specific marker for the assessment of vascularization in both normal and tumour tissues. Endoglin is a proliferation-associated and hypoxia-inducible protein abundantly expressed in angiogenic endothelial cells²⁹.

Endothelial cell proliferation rate of tumor vasculature is exponentially higher than that of the normal vessels. Therefore, micro vessel density in the tumor area, has become the most important predictor in tumor behavior²⁸.

Furthermore, circulating endothelial precursors, angioblast-like cells derived from bone marrow are reported to be present in the adult circulation, have recently been suggested contributing to tumor-derived blood vessels³⁰.

Tumor induced vessels form a conduit for the delivery of metabolites, but ultrastructurally they are abnormal. Many lack functional pericytes, they are dilated and convoluted, and they are exceptionally permeant due to the presence of the fenestrae and transcellular holes and lack of a complete basement membrane.

EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY

Furthermore, tumor vessel walls may be made up of both endothelial cells and tumor cells³¹.

In the majority of cancers, highly vascularized tumors showed a poor prognosis and the influence of tumor angiogenesis proved to be independent of the conventional prognostic indicators. The progression of oral epithelial dysplastic lesions to squamous cell carcinoma is characterized by an increase in neo-vascularization in the sub-epithelial lamina propria and can be considered as an indicator of malignant transformation³².

Angiogenesis, is essential for malignant tumour growth. Neo-vascularization allows growth of the tumour by supplying nutrient and oxygen, disposing metabolites and releasing growth factors that promote tumour cell proliferation, tumour progression in the host stroma and metastasis development. Process of angiogenesis is result of an imbalance among pro- angiogenic factors produced from both tumour and the normal cells. It has been stated that endothelial cells of the tumour vasculature undergo a very more rapid proliferation than quiescent endothelial cells in the vasculature of normal tissues. It has also been reported that that endothelial cells of tumour-associated neovasculature proliferate 20 to 2,000 times more rapidly than the endothelial cells of normal tissue³³.

Quantification of angiogenesis can be done by evaluating mean vascular density using CD105 (Endoglin) expression and mast cell density using toluidine blue stain. Microvessel density in a high power field can be considered proportional to the tumor growth.

EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY

In various types of tumour microenvironments, dysregulated angiogenesis plays an important role in supplying oxygen and nutrients to cancerous cells, leading to tumour size growth.

Vascularization is essential for tumor growth and metastasis. Preceding tumour growth beyond a certain size, hypoxic conditions initiate spontaneous secretion of angiogenic stimulators, which is an important step for transition of a benign tumour to a malignant state³⁴.

Insufficient supply of blood, causes tumor cells to undergo apoptosis and necrosis. Distinct tissue distribution and known functional integration with TGF- β system, highlights the fact that endoglin is involved in angiogenesis.

Angiogenic markers should ideally detect quality and quantity of newborn vessels. High levels of endoglin expression on actively proliferating tumoral endothelial cells on the luminal surface allows for immunoscintigraphy of tumors, which can be used for the diagnostic purposes³⁵.

The ability to visualize and quantify tumor angiogenesis may not only allow antiangiogenic treatment monitoring in patients with cancer but may also be an appropriate approach for screening and detection of cancer at an early, still curable stage, just after the angiogenic switch has occurred in the tumor progression. Several molecular angiogenic markers overexpressed in tumors like $\alpha_v\beta_3$ Integrin, endoglin, and vascular endothelial growth factor (VEGF) receptor (VEGFR), are the best-characterized molecular markers of tumor angiogenesis³⁶.

EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY

The proposition that CD105 may be associated with tumour angiogenesis developed from the fact that CD105 was strongly up-regulated in the endothelium of various tumour tissues compared with that in the normal tissue³⁷.

The number of blood vessels is more in a growing OPMD, as compared to healthy oral mucosa, and it further increases in an enlarging oral squamous cell carcinoma (OSCC)³⁸.

Previous studies done by Shieh YS et al³⁹ and Iamaroon A et al⁴⁰ have shown that vascularity increased progressively from normal mucosa through dysplasia to carcinoma, which suggests that disease progression in the oral mucosa is accompanied by angiogenesis.

Shivakumar Madappa Shivamallappa⁴¹ in their study implied that, in the development of OSCC, angiogenic phenotypic change occurs in carcinomas rather than pre-cancerous stage.

Previous studies have shown that vascularization increased with an increasing degree of dysplasia by microvascular volume^{32,41}.

Mast cells have surface receptors which when stimulated may release pro-angiogenesis factors, such as vascular endothelial growth factor (VEGF), basic fibroblastic growth factor, transforming growth factors, IL-8, matrix metalloproteinase, tryptase and chymase that participate in the neoangiogenesis or vascular formation process. It has been hypothesized that the angiogenic phenotype may result from the production of these growth factors by the tumor cells and/or the down-regulation of negative modulators, like thrombospondin-1 (TSP-1) in tissues with dormant vasculature⁴².

EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY

MAST CELLS

Mast cells (MCs) are bone marrow-derived tissue-homing leukocytes, first described by Paul Ehrlich. They are highly versatile cells playing important role in large spectrum of biological settings, including inflammation, angiogenesis, tissue repair, tissue remodelling and cancer⁴³.

They are component of the immune system which play an important role in host defence. They reside in all the vascularised tissues, especially in all the connective tissues and mucosal surfaces of the human body⁴⁴.

Stem Cell Factor plays an important role in the survival of mast cells because it stimulate directional motility of mucosal and connective tissue type of mast cells⁴⁵.

Shoshana D, Glalinsky T, Nechustan H⁴⁶ in their study demonstrated tumour growth induction secondary to activation of mast cell. Diverse mechanisms such as the release of pro-angiogenic inducers and activation of CD30 by mast cells have been reported.

Mast Cells are heterogeneous in shape, varying from round, oval, or spindle-shaped, about 12 microns (diameter) with 50 - 100 packed granules. It is often difficult to distinguish fibroblasts from these cells in routine hematoxylin and eosin (H&E) stain owing to its similarities in staining characteristics. Hence, a selective metachromatic stain of 1% toluidine blue was used first in 1856 by William Henry Perkin (British chemist) who illustrated purplish-red MCs and their nuclei appearing sky blue in color⁴⁷.

Mast cells are easily also recognized in light microscopy by toluidine blue staining because metachromatic granules that fill the cytoplasm. In normal oral tissues, mast

EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY

cells have been found to be present in the connective tissue of gingiva, tongue and lining mucosa, also reported in normal periodontal ligament and pulp although in very low densities. The numerous cytoplasmic granules in the mast cells bind to basic dyes such as toluidine blue and show metachromatic staining properties⁴⁸.

Under physiological conditions, they are particularly prominent near capillaries and also lymphatic channels. In many inflammatory disorders, they are characterized by profound vascular remodelling, and the infiltrate exhibits numerous mast cells which show structural features of degranulating elements. In various tumors, mast cells appear at the edges of invasive tumors, facilitate angiogenesis by releasing preformed mediators or by triggering proteolytic release of extracellular matrix bound angiogenic compounds⁴⁹.

In the early phase of hyperplasia and dysplasia, infiltrating mast cells degranulate and activate dermal fibroblasts which intensify angiogenesis. They also activate progelatinase B (matrix metalloproteinase family), involved in extracellular remodelling and angiogenic regulation and progressively intensify angiogenesis by releasing sequestered angiogenic activators. As the neoplastic sequence progresses, angiogenic growth factor gene expression is upregulated in the cancer cells. This is progression to the second cancer phase where tumor cells directly control their angiogenic phenotype instead of manipulating inflammatory cells to indirectly affect neovascularization⁵⁰.

Rakesh S et al⁴⁸ in their study reported that mast cells and their regulatory role in angiogenesis and inflammation by the release of mediators may play an important role in tumor progression, facilitating the transformation of oral leukoplakia into invasive carcinoma.

EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY

Mast Cells accumulate in inflammatory foci around the blood vessels and later around the tumor margins and release pro-angiogenic factors which favor tumor progression^{51,52}.

Mast Cells are immune cells which are a good source of proteases (tryptase and chymase) which directly degrade the extracellular matrix through their proteolytic activity, indirectly facilitates invasion and metastasis through extracellular matrix remodelling also stimulates angiogenesis. They hence impact the progression of dysplastic lesions to malignancy. Mast cells can be easily quantified using a simple easily available histochemical stain like Toluidine blue, Alcian blue etc⁵³.

Metachromatic dyes such as toluidine blue, causes more clearer visual detection of mast cells, hence this could prove to be a reliable method to be used for the same. The reliability of this technique is strengthened by the non-significant inter observer variability.

TOLUIDINE BLUE STAIN

Chairside techniques that are assessed to improve early detection and diagnosis of oral malignancy include toluidine blue, vizilite plus with toluidine blue, velscope brush biopsy, etc. Toluidine blue is a cationic metachromatic dye that may selectively bind to free anionic groups such as sulfate, phosphate, and carboxylate radicals of large molecules. It has been used for decades as an aid to the identify mucosal abnormalities in the oral cavity. Toluidine blue, also known by its chemical name tolunium chloride, is a basic metachromatic dye that is known for its property of differentially staining malignant neoplasm but not normal epithelium⁵⁴. It can be used not only as a supravital stain; but also to stain tissue biopsies.

EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY

Mast cells stain metachromatically with toluidine blue with greater intensity in cells having smaller granules. Most stains for mast cells detection rely on the cell's content of heparin, other glycosaminoglycans, and esterase. Mast cells are found in the connective tissue and their cytoplasm contains granules (metachromatic) composed of heparin and histamine. Mast cells are difficult to differentiate with Haematoxylin and Eosin, but metachromatically stain with toluidine blue. Mast cell granules appear purplish blue with nuclei- sky blue with the background blue (orthochromatic staining)⁵⁵.

Metachromasia is a phenomenon whereby a dye may absorb light at different wavelengths depending on its concentration and surroundings. The dye, which exhibits this phenomenon without changing its chemical structure, is said to be metachromatic in nature. When the tissues are stained with a concentrated basic dye solution, like toluidine blue, the bound dye molecules are close enough to form dimeric and polymeric aggregates. In dilute solutions, dyes such as toluidine blue are monomeric and it exhibits blue colour. In a concentrated or hypertonic solution, dye molecules polymerize and exhibits a metachromatic color shift⁵⁶.

Hence, effect of polyanions, because of their high-density negative charge, is to functionally polymerize toluidine blue molecules in tissues and metachromatic staining reaction is a strong predictor of tissue polyanions⁵⁷. Mast Cells accumulate in inflammatory foci around the blood vessels and later around the tumor margins⁵⁸.

Toluidine blue is an easily available and economical metachromatic dye. Various studies have reported the use of toluidine blue as screening tool for oral malignant and pre malignant lesions with sensitivity and specificity between the ranges of 93.5%, 97.8%, 73.3% and 92.9%⁵⁹.

EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY

Metachromatic property of toluidine blue imparts purple or reddish-blue color to the sulphated granules (mostly heparin) of the mast cell, depending on the degree of polymerization of the dye, making it easier to recognize. More recently it has been recommended as an adjunctive method to assist in early detection of oral premalignant lesions and oral squamous cell carcinoma, also in determining oral premalignant lesion at risk of progression to carcinoma⁶⁰.

MAST CELLS IN EPITHELIAL DYSPLASIA

Mast cells secrete various proteolytic enzymes which degrade the extracellular matrix and create space for developing blood vessels⁶¹.

Among the host immune cells, mast cells have been implicated in tumour progression as it promote angiogenesis. Several proangiogenic and angiogenic factors released by these cells are-histamine, heparin, chymase, basic fibroblastic growth factor, vascular endothelial growth factor and transforming growth factor⁶².

Study by Jandinski JJ, Sonis S, Doku HC⁶³ in their study revealed increase in number of mast cells from normal to hyperkeratotic and dyskeratotic tissues, but this increase was not significant while the number of mast cells increased significantly from normal tissue to low grade carcinoma. They suggested that this increase in number is due to antigenic stimulation. Further, they revealed that number of mast cells decreased in medium to high grade carcinomas, and attributed this to an unfavourable cellular environment.

Biviji AT⁶⁴ in their study showed an increase in the number of mast cells/unit microscopic field in oral leukoplakia (which represents increase in the thickness of the epithelium, its keratinization without dysplasia) compared to normal mucosa. They

EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY

concluded that pharmacologically active agents in the mast cells might contribute to inflammatory reaction, which is seen in leukoplakia.

Flynn E, Schwartz j, Shklar G⁶⁵, reported direct co-relation between sequential mast cell infiltration, activation and distinct stages of hyperkeratosis, dysplasia, carcinoma in-situ in the oral cavity and implicated role of mast cells in configuring the angiogenic phenotype in premalignant lesions.

A hypothesis was suggested that hypoxia might induce tumour cells to release the angiogenic factors, which in turn could attract mast cells to migrate into the hypoxic areas of the tumour, and after migration into the hypoxic areas, mast cells might produce stimulating factors that help in further angiogenesis⁶⁶. As mentioned- Histamine, heparin, chymase, basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), and transforming growth factor- β (TGF- β) etc. are pro-angiogenic factors that either directly promote angiogenesis by stimulating the migration and/or proliferation of endothelial cells or indirectly through degradation of the extracellular matrix⁶².

Hegde Veda and Marla Vinay⁶¹ in their study concluded that tumours that are rapidly growing may have a high nutritive demand that is provided by the vasculature, and when the epithelium is altered as in oral epithelial dysplasia, recruitment of inflammatory cells is noted. The inflammatory and mast cells that migrate to areas of altered epithelium may stimulate angiogenesis by secreting proangiogenic and angiogenic components, either directly or indirectly prior to invasion. Once invasion is established as in oral squamous cell carcinoma, the role of mast cells is probably shifted from angiogenesis to further promoting invasion.

EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY

Increased mast cells are also been associated with squamous cell carcinomas. A correlation between mast cell numbers and poor outcome was reported by **Iamaroon et al**⁴⁰ on oral squamous cell carcinoma.

As the neoplastic sequence progresses, angiogenic growth factor gene expression is upregulated in cancer cells and this is the progression to the second cancer phase where tumor cells directly control their angiogenic phenotype instead of manipulating inflammatory cells to indirectly affect neovascularization⁶⁷.

A study done by **Dantas RCM et al**⁶⁸, showed no significant difference in MCD (Mast cell density) between mild and moderate OED samples, and their findings also indicated that MCs are present in the tumor microenvironment and may be associated with a better prognosis.

In the study done by **Telagi N et al**⁶⁹, increased numbers of mast cells were seen in oral epithelial dysplasia, oral submucous fibrosis and OSCC, and the malignant lesions showed highest number of mast cells as compared with the premalignant lesions.

Sathyakumar M et al⁷⁰ in their study showed an exponential increase in microvessel density as mast cell density increased. There was significant role of mast cells in angiogenesis as it progresses from normal mucosa to dysplasia. The number of mast cells and microvessel can be used as indicators of the disease progression.

Hegde Veda and Marla Vinay⁶¹ in their study showed an increase in mast cell in response to factors released by the tumour cells, and in response to infections and microvessel density from normal oral mucosa to oral epithelial dysplasia to oral squamous cell carcinoma.

EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY

CD105

Endoglin, a 180 kDa transmembrane protein, which occurs in two isoforms (L and S) because of alternate splicing of the transcript, and it shows higher expression in proliferating endothelial cells. CD105 expression in tumor and normal endothelium are highly correlated⁷¹.

Amol Gadbail et al⁷² conducted study to determine mutations in the ENG gene, which encodes CD105, associated with hereditary hemorrhagic telangiectasia type 1 (HHT1), a genetic disorder which is characterized by vascular malformations and recurrent bleeding. Given its dual role in promoting vascular integrity and being implicated in pathological angiogenesis, CD105 is a critical for understanding the vascular biology and developing therapeutic strategies.

This auxiliary TGF- β receptor transmembrane glycoprotein is expressed on activated endothelial cells participating in neoangiogenesis. Evaluation of MVD using endoglin can be an effective tool in quantification of neovascularization in carcinomas and also determines the metastatic potential of the tumor and its prognosis⁷³.

Human endoglin is a 633 amino acid, 180 kDa homodimeric disulfide-linked hypoxia-inducible transmembrane glycoprotein and it contains a large extracellular domain, a hydrophobic transmembrane domain, and a short intracellular domain. The extracellular domain contains an Arg-Gly-Asp (RGD) tripeptide, and four *N*-linked glycosylation sites and a region of O-linked glycosylation^{74,75}. The intracellular domain contains many serine and threonine residues, some of which are phosphorylation sites⁷⁶. The endoglin gene is 40 kb long and it is located on human chromosome 9q34⁷⁷.

EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY

Gene transcription results in a mRNA product with a length of 3.4 kb and 14 exons; exons 1 to 12 encode the extracellular domain, and the exon 13 encodes the transmembrane domain, and the exon 14 encodes the cytoplasmic domain. Endoglin was assigned the cluster of differentiation number 105 at the fifth International Workshop on Human Leukocyte Differentiation Antigens and thus is also known as CD105⁷⁸.

Endothelial cells in normal quiescent endothelium are stable, have a very low turnover rate, with a doubling time of more than 1,000 days. Angiogenic endothelium, in contrast, has a rapid turnover, has been termed “activated”. One potential marker of this activated endothelium is endoglin. In several malignancies, endoglin is found on the peritumoral and intratumoral vessels⁷⁹.

TGF- β signaling in endothelial cells can mediate variety of processes, like proliferation, migration, and adhesion. TGF- β is expressed in endothelial cells of healing wounds, embryos, infected tissues, psoriatic skin, inflamed synovial arthritis, and solid tumors. Low doses of TGF β in vitro (0.25-0.70 ng/mL) stimulate endothelial cell proliferation and migration, whereas higher doses inhibit them, which endothelial cell quiescence⁸⁰.

Stimulatory phenotype in endothelial cells is mediated by ALK1-dominated signaling, and endoglin expression is necessary for the activation of this pathway. ALK1 activation causes increase in endothelial cell proliferation and migration as well as transcription of the proangiogenic genes Id1, interleukin- 1 receptor-like 1, and endoglin itself⁸¹.

In endoglin-expressing cells, endoglin is included as a dimer in this receptor complex, and TbetaR-II then phosphorylates either ALK1 or ALK5 in a highly conserved

EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY

glycine/serine-rich cytoplasmic domain, causing a conformational change in the receptor. Signaling is propagated to the nucleus via phosphorylation of Smad proteins, which act as transcriptional coactivators or corepressors. In endothelial cells, endoglin expression is up-regulated by hypoxia, TGF- β stimulation, and irradiation in vitro, whereas endoglin is down-regulated by tumor necrosis factor- α ⁸².

This (CD105) transmembrane phosphorylated glycoprotein, a fundamental constituent of the TGF- β receptor signaling pathway. The proliferation and differentiation of cells is modulated by this pleiotropic cytokine, which is fundamental for neo-angiogenesis and vascular growth. Multiplying endothelial cells show upregulation of CD105, and so is considered as a principal marker of proliferation of endothelial cells of newly formed blood vessels that is neoangiogenesis²⁹.

EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY

According to Nikolaos A. Dallas et al⁷⁹, expression of CD105 expression in normal human tissue is shown in Table 2:

Table 2. Endoglin (CD105) expression in normal human tissues

Location	Cell type
Cardiovascular system	Endothelial cells Vascular smooth muscle cells
Hematologic system	Bone marrow stromal fibroblasts Progenitor B cells Erythroid precursors Circulating CD34 ⁺ cells
Reticuloendothelial system	Interstitial macrophages in splenic red pulp Activated monocytes Differentiated macrophages Follicular dendritic cells in lymphoid organs
Genitourinary system	Kidney extraglomerular interstitial cells Kidney glomerular mesangial cells Seminiferous tubule basal cells
Embryo	Syncytiotrophoblasts of placenta Embryonic heart mesenchymal cells

There is a strong association of Endoglin expression in tumor endothelium than in normal endothelium. Hence, used as a better immunohistochemical marker in assessing the microvascular density than any other endothelial marker.

CD105 IN EPITHELIAL DYSPLASIA

Several reports have shown that CD105 expression was related to metastasis, whereas only few studies investigating CD105 expression in head and neck regions have been reported. Endoglin CD 105 expression was studied by many workers in various oral lesions like, oral submucous fibrosis, angiogenic squamous dysplasia-like phenomenon in oral epithelial precursor lesions .

EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY

According to **Raunak Kumar Das, et al**⁸², expression of Endoglin CD 105 in normal oral mucosa was completely absent. The expression of CD 105 in epithelial dysplasia, in premalignant lesions, though variable, but is expressed faintly in oral submucous fibrosis, it became remarkable in dysplastic conditions, with maximum presence in severe oral submucous fibrosis condition, which indicates that CD105 expression, a marker for tumour vasculature, was upregulated in dysplastic conditions.

Quantification of MVD with CD105 in a study by **Maharudrappa Basnaker et al**²⁸ reveals that the highest density was present in tumour specimens, than in normal healthy mucosa and dysplastic epithelium.

According to **Daniel GE Thiem et al**⁸³, microvessel density (MVD) is a parameter for evaluation of new angiogenetic patterns, which has shown to be a prognostic factor for several malignant lesions including oral invasive carcinoma, and angiogenesis increases during the transition from OL through dysplasia to OL-OSCC.

In the study done by **Neda Kargahi et al**⁸⁴, MVD was used for quantitative angiogenesis assessment and evaluation of CD105 positive microvessels and it was significantly higher in oral invasion carcinoma than in pre-cancerous mucosa, and there was no expression in normal mucosa. There was also a significant increase from pre-cancerous mucosa to malignant lesions. Therefore, evaluation of CD105 marker can be a helpful method to distinguish the premalignant lesions from malignant ones.

Amina Fouad Farag et al⁸⁵ in their study showed, MVD increased with disease progression from the low risk to the high risk OED cases then from the low grade to the high grade OSCC cases. When compared to CD31, CD105 as a marker had a greater specificity for tumor-induced angiogenesis and provided more accurate measures and easier measuring protocol with no discrepancies.

EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY

A Bhargav Kumar Gudipati et al⁸⁶ in their study showed that Mean Vascular Density (MVD) was not significant between three different groups of OSCC but there was significant difference between normal mucosa and different grades of dysplasias when compared with OSCC. WDSOC showed high amount of MVD compared to all the other groups.

Timely diagnosis and treatment can prevent the progression of OPMD to oral cancer. With advancements in science and technology, numerous novel techniques have been developed that have advantages over currently practiced conventional diagnostic methodologies⁸⁷. Conventional diagnostic technique involves histopathological evaluation of tissues. Immunohistochemical markers are not only diagnostic but they also predictive and prognostic. Hence quantification of Endoglin in oral epithelial dysplasia can be used as predictive or a prognostic marker.

In the present study, toluidine blue stain will be used for the identification and quantification of MCs. Expression of Endoglin and Mast cells in the different grades of oral epithelial dysplasia can thereby add as predictive markers.



*MATERIALS
AND
METHODS*



EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY

MATERIALS AND METHOD

This retrospective comparative study was conducted using the archival slides of pre-diagnosed cases from the Department of Oral Pathology and Oral Microbiology, Babu Banarasi Das College of Dental Sciences, BBD University, Lucknow

Study sample: 10 cases each of different grades (Mild dysplasia, moderate dysplasia and severe dysplasia) of Oral epithelial dysplasia and pyogenic granuloma as control, were immunohistochemically analysed for expression of Endoglin (CD-105) to assess microvessel density and mast cell density using toluidine blue stain.

Pyogenic Granuloma (PG) was used as control because it is an inflammatory condition which is known to have increased number of blood vessels than the normal oral mucosa. Normal oral tissues showed lesser number of blood vessels; hence, PG served as a better control.

Eligibility Criteria:

Inclusion criteria:

- Cases previously diagnosed and reported as mild, moderate or severe Oral epithelial dysplasia
- Cases previously diagnosed and reported as Pyogenic granuloma.

Exclusion Criteria:

- Cases of previously diagnosed Oral Squamous Cell Carcinoma

Sampling Method: Random Sampling

Methodology:

One section each were taken to stain with Hematoxylin & Eosin, Toluidine Blue and CD105 respectively, thereby divided into 3 groups.

EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY

Group 1 (10 samples): Mild dysplasia

Group 2(10 samples): Moderate dysplasia

Group 3(10 samples): Severe dysplasia

Control sections (10 samples): Pyogenic Granuloma

A total of 40 formalin-fixed, paraffin-embedded tissue samples were collected and mounted on slides by consecutive, non-probability sampling technique.

Methodology for CD105

One section of 3-4 μm thickness was taken from each block. CD 105 (BioGenex) antibody was applied onto sections on Poly-L-lysine coated slides. Antigen retrieval was done by transferring the slides to citrate buffer of pH 6.2 and autoclaved at 15 lbs pressure for 30 min. The slides were treated with 3% hydrogen peroxidase for 10 min to quench endogenous peroxidase activity. Prediluted primary antibody, namely mouse monoclonal CD105 antibody (BioGenex), was added to the sections and incubated for 60 min in a hydrated chamber. The sections were then washed with tris buffer for 2–3 min and a super-enhancer reagent was added and incubated for 30 min. Then, the sections were washed with tris buffer for 2–3 min and incubated for 60 min with a secondary antigen that is polymer-Horseradish peroxidase immunohistochemistry, 3,3' (HRP IHC) detection system. Chromogen Diaminobenzidine (DAB) was added and incubated for 20 min, then washed with tris buffer and counterstained with hematoxylin. Specimens of pyogenic granuloma known to express CD105 positive cells were used as positive control. CD105 staining is localized to cell membranes of the endothelium of blood vessels. Prior to assessment, slides were dehydrated with immersion in 70%, 90% and 100% ethanol

EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY

for 5 min each respectively, followed by Xylene immersion (5 min) and mounted using DPX⁷³.

Scoring criteria for CD105:

Stained sections were interpreted microscopically under the penta Head (Olympus Magnus MLXi Plus). Slides were first be screened using a low-magnification objective lens. The areas with the highest number of positively stained vessels (hot spots) were identified and selected. At x400 magnification (0.0625 mm²), the microvessels (recognized by brown cytoplasmic staining by CD105) were counted using oculometer grid with 100 subdivisions. This was done for 05 fields and average of the 05 fields were taken. The criteria adopted by Weidner⁸⁸, were used and only strong positively stained endothelial cell or cell cluster, which was easily separated from adjacent micro vessel and other connective tissue elements were considered as a distinct countable micro vessel. Two observers simultaneously evaluated all the slides by penta head microscope (Olympus Magnus MLXi Plus). Each micro vessel which was agreed upon by both observers were included in the count. Micro vessel density is defined as the number of CD105 (Endoglin) stained vessels per mm².

Formula for calculation of microvessel density= (Number of microvessels in x400 grid field)/Area of ocular grid (0.0625 mm²)⁷⁰.

Methodology for Toluidine Blue⁸⁹

Toluidine Blue staining was done for all grades of epithelial dysplasia and mast cells were assessed.

Tissues fixed in 10% Formalin were cut at 5 um paraffin sections.

Solutions and Reagents:

Toluidine Blue Stock Solution:

**EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL
EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF
MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY**

Toluidine blue O (Sigma) ----- 1 g

70% alcohol ----- 100 ml

Mixed to dissolve.

Sodium Chloride (1%):

Sodium chloride ----- 0.5 g

Distilled water ----- 50 ml

Mixed to dissolve (made this solution fresh each time). Adjust pH to 2.0~2.5

using glacial

acetic acid or HCl.

Toluidine Blue Working Solution (pH 2.0~2.5):

Toluidine blue stock solution ----- 5 ml

1% Sodium chloride, pH 2.3 ----- 45 ml

Mixed well. The pH should be around 2.3 and less than 2.5

Made this solution fresh and discarded after use. pH higher than 2.5 will make staining less

contrast.

Procedure:

1. Deparaffinize and hydrate sections to distilled water.
2. Stain sections in toluidine blue working solution for 2-3 minutes.
3. Wash in distilled water, 3 changes.
4. Dehydrate quickly through 95% and 2 changes of 100% alcohol (10 dips each since stain fades quickly in alcohol).
5. Clear in xylene or xylene substitute, 2 changes, 3 minutes each.

**EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL
EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF
MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY**

6. Coverslip with resinous mounting medium.

Identification & Scoring criteria for Mast cells:

Mast cells ----- Violet/Red purple

Background ----- Blue

Stained sections were interpreted microscopically under the penta head microscope (Olympus Magnus MLXi Plus). Slides were first be screened using a low-magnification objective lens. The areas with the highest number of positively stained vessels (hot spots) were identified and selected. Atx400 magnification (0.0625 mm^2), the mast cells (recognized by blue cytoplasmic staining by Toluidine blue) were counted using oculometer grid with 100 subdivisions. This was done for 05 fields and average of the 05 fields were taken. The criteria adopted by Weidner⁸⁸, was used and only strongly stained mast cells were considered. Two observers simultaneously evaluated all the slides by penta head microscope (Olympus Magnus MLXi Plus). Each mast cell which were agreed by both observers was included in the count. Mast cell density is defined as the number of mast cells per mm^2 .

Formula for calculation of mast cell density= (Number of mast cells in x400 grid field)/Area of ocular grid (0.0625 mm^2)⁷⁰.



LEGENDS



**EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL
EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF
MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY**

LEGENDS

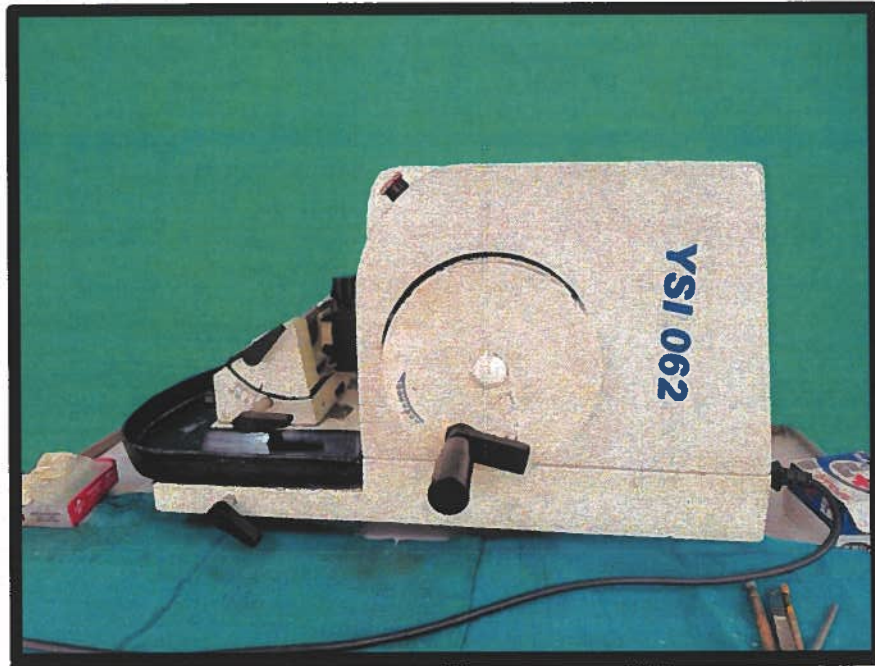


Figure 1: Semi-Automatic Rotary Microtome (Yorco YSI 062)



Figure 2: Decloaking chamber

**EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL
EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF
MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY**



Figure 3: Reagents used for Hematoxylin & Eosin staining

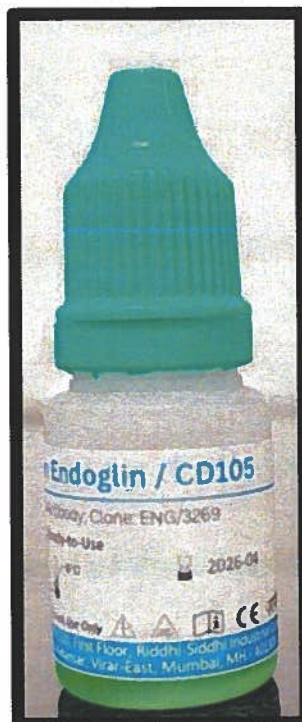


Figure 4: CD 105 antibody (BioGenex) used for Immunohistochemical staining

**EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL
EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF
MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY**



Figure 5: Other reagents used for Immunohistochemical staining



Figure 6: Tris buffer

**EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL
EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF
MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY**

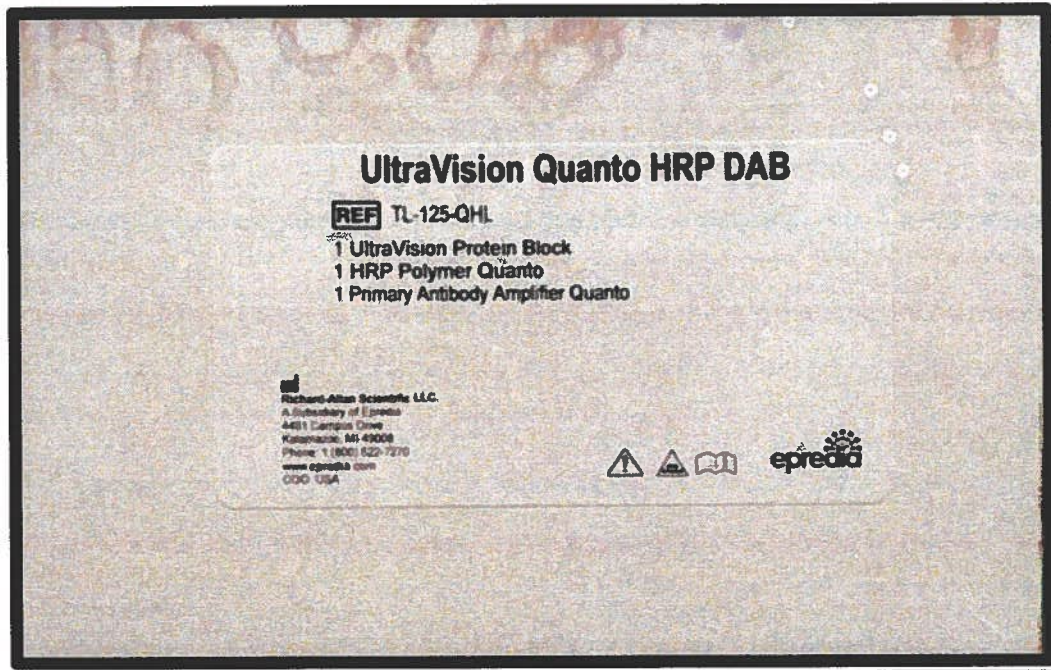


Figure 7: Ultra Vision HRP DAB

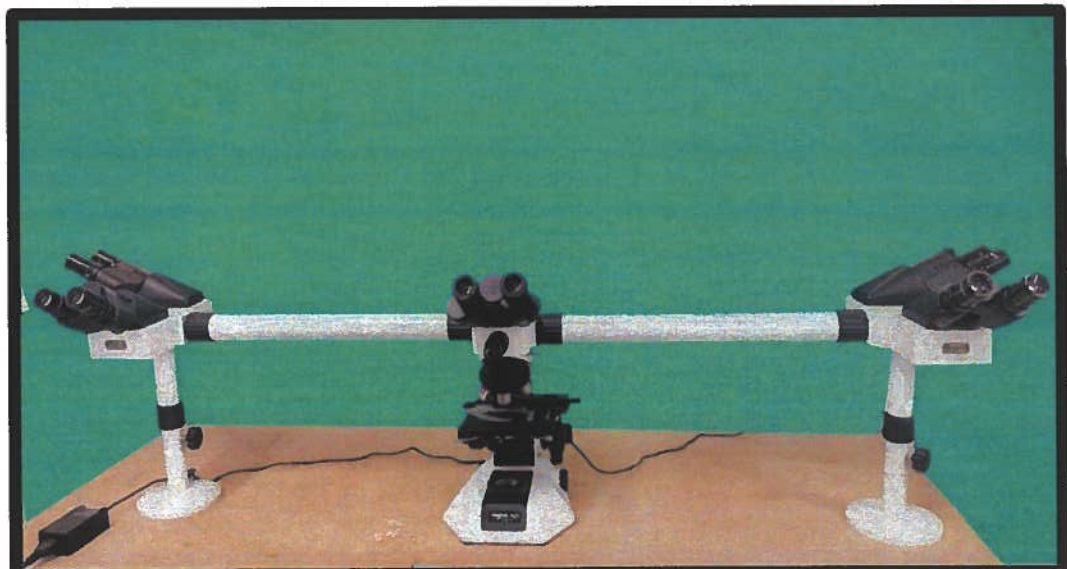


Figure 8: Pentahead microscope (Olympus Magnus MLXi Plus)

**EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL
EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF
MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY**

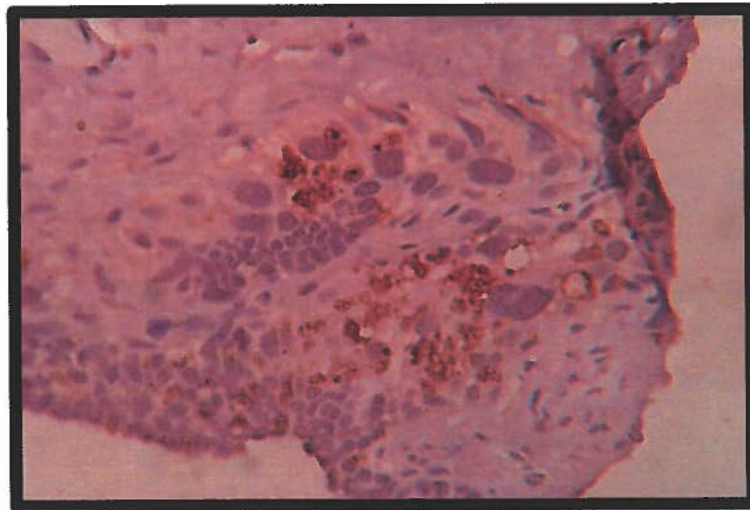


Figure 9: Control Section for CD105

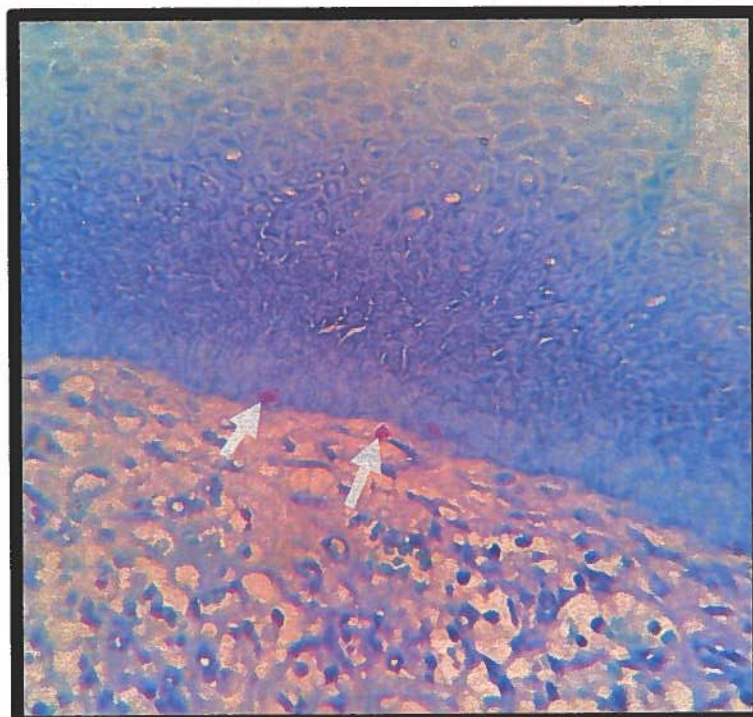


Figure 10: Photomicrograph showing mast cells in Mild dysplasia (Toluidine Blue stain) (x400)

**EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL
EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF
MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY**

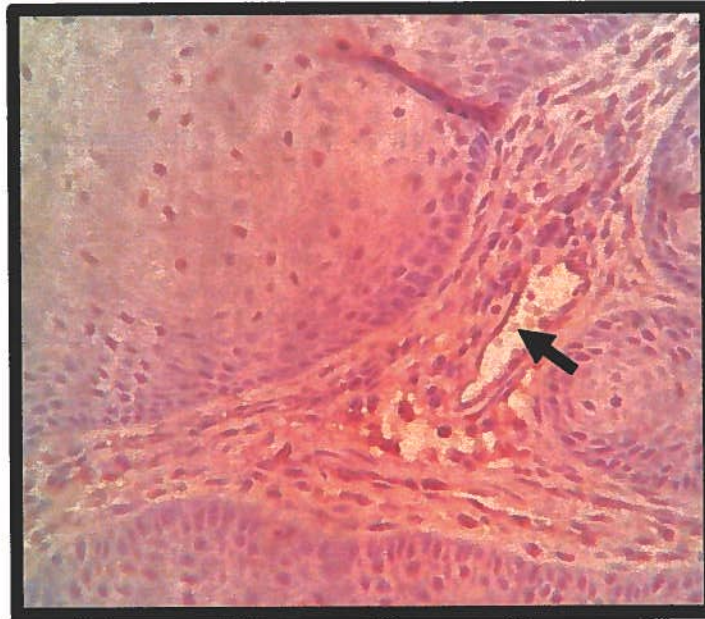


Figure 11: Photomicrograph showing CD 105 positive endothelial cells in Mild dysplasia (x400)

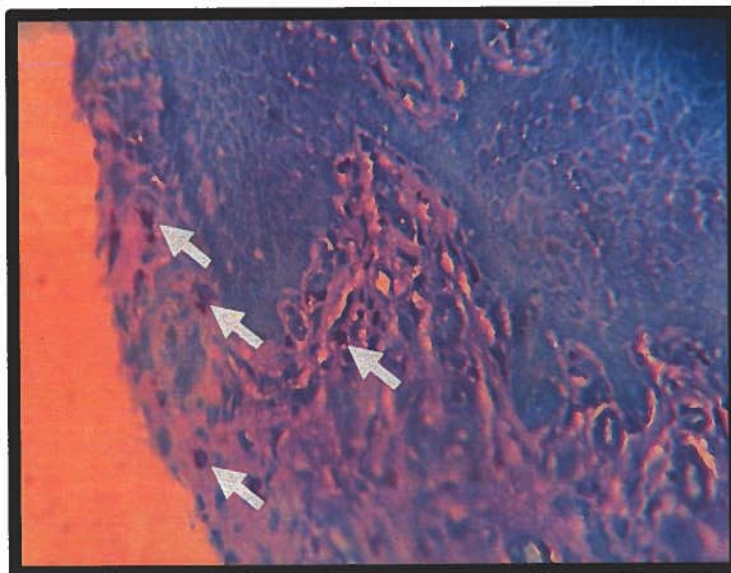


Figure 12: Photomicrograph showing mast cells in Moderate dysplasia (Toluidine Blue stain) (x400)

**EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL
EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF
MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY**

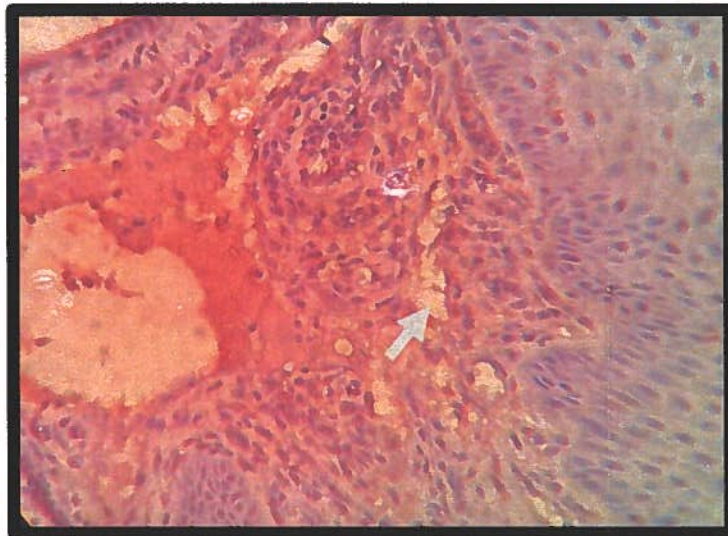


Figure 13: Photomicrograph showing CD 105 positive endothelial cells in Moderate dysplasia (x400)

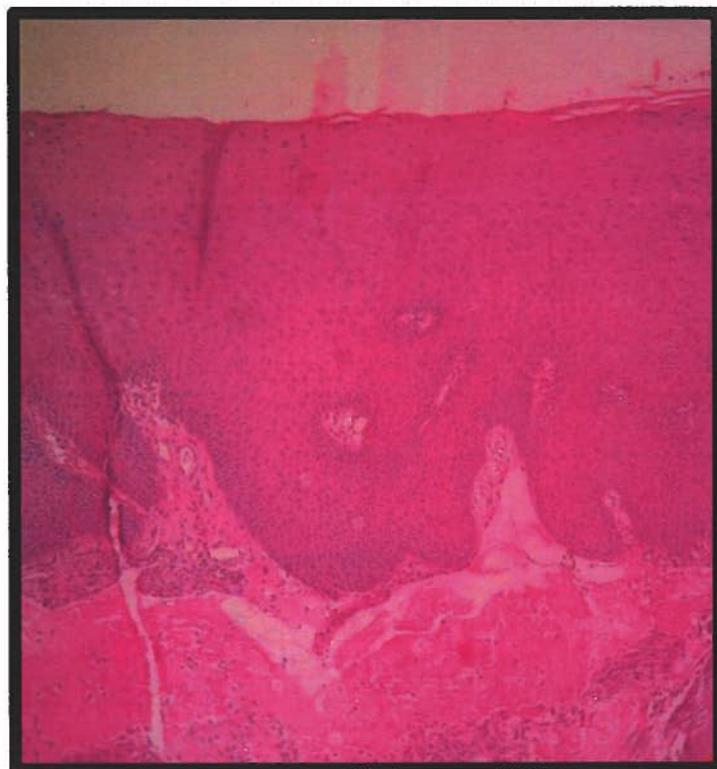


Figure 14: Photomicrograph showing H & E stained section of Severe dysplasia (x400)

**EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL
EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF
MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY**

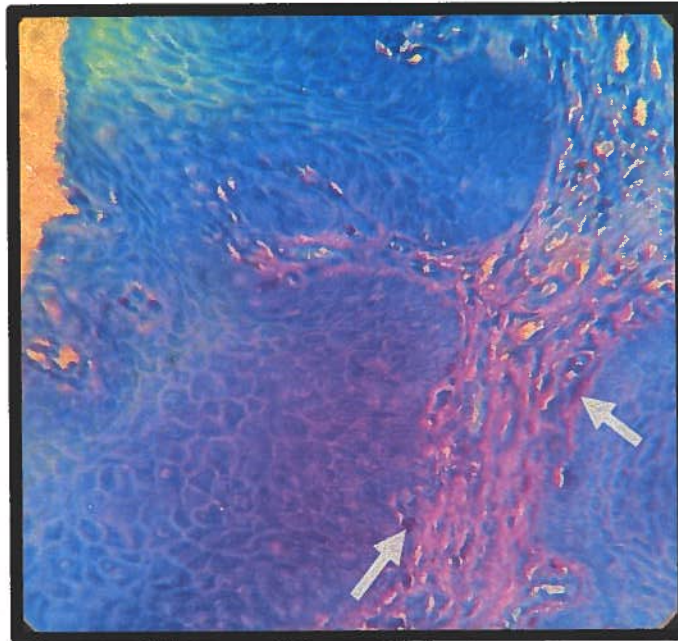


Figure 15: Photomicrograph showing mast cells in Severe dysplasia (Toluidine Blue stain)(x400)

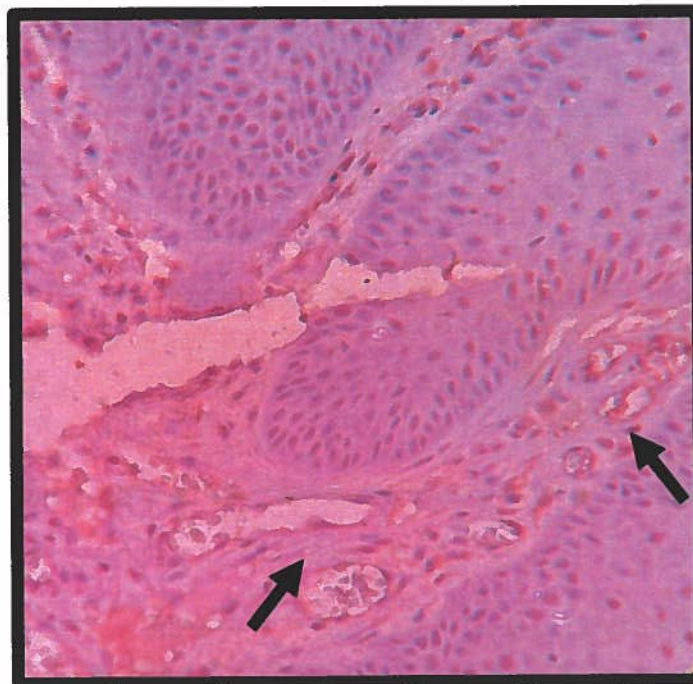


Figure 16: Photomicrograph showing CD 105 positive endothelial cells in Severe dysplasia (x400)



*OBSERVATIONS
AND
RESULTS*



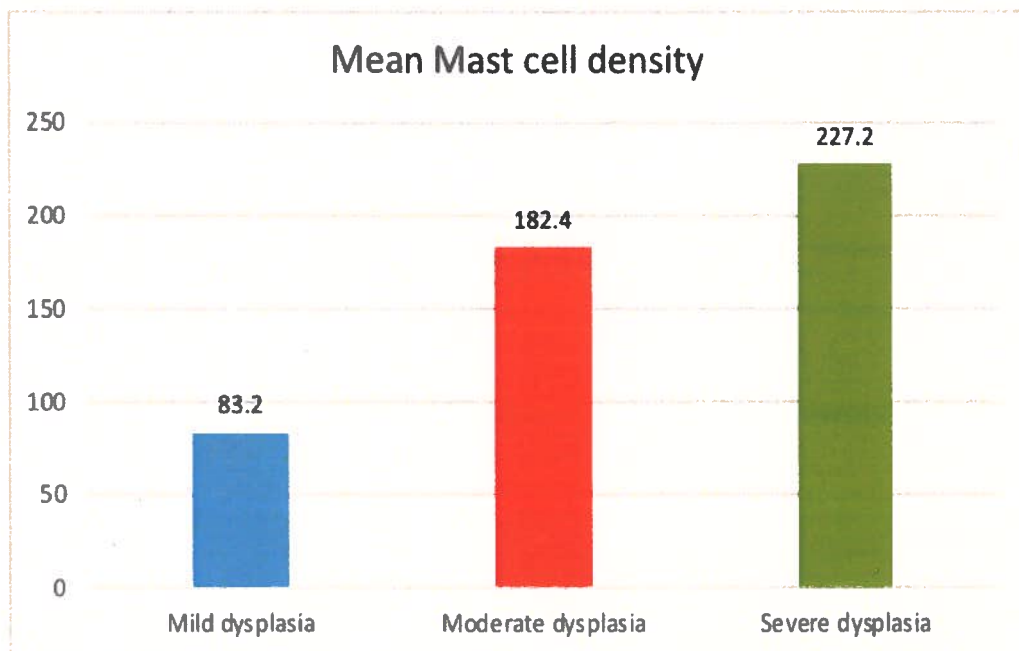
**EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL
EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF
MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY**

OBSERVATIONS AND RESULTS

Table 3: Descriptives of Mast cell density with respect to different grades of dysplasia

Mast cell density					
	N	Mean	Std. Deviation	95% Confidence Interval for Mean	
				Lower Bound	Upper Bound
Mild dysplasia	10	83.2000	6.74619	78.3741	88.0259
Moderate dysplasia	10	182.4000	8.26236	176.4895	188.3105
Severe dysplasia	10	227.2000	6.74619	222.3741	232.0259

Table 3 showed the descriptives of Mast cell density with respect to different grades of dysplasia. Mild dysplasia cases showed the minimum mast cell density while the severe dysplasia cases showed the maximum mast cell density.



Graph 1: Mean Mast cell density with respect to different grades of dysplasia

**EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL
EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF
MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY**

Table 4: Intergroup comparison of Mast cell density

ANOVA					
Mast cell density					
	Sum of Squares	df	Mean Square	F	P value
Between Groups	108612.267	2	54306.133	1022.786	<0.001, S
Within Groups	1433.600	27	53.096		
Total	110045.867	29			

Intergroup comparison of Mast cell density was done using one way ANOVA test.

Overall, a statistically significant difference was observed. In other words, it can be said that the mean mast cell density was significantly different among all the three types of dysplasia.

**EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL
EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF
MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY**

Table 5: Post hoc pairwise comparisons of mean Mast cell density using Tukey's test

Multiple Comparisons						
Dependent Variable: Mast cell density						
Tukey HSD						
(I)	(J)	Mean	Std.	P value	95% Confidence Interval	
dysplasia	dysplasia	Difference (I-J)	Error		Lower Bound	Upper Bound
Mild dysplasia	Moderate dysplasia	-99.20000*	3.2587 2	<0.001, S	-107.2797	-91.1203
	Severe dysplasia	- 144.00000*	3.2587 2	<0.001, S	-152.0797	-135.9203
Moderate dysplasia	Mild dysplasia	99.20000*	3.2587 2	<0.001, S	91.1203	107.2797
	Severe dysplasia	-44.80000*	3.2587 2	<0.001, S	-52.8797	-36.7203
Severe dysplasia	Mild dysplasia	144.00000*	3.2587 2	<0.001, S	135.9203	152.0797
	Moderate dysplasia	44.80000*	3.2587 2	<0.001, S	36.7203	52.8797

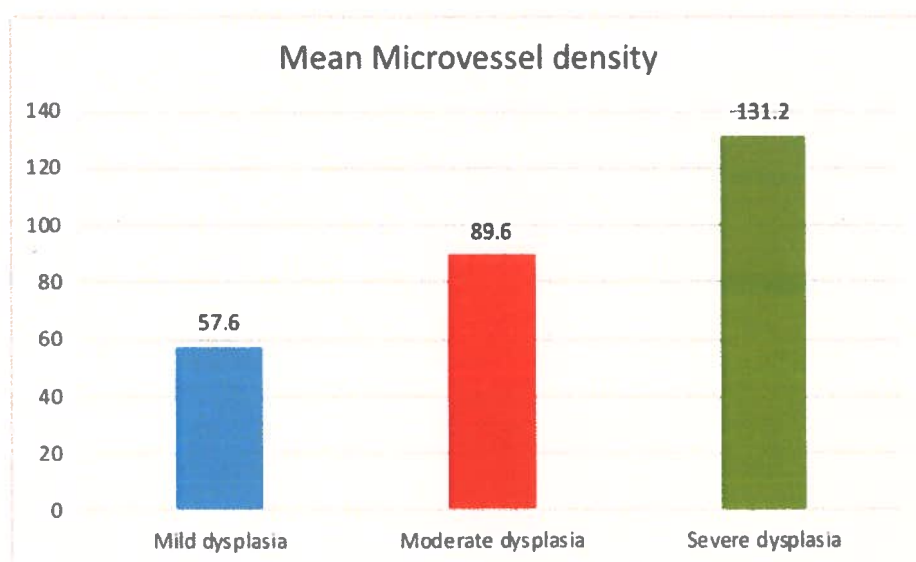
Post hoc pairwise comparisons of mean mast cell density using Tukey's test. It showed that the mean mast cell density of cases of mild dysplasia was significantly lower than that of moderate dysplasia, which was further significantly lower than that among severe dysplasia cases.

**EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL
EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF
MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY**

Table 6: Descriptives of Microvessel density with respect to different grades of dysplasia.

Microvessel density					
	N	Mean	Std. Deviation	95% Confidence Interval for Mean	
				Lower Bound	Upper Bound
Mild dysplasia	10	57.6000	8.26236	51.6895	63.5105
Moderate dysplasia	10	89.6000	8.26236	83.6895	95.5105
Severe dysplasia	10	131.2000	6.74619	126.3741	136.0259

Table 6 showed the descriptives of Microvessel density with respect to different grades of dysplasia. Mild dysplasia cases showed the minimum microvessel density while the severe dysplasia cases showed the maximum microvessel density.



Graph 2: Mean Microvessel density with respect to different grades of dysplasia

**EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL
EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF
MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY**

Table 7: Intergroup comparison of Microvessel density using one way ANOVA test

ANOVA					
Microvessel density					
	Sum of Squares	df	Mean Square	F	P value
Between Groups	27238.400	2	13619.200	224.438	<0.001, S
Within Groups	1638.400	27	60.681		
Total	28876.800	29			

Intergroup comparison of Microvessel density was done using one way ANOVA test.

Overall, a statistically significant difference was observed. In other words, it can be said that the mean microvessel density was significantly different among all the three types of dysplasia.

**EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL
EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF
MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY**

Table 8: Post hoc pairwise comparisons of mean Microvessel density using Tukey's test

Multiple Comparisons						
Dependent Variable: Microvessel density						
Tukey HSD						
(I) dysplasia	(J) dysplasia	Mean Difference (I-J)	Std. Error	P value	95% Confidence Interval	
					Lower Bound	Upper Bound
Mild dysplasia	Moderate dysplasia	-32.00000*	3.4837 2	<0.001, S	-40.6376	-23.3624
	Severe dysplasia	-73.60000*	3.4837 2	<0.001, S	-82.2376	-64.9624
Moderate dysplasia	Mild dysplasia	32.00000*	3.4837 2	<0.001, S	23.3624	40.6376
	Severe dysplasia	-41.60000*	3.4837 2	<0.001, S	-50.2376	-32.9624
Severe dysplasia	Mild dysplasia	73.60000*	3.4837 2	<0.001, S	64.9624	82.2376
	Moderate dysplasia	41.60000*	3.4837 2	<0.001, S	32.9624	50.2376

Post hoc pairwise comparisons of mean Microvessel density using Tukey's test. It showed that the mean microvessel density of cases of mild dysplasia was significantly lower than that of moderate dysplasia, which was further significantly lower than that among severe dysplasia cases.

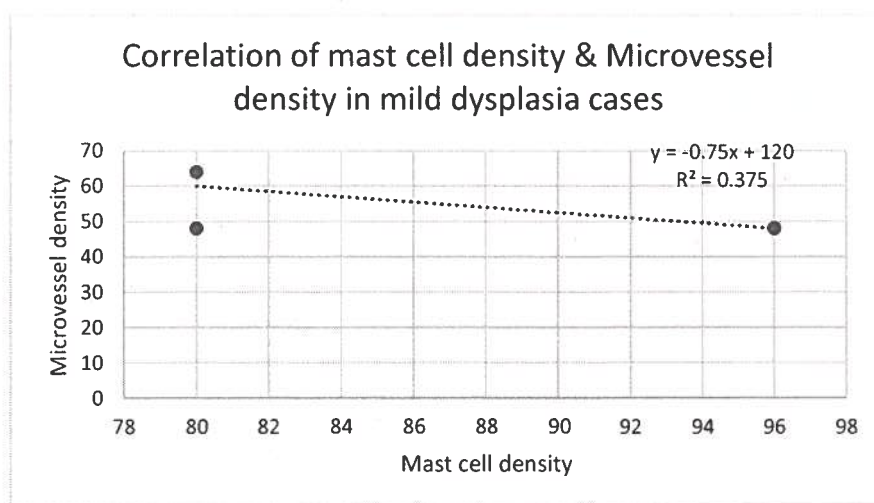
**EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL
EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF
MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY**

Table 9: Correlation between Mast cell density & Microvessel density among dysplasia cases

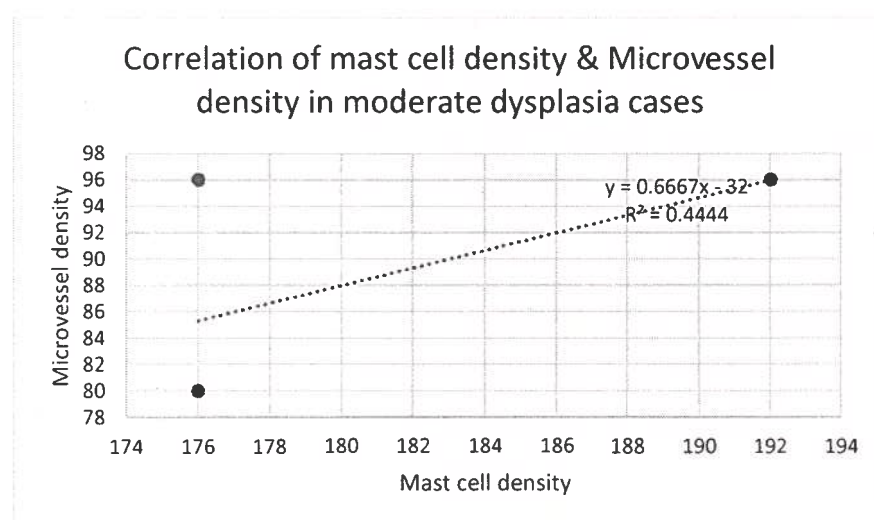
	Correlation coefficient	P value
Mild dysplasia	-0.612	0.06, NS
Moderate dysplasia	0.667	0.035, S
Severe dysplasia	1.000	<0.001, S
Overall	0.934	<0.001, S

This table presents the correlation coefficients and corresponding p-values for the relationship between Mast cell density & Microvessel density among different grades of dysplasia (mild, moderate, severe) and overall. It showed that w.r.t mild dysplasia cases, a moderate negative but statistically not significant, correlation was found between mast cell density & microvessel density. With respect to moderate dysplasia cases, a moderate positive correlation was found which was statistically significant also. With respect to severe dysplasia cases, a perfect positive & statistically significant correlation was found. Overall, a very strong positive correlation (statistically significant) was found between Mast cell density & Microvesel density.

EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY

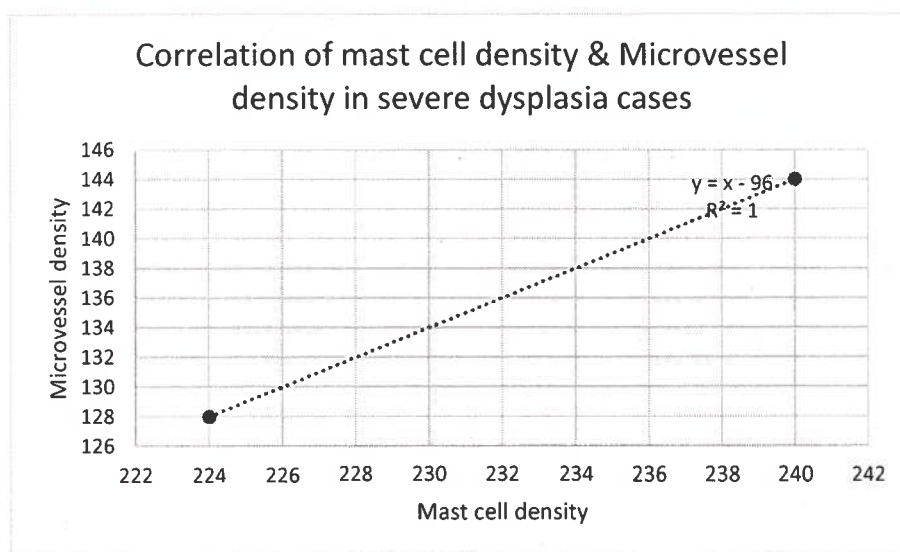


Graph 3: Scatter plot showing the correlation of Mast cell density & Microvessel density among mild dysplasia cases

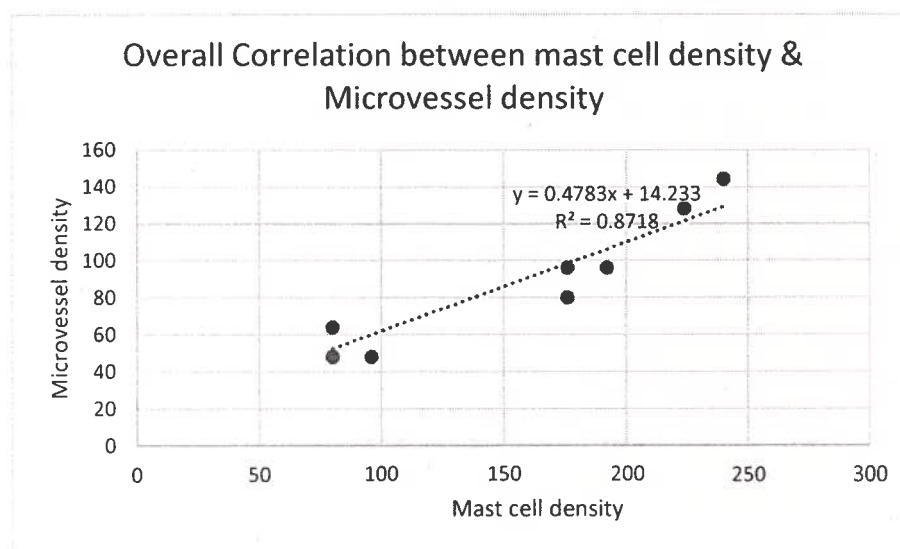


Graph 4: Scatter plot showing the correlation of Mast cell density & Microvessel density among moderate dysplasia cases

EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY



Graph 5: Scatter plot showing the correlation of Mast cell density & Microvessel density among severe dysplasia cases



Graph 6 : Scatter plot showing the overall correlation of mast cell density & microvessel density among all dysplasia cases



DISCUSSION



EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY

DISCUSSION

Oral carcinogenesis typically follows a two-step process which initially presents a precursor lesion which World Health Organization (WHO), has defined as "altered epithelium with an increased likelihood for progression to squamous cell carcinoma". The cytological and architectural changes exhibited by the altered epithelium collectively is grouped under the term dysplasia¹⁴.

Neo-angiogenesis (formation of new vascular channels by division of endothelial cells and resultant sprouting of new capillaries) is an essential step not only in carcinogenesis but also in the growth of dysplastic epithelial lesions. Blood vessels provide nutrition to the actively dividing dysplastic epithelial cells that in turn helps them grow into frank carcinomas and gradually to metastasize.

Angiogenesis is a phenomenon associated with physiological (embryogenesis, wound healing) and pathological events(neoplasia). It is determined by a balance between pro angiogenic (TGF β , FGF, TNF) and anti-angiogenic (Angiostatin, Platelet factor) factor⁹⁰. Pro angiogenic factors are produced by host cells (mast cells, VEGF) and tumour cells. Angiogenesis which is directly proportional to the microvascular density (MVD) can be assessed by marking the proliferating endothelial cells using immunohistochemical expression of various endothelial markers like CD31, CD34, VEGF-A, Endoglin etc. Several markers have been used in the past; but we chose Endoglin (CD105), a cluster differentiation factor in the present study as it specifically stained the activated endothelial cells as compared to CD34 that stained all endothelial cells^{86, 91}.

Endoglin is a potent pleiotropic angiogenic factor expressed by activated endothelial cells during new blood vessel formation and is considered the most specific marker

EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY

for tumor angiogenesis. It is also elevated in areas of healing wounds & inflammation and is associated with tumor metastasis and shorter survival. Endothelial cell proliferation rate of tumor vasculature is exponentially higher than that of the normal vessels. Thus, the micro vessel density within the tumor may serve as a key indicator of its behavior. Endoglin is a 180 Kda trans-membrane protein, highly expressed in activated endothelial cells; also utilized as membrane marker for identifying blood vessels. Endoglin serves as receptor protein for TGF β signaling which induces endothelial cell proliferation, its migration and fibrogenesis⁹². CD105 expression can be used to assess angiogenesis by quantifying the micro vessel density. Anti CD105 antibodies can be used to control angiogenesis in cancer therapies. Hence, it's use as a prognostic and therapeutic target in cancer needs to be studied further.

We found microvessel density (MVD) increasing proportionately with severity in grades of epithelial dysplasia. Intergroup comparison of micro vessel density was done with one-way ANOVA test where a statistically significant difference was observed in the MVD in all grades of dysplasia. Post hoc pairwise comparisons of mean MVD using Tukey's test revealed that the mean MVD in mild dysplasia were significantly lower than that seen in moderate dysplasia which was further significantly lower than that among severe dysplasia. Our results were similar to those reported by **Sathyakumar M et al**⁷⁰.

Amol Gadbaile et al⁷² in their study suggested that tumour angiogenesis is not necessarily a trait of invasive neoplasm but may be an early event during tumour formation. The angiogenic switch occurs in the dysplastic stage preceding tumour development in experimental study models of oral cancer. Potentially malignant tissues show an increased endothelial proliferation and hence proportionately high

EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY

micro vessel density⁹³. **Gandolfo M et al**⁹⁴ reported an increased VEGF expression which corresponds to sub-epithelial vascularization in leukoplakia with dysplastic changes. Architectural changes in blood vessels and impaired lymphatic network coupled with increased interstitial fluid pressure in premalignant tissues were reported by **Hagendorn J et al**⁹⁵. We believe the aforementioned factors could increase the MVD on account of compensatory measures by the host. We also hypothesize, the increased MVD in dysplasia is due to metabolic changes in the epithelial cells brought about by effects of tobacco in leukoplakia and hypoxia in oral sub mucous fibrosis (Both lesions are associated with epithelial dysplasia).

Yet another indicator of angiogenesis is the presence of mast cells. In vitro studies have shown that mast cells accumulate near sites of vascular tube formation⁹⁶. Mast cell density has been shown to modulate micro vessel density in malignant tumors⁹⁰. Mast cells arise in the bone marrow, are seen in the circulating blood and releases chemical mediators of inflammation through degranulation⁹⁷. Activated mast cells play a role in mitogenesis, extracellular matrix degradation, angiogenesis, upregulation of microvascular hyperpermeability and recruitment of macrophages. **Telagi N et al**⁶⁹ in their study, grouped mast cells into typical (clear visualisation of nucleus, cell membrane and granules), atypical (clear visualisation of nucleus and granules without clarity of the cytoplasm) and granular (aggregations of granules without presence of nucleus). They reported a proportionate increase of degranulated mast cells in OSMF, OSCC and Oral leukoplakia with increase in inflammatory infiltration. They found more granulating type of mast cells in OSCC and more atypical mast cells in Oral epithelial dysplasia. Activated mas cells secrete mediators that induce angiogenesis (VEGF), tumour proliferation, degradation of ECM etc.

EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY

ECM degradation is aided by chymases and tryptases secreted by Mast Cells which provide space for the budding capillaries⁹⁸. Mast cell growth factor which induces mast cell migration is synthesised by endothelial cells⁹⁹ and epidermal keratinocytes¹⁰⁰. Hence, we believe, the increase in number of endothelial cells will also correlate with the mast cell numbers. Mast cell density (MCD) was assessed by identifying the mast cells using toluidine blue, an easily available metachromatic dye. We found mast cell density (MCD) increasing proportionately with severity in grades of epithelial dysplasia. Intergroup comparison of mast cell density was done with one-way ANOVA test where a statistically significant difference was observed in the MCD in all grades of dysplasia. Post hoc pairwise comparisons of mean MCD using Tukey's test revealed that the mean MCD in mild dysplasia were significantly lower than that seen in moderate dysplasia which was further significantly lower than that among severe dysplasia. Our study showed similar results as in previous published literature by **Dantas RCM et al**⁶⁸ and **Sathyakumar M et al**⁷⁰. Interleukin-1 & Histamine released by mast cells increases in epithelial dysplasia¹⁰¹.

Tobacco is the prime etiological factor in oral potentially malignant disorders which show epithelial dysplasia. Carcinogens from tobacco may induce cytotoxicity or mutagenicity in the oral keratinocytes which in turn induces a host cell inflammatory reaction that upregulates mast cell numbers⁷⁰. In addition, epidermal keratinocytes are also a source of mast cell growth factor¹⁰⁰. We believe, the increased proliferative rates in epithelial dysplasia, could therefore up regulate the MCD with increase in grades.

In the present study, we recorded a proportionate increase of MCD and MVD in the different grades of epithelial dysplasia. On assessing the correlation coefficients and

EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY

corresponding p-values we found, a moderate negative but statistically not significant, correlation between mast cell density & microvessel density in cases of mild dysplasia. In moderate dysplasia, a moderately positive correlation was found which was statistically significant. However, in cases of severe dysplasia, a perfectly positive & statistically significant correlation was found. In general, a strongly positive correlation (statistically significant) was found between mast cell density & microvessel density in cases of dysplasia. Several published literature have shown similar correlation between MCD and MVD^{62,70,102,103}.

The results of the present study revealed that angiogenesis can be quantified through assessment of micro vessel density using Endoglin as a marker to predict the risk of neoplastic transition in oral epithelial dysplasia. Also, an exponential increase of mast cell density was observed with toluidine blue in increasing grades of dysplasia. A statistically significant correlation between microvascular density and mast cell density in epithelial dysplasia is also noteworthy in our present study. This can be used to improvise early therapeutic strategies to prevent the dysplastic cells from crossing the “point of no return” in the cell cycle.



CONCLUSION



**EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL
EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF
MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY**

CONCLUSION

The present study was conducted using 10 sample each of previously diagnosed cases of oral epithelial dysplasia of all grades which were retrieved from the archives. Three section each of every tissue was sectioned and stained with H & E, Toluidine Blue and CD105 (Endoglin). 10 samples of Pyogenic Granuloma was used as control.

Micro vascular density was assessed using Endoglin and Mast cell density was assessed using Toluidine Blue.

We found statistically significant increase with increase in severity of epithelial dysplasia in the both the parameters. Hence, we conclude the assessment of these variables can be used to improvise early therapeutic strategies for better treatment outcomes for the patients.



BIBLIOGRAPHY



**EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL
EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF
MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY**

BIBLIOGRAPHY

1. Gelband H, Jha P, Sankaranarayanan R, Horton S, editors. Cancer: Disease Control Priorities. 3rd ed. Ch. 5. Vol. 3. Washington, (DC): The International Bank for Reconstruction and Development/The World Bank; 2015.
2. Sankaranarayanan R et al. Effect of screening on oral cancer mortality in Kerala, India: A cluster-randomised controlled trial. *Lancet*. 2005;365:1927–33.
3. Reibel J. Prognosis of oral pre-malignant lesions: Significance of clinical, histopathological, and molecular biological characteristics. *Crit Rev Oral Biol Med*. 2003;14:47–62.
4. I R Kramer et al. Definition of leukoplakia and related lesions: an aid to studies on oral precancer. *Oral Surg Oral Med Oral Pathol*. 1978;46:518–39.
5. Warnakulasuriya S, Johnson NW, Vander Waal. Nomenclature and classification of potentially malignant disorders of the oral mucosa. *J Oral Pathol Med*. 2007;36:575–80.
6. Odell, E., Kujan, O, Warnakulasuriya, S, & Sloan, P. Oral epithelial dysplasia: Recognition, grading and clinical significance. *Oral Diseases*. 2021;00:1–30.
7. Van der Waal I. Potentially malignant disorders of the oral and oropharyngeal mucosa; terminology, classification and present concepts of management. *Oral Oncol*. 2009;45:317–23.
8. Warnakulasuriya S, Kujan O, Aguirre-Urizar JM, et al.: Oral potentially malignant disorders: a consensus report from an international seminar on nomenclature and classification, convened by the WHO Collaborating Centre for oral cancer. *Oral Dis*. 2021;27:1862-80.

**EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL
EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF
MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY**

9. El-Naggar, A.K., Chan, J.K.C., Grandis, J.R., Takata, T. and Slootweg, P.J., Eds., WHO Classification of Head and Neck Tumours, IARC, Lyon, 112.
10. Sonia Gupta et al, Journal of Clinical and Diagnostic Research. 2017 Dec, Vol-11(12): EC29-EC34.
11. Varun Rastogi et al. An Insight to Oral Epithelial Dysplasia. International Journal of Head and Neck Surgery. 2013;4(2):74-82
12. Neville BW, et al. Oral and maxillofacial pathology. London: WB Saunders Company 2001:343.
13. Warnakulasuriya S, Reibel J, Bouquot J, Dabelstein E. Oral epithelial dysplasia classification systems: Predictive value, utility, weaknesses and scope for improvement. J Oral Path Med. 2008;37:127-33.
14. Barnes L, Eveson JW, Reichert P, Sidransky D, editor. World Health Organization Classification of Tumours, Pathology & Genetics of Head and Neck Tumours. 1. Lyon: IARC Press; 2005.
15. Shivamallappa SM, Venkatraman NT, Shreedhar B, Mohanty L, Shenoy S. Role of angiogenesis in oral squamous cell carcinoma development and metastasis: an immunohistochemical study. Int J Oral Sci. 2011;3(4):216-24.
16. Marioni G, D'Alessandro E, Giacomelli L, Sta eri A. CD105 is a marker of tumour vasculature and a potential target for the treatment of head and neck squamous cell carcinoma. J Oral Pathol Med. 2010;39(5):361-7.
17. Saede Atarbashi-Moghadam et al. Comparison of Mast Cell Count in Oral Epithelial Dysplasia and Squamous Cell Carcinoma. J Dent Shiraz Univ Med Sci. December. 2024; 25(4): 369-373

**EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL
EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF
MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY**

18. Yokoyama M. Alterations in stromal reaction during tumor progression in oral mucosa. *Journal of hard tissue biology*. 2011;20(1):23-30.
19. Rojas IG, Spencer ML, Martínez A, Maurelia MA, Rudolph MI. Characterization of mast cell subpopulations in lip cancer. *J Oral Pathol Med*. 2005; 34: 268–273
20. Pitiyage G, Tilakaratne WM, Tavassoli M, Warnakulasuriya S. Molecular markers in oral epithelial dysplasia: Review. *J Oral Pathol Med*. 2009;38:737–52.
21. Shah AA, Kamal MA, Akhtar S. Tumor angiogenesis and VEGFR-2: mechanism, pathways and current biological therapeutic interventions. *Current Drug Metabolism*. 2021;22(1):50-9.
22. Bussolino F, Mantovani A, Persico G. Molecular mechanisms of blood vessel formation. *Trends Biochem Sci*. 1997;22:251–256.
23. Michael Papetti and Ira M. Herman. *Am J Physiol Cell Physiol* 282: C947–C970, 2002; 10.1152/ajpcell.00389.2001.
24. Holash J et al. Vessel cooption, regression, and growth in tumors mediated by angiopoietins and VEGF. *Science* 284: 1994–1998, 1999.
25. Sokmen S, Sarioglu S, Fuzun M et al. Prognostic significance of angiogenesis in rectal cancer: a morphometric investigation. *Anticancer Res*. 2001;21: 4341–4348.
26. Folkman J. Angiogenesis and breast cancer. *J Clin Oncol*. 1994;12:441–443.
27. Weidner N. Intratumor microvessel density as a prognostic factor in cancer. *Am J Pathol*. 1995;147:9–19.
28. Maharudrappa Basnaker, Shashikant SR, Satish BNVS. Expression of endoglin (CD105) and Micro vessel density in Oral dysplasia and Squamous cell carcinoma. *Journal of clinical and diagnostic research*. 2014; 8(9):p. ZC91–ZC94.

**EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL
EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF
MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY**

29. Nassiri F et al. Endoglin (CD105): a review of its role in angiogenesis and tumor diagnosis, progression and therapy. *Anticancer Res.* 2011;31(6):2283-90.
30. Rafii S. Circulating endothelial precursors: mystery, reality, and promise. *J Clin Invest.* 2000;17-19
31. Chang YS et al. Mosaic blood vessels in tumors: frequency of cancer cells in contact with flowing blood. *Proc Natl Acad Sci USA.* 97: 14608-14613, 2000.
32. MacLuskey M et al. Apoptosis, proliferation, and angiogenesis in oral tissues possible relevance to tumour progression. *J Pathol.* 2000;91(4):368-375.
33. G. Marioni et al. CD105-assessed micro-vessel density is associated with malignancy recurrence in laryngeal squamous cell carcinoma. *EJSO.* 2006;36(10):1149-1153.
34. R. Bansal et al. Therapeutic progression of quinazolines as targeted chemotherapeutic agents. *Eur. J. Med. Chem.* 2021. 211:113016.
35. Legan M. New marker of angiogenesis CD105(endoglin):Diagnostic, prognostic and therapeutic use. *Radiol Oncol.* 2005;39:253-9.
36. Nirupama Deshpande , Ying Ren , Kira Foygel , Jarrett Rosenberg, Jürgen K Willmann. *Radiology.* 2011;258(3):804-811.
37. Kumar, P., Wang, J. M., and Bernabeu, C. CD105 and angiogenesis. *J. Pathol.* 1996.178: 363-66.
38. Gupta, S.; Shetty, D.; Gulati, N.; Juneja, S.; Jain, A. Potentiated Action on the Progression of OSMF by Hypoxia Mediated Signaling Pathway by the Epithelial Mesenchymal Transition and Angiogenic Apparatus. *J. Cancer Res. Ther.* 2023;19:S389-S396.

**EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL
EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF
MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY**

39. Shieh YS, Lee HS, Shiah SG et al. Role of angiogenic and non-angiogenic mechanisms in oral squamous cell carcinoma: correlation with histologic differentiation and tumor progression. *J Oral Pathol Med.*2004; 33: 601–606.
40. Iamaroon A, Pongsiriwet S, Jittidecharaks S et al. Increase of mast cells and tumor angiogenesis in oral squamous cell carcinoma. *J Oral Pathol Med.*2003; 32: 195–199.
41. Shivakumar Madappa Shivamallappa, Narayan Tondikulam Venkatraman, Balasundari Shreedhar, Leeky Mohanty, Sadhana Shenoy. Role of angiogenesis in oral squamous cell carcinoma development and metastasis: an immunohistochemical study. *Int J Oral Sci.*2011; 3:216-224.
42. Dastpak M, Nafarzadeh S, Khafri S. A comparative study on the mast cells count in oral squamous cell carcinoma and normal oral mucosa. *Caspian J Dent Res.* 2015;4:17–22.
43. Ribatti D, Crivellato E. The controversial role of mast cells in tumour growth. *Int Rev Cell Mol Biol.* 2009;275:89-131.
44. Marichal T, Tsai M, Galli SJ. Mast cells: potential positive and negative roles in tumor biology. *Cancer Immunol Res* 2013; 1: 269-79.
45. Gurish MF, Boyce JA. Mast cell growth, differentiation, and death. *Clin Rev Allergy Immunol.* 2002; 22:107-18.
46. Shoshana D, Glalinsky T, Nechustan H. Mast cells and cancer-No longer just basic science. *Crit Rev Oncol/Hematol.* 2008;68:115-130.
47. Sridharan G, Shankar AA. Toluidine blue: a review of its chemistry and clinical utility. *J Oral Maxillofac Pathol.* 2012;16:251–255.
48. Rakesh S, Vidya M, Janardhanan M, Vinod Kumar RB, Savithri V. Analysis of mast cell counts in oral leukoplakia. *Oral Maxillofac Pathol J.* 2012;3(1):181-5.

**EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL
EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF
MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY**

49. Crivellato E, Nico B, Ribatti D. Mast cell contribution to tumor angiogenesis: A clinical approach. *Eur Cytokine Netw.* 2009;20:197-206.
50. Anuradha A, Kiran Kumar Naik B, Vijay Srinivas G, Devi RS, Puneet HK. Incidence of mast cells in oral squamous cell carcinoma: A short study. *J Oncol.* 2014;2014:614291.
51. Mohtasham N, Babakoochi S, Salehinejad J, Montaser-Kouhsari L, Shakeri MT, Shojae S, Sistani NS, Firooz A. Mast cell density and angiogenesis in oral dysplastic epithelium and low- and high-grade oral squamous cell carcinoma. *Acta Odontol Scand.* 2010;68:300-4.
52. I. Feoktistov, S. Ryzhov, A. E. Goldstein, and I. Biaggioni, "Mast cell-mediated stimulation of angiogenesis," *Circulation Research*, vol. 92, no. 5, pp. 485–492, 2003.
53. Gaje PN, Amalia Ceausu R, Jitariu A, Stratul SI, Rusu LC, Popovici RA, Raica M. Mast cells: key players in the shadow in oral inflammation and in squamous cell carcinoma of the oral cavity. *Biomed Res Int.* 2016: 9235080.
54. Güneri P, Epstein JB, Ergün S, Boyacıoğlu H. Toluidine blue color perception in identification of oral mucosal lesions. *Clin Oral Investig.* 2011;15:337-45.
55. Ribatti D. The Staining of Mast Cells: A Historical Overview. *Int Arch Allergy Immunol.* 2018;176(1):55- 58
56. Spicer SS. Basic protein visualized histochemically in mucinous secretions. *Exp Cell Res.* 1962; 28 :480-8.
57. Sridharan G, Shankar AA. Toluidine blue : A review of its chemistry and clinical utility. *JOMFP.* 2012; 16:251-5.
58. Daisy Laishram et al. Mast cells and angiogenesis in malignant and premalignant oral lesions: An immunohistochemical study. *JOMFP.* 2017;21(2):229–238

**EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL
EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF
MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY**

59. Junaid M, Choudhary MM, Sobani ZA, Murtaza G, Qadeer S, Ali NS, et al. A comparative analysis of toluidine blue with frozen section in oral squamous cell carcinoma. *World J Surg Oncol*. 2012;10:57.
60. Epstein JB, Guneri P. The adjunctive role of toluidine blue in detection of oral premalignant and malignant lesions. *Curr Opin Otolaryngol Head Neck Surg*. 2009;17:79-87.
61. Hegde Veda, Marla Vinay. Mast cells and angiogenesis in oral epithelial dysplastic lesions and oral Squamous cell carcinoma. *Int J Med Res Health Sci*. 2015;4(1): 46-52
62. Michailidou EZ, Markopoulos AK, Antoniadis DZ. Mast cells and angiogenesis in oral malignant and premalignant lesions. *Open Dent J*. 2008;2:126-32. doi: 10.2174/1874210600802010126. PMID: 19444318; PMCID: PMC2606660.
63. Jandinski JJ, Sonis S, Doku HC. The incidence of mast cells in selected oral lesions. *Oral Surg*. 1972;3(2):245-48.
64. Biviji AT. Mast cells in normal and leukoplakic buccal mucosa. *J Ind Dent Assoc*. 1973; 45: 189- 91.
65. Flynn E, Schwartz j, Shklar G. Sequential mast cell infiltration and degranulation during experimental carcinogenesis. *J Cancer Res Clin Oncol*. 1991; 117:115- 22.
66. Kalra M, Rao N, Nanda K, Rehman F, Girish KL, Tippu S et al. The role of mast cells on angiogenesis in oral squamous cell carcinoma. *Med Oral Patol Oral Cir Bucal*. 2012;17(2):190- 6.
67. Culling CF, Allison TR. *Cellular Pathology Technique*. 4th ed. London: Butterworths 1985.

**EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL
EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF
MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY**

68. Dantas RCM, de Souza RO, Valverde LF, Vidal MTA, Sales CBS, Sousa LP, Dos Santos JN, Ramos EAG, Gurgel Rocha CA. Evaluation of Mast Cell Density in the Tumor Microenvironment in Oral Epithelial Dysplasia and Oral Squamous Cell Carcinoma. *Appl Immunohistochem Mol Morphol*. 2017;25(10):e83-e88. doi: 10.1097/PAI.0000000000000587. PMID: 29116959.
69. Telagi N, Ahmed Mujib BR, Kulkarni PG, Naik R. The master switch: Comparative study of mast cell in oral epithelial dysplasia, oral submucous fibrosis and oral squamous cells carcinoma and their association with inflammation and angiogenesis. *J Oral Maxillofac Pathol* 2015;19:25-9.
70. Sathyakumar M, Sriram G, Saraswathi T, Sivapathasundharam B. Immunohistochemical evaluation of mast cells and vascular endothelial proliferation in oral precancerous lesion-leukoplakia. *J Oral Maxillofac Pathol*. 2012 Sep;16(3):343-8. doi: 10.4103/0973-029X.102481. PMID: 23248463; PMCID: PMC3519206.
71. Srimahalakshmi A, Arunalatha P. CD 105 (Endoglin) Expression in Oral Squamous Cell Carcinoma. *IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)*. 2018;2279- 0861(17):57-63.
72. Amol Gadgail et al. Ki67, CD105 and alphaSMA expression better relates the binary oral epithelial dysplasia grading system of World Health Organisation. doi: 10.1111/jop.12612.
73. Niva Mahapatra et al. Study of expression of Endoglin(CD105) in oral squamous cell carcinoma. *J Oral Maxillofac Pathol*. 2021; 25-552.

**EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL
EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF
MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY**

74. Gougos A and Letarte M: Identification of a human endothelial cell antigen with monoclonal antibody 44G4 produced against a pre-B leukemic cell line. *J Immunol.* 1998; 141: 1925-1933.
75. Gougos A and Letarte M: Primary structure of endoglin, an RGD-containing glycoprotein of human endothelial cells. *J Biol Chem.* 1990;265: 8361-8364.
76. Koleva RI, Conley BA, Romero D, Riley KS, Marto JA, Lux A and Vary CP: Endoglin structure and function: Determinants of endoglin phosphorylation by transforming growth factor-beta receptors. *J Biol Chem.* 2006;281:25110-25123.
77. Rius C, Smith JD, Almendro N, et al. Cloning of the promoter region of human endoglin, the target gene for hereditary hemorrhagic telangiectasia type 1. *Blood.* 1998;92:4677-90.
78. Cheifetz S, Bellon T, Cales C, et al. Endoglin is a component of the transforming growth factor-h receptor system in human endothelial cells. *J Biol Chem.* 1992; 267:19027-30.
79. Nikolaos A. Dallas et al. Endoglin (CD105): A Marker of Tumor Vasculature and Potential Target for Therapy. *Clin Cancer Res.* 2008;14 (7): 1931–1937.
80. Goumans MJ, Valdimarsdottir G, Itoh S, Rosendahl A, Sideras P, Ten Dijke P. Balancing the activation state of the endothelium via two distinct TGF-h type I receptors. *EMBO J.* 2002;21:1743-53.
81. Bobik A. Transforming growth factors and vascular disorders. *Arterioscler Thromb Vasc Biol.* 2006;26: 1712-20.
82. Raunak Kumar Das, et al, Assessment of malignant potential of oral submucous fibrosis through evaluation of p63, E-cadherin and CD105 expression. *J Clin Pathol.* 2010; 63:894-99.

**EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL
EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF
MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY**

83. Daniel GE Thiem, Schamiem Schneider , Narayan T. Venkatraman, et al. Semiquantifiable angiogenesis parameters in association with the malignant transformation of oral leukoplakia. *J Oral Pathol Med.* 2017;46:710-6.
84. Neda Kargahi et al. Immunohistochemically Detection of Angiogenesis in Oral Pre-Cancerous Lesions Compared with Oral Invasive Carcinomas. *Asian Pac J Cancer Prev.*19 (7); 1805-1808.
85. Amina Fouad Farag, Rehab Fawzi Kasem, Nahed Emad El-Din Abo-Azma and Mohsen Kazem Abd El- Latif. Evaluation of angiogenesis in oral epithelial dysplasia and oral squamous cell carcinoma: an immunohistochemical comparison between anti-cd105 and anti-cd31 antibodies. *Int. J. Adv. Res.* 6(1), 338-347.
86. A Bhargav Kumar Gudipati et al. An Immunohistochemical Assessment of Endoglin (CD105) Expression in Oral epithelial Dysplasia's and Oral Squamous Cell Carcinoma. *American Journal of Clinical Case Reports.* 2023; 4 | Article 1077.
87. E. A. Baker, D. J. Leaper, J. P. Hayter, A. J Dickenson. The matrix metalloproteinase system in oral squamous cell carcinoma. *British Journal of Oral and Maxillofacial Surgery.* 2006;44(6):482–486.
88. Weidner N, Carroll P.R., Flax J., Blumenfeld W., Folkman J. Tumor angiogenesis correlates with metastasis in invasive prostrate carcinoma. *Am J Pathol.* 1993; 143(2): 401-409.
89. https://www.ihcworld.com/_protocols/special_stains/toluidine_blue.htm
90. Khan Y, Rehani S, Sharma M. To evaluate the role of mast cells on angiogenesis in various grades of oral squamous cell carcinoma: A histochemical study. *J Oral Maxillofac Pathol.* 2024;28(3):403-408. doi: 10.4103/jomfp.jomfp_17_24. Epub 2024 Oct 15. PMID: 39670125; PMCID: PMC11633918.

**EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL
EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF
MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY**

91. Pammar, Chandrashekhar; Nayak, Ramakan S.; Kotrashetti, Vijayalakshmi S.; Hosmani, Jagadish. Comparison of microvessel density using CD34 and CD105 in oral submucous fibrosis and its correlation with clinicopathological features: An immunohistochemical study. *Journal of Cancer Research and Therapeutics*. 2018; 14(5): 983-988. DOI: 10.4103/0973-1482.181186.
92. Shetty S, Pereira T, Tamgadge S, Kale S, Kamat P, Kumar S. ENDOGLIN (CD105): A marker of tumour vasculature for assessment of malignant potential of oral submucous fibrosis. *J Oral Maxillofac Pathol*. 2024;28(4):589-595. doi: 10.4103/jomfp.jomfp_67_24. Epub 2024 Dec 31. PMID: 39949694; PMCID: PMC11819617.
93. Aromando RF, Raimondi AR, Pérez MA, Trivillin VA, Schwint AE, Itoiz ME. Angiogenesis in potentially malignant lesions and carcinomas during experimental oral carcinogenesis: a preliminary study in the hamster cheek pouch. *Anticancer Res*. 2014;34(11):6381-8. PMID: 25368237.
94. Gandolfo M, Keszler A, Lanfranchi H, Itoiz ME: Increased subepithelial vascularization and VEGF expression reveal potentially malignant changes in human oral mucosa lesions. *Oral Surg Oral Med Oral Pathol Oral Rad and Endodontol*. 2011;111: 486-493.
95. Hagendoorn J, Tong R, Fukumura D, Lin Q, Lobo J, Padera TP, Xu L, Kucherlapati R, Jain LK: Onset of abnormal blood lymphatic vessel function and interstitial hypertension in early stages of carcinogenesis. *Cancer Res*. 2006; 66(7): 3360-3364.

**EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL
EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF
MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY**

96. Jippo T, Tsujino K, Kim HM, et al. Expression of mast-cell-specific proteases in tissues of mice studied by in situ hybridization *Am J Pathol.* 1997; 150(4): 1373-82.
97. Sudhakar R, Ramesh V, Balamurali PD, Nirima O, Premalatha B, Karthikshree V. Incidence of mast cells in oral inflammatory lesions: A pilot study. *J Oral Maxillofac Pathol.* 2005;9:12-15.
98. Pansrikaew P, Cheewakriangkrai C, Taweewisit M, Khunamornpong S, Siriaunkgul S. Correlation of mast cell density, tumor angiogenesis, and clinical outcomes in patients with endometrioid endometrial cancer. *Asian Pac J Cancer Prev.* 2010;11(3):623-6. PMID: 21039027.
99. Rochelle R. Weiss, Diana Whitaker-Menezes, Jack Longley, Jeff Bender, George F. Murphy, Human Dermal Endothelial Cells Express Membrane-Associated Mast Cell Growth Factor, *Journal of Investigative Dermatology.*1995;104(1):101-106, <https://doi.org/10.1111/1523-1747.ep12613587>.
100. Kligman LH, Murphy GF. Ultraviolet B radiation increases hairless mouse mast cells in a dose-dependent manner and alters distribution of UV-induced mast cell growth factor. *Photochem Photobiol.* 1996;63:123-7.
101. Atarbashi-Moghadam S, Niazmand M, Vafadar S, Gholami Toghechi S. Comparison of Mast Cell Count in Oral Epithelial Dysplasia and Squamous Cell Carcinoma. *J Dent (Shiraz).* 2024;25(4):369-373. doi: 10.30476/dentjods.2024.99652.2164. PMID: 39713112; PMCID: PMC11662177.
102. Nakandala K, Suraweera A, Jayasooriya P. Correlation of elevated mast cell and micro-vessel densities with lymph node metastasis in oral squamous cell carcinoma. *Stomatological Dis Sci.* 2018;2:4. <http://dx.doi.org/10.20517/2573-0002.2017.17>

**EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL
EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF
MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY**

103. Sundararajan A, Muthusamy R, Gopal Siva K, Harikrishnan P, Kumar SCK, Rathinasamy SK. Correlation of Mast Cell and Angiogenesis in Oral Lichen Planus, Dysplasia (Leukoplakia), and Oral Squamous Cell Carcinoma. Rambam Maimonides Med J. 2021;29;12(2):e0016. doi: 10.5041/RMMJ.10438. PMID: 33938803; PMCID: PMC8092953.



APPENDICES



**EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL
EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF
MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY**

APPENDICES

ANNEXURE – I



BABU BANARASI DAS COLLEGE OF DENTAL SCIENCES

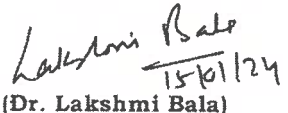
(Faculty of BBD University, Lucknow)

INSTITUTIONAL RESEARCH COMMITTEE APPROVAL

The project titled "**Expression of Endoglin in different grades of Oral Epithelial Dysplasia and its correlation with presence of mast cells: A retrospective comparative study.**" submitted by **Dr. DEEP SHARMA** Postgraduate student in the **Department of Oral Pathology & Microbiology** for the Thesis Dissertation as part of MDS Curriculum for the academic year 2022-2025 with the accompanying proforma was reviewed by the Institutional Research Committee in its meeting held on **16th October, 2023** at BBDCODS.

The Committee has granted approval on the scientific content of the project. The proposal may now be reviewed by the Institutional Ethics Committee for granting ethical approval.


(Prof. Dr. Puneet Ahuja)
Chairperson (IRC)
Principal
BBD College of Dental Sciences
BBD University, Lucknow


(Dr. Lakshmi Bala)
Co-Chairperson (IRC)
HOD Biochemistry
BBD College of Dental Sciences
BBD University, Lucknow.

**EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL
EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF
MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY**

ANNEXURE – II



BABU BANARASI DAS UNIVERSITY
BBD City, Faizabad Road, Lucknow – 226028 (INDIA)

Dr. Lakshmi Bala

Professor and Head, Deptt of Biochemistry and

Member-Secretary, Institutional Ethics Committee (IEC) of BBD University, Lucknow

Communication of the Decision of the VIIIth Institutional Ethics Committee Meeting

IEC Code: 14

BBDU/MDS/14/2024

Date: 07/03/2024

Title of the Project: Expression of Endoglin in different grades of Oral Epithelial Dysplasia and its correlation with presence of mast cells: A retrospective comparative study

Principal Investigator: Dr. DEEP SHARMA

Department: Department of Oral Pathology & Microbiology

Name and Address of the Institution: BBD University, Lucknow

Type of Submission: New, MDS Dissertation proposal

Dear Dr. Deep Sharma

The meeting of the Institutional Ethics Committee (IEC) was held on 06-02-2024 in Conference room, First Floor, BBDCODS, BBD University, Lucknow. Following members were present:

- | | | |
|---|---|------------------|
| 1 | Dr. Chandishwar Nath
Rtd. Chief Scientist, CDRI, Lucknow. | Chairman |
| 2 | Dr. JS Srivastava,
Rtd. Chief Scientist, CDRI, Lucknow. | Member |
| 3 | Dr. Manodeep Sen,
Professor, Department of Microbiology, RMLIMS, Lucknow | Member |
| 4 | Dr. Shaleen Chandra, Professor & Dean, Dental Sciences, Atal Bihari
Vajpai Medical University (ABVM University), Lucknow | Member |
| 5 | Dr. Manuka Khanna,
Professor, Deptt. of Political Science, Lucknow University, Lucknow | Member |
| 6 | Mr Abhishek Chaudhary, Advocate, Lucknow | Member |
| 7 | Dr. Puneet Ahuja, Professor of Oral Pathology and Principal, BBD
College of Dental Sciences, BBD University, Lucknow | Member |
| 8 | Dr. Lakshmi Bala, Professor & Head, Department of Biochemistry,
BBD College of Dental Sciences, BBD University, Lucknow | Member-Secretary |

**EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL
EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF
MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY**

The committee reviewed and discussed your submitted documents of the research proposal in the meeting.
Decisions: The committee provided waiver to obtain consent for the above proposal.

Lakshmi Bala
07/03/24
(Dr. Lakshmi Bala)
Member-Secretary IEC
BBD University
Ethics Cell (Vth Floor, BBDCODS)
ethics@bbdu.ac.in

Dr. LAKSHMI BALA
Member-Secretary
Institutional Ethics Committee
BBD University, Lucknow-28

**EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL
EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF
MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY**

ANNEXURE – III

OBSERVATIONS

Cases	Toludine Blue – No of Mast Cells	Toludine Blue – No of Mast Cells	CD 105-No of Positive Blood Vessels	CD 105-No of Positive Blood Vessels
	Observer 1	Observer 2	Observer 1	Observer 2
MED 01	5, 6,6, 5,5	6, 5,5, 5,5	4,3,3,4,4	4,4,3,4,3
MED 02	5, 6,5,6,6	6, 6,4,5,4	3,3,3,4,3	3,2,2,4,4
MED 03	6, 6,5,5,5	5, 5,6, 5,5	2,3,3,4,4	4,3,3,4,4
MED 04	5, 5,5,5,6	5, 5,5,5,5	3,3,3,2,4	4,3,3,2,2
MED 05	5, 4,5,5,5	4, 6,5,5,5	4,2,4,4,4	3,3,4,4,4
MED 06	6, 6,5,6,6	6, 5,5,5,6	4,2,3,4,3	4,4,4,4,3
MED 07	5, 4,5,5,4	5, 4,6,5,5	3,2,3,4,4	4,3,3,4,2
MED 08	5, 5,5, 5,5	5, 6,5,6,5	4,3,3,2,4	4,3,3,2,4
MED 09	6, 6,5,5,5	5, 4,5,5,5	4,3,3,3,2	3,2,4,4,4
MED 10	6, 6,6, 5,5	6, 5,5,5,6	3,2,4,3,4	4,3,3,4,2
MOED 01	12,10,11,12,12	10,10,12,11,10	8,6,5,4,7	5,6, 5, 6, 5
MOED 02	9,8,12,11,12	11,10,11,12,12	5,6, 5, 5, 6	8,6, 5, 6, 6
MOED 03	10,12,12,9,11	10,8,12,11,9	6,6, 5, 5, 6	6,5, 5, 6, 6
MOED 04	10,12,12,11,10	9,12,12,9,10	5,5, 5, 5, 5	5,5, 5, 6, 5
MOED 05	12,12,12,11,12	12,12,9,11,10	6,6, 5, 6, 6	6,6, 6, 5, 6
MOED 06	12,12,12,12,12	10,12,12,10,12	6,5, 5, 6, 6	5,6, 5, 5, 5
MOED 07	10,8,12,11,12	10,10,11,11,12	5,6, 5, 5, 6	6,6, 5, 6, 5
MOED 08	10,12,12,10,11	12,8,9,11,12	6,6, 5, 5, 6	6,5, 5, 6, 6

**EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL
EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF
MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY**

MOED 09	10,12,12,11,10	12,12,12,11,10	5,5, 6, 5, 5	5,5, 5, 5,65
MOED 10	10,10,12,11,12	10,8,12,10,12	6,6, 5, 5, 5	5,6, 5, 6, 5
SED 01	14, 14, 14, 14, 15	14, 13, 14, 14, 13	7, 8,8,9,7	9, 9,8,9,8
SED 02	13, 12, 14, 14, 15	15, 13, 14, 14, 15	8, 9,8,9,8	8, 8,9,9,7
SED 03	14, 15, 14, 15, 15	14, 15, 14, 14, 15	8, 8,9,9,9	9, 8,9,9,8
SED 04	12, 15, 14, 14, 13	15, 12, 14, 14, 13	7, 8,9,9,7	8, 8,9,9,8
SED 05	15, 12, 14, 14, 14	15, 14, 14, 15, 15	9, 8,8,9,8	8, 8,8,9,9
SED 06	12, 12, 14, 14, 15	15, 13, 14, 14, 12	9, 9,8,9,9	9, 9,9,9,8
SED 07	15, 13, 14, 14, 13	14, 12, 14, 14, 14	9, 9,8,9,7	9, 8,9,9,9
SED 08	15, 13, 14, 15, 15	15, 14, 14, 14, 14	7, 8,9,9,7	9, 8,9,9,9
SED 09	15, 14, 14, 14, 13	14, 15, 14, 14, 14	9, 9,9,9,7	8, 8,9,9,7
SED 10	14, 15, 14, 14, 15	15, 15, 14, 14, 14	8, 8,8,9,8	7, 8,8,9,9

**EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL
EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF
MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY**

ANNEXURE – IV

FORMULA USED FOR ANALYSIS

Data was entered into Microsoft Excel spreadsheet and then checked for any missing entries. It was analysed using Statistical Package for Social Sciences (SPSS) version 21. All the variables were continuous variables like Gloss values and surface roughness values, which were summarized as mean and standard deviation. Graphs were prepared on Microsoft Excel.

Normality of the data was checked by **Shapiro Wilk test**. Data was found to be normal. Keeping in view the nature (continuous) & distribution (normal) of data, inferential statistics were performed using **parametric tests of significance**.

Inferential statistics were performed using **One way Analysis of Variance & Independent t Test**. CPost hoc pairwise comparison was done using **Post hoc Tukey's test**. The level of statistical significance was set at 0.05.

The following formulas were employed for calculation for various parameters:

Mean

The mean (also known as average), is obtained by dividing the sum of observed values by the number of observations, n . The formula for the mean is given below as :

$$\bar{X} = \frac{\sum_{i=1}^{i=n} X_i}{n}$$

Where, $\bar{X}$ = Mean

$\sum X$ = Sum of all individual observations

n = Total number of observations

EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY

Standard Deviation

The standard deviation gives an idea of how close the entire set of data is to the average value. Data sets with a small standard deviation have tightly grouped, precise data. Data sets with large standard deviations have data spread out over a wide range of values. The formula for standard deviation is given below as:

$$\sigma = \sqrt{\frac{1}{n-1} \sum_{i=1}^{i=n} (X_i - \bar{X})^2}$$

Where, σ = Standard deviation

$\bar{X}$ = Mean

X_i = Individual observation

$\sum X$ = Sum of all individual observations

$\sum (X_i - \bar{X})$ = Sum of differences of every observation from the mean value

n = Total number of observations

Analysis of Variance Test:

This test is used to compare more than two means simultaneously. The critical ratio which is calculated for performing ANOVA test is called as F ratio. It is calculated as ratio of between-groups variance to within groups variance.

$$F \text{ ratio} = \frac{\text{Between-groups variance}}{\text{Within-groups variance}}$$

$$F \text{ ratio} = \frac{\text{Mean square Between-groups}}{\text{Mean square Within-groups}}$$

**EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL
EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF
MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY**

Post hoc Tukey's Test:

Tukey's post-hoc test is a method that is used to determine which groups among the sample have significant differences. This method calculates the difference between the means of all the groups. Tukey's test values are numbers which act as a distance between the groups.

Formula is as follows:

$$M = \frac{M_1 - M_2}{\sqrt{MS_w \left(\frac{1}{n} \right)}}$$

treatment/group
mean
n = number per
treatment/group

**EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL
EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF
MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY**

ANNEXURE – V

PLAGIARISM REPORT



Page 1 of 9 - Cover Page

Submission ID tm:oid=26066:449973985

Deep Sharma

RE-2022-540914

Batch

Batch

Universidad del Valle

Document Details

Submission ID

tm:oid=26066:449973985

6 Pages

Submission Date

Apr 17, 2025, 4:47 PM GMT+5:30

1,452 Words

Download Date

Apr 17, 2025, 4:51 PM GMT+5:30

8,637 Characters

File Name

RE-2022-540914.pdf

File Size

91.6 KB



Page 1 of 9 - Cover Page

Submission ID tm:oid=26066:449973985

EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY



Page 2 of 9 - Integrity Overview

Submission ID tm:oid::26066-449973985

8% Overall Similarity

The combined total of all matches, including overlapping sources, for each database.

Filtered from the Report

- Bibliography
- Quoted Text

Match Groups

- 13 Not Cited or Quoted 8%**
Matches with neither in-text citation nor quotation marks
- 0 Missing Quotations 0%**
Matches that are still very similar to source material
- 0 Missing Citation 0%**
Matches that have quotation marks, but no in-text citation
- 0 Cited and Quoted 0%**
Matches with in-text citation present, but no quotation marks

Top Sources

- 3% Internet sources
- 7% Publications
- 1% Submitted works (Student Papers)

Integrity Flags

0 Integrity Flags for Review

No suspicious text manipulations found.

Our system's algorithms look deeply at a document for any inconsistencies that would set it apart from a normal submission. If we notice something strange, we flag it for you to review.

A Flag is not necessarily an indicator of a problem. However, we'd recommend you focus your attention there for further review.



Page 2 of 9 - Integrity Overview

Submission ID tm:oid::26066-449973985

EXPRESSION OF ENDOGLIN IN DIFFERENT GRADES OF ORAL EPITHELIAL DYSPLASIA AND ITS CORRELATION WITH PRESENCE OF MAST CELLS: A RETROSPECTIVE COMPARATIVE STUDY



Page 3 of 9 - Integrity Overview

Submission ID trn:oid::26066.449973985

Match Groups

- 13 Not Cited or Quoted 8%**
Matches with neither in-text citation nor quotation marks
- 0 Missing Quotations 0%**
Matches that are still very similar to source material
- 0 Missing Citation 0%**
Matches that have quotation marks, but no in-text citation
- 0 Cited and Quoted 0%**
Matches with in-text citation present, but no quotation marks

Top Sources

- 3%** **Internet sources**
- 7%** **Publications**
- 1%** **Submitted works (Student Papers)**

Top Sources

The sources with the highest number of matches within the submission. Overlapping sources will not be displayed

1	Internet		
core.ac.uk			2%
2	Publication		
Amol Ramchandra Gadgil, Minal S. Chaudhary, Sachin C. Sarode, Madhuri Gawa...			2%
3	Publication		
Shigenobu Kaneko. "Use of Bromodeoxyuridine, Proliferating Cell Nuclear Antige...			1%
4	Publication		
Pusa Nela Gaje, Raluca Amalia Ceausu, Adriana Jitariu, Stefan Ioan Stratul et al. "...			<1%
5	Internet		
www.cancerjournal.net			<1%
6	Internet		
journals.plos.org			<1%



Page 3 of 9 - Integrity Overview

Submission ID trn:oid::26066.449973985