"EVALUATION OF IMMUNOEXPRESSION OF CDK14 IN NORMAL ORAL MUCOSA, ORAL EPITHELIAL DYSPLASIA AND ORAL SQUAMOUS CELL CARCINOMA"

By

REG. NO. IH0220004

Dissertation

Submitted to KLE Academy of Higher Education and Research

(KAHER), Belagavi

In Partial Fulfillment of the Requirements for the Degree Of

MASTER OF DENTAL SURGERY

In

ORAL & MAXILLOFACIAL PATHOLOGY & ORAL MICROBIOLOGY

(BRANCH - IV)

DEPARTMENT OF ORAL & MAXILLOFACIAL PATHOLOGY & ORAL MICROBIOLOGY

KAHER'S KLE VISHWANATH KATTI INSTITUTE OF DENTAL SCIENCES, KAHER, NEHRU NAGAR, BELAGAVI -10, KARNATAKA.

2020 -2023

KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH, BELAGAVI, KARNATAKA

ENDORSEMENT BY THE HOD, PRINCIPAL/HEAD OF THE INSTITUTION

This is to certify that the dissertation **"EVALUATION OF IMMUNOEXPRESSION OF CDK14 IN NORMAL ORAL MUCOSA, ORAL EPITHELIAL DYSPLASIA AND ORAL SQUAMOUS CELL CARCINOMA"-** is a bonafide work done by **REG. NO. IH0220004**

27/12/22

Dr. Punnya S Rao M.D.S, DNB, PGBE, FFO, PhD
Professor and Head
Department of Oral & Maxillofacial Pathology & Oral Microbiology
KLE VK Institute of Dental Sciences,
Nehru Nagar, Belagavi- 590010

Date: 27.12.22
Place: Belagavi.

Professor and Head
Department of Oral Pathology,
KLE V. K. Institute of Dental Sciences,
Belagavi

Dr. Alka D. Kale M.D.S, Ph.D.
Principal
KLE VK Institute of Dental Sciences
Nehru Nagar, Belagavi-590010.

Date: 27/12/22
Place: Belagavi.

PRINCIPAL
KLE V.K. Institute of Dental Sciences
Nehru Nagar, BELAGAVI-590010.

UNDERTAKING

I, **REG. NO. IH0220004** hereby declare that the information and data mentioned in my dissertation entitled **"EVALUATION OF IMMUNOEXPRESSION OF CDK14 IN NORMAL ORAL MUCOSA, ORAL EPITHELIAL DYSPLASIA AND ORAL SQUAMOUS CELL CARCINOMA"** belongs to me and is original.

I am aware of the definition of plagiarism as detailed below:

- An act or instance of using or closely imitating the language and thoughts of another author without authorization and the representation of that author's work as one's own, as by not crediting the original author.
- A piece of writing or other work reflecting such unauthorized use or imitation.
- The deliberate or reckless representation of another's words, thoughts or ideas as one's own without attribution in connection with submission of academic work, whether graded or otherwise.

I hereby declare that the thesis prepared by me is original one and does not involve plagiarism anywhere. In case at later stage it is found that I have indulged in plagiarism, then I am solely responsible for the same and the Institution is at liberty to take any disciplinary action against me including cancellation of dissertation or any other penalties imposed by the University.

Date:

Place: Belagavi

UNDERTAKING

I, **REG. NO. IH0220004** post-graduate student from Department of Oral & Maxillofacial Pathology & Oral Microbiology has completed research work on the topic **"EVALUATION OF IMMUNOEXPRESSION OF CDK14 IN NORMAL ORAL MUCOSA, ORAL EPITHELIAL DYSPLASIA AND ORAL SQUAMOUS CELL CARCINOMA",** in the year 2020-2023

I have been given to understand that any research work I undertake for the purpose of dissertation, oral presentations or publications during my study course shall be property of the KAHER Vishwanath Katti Institute of Dental Science, Belagavi. Hence, I hereby declare that the name of department institute and university shall be mentioned in my publications. The authorship shall be according to the guideline informed to me.

Date:

Place: Belagavi **REG. NO. IH0220004**

PLAGIARISM CHECK CERTIFICATE

Scientific Correspondence and Review Committee

KLE VK Institute of Dental Sciences

A Constituent Unit of KLE Academy of Higher Education and Research
(Deemed-to-be-University u/s 3 of the UGC Act, 1956)
Nehru Nagar, Belagavi - 590 010, Karnataka State

Accredited 'A' Grade by NAAC (2nd Cycle) | Placed in Category 'A' by MHRD (GoI)

☎: 0831-2470362
FAX: 0831-2470640
Web: http://www.kledental-bgm.edu.in
E-mail:principal@kledental-bgm.edu.in

Date : 24-12-2022 Serial No. : 128

PLAGIARISM CHECK REPORT

Name of the Applicant :
UG / PG / Ph.D / Staff : POSTGRADUATE STUDENT
Batch & Year : 2020-23
Department : ORAL PATHOLOGY AND MICROBIOLOGY

The soft copy of Research Work / Manuscript by ..... entitled "EVALUATION OF IMMUNOEXPRESSION OF CDK4 IN NORMAL ORAL MUCOSA, ORAL EPITHELIAL DYSPLASIA AND ORAL SQUAMOUS CELL CARCINOMA"

under the guidance of .....has been submitted for Anti-Plagiarism check to the Scientific Correspondence & Review Committee of KLE VK Institute of Dental Sciences using "Turn-it-in" software.

The scan has been carried out and the scanned output reveals a Similarity Index of .....6.....%, which is **within / not within** the acceptable limits of 10% as per the UGC guidelines.

Member Secretary
Scientific Correspondence and Review Committee
KLEVK Institute of Dental Sciences
KAHER-Belagavi

Chairman
Scientific Correspondence and Review Committee
KLEVK Institute of Dental Sciences
KAHER - Belagavi

LIST OF ABBREVATIONS

SR. NO.	ABBREVATIONS	FULL FORM
1.	%	Percentage
2.	i.e.	That is
3.	No.	Number
4.	OM	Oral Mucosa
5.	OSCC	Oral squamous cell carcinoma
6.	OED	Oral epithelial dysplasia
7.	CDK14/PFTK1	Cyclin dependent kinases 14
8.	IHC	Immuno-histochemistry
9.	LRP6	Low-density lipoprotein receptor-related protein 6
10.	MRD	Minimal residual disease,
11.	CCNY	Cyclin Y
12.	CCND3	Cyclin D3
13.	CDKs	Cyclin Dependent Kinases
14.	ESCC	Oesophageal squamous cell carcinoma
15.	HCC	Hepatocellular Carcinoma
16.	CRC	Colorectal carcinoma
17.	Cdc2	Cell division cycle 2
18.	PCP	Planar cell polarity pathway
19.	WDSCC	Well differentiated squamous cell carcinoma
20.	MDSCC	Moderately differentiated squamous cell carcinoma
21.	PDSCC	Poorly differentiated squamous cell carcinoma
22.	GBS	Gingivo-buccal sulcus

ABSTRACT

INTRODUCTION: Oral Squamous Cell Carcinoma (OSCC) constitutes the most prevalent oral cavity cancer. It exhibits an elevated level of local aggressiveness, invasion and involvement of lymphatics, as well as a significant mortality rate. OSCC is mostly preceded by the appearance of precursor lesions with varying degrees of malignant transformation potential. OSCC development and progression are regulated by a variety of molecular mechanisms and pathways. CDK14 belongs to the cyclin-dependent kinase (CDK) menage, which is involved in cancer tumorigenesis. It is found expressed in different types of tumours, including ovarian cancer, hepatocellular carcinoma (HCC), gastric carcinoma, breast cancer, and oesophageal squamous cell carcinoma (ESCC). However, its expression and role in normal oral mucosa (OM), Oral epithelial dysplasia (OED), and OSCC unknown. As a result, the study was directed to examine the expression of CDK14 in normal oral mucosa, OED, and OSCC.

MATERIAL AND METHODOLOGY: Total 120 samples were taken for this study. 40 each from surface of OM, OED and OSCC using archived tissue blocks. Immunohistochemistry was done using CDK14 marker.

RESULTS: Chi-Square test was performed. Our results disclosed a statistically significant association of the immunoexpression of CDK14 with OM, OED and OSCC. We also noticed statistical significant association of expression of CDK14 between the normal OM and OED & between the normal OM and OSCC.

CONCLUSION: Immuno-histochemical analysis demostrated that CDK14 expression was upregulated in OED and OSCC than in normal oral mucosa tissues.

KEYWORDS: Oral Squamous Cell Carcinoma, OSCC, CDK14, PFTK1 Oral normal mucosa, Oral epithelial dysplasia , OED

LIST OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

With more than 3 lakhs new occurrences every year, OSCC is a deforming disease that is becoming increasingly common in the 21st century, especially in younger people.[1]

The normal stratified squamous oral mucosa undergoes a complex multi-step multifactorial process of transformation into premalignant and then malignant tissue as a result of genetic change that disrupts the normal activity of oncogenes and tumour suppressor genes.[1]

"Epithelial precursor lesions" are "altered epithelia with an enhanced chance of development to squamous cell carcinoma".[2]Because the majority of OSCC are initiated by clinically premalignant lesions and disorders characterised by different grades of epithelial dysplasia, it is regarded as an cheif histological indicator of premalignancy.[3] It has been discovered that OSCCs can develop from oral epithelial dysplasia. Using the pre-malignancy window to detect malignant transformation at the earliest possible stage and intervening effectively to stop disease progression are critical for improving patient survival and lowering morbidity after OSCC diagnosis.[3]

There are numerous molecules that are crucial to the development of OSCC. The prognosis of this disease will therefore benefit from researching these molecular pathways.

Family of 20 protein kinases called cyclin-dependent kinases (CDKs) regulates a number of biological processes which includes cell cycle progression, transcription, differentiation , and many others. One of these is CDK14, also widely recognized as PFTAIRE1 or PFTK1.

CDK14 or PFTK1, is associated with cell division cycle 2 (cdc2). It is the newest member of CDK family. Its gene is located on chromosome 17q21.13. It regulates the G1/S and G2/M phases of cell cycle.[4]

Cyclin D3 (CCND3) and cyclin Y(CCNY) are the proteins that interact with CDK14 to produce an effect.[5] Cyclin Y and its association with CDK14 increases its activity & attracted towards plasma membrane. The primary job of CDK14 is to control the cell cycle through a unique interaction with CCND3 and cyclin Y.[6] The cell cycle inhibitor p21, cyclin D3 and cyclin Y form a ternary complex, which phosphorylates Retinoblastoma (Rb) the tumour suppressor, for the G1/S transition.[6] Studies have revealed that CDK14 frequently functions as an oncogene because it is a regulatory component that controls cell proliferation and progression of cell cycle.[7]

Various study found that CDK14 has been overexpressed in ESCC, breast carcinoma, ovarian cancer, HCC and colorectal cancer (CRC). Its expression was linked to HCC and CRC's propensity for invasion and cell migration, as well as a poor prognosis and treatment resistance in ESCC patients. But no research has yet been done on the connection between CDK14 and OSCC.[8]

Immunohistochemistry (IHC) study of ESCC tissues confirmed that, when compared to normal tissues and cell lines, the immune-expression of CDK14 in tumour is elevated. IHC staining showed a strong correlation between CDK14 and clinical pathological factors as tumour size, tumour grade, and tumour invasion, as well as Ki-67.[4]

Thus, the present study aimed to evaluate CDK14 expression & its correlation in oral normal mucosa, OED and OSCC.

AIM OF THE STUDY:

Evaluation of Immunoexpression of CDK14 in normal oral mucosa, oral epithelial dysplasia and oral squamous cell carcinoma.

OBJECTIVES:

- To assess the immunoexpression of CDK14 in normal oral mucosa, oral epithelial dysplasia, oral squamous cell carcinoma.
- To compare the immunoexpression of CDK14 in normal oral mucosa, oral epithelial dysplasia and oral squamous cell carcinoma.
- To co-relate the Immunoexpression of CDK14 with clinicopathological and prognostic parameters.

REVIEW OF LITERATURE

ORAL EPITHELIAL DYSPLASIA

Introduction of theory of epithelial dysplasia in oral pathology was done 60 years ago, because some oral lesions, now known as oral potentially malignant disorders (OPMDs), had the potential to progress to oral cancer.[9]

Oral potentially malignant disorders, a clinical diagnosis for which the histological diagnosis may be hyperplasia, hyperkeratosis, OED or OSCC. [10,11]

Dysplasia refers to abnormal changes in the epithelium. Removal of underlying inciting stimulus, may revert dysplastic changes to normal. WHO monograph on head and neck tumours (2005) defines "epithelial precursor lesions" as "altered epithelium with an increased likelihood of progression to squamous cell carcinoma."[12]

OED is characterised by cytological and architectural changes that reflect the defects in normal surface epithelial maturation and stratification.[10,11] The histopathological changes in a chronic, progressive, and premalignant disorder of the oral mucosa are referred to as OED.[13]Epithelial dysplasia only describes histological changes and doesn't have any clinical morphological equivalent.[9]

OED is a histologic marker of premalignancy that predicts an increased risk development of OSCC.[13] Richart and Barron first reported the risk of epithelial dysplasia (ED) changing into carcinoma in 1969.[14]Over the time, it was discovered that OPMD with OED transformed very often into OSCC than lesions without

dysplasia. Furthermore, potentially malignant disorders and oral epithelial dysplasia were observed in the mucosa surrounding invasive OSCC.[15,16]

Various changes in the stratified squamous epithelium of oral lesion are used to establish the diagnosis of OED and is used to assess the probability of malignant transformation.[13,17] The "Grade" of OED refers to the severity of dysplastic characteristics.[13] Till date various grading systems have been proposed and out of which WHO 2017 is most recent one(Table 1). OED has been graded using a variety of dysplastic architectural and cytological characteristics in various combinations (Table 2).

Grading Systems of Oral epithelial dysplasia (Table 1)

Year	Author
1969	Smith and Pindborg photographic methods
1971	Mehta et al
1976	Bancozy and Csiba
1978	WHO
1980	Kramer
1981	Burkhardt and Maerkar
1983	Shafer
1995	Lumermann H et al
1995	Neville et al
1996	Speight PM et al
2002	Kuffer and Lombardi
2003	Ljunljana
2003	Brothwell DJ
2005	WHO system
2005	Binary system
2017	WHO system

World Health Organization (WHO) criteria for epithelial dysplasia (2017)[17]

(Table 2)

Architectural changes	Cellular changes
Irregular epithelial stratification	Abnormal variation in nuclear size (anisonucleosis)
Loss of polarity of basal cells	Abnormal variation in nuclear shape (nuclear pleomorphism)
Drop-shaped rete ridges	Abnormal variation in cell size (anisocytosis)
Increased number of mitotic figures	Abnormal variation in cell shape (cellular pleomorphism)
Abnormal superficial mitosis	Increased nuclear-cytoplasmic ratio
Premature keratinization in single cells (Dyskeratosis)	Atypical mitotic figures
Keratin pearls within rete ridges	Increased number and size of nucleoli
Loss of epithelial cell cohesion	Hyperchromasia

The malignant transformation of dysplastic lesions is still poorly understood, according to Califano et al., 1996 and Leemans et al., 2018. Oral carcinoma develops through the combination of key molecular changes in tumour suppressor genes and oncogenes, possibly in a sequence, till the final required change completes the cancer genotype and initiates invasion and supported by gradual increase in mutations and chromosomal aberrations and genetic signatures in dysplastic lesions, to predict transformation.[9]

ORAL SQUAMOUS CELL CARCINOMA

Oral cancer are a group of neoplasms affecting any part of the oral cavity, pharyngeal regions & salivary glands.[18] Cancer is a multi - stage process in which cell mutations accumulate and cause initiation, progression, proliferation maintenance, signalling, evasion of growth suppressors, cell death avoidance, continuous replication, angiogenesis, invasion, and metastasis. Cancer is caused by gene mutations, which cause changes in various pathways.[19]

However, this term is often used interchangeably with OSCC, which is estimated to account for more than 90% of all oral neoplasms.[18]

Histopathologically, it develops from OED, characterised by proliferation of dysplastic squamous cells, which degrades the basement membrane (BM). The degradation of BM causes destruction as well as distant invasion via metastasis. Local invasion of the tissue occurs via epithelial cell islets and cords.[20]

OSCC develops due to numerous molecular events caused by a combination of an individual's genetic predisposition and exposure to environmental carcinogens[21] such as tobacco, alcohol, chemical carcinogens, UV or ionising radiation, and microorganisms.[22-24] Chronic carcinogen exposure can harm larger portions of the genetic material. Mutations of oncogenes that promote cell survival and proliferation may result from genetic damage. Mutations such as DNA hypomethylation in general, hyper- or hypomethylation of specific genes such as cyclin D, and chromatin alterations.[25,26] Recent research has looked into oncogenic signalling pathways like glycogen synthase kinase 3 (GSK-3), c-Myc, AKT, β-catenin, Rb and p53, and (NF-kB) and suggested their part in the progression of oral cancer.[27] The nuclear factor kappa B (NF-kB), PI3K-AKT, and Wnt pathways were discovered to be three major

interconnected pathways. Cyclin D1 (CCND1), Rb, p53, FLJ10540, and TC21 are the most frequently mutated genes. The most common molecular pathways involved in the pathogenesis of oral cancer are the NF-kB, PI3K-AKT, and Wnt pathways.[27]

Various molecular changes that occur during carcinogenesis are thought to be the initial events that will be reflected later histopathologically and clinically. Neoplastic cells influence tumour behaviour by undergoing several molecular changes.[28]Because of their altered characteristics, these abnormal cells exhibit a variety of morphological and functional abnormalities. Protein expression in OSCC may be altered, such as being reduced or overexpressed, as a result of genetic and epigenetic changes.[28]OSCC could develop due to these alterations in oncogenes and tumour suppressor genes such as "cyclin D1,p53, Retinoblastoma(Rb), epidermal growth factor receptor," among others.[29] Because of the molecular mechanisms governing OSCC, a vast number of biomarkers are being employed to predict and characterize the disease behavior. Based on the biological roles , these biomarkers are divided into five groups : a) cell proliferation and cell cycle progression; b) tumor suppression and apoptosis ;c) hypoxia; d)angiogenesis and e) cell adhesion and matrix degradation.[30]

There are various biomarkers which transforms a normal cell into malignant cell.[22] One among them is CDK14. A Cyclin-dependent kinase, CDK14 also called as PFTK1/PFTAIRE protein kinase 1, interacts with CCND3 and CCNY to regulate progression and proliferation of the cell cycle.[31,32]

CDK14

CDK14, a newly discovered CDK that is of Cdc2-related protein kinase family.[38]It has an N-terminal domain of ~ 140 amino acids, a kinase conserved domain of ~300 amino acids, and a C-terminal domain of ~30 amino acids. [39]Despite the fact that the N-terminal region contains two predicted nuclear localization signals, it is cytoplasmic in Hela cells (NLSs). [37]CDK14, also known as PFTK1, is present in high levels in brain, heart, pancreas, kidney, testis, and ovary and minimal in the lung, liver, or placenta.[33]

CDKs are Ser/Thr protein kinases that all share the PSTAIRE motif, which is found in the kinase domain's subdomain III. This motif is involved in CDK-cyclin binding and is used to classify other recently discovered CDK-related kinases such as PCTAIRE, PITSLRE, PFTAIRE, PITAIRE, KKIALRE, PISSLRE, MAK, and MRK.[34]

Cdc2-related CDKs are regulators of yeast and human growth and differentiation. The Cdk's family regulate transitions between successive cell cycle in eukaryotic cells.[33] Cdc2 and Cdk2 are functionally homologous to yeast cdc2/Cdc28 and it participates in the cell cycle functions. The fission yeast cdc2 protein kinase is required for the control of the mitotic cell cycle, as well as the transition from G1 to S phase. [33]

CDK14 is a CDK that regulates progression and proliferation of cell cycle by interacting with cyclin proteins such as CCND3[40] & CCNY.[41-43]

According to Etonia Y-T. Pang et al, PFTK1 is a novel member i.e. upregulated in HCC and correlates with metastatic and motile phenotypes.[35]

Cell cycle regulation by the CDK14-CCND3 complex, according to Fang Shu et al, is presumably tightly linked to cell growth and stimulation.[26] The CDK14 is activated by CCND3 and inhibited by p21.[33] The Rb protein is essential for cell cycle regulation because it phosphorylation in the cell cycle, leads to uncontrolled proliferation.[11]Fang Shu et al. found that in order to function, CDK14 could phosphorylate Rb. [33]

Interaction of CDK14 with CCN, a important functions in Drosophila embryogenesis[44], has been discovered to mediate the phosphorylation of LRP6 in Drosophila.[44-46]The phosphorylation of transmembrane receptor LRP6 is well recognised to be a significant and as an early step in the canonical Wnt signalling cascade[47-49], implying a possible role of CDK14-CCNY complex in controlling Wnt pathway.[50]

Wnt pathway is implicated in many human diseases together with cancer, and is involved in various biological processes.[51-53] Wnt signalling is branched into two pathways the canonical -catenin-dependent pathway and the non-canonical planar cell polarity (PCP) and Wnt/Ca^{2+} pathways. Non-canonical Wnt signals control cell polarity and motility by activating the Rho family of small GTPases and increasing intracellular Ca^{2+}. Canonical Wnt signals control cell proliferation and fate by stabilising and nuclear translocating β-catenin.[54]

In HCC cells, Tingting Sun et al discovered a interaction between 'CDK14 and cyclin Y'. CDK14-CCNY complex activates non-canonical Wnt pathway but does not activate the canonical Wnt/β-catenin pathway. [56] Actin polymerization and the formation of stress fibres are caused by subsequent activation of Rho GTPases in non-canonical Wnt pathways.[56] Evidence suggests that non-canonical Wnt pathway

components are frequently highly expressed in gastric, lung, and colon cancers.[59-62] Tingting Sun et al observed that combining CDK14 and cyclin Y activated non-canonical Wnt pathways while suppressing canonical Wnt pathway. Dvl2 expression was significantly increased in cells co-transfected with CDK14 and Cyclin Y.[56] Despite the fact that Dvl2 can activate both the non-canonical and canonical β-catenin pathways[63], it was also found that Naked1 protein expression was accelerated. When Naked1 binds to Dvl, it directs Dvl's protein activity to a non-canonical pathway, inhibiting Wnt/β-catenin signalling. [64,65] The PCP pathway and Ca^{2+} pathway are non-canonical Wnt pathways that result in the activation of the Rho family of small GTPases and actin polymerization.[63]

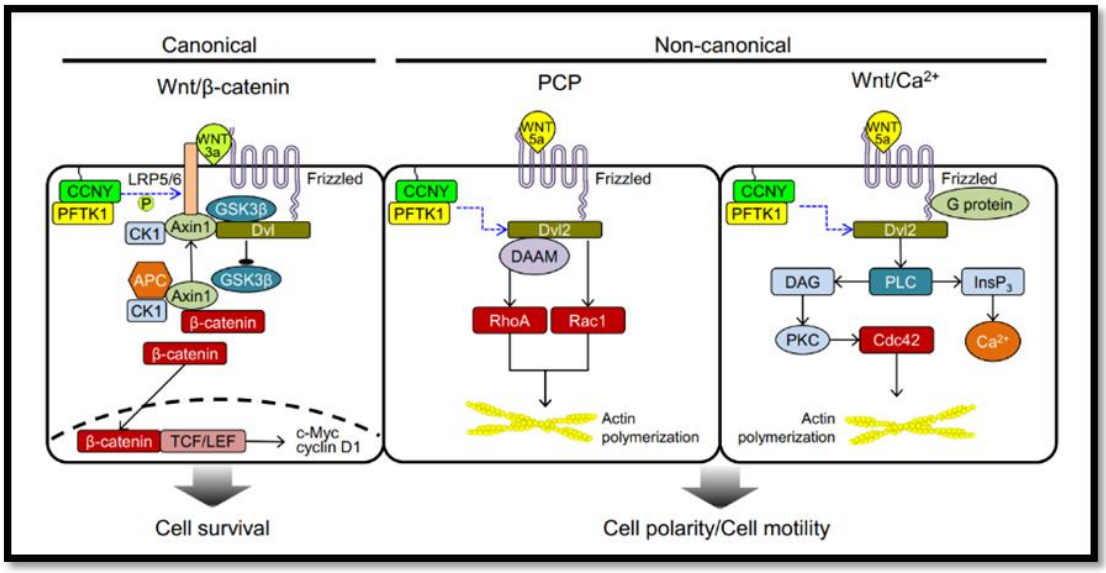


Figure 1: CDK14/CCNY participates in both canonical and non-canonical Wnt pathways. In Drosophila cells, PFTK1 interacts with CCNY and works together to phosphorylate LRP6, activating the Wnt/b-catenin cascades. The interaction of CDK14 and CCNY in HCC cells activates Dvl2 and the downstream RhoA, Rac1, and Cdc42 in the non-canonical PCP pathway and the Wnt/Ca2+ pathway, leading to actin polymerization.

Sun T, Co NN, Wong N. PFTK1 interacts with cyclin Y to activate non-canonical Wnt signaling in hepatocellular carcinoma. Biochemical and biophysical research communications. 2014 Jun 20;449(1):163-8.

Tingting Sun et al. demonstrated non-canonical Wnt pathway activation in CDK14 and CCNY co-transfected cells by showing enhanced activated forms of RhoA, Rac1, and Cdc42, as well as marked filamentous actin polymerization. Rho GTPases control cytoskeletal dynamics and the formation of filopodia and actin stress fibres, which may be essential in spreading of primary cancer cells (Fig 1).[67-69]This suggests that CDK14 with CCNY promotes invasiveness and cell motility by activating the non-canonical Wnt pathway.

METHODOLOGY

ETHICAL APPROVAL

The institutional ethical review committee gave its clearance to the study. Ethical clearance no. 1464 (Annexure I).

STUDY SAMPLE

For the retrospective analysis, 120 tissue blocks of cases of normal oral mucosa, OED and OSCC with histological confirmation were embedded in paraffin. According to the WHO classification, OED samples were further categorised into mild, moderate and severe dysplasia. The cases used in the study were located in the archives of the Department of Oral and Maxillofacial Pathology and Oral Microbiology at the VK Institute of Dental Sciences, part of the KLE Academy of Higher Education and Research (KAHER) in Belgaum. The tissue was stored as paraffin-embedded blocks, which were later subjected to immune-histochemical analysis. During third molar extraction and gingivectomy procedures, 40 Oral normal mucosa samples were collected.

From each block, two tissue sections measuring 4 µm each were removed and placed on slides coated with "amino propyl triexthosilane (APES)" (AnnexureIV). Hematoxylin and eosin was used to stain one slide (Annexure V). While anti-CDK14 was used to immunehistochemically stain the other slide.

SAMPLE SIZE ESTIMATION

$n = Z^2pq/d^2$

p = 65% (percentage of positivity)

q =35%

d = 15% (margin of error)

Z = 1.96 at 5% alpha error (95% confidence)

n = (1.96 x 1.96) (65) (35) / 15x15

= 39~40

Normal Mucosa	40
Oral epithelial dysplasia	40
Oral squamous cell carcinoma	40

DEMOGRAPHIC DETAILS

Demographic information for all three groups—including age, sex, site, habit, histological grade, and lymph node status—was collected from the departmental archives. Additionally, information on existence or lack of lymph node metastases in RND patients of OSCC was gathered.

STAINING PROCEDURE

An antibody against CDK14 was used for immune-staining. Using the PathnSitu Catalogue No. PEH002/USA PolyExcel HRP/DAB Detection System Two Step Universal Kit.

PRINCIPLE OF IMMUNOSTAINING

The Antigen/Antibody Reaction in Tissues Principle is the foundation of the PolyExcel HRP/DAB Detection System Two Step Universal Kit. The primary CDK14 antibody binds to the appropriate antigen in tissues. The primary antibody is conjugated with dextran polymer-backed secondary antibodies. Antigen-antibody complexes and the chromogen DAB (3'3'diaminobenzidine) interact to produce a coloured reaction result.

REAGENTS USED

1. Primary & secondary antibody:

A. Rabbit Polyclonal antibody to Human CDK 14 purified from primary cultures

Catalogue number: # PA5-53293

Specificity: Human

Class: Polyclonal

Company: Invitrogen

B. Secondary Kit contains

- Peroxide block (H_2O_2): This blocks the activity of endogenous peroxidase by including 3 percent hydrogen peroxide in water.
- PolyExcel target binder: This is a universal protein that helps in binding to primary antibody.

- PolyExcel HRP Reagent: This includes stabilisers, proclin 300, enzyme polymer in phosphate buffered saline, and anti-mouse or anti-rabbit antibody labelled with IgG.
- Liquid DAB Chromogen: DAB chromogen that has enhanced sensitivity with HRP as colorimetric agent.
- Stable DAB substrate buffer: This buffer contains Tris-buffer along with peroxide as well as stabilizers. It is used along with DAB chromogen.

3. Buffers:

- Citrate buffer: for antigen retrieval (Annexure VI)
- This was employed to reveal antigen-binding sites in the tissues with pH 6 by a process called heat-induced epitope retrieval (HIER).

4. Xylene for cleaning/ dewaxing.

5. Graded alcohol solution (100%, 90%, 80%, 70%, and 50%) for dehydrating.

6. Distilled water-wash

7. Harris Hematoxylin – counter stain

8. Mounting medium, DPX

9. Other equipment's used:

- APES coated glass slides
- Humidifying chamber
- Wash bottles
- Absorbent wipes (tissue papers)
- EZ retriever system V.2.1 (for HIER)
- Calibrated test tube
- Plastic Pasteur pipette (provided with detection kit to mix DAB chromogen & buffer)

- Cover slips
- Micropipettes
- Refrigerator (4°C, -20°C)
- Semi-automatic microtome (Leica RM 2145)
- Slide warmer
- Water bath
- Multi-viewer Microscope

IHC STAINING PROTOCOL:

1. Sectioning: APES-coated slides were mounted with 4µm sections of formalin-fixed, paraffin-embedded tissues.
2. Deparaffinization: Slides were treated with two changes of xylene for 15 minutes each after being deparaffinized by heating on a slide warmer at 60°C for an hour.
3. The slides were treated with one change each of 100% alcohol followed by graded alcohol 90%, 80% and 70% for 10 min each.
4. Slides were then rinsed with distilled water.
5. Heat Induced Epitope Retrieval: A staining trough filled with citrate buffer (pH 6) was placed in a pressure cooker (**Table 3**).

Table 3: Primary antibody retrieval method

Primary antibody	Method of antigen retrieval
CDK14	**Pressure cooker HIER** Preheating buffer : 5mins After placing the slides : 10mins

6. After this step, slides were allowed to cool to room temperature followed by distilled water wash for 5 minutes and later followed by PBS rinse for 5 minutes.

IMMUNOHISTOCHEMICAL STAINING:

Endogenous peroxidase activity was blocked by incubating with peroxidase block for 10 minutes and washed with wash buffer (PBS) for 5 minutes

↓

Slides were incubated with primary polyclonal antibody against CDK14 for overnight incubation at 4 degree Celsius. After that slides were rinse with PBS for 5 minutes each

↓

Target binder was added to promote Ag-Ab reaction and incubated for 30 minutes in humidifying chamber. This was followed by PBS rinse for 5 minutes

↓

Slides incubation was done with Poly HRP for 30 minutes and it was followed by 2 change of in PBS for 5 minutes each

↓

Incubation was done with fresh substrate/chromogen mix of 3,3'Diaminobenzidine (DAB) mixed with buffer (i.e 25 μl concentrated DAB in 500 μl of substrate buffer for 10 slides) for upto 10 minutes. This step enables visualization of antigen-antibody reaction as a brown colored end product. After that slides were dipped in distilled water

↓

Slides were counterstained with Harris Hematoxylin upto 1-2 minutes.

↓

Under running tap water, bluing was carried out for upto 10 minutes.

↓

After that slides were dehydrated and mounted with DPX.

ANALYSIS OF DATA:

From the archival registers, the clinical information for the cases was gathered and calculated. The OED and OSCC stained in H and E slides were Graded based on WHO criteria.

Following immunological staining, the slides were examined by two oral pathologists, whose findings were tabulated. To obtain a consensus, any discrepancy was once again evaluated in the penta-headed multi-viewing microscope.

The three groups were analyzed on three main criteria; intensity, localization, percentage of positive staining and extent. (**Table 4-7**)

CRITERIA OF ANALYSIS :

Table 4: Criteria used for intensity of CDK14 in both epithelium and Stroma

Intensity	Criterion of Intensity
0	None
1	Mild
2	Intense

Table 5: Criteria used for localisation of CDK14 in both epithelium and Stroma

Location	Criterion of Location
0	Absent
1	Nuclear
2	Cytoplasmic
3	Nuclear + Cytoplasmic

Table 6: Criteria used for Percentage of positivity of CDK14 in both epithelium and Stroma

Percentage staining score	Percentage of cells
0	Absent
1	1-25%
2	25-50%
3	>50%

Table 7: Criteria used for Extent of CDK14 in epithelium

Extent score	Extent
0	Absent
1	Basal +Suprabasal cell layer
2	Spinous cell layer
3	Granular cell layer
4	Corneal cell layer

ASSESSMENT OF VARIOUS HISTO-PATHOLOGICAL PARAMETERS IN OSCC CASES:

Various Histopathological parameters were evaluated according to different criteria such as Pattern of invasive front (POI), Tumor budding (TB), depth of invasion (DOI), type of Connective tissue stroma, degree of inflammation, lymphovascular invasion (LVI), and perineural invasion (PNI). A H & E stained segment of OSCC instances was used to investigate each of these factors.

1. **Tumor Budding:** According to Wang et al's criteria, it was evaluated.[63]It was determined that it was the development of single tumour cells or the presence of small clusters with five or less cells before the tumor's invasive front. Intensity of bud growth was categorised as low if there were fewer than five buds per field and high if there were more than five buds per field. Under low power (4x), the sections were scanned to look for high density blossoming areas. The maximum count seen for each slide was stated as the number of tumour buddings for that specific case, which was followed by counting of tumour buds under higher magnification (40x). (**Table 8)**

Table 8: Tumor budding evaluation criteria

Tumor budding	Criteria
Low intensity	<5 buds/field
High intensity	>5 buds/field

2. **Depth of Invasion:** According to Angadi et al[67], it was. To search for DOI, sections were scanned at a 10x magnification. It received grades in four areas (Table 9). Each instance received a certain grade.

Table 9: DOI Evaluation criteria

DOI grade	Criteria
1	Carcinoma insitu/ questionable invasion
2	Definite invasion involving lamina propria
3	Invasion below the lamina propria adjacent to muscle, salivary gland and periosteum
4	Extensive and deep invasion replacing most of the stromal tissue in the jaw

3. **Type of invasive front:** According to Angadi et al[67], invasive front was graded into 4 categories as mentioned in the table below (Table10). Each case was assigned with specific grade following the evaluation.

Table 10: Type of invasive front evaluation criteria

Invasive Front	Criteria
1	Pushing well- delineated borders
2	Infiltrative solid cords/ bands/strands
3	Small groups or cords of infiltrative cells(nests)
4	Marked cellular dissociation in small groups of cells or single cells

4. **Type of stroma:** the evaluation used the Angadi et al criteria[67]. Tumor islands' surrounding stroma was evaluated and classified into 4 different categories (table 11). Each case was given a specific kind.

Table 11 : Type of stroma evaluation criteria

Stroma	Criteria
1	Abundant
2	Dense
3	Delicate
4	None

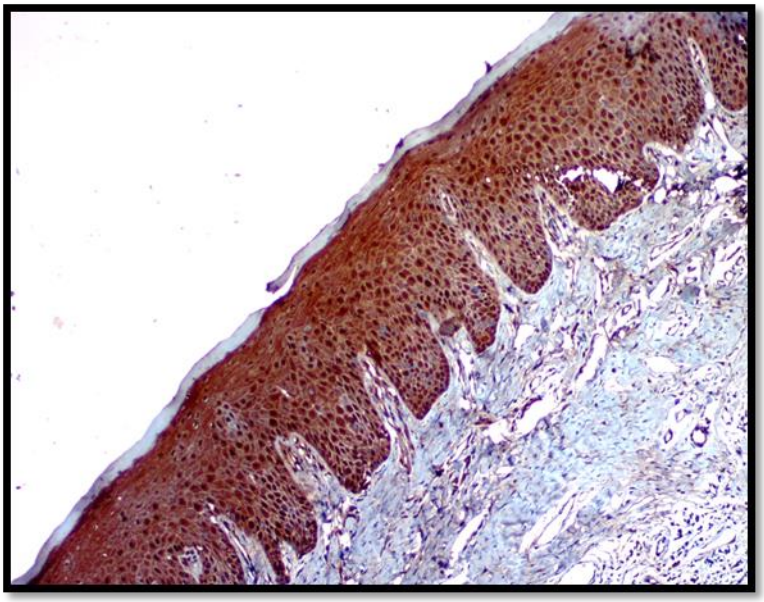
5. **Degree of Inflammation:** This was assessed using the Angadi et al.[67] methodology. In order to detect any inflammatory components, the surrounding stroma was examined and divided into 4 categories (Table 12). Based on the following factors, a specific grade was assigned to each instance.

Table 12 : Degree of inflammation evaluation criteria

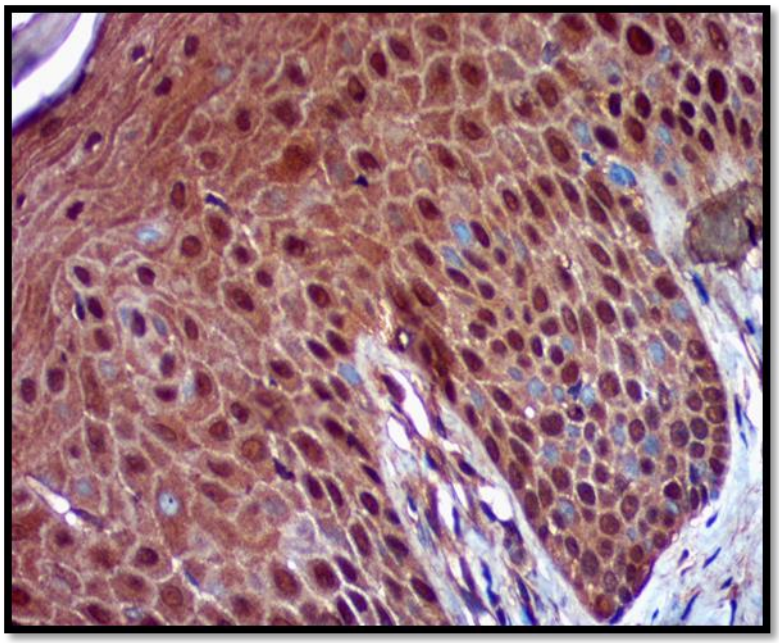
Inflammation	Criteria
1	Marked
2	Moderate
3	Slight
4	None

6. **Lympho-vascular invasion:** High magnification was used to inspect the slice for tumour cells along the lympho-vascular pathways.
7. **Perineural invasion:** To check for the presence of tumour cells within the perineural space of nerve fascicles, a section was examined under a high power microscope.

1. PHOTOMICROGRAPHS OF CDK14 IN ORAL MUCOSA



1.a. Oral mucosa

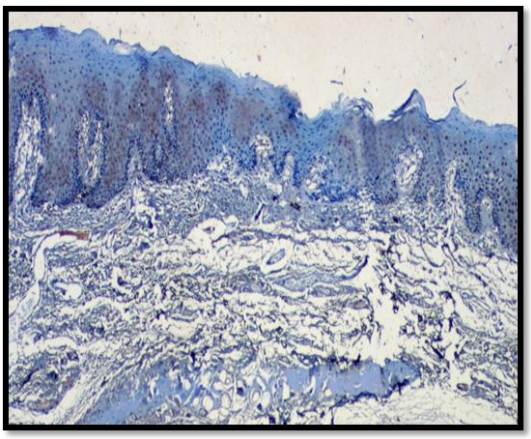


1.b. Oral mucosa

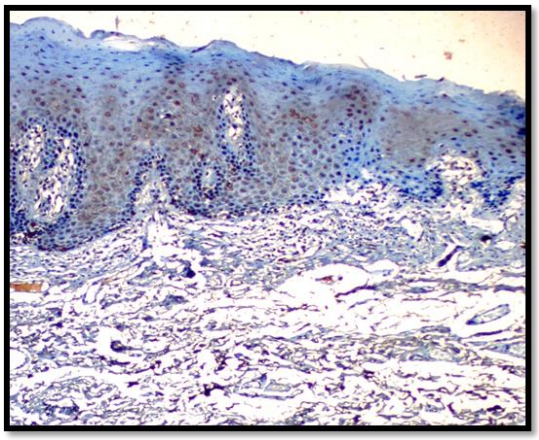
1.a Immunoexpression of CDK14 is intense and both nuclear and cytoplasmic in normal oral mucosa (10x)

1.b Immunoexpression of CDK14 is intense and both nuclear and cytoplasmic in normal oral mucosa (40x)

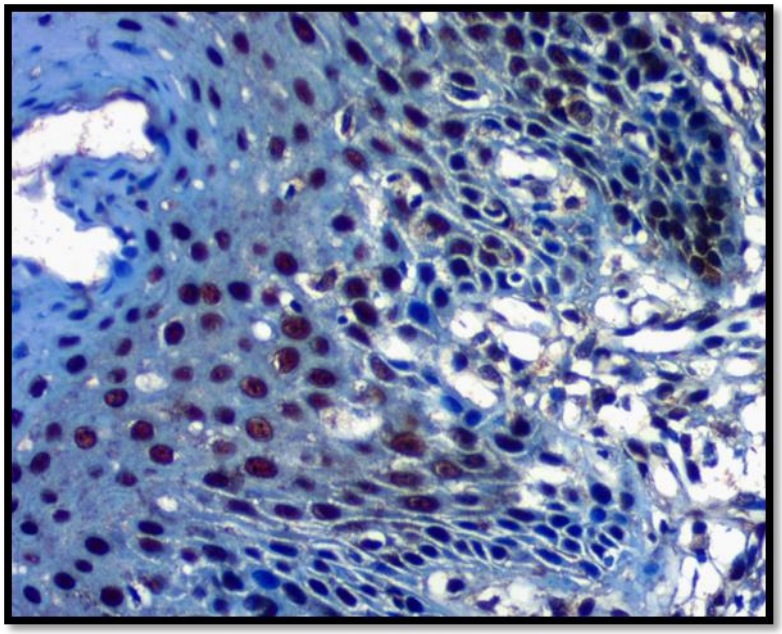
2. PHOTOMICROGRAPHS OF CDK14 IN MILD DYSPLASIA



2.a. Mild Dysplasia



2.b. Mild Dysplasia



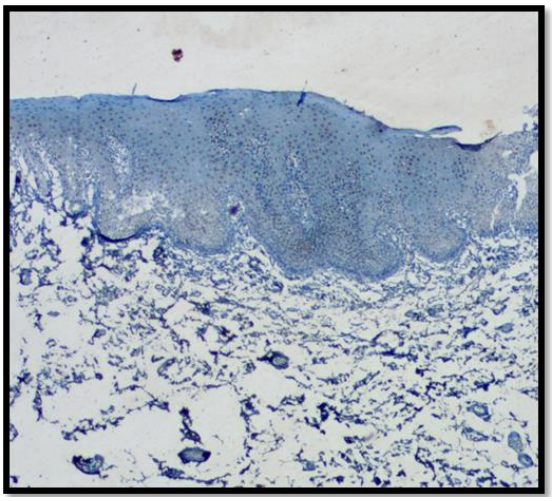
2.c. Mild Dysplasia

2.a Immunoexpression of CDK14 is mild and both nuclear and cytoplasmic in Mild dysplasia(4x)

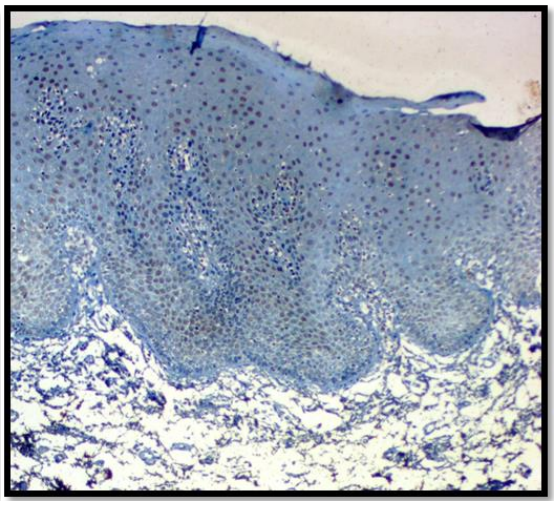
2.b Immunoexpression of CDK14 is intense and both nuclear and cytoplasmic in Mild dysplasia(10x)

2.c Immunoexpression of CDK14 is intense nuclear in Mild dysplasia (40x)

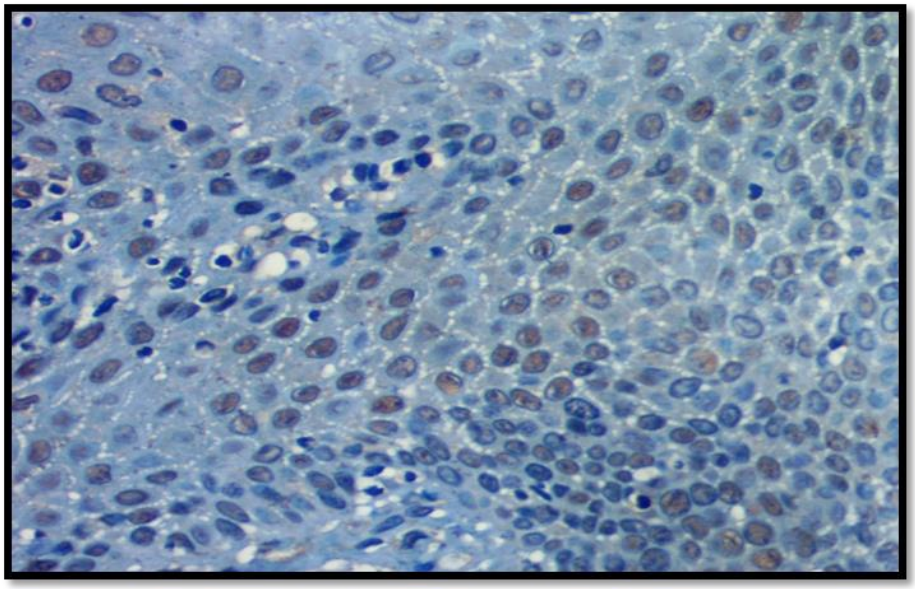
3. PHOTOMICROGRAPHS OF CDK14 IN MODERATE DYSPLASIA



3.a. Moderate Dysplasia



3.b. Moderate Dysplasia



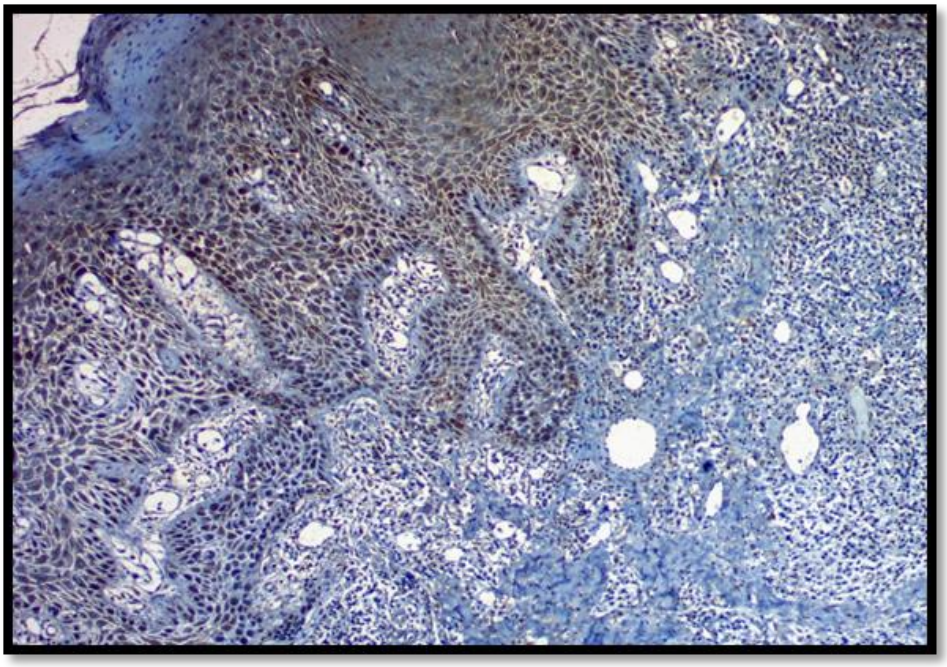
3.c. Moderate Dysplasia

3.a Immunoexpression of CDK14 is mild nuclear in Moderate dysplasia(4x)

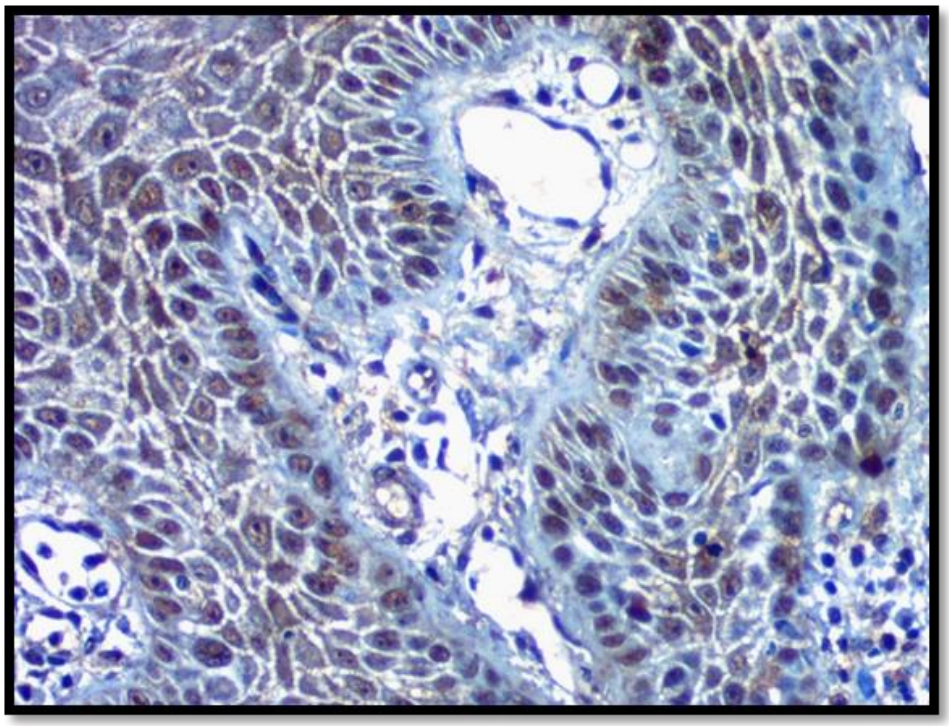
3.b Immunoexpression of CDK14 is mild nuclear in Moderate dysplasia(10x)

3.c Immunoexpression of CDK14 is mild nuclear in Moderate dysplasia (40x)

4. PHOTOMICROGRAPHS OF CDK14 IN SEVERE DYSPLASIA



4.a. Severe Dysplasia

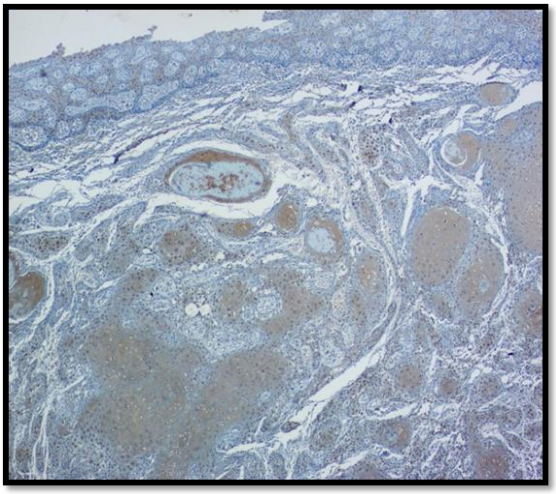


4.b. Severe Dysplasia

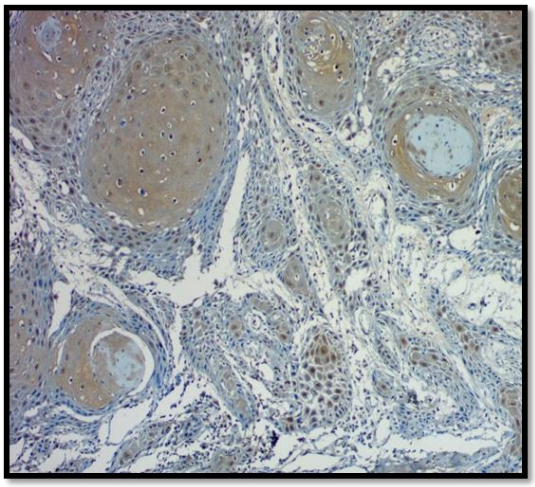
4.a Immunoexpression of CDK14 is intense nuclear in Severe dysplasia(10x)

4.b Immunoexpression of CDK14 is intense nuclear in Severe dysplasia (40x)

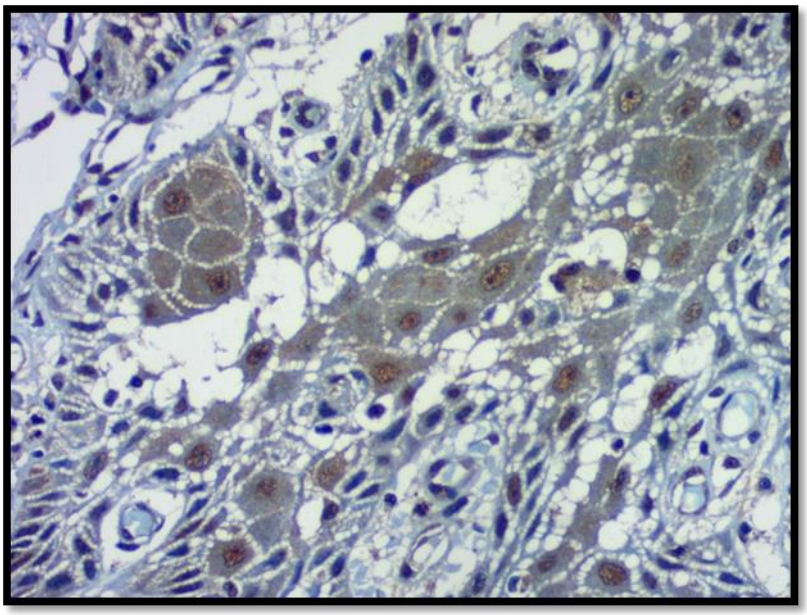
5. PHOTOMICROGRAPHS OF CDK14 IN ORAL SQUAMOUS CELL CARCINOMA



5.a. OSCC



5.b. OSCC



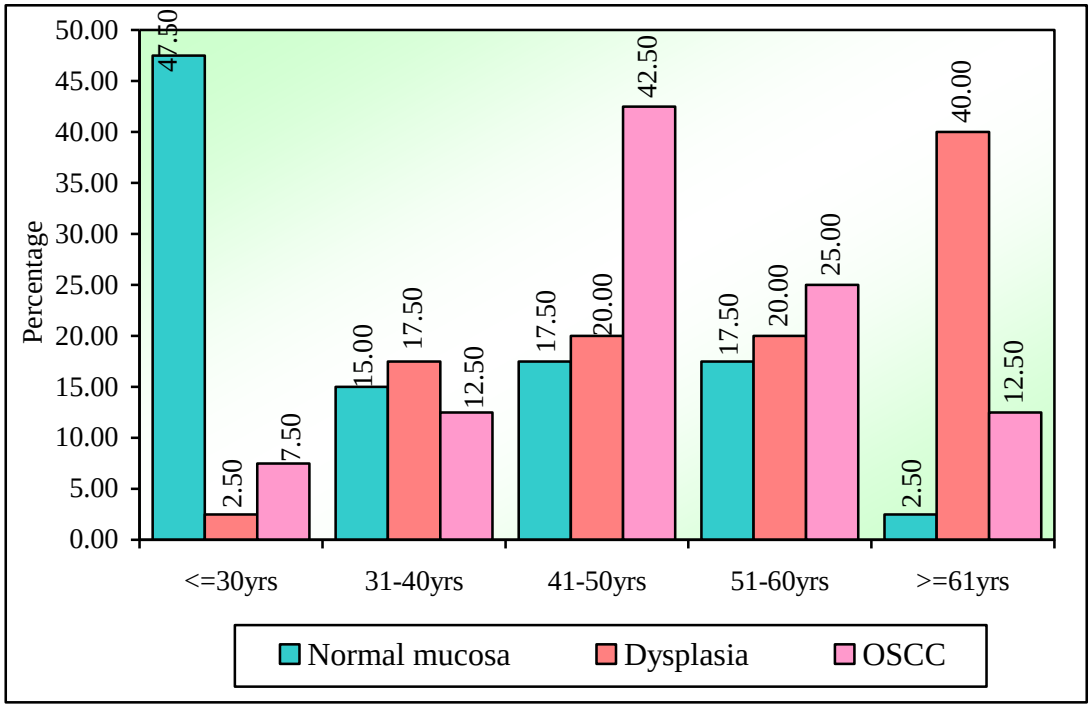
5.c. OSCC

5.a Immunoexpression of CDK14 is intense and both nuclear and cytoplasmic in OSCC4x)
5.b Immunoexpression of CDK14 is intense and both nuclear and cytoplasmic in OSCC(10x)

5.c Immunoexpression of CDK14 is intense both nuclear and cytoplasmic in OSCC (40x)

RESULTS

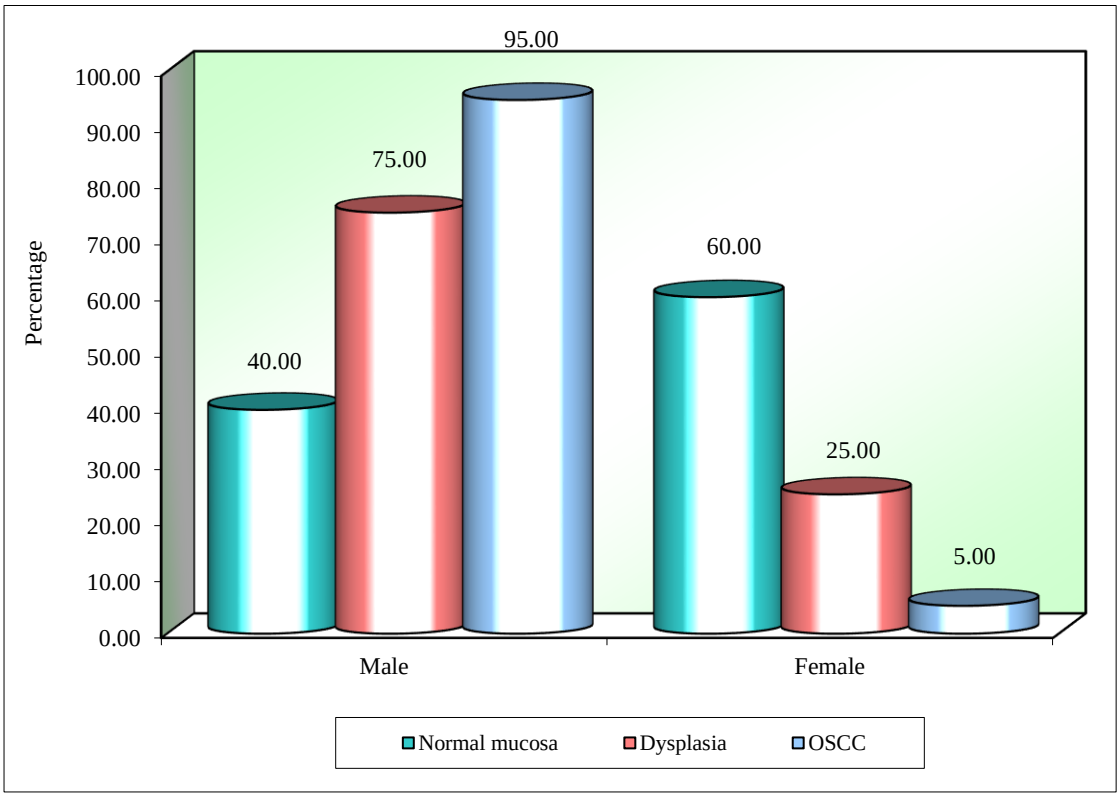
***Figure 2*: Comparison of OM, OED and OSCC group according to age groups**



Inference: Out of 120 cases in all the three study groups, in Oral Normal mucosa,19 patients were less than or equal to 30 years (47.5 %) , 6 patients were between the age group 31 to 40 years (15%),7 patients were between the age group 41 to 50 (17.5%), 7 patients were between the age group 51 to 60 years(17.5%) and 1 patient of age group less than and equal to 60 years (2.5%). In Oral epithelial dysplasia, 1 patient was less than or equal to 30 years (2.5 %) , 7 patients were between the age group 31 to 40 years (17.5%), 8 patients were between the age group 41 to 50 (20%), 8 patients were between the age group 51 to 60 years(20%) and 16 patient of age group less than and equal to 60 years (40%). In Oral squamous cell carcinoma, 3 patient was less than or equal to 30 years (7.5 %) , 5 patients were between the age group 31 to 40 years (12.5%), 17 patients were between the age group 41 to 50 (42.5%), 10 patients

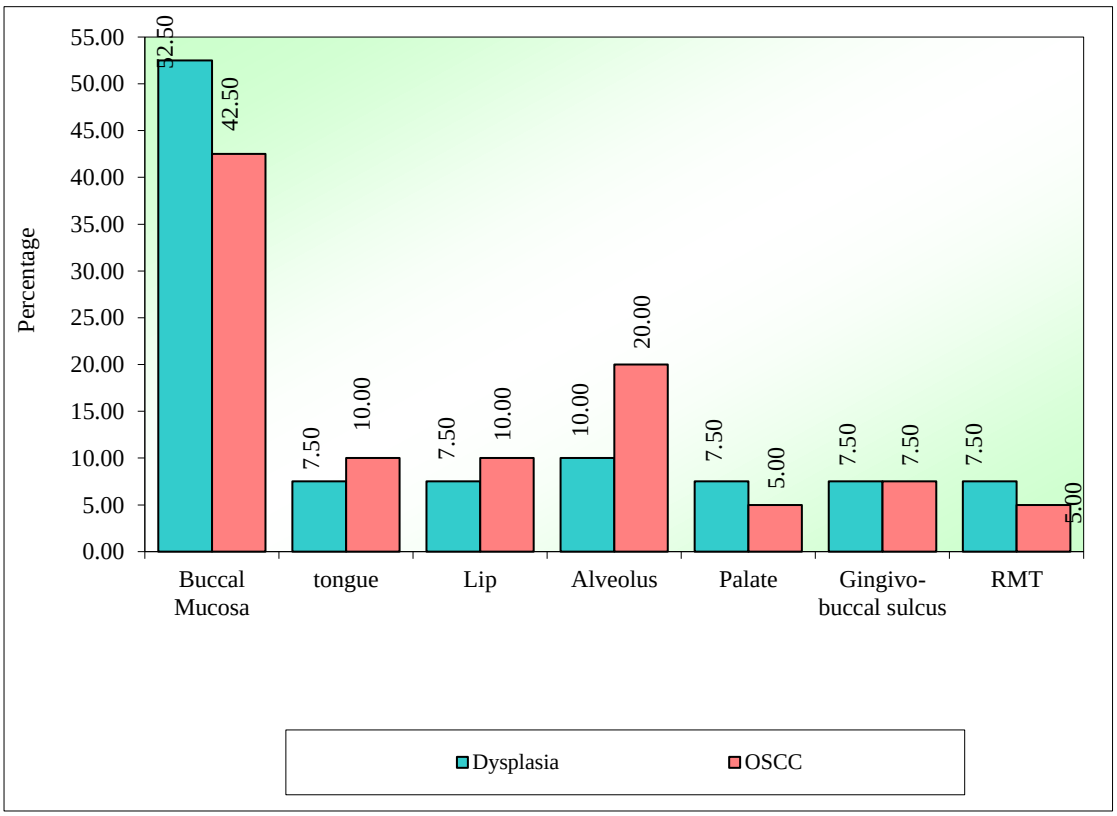
were between the age group 51 to 60 years(25%) and 5 patients of age group less than and equal to 60 years (12.5%). There was significant difference, p= 0.0001 seen between OED and OSCC with age groups.

***Figure 3*: Comparison of OM, OED and OSCC group according to gender**



Inference : The present case selection showed Female predominance in NM (60%) whereas OED and OSCC showed male predominance(75% and 95% respectively). There was significant difference, p= 0.0001 seen between OED and OSCC according to gender.

***Figure 4*: Comparison of OED and OSCC group with sites**



Inference: Most common site involved in OED and OSCC was buccal mucosa 38 (95%), followed by alveolus 12(30%), tongue 7 (17.5%), lip 7 (17.5%), gingiva-buccal sulcus sulcus 6 (15%), Palate 5 (12.5%) and RMT (12.5%). There was no significant difference was found between both groups with sites.

Table 13: Comparison of three groups with status of intensity of expression of CDK14

Intensity	Normal mucosa	%	Dysplasia	%	OSCC	%	Total
Absent	28	70.00	6	15.00	8	20.00	42
Mild	9	22.50	19	47.50	22	55.00	50
Intense	3	7.50	15	37.50	10	25.00	28
Total	40	100.00	40	100.00	40	100.00	120
Among all groups, Chi-square=34.4890, p=0.0001*							
Between Normal mucosa and Dysplasia, Z=-4.6044, p=0.0001*							
Between Normal mucosa and OSCC, Z=-3.9597, p=0.0001*							
Between Dysplasia and OSCC, Z=1.0537, p=0.2920							

*p<0.05

Inference : In Oral normal mucosa, 28 (70%) cases showed absence of expression, 9(22.5%) mild expression and 3(7.5%) showed intense expression. In OED, 6 (15%) cases showed absence of expression, 19(47.5%) mild expression and 15(37.5%) showed intense expression. In OSCC, 8 (20%) cases showed absence of expression, 22(55%) mild expression and 10(25%) showed intense expression.

Table 14: Comparison of three groups with location of expression of CDK14

Location	Normal mucosa	%	Dysplasia	%	OSCC	%	Total
Absent	28	70.00	6	15.00	8	20.00	42
Nuclear	3	7.50	21	52.50	1	2.50	25
Cytoplasm	2	5.00	3	7.50	18	45.00	23
Nuclear + Cytoplasm	7	17.50	10	25.00	13	32.50	30
Total	40	100.00	40	100.00	40	100.00	120
Among all groups, Chi-square=73.0190, p=0.0001*							
Between Normal mucosa and Dysplasia, Z=-3.5026, p=0.0005*							
Between Normal mucosa and OSCC, Z=-3.7672, p=0.0002*							
Between Dysplasia and OSCC, Z=-2.0015, p=0.0453*							

*p<0.05

Inference: In Oral normal mucosa, 28 (70%) cases showed absence of expression, 7(25%) cases showed nuclear as well cytoplasmic expression both, 3(7.5%) only nuclear expression and 2 (5%) cases showed only cytoplasmic expression . In OED, 6 (15%) cases showed absence of expression, 10(17.5%) cases showed nuclear as well cytoplasmic expression both, 21(52.5%) only nuclear expression and 3 (7.5%) cases showed only cytoplasmic expression. In OSCC, 8 (20%) cases showed absence of expression, 13(32.5%) cases showed nuclear as well cytoplasmic expression both, 1(2.5%) only nuclear expression and 18 (45%) cases showed only cytoplasmic expression.

Table 15: Comparison of three groups with percentage of positivity of expression of CDK14

Percentage	Normal mucosa	%	Dysplasia	%	OSCC	%	Total
Absent	28	70.00	6	15.00	8	20.00	42
1-25%	3	7.50	4	10.00	4	10.00	11
26-50%	7	17.50	11	27.50	9	22.50	27
> 50%	2	5.00	19	47.50	19	47.50	40
Total	40	100.00	40	100.00	40	100.00	120
Among all groups, Chi-square=36.6640, p=0.0001*							
Between Normal mucosa and Dysplasia, Z=-5.0229, p=0.0001*							
Between Normal mucosa and OSCC, Z=-4.6284 p=0.0001*							
Between Dysplasia and OSCC, Z=0.2358, p=0.8136							

*p<0.05

Inference: In Oral normal mucosa, 28 (70%) cases showed absence of expression, 3(7.5%) cases showed 1-25% percentage of positivity, 7(17.5%) cases showed 26-50% percentage of positivity and 2 (5%) cases showed >50% percentage of positivity. In OED, 6 (15%) cases showed absence of expression, 4(10%) cases showed 1-25% percentage of positivity, 11(27.5%) cases showed 26-50% percentage of positivity and 19 (47.5%) cases showed >50% percentage of positivity. In OSCC, 8 (20%) cases showed absence of expression, 4(10%) cases showed 1-25% percentage of positivity, 9(22.5%) cases showed 26-50% percentage of positivity and 19(47.5%) cases showed >50% percentage of positivity.

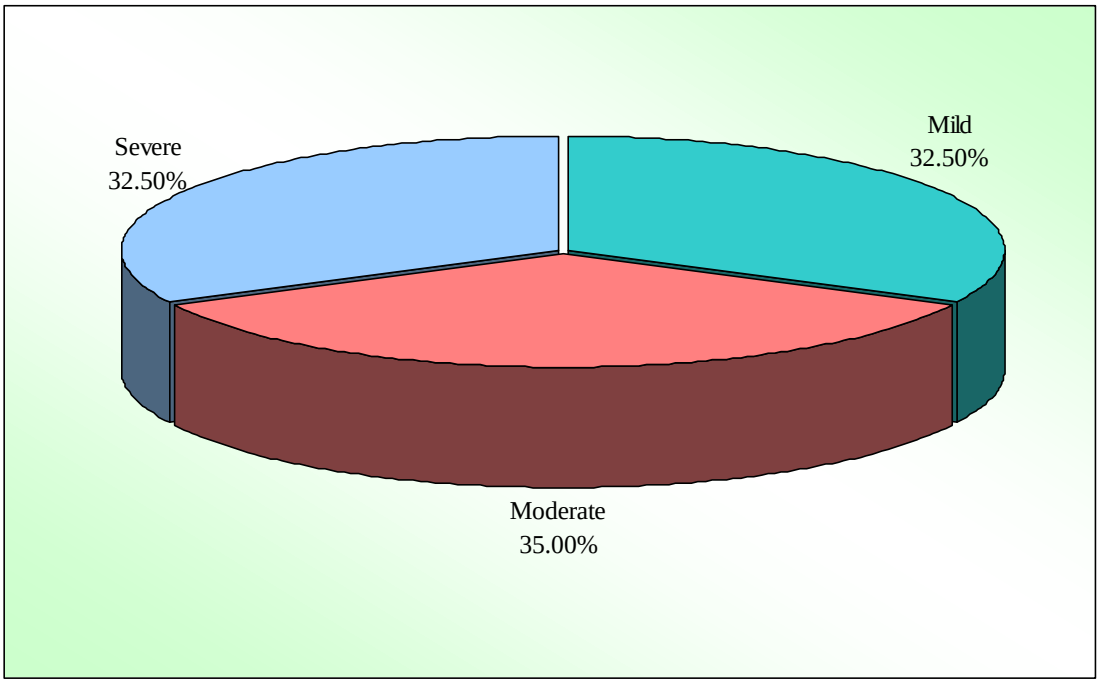
Table 16: Comparison of Normal mucosa group and OED group with extent of expression of CDK14 in epithelium

Extent	Normal mucosa	%	Dysplasia	%	Total
Absent	28	70.00	6	15.00	34
Basal + Suprabasal	5	12.50	2	5.00	7
Spinous	5	12.50	18	45.00	23
Granular	2	5.00	12	30.00	14
Corneal	0	0.00	2	5.00	2
Total	40	100.00	40	100.00	80
Chi-square=32.0120, p=0.0001*					

*p<0.05

Inference: In Oral normal mucosa, 28 (70%) cases showed absence of expression, 5(12.5%) cases showed basal and suprabasal cell layer positivity to CDK14, 5(12.5%) cases showed positivity till spinous cell layer , 2 (5%) cases showed positivity till granular cell layer , 0 cases of the showed positivity till corneal cell layer. In OED, 6 (15%) cases showed absence of expression, 2(5%) cases showed basal and suprabasal cell layer positivity to CDK14, 18(45%) cases showed positivity till spinous cell layer , 12 (30%) cases showed positivity till granular cell layer , 2 cases of the showed positivity till corneal cell layer.

***Figure 5*: Distribution among the oral epithelial dysplasia grades**



Inference: Out of 40 cases of oral epithelial dysplasia , 13 (32.5%) cases were Mild dysplasia, 14 (35%) cases were Moderate dysplasia and 13 (32.5%) cases were severe dysplasia.

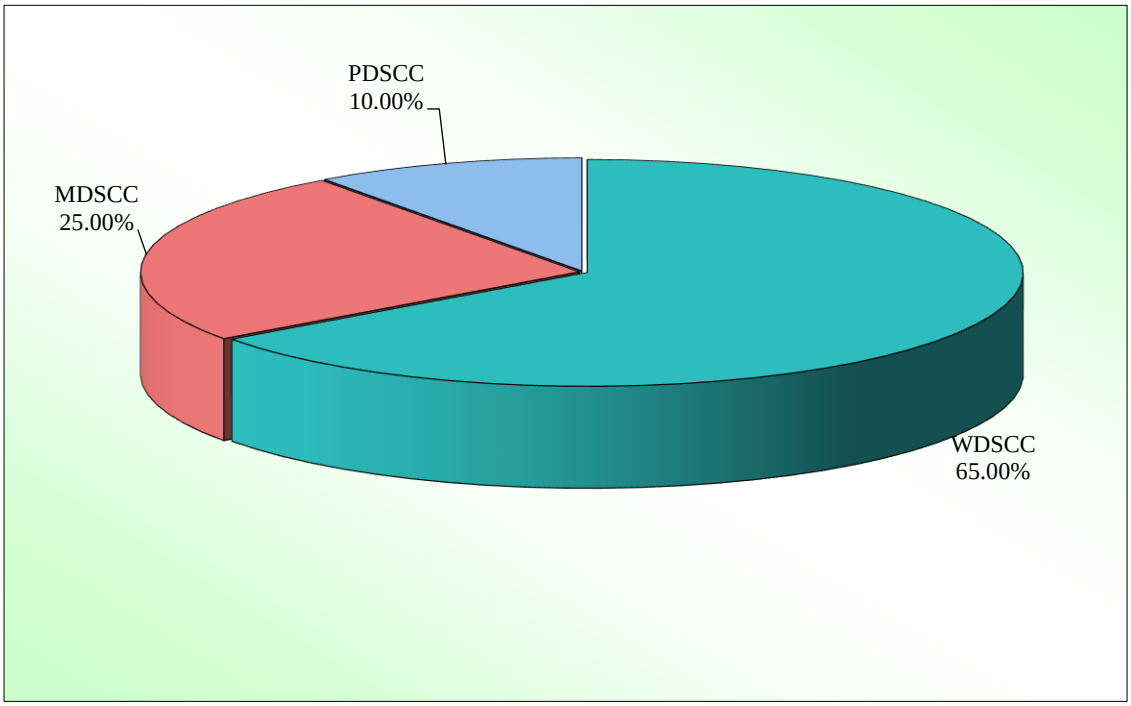
Table 17: Association between Expression of CDK14 with intensity, location, percentage of positivity and extent in Oral epithelial dysplasia grades

	Mild	%	Moderate	%	Severe	%	Total	%	χ^2	p-value
Intensity										
Absent	1	16.67	2	33.33	3	50.00	6	15.00	5.7590	0.2180
Mild	4	21.05	9	47.37	6	31.58	19	47.50		
Intense	8	53.33	3	20.00	4	26.67	15	37.50		
Location										
Absent	1	16.67	2	33.33	3	50.00	6	15.00	5.2200	0.5160
Nuclear	8	38.10	8	38.10	5	23.81	21	52.50		
Cytoplasm	1	33.33	2	66.67	0	0.00	3	7.50		
Nuclear + Cytoplasm	3	30.00	2	20.00	5	50.00	10	25.00		
Percentage										
Absent	1	16.67	2	33.33	3	50.00	6	15.00	5.0090	0.5430
1-25%	2	50.00	2	50.00	0	0.00	4	10.00		
25-50%	2	18.18	5	45.45	4	36.36	11	27.50		
> 50%	8	42.11	5	26.32	6	31.58	19	47.50		
Extent										
Absent	1	16.67	2	33.33	3	50.00	6	15.00	12.4540	0.1320
Basal + Suprabasal	2	100.00	0	0.00	0	0.00	2	5.00		
Spinous	6	33.33	8	44.44	4	22.22	18	45.00		
Granular	2	16.67	4	33.33	6	50.00	12	30.00		
Corneal	2	100.00	0	0.00	0	0.00	2	5.00		
Total	13	32.50	14	35.00	13	32.50	40	100.00		

*$p<0.05$

Inference: No statistical significant association was found between the grades of OED i.e. mild, moderate and severe dysplasia and various parameters of immunoexpression of CDK14.

***Figure 6*: Distribution of grades of Oral squamous cell carcinoma**



Inference: Out of 40 cases of Oral squamous cell carcinoma, 26 (65%) cases were of Well differentiated squamous cell carcinoma, 10 (25%) cases were of Moderately differentiated squamous cell carcinoma and 4 (10%) cases were of Poorly differentiated squamous cell carcinoma.

Table 18: Association between expression of CDK14 with intensity, location and percentage in OSCC grades

	WDSCC	%	MDSCC	%	PDSCC	%	Total	%	χ^2	p-value
Intensity										
Absent	2	25.00	5	62.50	1	12.50	8	20.00	11.2670	0.0240*
Mild	16	72.73	5	22.73	1	4.55	22	55.00		
Intense	8	80.00	0	0.00	2	20.00	10	25.00		
Location										
Absent	2	25.00	5	62.50	1	12.50	8	20.00	11.9880	0.0620
Nuclear	1	100.00	0	0.00	0	0.00	1	2.50		
Cytoplasm	11	61.11	5	27.78	2	11.11	18	45.00		
Nuclear + Cytoplasm	12	92.31	0	0.00	1	7.69	13	32.50		
Percentage										
Absent	2	25.00	5	62.50	1	12.50	8	20.00	13.3780	0.0370*
1-25%	2	50.00	2	50.00	0	0.00	4	10.00		
25-50%	7	77.78	2	22.22	0	0.00	9	22.50		
> 50%	15	78.95	1	5.26	3	15.79	19	47.50		
Total	13	32.50	14	35.00	13	32.50	40	100.00		

*$p<0.05$

Inference: Statistically significant association was found between histopathological grades of OSCC i.e. WDSCC, MDSCC & PDSCC and intensity of expression of CDK14 (p=0.0240). Also Significant association was seen with WDSCC, MDSCC & PDSCC and percentage of positivity of expression of CDK14.

Table 19: Association between Lymphnode metastasis (LNM) with clinicopathological , histopathological parameters and expression of CDK14 of OSCC group

	Absent	%	Present	%	Total	%	χ^2	p-value
Age groups								
<=39yrs	3	50.00	3	50.00	6	15.00	0.6810	0.8780
40-49yrs	12	66.67	6	33.33	18	45.00		
50-59yrs	5	55.56	4	44.44	9	22.50		
>=60yrs	4	57.14	3	42.86	7	17.50		
Habits								
With habit	21	61.76	13	38.24	34	85.00	0.2940	0.5880
Without habit	3	50.00	3	50.00	6	15.00		
Total	24	60.00	16	40.00	40	100.00		
Tumor budding								
Low	19	73.08	7	26.92	26	65.00	5.2930	0.0210*
High	5	35.71	9	64.29	14	35.00		
Depth of invasion								
Carcinoma insitu/questionable invasion	0	0.00	0	0.00	0	0.00	0.8330	0.6590
Disticnt invasion involving lamina propria	2	50.00	2	50.00	4	10.00		
invasion below the lamina propria adjacent to muscle , salivary gland and periosteum	21	60.00	14	40.00	35	87.50		

extensive and deep invasion replacing most of the stromal tissue in the jaw bone	1	100.00	0	0.00	1	2.50		
Type of invasive								
Pushing well delineated borders	2	50.00	2	50.00	4	10.00	0.3350	0.9530
infiltrative solid cords/bands/strands	8	57.14	6	42.86	14	35.00		
Small groups or cords of infiltrative cells (nests)	7	63.64	4	36.36	11	27.50		
Marked cellular dissociation in small groups of cells or single cells	7	63.64	4	36.36	11	27.50		
Type of stroma								
Abundant	17	58.62	12	41.38	29	72.50	0.0990	0.9520
Dense	2	66.67	1	33.33	3	7.50		
Delicate	5	62.50	3	37.50	8	20.00		
Extent of inflammation								
marked	17	60.71	11	39.29	28	70.00	1.7560	0.4160
Moderate	5	50.00	5	50.00	10	25.00		
Slight	2	100.00	0	0.00	2	5.00		
Lymphovascular Invasion								
Absent	22	64.71	12	35.29	34	85.00	2.0920	0.1480
Present	2	33.33	4	66.67	6	15.00		
Perineural invasion								
Absent	23	60.53	15	39.47	38	95.00	0.0880	0.7670
Present	1	50.00	1	50.00	2	5.00		

Intensity								
Absent	3	37.50	5	62.50	8	20.00	2.3010	0.3160
Mild	15	68.18	7	31.82	22	55.00		
Intense	6	60.00	4	40.00	10	25.00		
Location								
Absent	3	37.50	5	62.50	8	20.00	2.7000	0.4400
Nuclear	1	100.00	0	0.00	1	2.50		
Cytoplasm	12	66.67	6	33.33	18	45.00		
Nuclear + Cytoplasm	8	61.54	5	38.46	13	32.50		
Percentage								
Absent	3	37.50	5	62.50	8	20.00	5.0190	0.1700
1-25%	2	50.00	2	50.00	4	10.00		
25-50%	8	88.89	1	11.11	9	22.50		
> 50%	11	57.89	8	42.11	19	47.50		
Total	24	60.00	16	40.00	40	100.00		

*$p<0.05$

Inference: Tumor budding was the only parameter that showed a significant association with lymph node metastasis.

DISCUSSION

According to the recent studies and statistics, lip & oral cavity cancers are the eighth most common cancer.[65] 90% of oral cavity tumours are OSCC.[66]

Before developing cancer, the lesion undergoes a preneoplastic changes. Precancerous lesions may be the source of this carcinogenesis. However, not every potentially malignant lesions transforms into cancer.[67]

The typical method of diagnosing OSCC is clinical and histopathological examination of tissue.[68] Clinical examination may result in the expulsion of some lesions and the inability to identify if a tumour is benign or malignant. As a result, the OSCC is mostly detected in the later stage.[69]

Early-invasive OSCC shows survival of 81% when compared to advanced disease, demonstrating the predictive value of its prognostics. [70]

There are two types of methods used to characterise and diagnose tumours: Molecular and Histopathological. Biopsy, FNAC, cytological smear, IHC and flow cytometry are histopathological methods, while the molecular methods is derived from the results of FISH or PCR.[71]

Molecular-based methodologies can recognise the cancer cells, its behaviour, MRD and genetic propensity in cancer formation, as well as speed up the decision-making process for the best treatment option. Cancer biomarkers are molecules that are produced in the body by malignant cells and other cells in response to an pre-existing tumour.[72]

Biomarker analysis can be beneficial in a variety of clinical settings, from predicting disease risk to evaluating prognosis and treatment response.[73] Recently, there has been a lot of emphasis on biomarkers, which might play a significant role in diagnosis.[74] Understanding these biomarkers is essential to provide the effective targeted therapy.

Several biomarkers have been linked to PMDs, including the cyclin-dependent inhibitor p16, ki67, and p53. Wnt pathways contribute to the emergence and progression of PMDs.[75] Wnt pathway is frequently linked to pathogenesis of OSCC, making it a target for anti-cancer therapy.[71]

CDKs are essential regulators of the eukaryotic cell cycle. The PFTK1 gene, also known as 'PFTAIRE1' or CDK14, is a newly discovered CDK family member. CDK14 promotes cell cycle as a classical CDK , but it also responsible for regulating several pathways and cellular mechanisms as an oncogene.[32]

According to Pang et al, 2007 & Leung et al, 2011, they observed that CDK14 enhances invasiveness and cell motility in HCC.[5]

Li et al. stated that according to TCGA data, CDK1 and CDK14 are significantly up - regulated in HCC, implying that their positive modulations in Wnt/β -catenin signalling may contribute to HCC progression.[76]

CDK14 may be involved in CRC cell proliferation, invasion, and EMT progression, according to Zhu et al. According to their findings, PFTK1 downregulation prevents CRC cell migration/invasion and EMT by suppressing the Shh signalling pathway and may act as an oncogene in colon cancer progression.[77]

CDK14 is overexpressed in breast cancer patients, and Gu et al found that knocking it down prevents the proliferation of breast cancer cells. In gastric cancer, Yang et al. discovered that overexpression of CDK14 increased cell proliferation. Chen et al. claim that CDK14 affects tumour cell growth, which could result in medication resistance in ESCC.[4]

Though there many studies established with CDK14 other carcinomas, its expression and clinical significance in OSCC, however, have yet to be reported. The current study looked at the immunoexpression of CDK14 in oral normal mucosa, OED and OSCC, as well as its relationship to other parameters.

The study included 120 paraffin embedded tissue blocks of histologically proven cases of OED, 40 and OSCC 40 cases. For control, 40 cases normal mucosa tissue was taken during third molar extraction and gingivectomy procedures. All these sections were stained immune-histochemically by CDK14 marker using a standard protocol.

Clinico-pathological Details

In the present study, age group of OED patients was ranging from 22 to 75 years. A peak incidence was noted in 5th & 6th decade of life.[78] This data is in accordance with the available literature, were in OED is most commonly seen in middle aged and elderly individuals. OSCC patients were in the age range of 30 to 76 years. Highest incidence was noted in 4th to 5th decade of life.

Chi-square test was done to compare between the age groups of OED and OSCC and was statistically significant (p=0.0001).

OED and OSCC study groups showed male predominance as compared to females. This is consistent with reports in the literature, which show that males are frequently affected than females as a result of their habitual behaviour. Majority of the cases in the study groups were associated with habits, such as chewing tobacco, more smoking, areca nut chewing and to certain extent alcohol consumption. This is consistent with the reports in the literature.

A chi-square test was done to compare between the gender of OED and OSCC and it was found to be statistically significant (p=0.0001).

OED on the buccal mucosa was often observed in the current investigation. According to certain research, tongue, floor of the mouth, and lower lip were the most often affected areas by OED.

Our study showed, buccal mucosa as most frequently affected site in OSCC which was in accordance to Babu et al., 2021.[79] They studied the epidemiological trends in OSCC, in the Southern part of India, which showed a M:F ratio of 0.7: 0.3, inferring to male predominance, with maximum no. of cases occurring in the 4th and 6th decade of life and the most commonly affected site being buccal mucosa i.e., in 40% of the cases.[79]

Histo-pathologic details

According to WHO three-tier grading of oral dysplasia, OED cases of our study was graded into Mild, moderate and severe dysplasia. Among 40 cases , 13 were mild , 14 were moderate and 13 were severe dysplasia.

In the present study, OSCC cases were categorized into three grades by Bryne's grading system. Out of 40 cases of 26 cases were WDSCC, 10 cases were MDSCC whereas, 4 cases were PDSCC. There was unequal distribution of OSCC cases according to the grades. Various histopathological parameters were assessed. Depth of invasion was evaluated and classified into 4 types, out of 40 cases 4 cases showed type 2 DOI and 35 cases showed type 3 DOI and 1 case showed type 4 . In type of invasive front of tumor,4 cases showed type 1, 14 cases exhibited type 2, 11 cases exhibited type 3 and 11 case exhibited type 4 invasive front. Stroma surrounding the tumor islands were assessed and classified into four types. Out of 40 cases, 29 cases showed abundant stroma, 8 cases showed delicate stroma and 3 cases showed dense stroma. Extent of inflammatory reaction was also observed and categorized into four different types. Among 40 cases, 10 cases exhibited moderate inflammation, 2 cases with slight inflammation, 28 cases with marked inflammation. Lympho-vascular invasion was evaluated in all OSCC cases.Tumor cells within the blood vessels and lymphatic channels forming tumor emboli were assessed. Out of 40 cases of OSCC, 4 cases showed LVI. Another histo- pathological parameter i.e, tumor budding was analyzed according to Wang et al criteria. 26 cases showed low budding intensity and 14 cases showed high budding intensity.

CDK14 Immunoexpression

Intensity:

In Oral normal mucosa, 28 (70%) cases showed absence of expression, 9(22.5%) mild expression and 3(7.5%) showed intense expression. In OED, 6 (15%) cases showed absence of expression, 19(47.5%) mild expression and 15(37.5%) showed intense expression. In OSCC, 8 (20%) cases showed absence of expression,

22(55%) mild expression and 10(25%) showed intense expression. There was statistically significant difference in CDK14 expression in terms of intensity among the three groups. CDK14 expression was seen to be higher in OSCC and OED tissues than normal tissues.

Location:

In Oral normal mucosa, 28 (70%) cases showed absence of expression, 7(25%) cases showed nuclear as well cytoplasmic expression both, 3(7.5%) only nuclear expression and 2 (5%) cases showed only cytoplasmic expression . In OED, 6 (15%) cases showed absence of expression, 10(17.5%) cases showed nuclear as well cytoplasmic expression both, 21(52.5%) only nuclear expression and 3 (7.5%) cases showed only cytoplasmic expression. In OSCC, 8 (20%) cases showed absence of expression, 13(32.5%) cases showed nuclear as well cytoplasmic expression both, 1(2.5%) only nuclear expression and 18 (45%) cases showed only cytoplasmic expression. CDK14 was diffusely located in the cytoplasm and nucleus and also shuttle between nucleus and cytoplasm. There was statistically significant difference in CDK14 expression in terms of location among the three groups.

Percentage of positivity:

In Oral normal mucosa, 28 (70%) cases showed absence of expression, 3(7.5%) cases showed 1-25% percentage of positivity, 7(17.5%) cases showed 26-50% percentage of positivity and 2 (5%) cases showed >50% percentage of positivity. In OED, 6 (15%) cases showed absence of expression, 4(10%) cases showed 1-25% percentage of positivity, 11(27.5%) cases showed 26-50% percentage of positivity and 19 (47.5%) cases showed >50% percentage of positivity. In OSCC, 8 (20%) cases

showed absence of expression, 4(10%) cases showed 1-25% percentage of positivity, 9(22.5%) cases showed 26-50% percentage of positivity and 19(47.5%) cases showed >50% percentage of positivity. There was statistically significant difference in CDK14 expression in terms of Positivity among the three groups. Positivity of CDK14 expression was seen to be higher in OSCC and OED tissues than normal tissues.

Extent of epithelium:

In Oral normal mucosa, 28 (70%) cases showed absence of expression, 5(12.5%) cases showed basal and suprabasal cell layer positivity to CDK14, 5(12.5%) cases showed positivity till spinous cell layer , 2 (5%) cases showed positivity till granular cell layer , 0 cases of the showed positivity till corneal cell layer. In OED, 6 (15%) cases showed absence of expression, 2(5%) cases showed basal and suprabasal cell layer positivity to CDK14, 18(45%) cases showed positivity till spinous cell layer , 12 (30%) cases showed positivity till granular cell layer , 2 cases of the showed positivity till corneal cell layer. There was statistically significant difference in CDK14 expression in terms of extent among the two groups.

Association of various parameters CDK14 immunoexpression between the Oral epithelial Dysplasia groups

Chi square test was done to assess the association of immunoexpression of CDK14 various parameters (intensity, location, percentage of positivity and extent) with grades with dysplasia (mild, moderate, severe). There was no statistical significant difference found between the groups.

Association of various parameters CDK14 immunoexpression between OSCC groups

Chi square test was done to assess the association of immunoexpression of CDK14 various parameters (intensity, location, percentage of positivity) with grades with OSCC(WDSCC, MDSCC, PDSCC). There was statistical significant difference found between the groups with the intensity (p =0.0240*) and percentage of positivity (p=0.0370*).

Association of various Clinico and histo-pathological parameters along with CDK14 expression for LNM

No other Clinico and histo-pathogical parameters were significant other than , tumor budding which showed significant association with LNM

Our findings provide new evidence for the role of CDK14 in the development of OSCC for the first time. However, more research is required to determine the precise mechanism by which CDK14 affects the proliferation, migration, and invasion ability of oral cancer cells.

SUMMARY

- The current study's goal was to evaluate the immunoexpression of CDK14 in OM, OED and OSCC.
- A total of 120 sample, 40 of each OM , OED and OSCC, APES gel coated slides with 4µm tissue section were taken for IHC.
- IHC analysis of each slide was done according to the parameters such as intensity, location and percentage of positivity of CDK14
- Chi square test was done for statistical analysis. Association of expression of CDK14with various clinicopathogical and histopathological parameters are also done.
- Results showed a statistical significant association of the immunoexpression of CDK14 with Normal OM, OED and OSCC. Statistically significant association of expression of CDK14 between the normal OM and OED & between the normal OM and OSCC was observed.

CONCLUSION

This was the first study that has attempted to detect CDK14 expression in OM, OED and OSCC. Its upregulated expression in cases of OED & OSCC compared to normal mucosa indicates that the CDK14 plays an essential part in carcinogenesis of oral tissues. Though we discovered a significant association between CDK14, OED and OSCC, the mechanism of its function in proliferation, migration & invasion have to be further investigated in detail. As a result, we believe that CDK14 plays an important role for oral cancer formation and its progression. The present study proposes that CDK14 is a newly discovered biomarker for future targeted therapy in OSCC.

LIMITATIONS

1. The limited sample size in the subgroups may have affected some the findings, thus needs corroboration with larger sample size
2. Follow up data related to OED cases turning into malignancy was unavailable.
3. Because of the limited clinicopathological data, TNM staging and tumor size was not co-related with any of the parameters.

FUTURE SCOPE:

The expression profile of CDK14 has been detected in normal oral mucosa, OED and OSCC, but still our observation needs to be further validated. As there is no literature regarding the expression of CDK14 in oral tissues, further studies should be carried out to detect the cancer progression to aid better treatment approaches. Moreover, the role of molecule CDK14 still needs to be established through other advanced molecular techniques so as to reach this molecule as a target for therapy in OSCC.

BIBLIOGRAPHY

1. Thomson PJ. Perspectives on oral squamous cell carcinoma prevention—proliferation, position, progression and prediction. Journal of Oral Pathology & Medicine. 2018 Oct;47 9:803-7.

2. Barnes L, Eveson JW, Reichart P, Sidransky S. World Health Organisation Classification of Tumours. Pathology and Genetics. Head and Neck Tumours. Lyon, France: International Agency for Research on Cancer Press; 2005.

3. Warnakulasuriya S. Living with oral cancer: epidemiology with particular reference to prevalence and life-style changes that influence survival. Oral oncology. 2010 Jun 1;46 6:407-10.

4. Chen L, Wang Y, Jiang W, Ni R, Wang Y, Ni S. CDK14 involvement in proliferation migration and invasion of esophageal cancer. Annals of Translational Medicine. 2019 Nov;7 22.

5. Miyagaki H, Yamasaki M, Miyata H, Takahashi T, Kurokawa Y, Nakajima K, Takiguchi S, Fujiwara Y, Ishii H, Tanaka F, Mori M. Overexpression of PFTK1 predicts resistance to chemotherapy in patients with oesophageal squamous cell carcinoma. British journal of cancer. 2012 Feb;106 5:947-54

6. Yang L, Zhu J, Huang H, Yang Q, Cai J, Wang Q, Zhu J, Shao M, Xiao J, Cao J, Gu X. PFTK1 promotes gastric cancer progression by regulating proliferation, migration and invasion. PLoS One. 2015 Oct 21;10 10:e0140451.

7. Sun Y, Wang P, Yang W, Shan Y, Zhang Q, Wu H. The role of lncRNA MSC-AS1/miR-29b-3p axis-mediated CDK14 modulation in pancreatic cancer proliferation and Gemcitabine-induced apoptosis. Cancer biology & therapy. 2019 Jun 3;20 6:729-39.

8. Zhang W, Liu R, Tang C, Xi Q, Lu S, Chen W, Zhu L, Cheng J, Chen Y, Wang W, Zhong J. PFTK1 regulates cell proliferation, migration and invasion in epithelial ovarian cancer. International journal of biological macromolecules. 2016 Apr 1;85:405-16.

9. Odell E, Kujan O, Warnakulasuriya S, Sloan P. Oral epithelial dysplasia: Recognition, grading and clinical significance. Oral Diseases. 2021 Nov 1

10. Ho PS, Chen PL, Warnakulasuriya S, Shieh TY, Chen YK, Huang I. Malignant transformation of oral potentially malignant disorders in males: a retrospective cohort study. BMC cancer. 2009 Dec;9(1):1-7.

11. Pindborg JJ, Reichart PA, Smith CJ, van der Waal I. World Health Organization: Histological Typing of Cancer and Precancer of the Oral Mucosa. Berlin: Springer-Verlag; 1997. Back to cited text no. 17

12. Barnes L, Eveson JW, Reichart P, Sidransky S. World Health Organisation Classification of Tumours. Pathology and Genetics. Head and Neck Tumours. Lyon, France: International Agency for Research on Cancer Press; 2005.

13. Lumerman H, Freedman P, Kerpel S. Oral epithelial dysplasia and the development of invasive squamous cell carcinoma. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology. 1995 Mar 1;79(3):321-9.

14. Richart RM, Barron BA. A follow-up study of patients with cervical dysplasia. Am J Obstet Gynecol. 1969;105:386-393.

15. Chierici G, Silverman S Jr, Forsythe B. A tumor registry study of oral squamous carcinoma. J Oral Med. 1968;23(3):1-98.

16. Wright A, Shear M. Epithelial dysplasia immediately adjacent to oral squamous cell carcinomas. Journal of Oral Pathology & Medicine. 1985 Aug;14(7):559-64.

17. Ranganathan K, Kavitha L. Oral epithelial dysplasia: Classifications and clinical relevance in risk assessment of oral potentially malignant disorders. Journal of oral and maxillofacial pathology: JOMFP. 2019 Jan;23(1):19.

18. Choi S, Myers JN. Molecular pathogenesis of oral squamous cell carcinoma: implications for therapy. J Dent Res 2008; 87: 14-32.

19. Lakshminarayana S, Augustine D, Rao RS, Patil S, Awan KH, Venkatesiah SS, Haragannavar VC, Nambiar S, Prasad K. Molecular pathways of oral cancer that predict prognosis and survival: A systematic review. Journal of carcinogenesis. 2018;17.

20. Fuentes B, Duaso J, Droguett D, et al: Progressive extracellular matrix disorganization in chemically induced murine oral squamous cell carcinoma. ISRN Pathology. 2012

21. Califano J, van der Riet P, Westra W, et al. Genetic progression model for head and neck cancer: implications for field cancerization. Cancer Res 1996; 56: 2488-92.

22. Markopoulos A, Albanidou-Farmaki E, Kayavis I. Actinic cheilitis: clinical and pathologic characteristics in 65 cases. Oral Diseases 2004; 10: 212-6.

23. Preston DL, Ron E, Tokuoka S, et al. Solid cancer incidence in atomic bomb survivors: 1958-1998. Radiat Res 2007; 168: 1-64.

24. O'Grady JF, Reade PC. Candida albicans as a promoter of oral mucosal neoplasia. Carcinogenesis 1992; 13: 783-6.

25. Huang M, Spitz MR, Gu J, et al. Cyclin D1 gene polymorphism is a risk factor for oral premalignant lesions. Carcinogenesis 2006; 27: 2034-7.

26. Díez-Pérez R, Campo-Trapero J, Cano-Sánchez J, et al. Methylation in oral cancer and pre-cancerous lesions (Review).Oncol Rep 2011; 25(5): 1203-9.

27. Lakshminarayana S, Augustine D, Rao RS, Patil S, Awan KH, Venkatesiah SS, Haragannavar VC, Nambiar S, Prasad K. Molecular pathways of oral cancer that predict prognosis and survival: A systematic review. Journal of carcinogenesis. 2018;17.

28. Choi S, Myers JN. Molecular pathogenesis of oral squamous cell carcinoma: implications for therapy. Journal of dental research. 2008 Jan;87(1):14-32.New Insights Into Oral Squamous Cell Carcinoma: From Clinical Aspects to Molecular Tumorigenesis

29. Ricci S, Pinto F, Auletta A, Giordano A, Giovane A, Settembre G, Boccellino M, Boffo S, Di Carlo A, Di Domenico M. The enigmatic role of matrix metalloproteinases in epithelial-to-mesenchymal transition of oral squamous cell carcinoma: Implications and nutraceutical aspects. Journal of cellular biochemistry. 2019 May;120(5):6813-9.Shu F, Lv S, Qin Y, Ma X, Wang X, Peng X, et al. Functional characterization of human PFTK1 as a cyclin-dependent kinase. Proc Natl Acad Sci. 2007;104(22):9248–53.

30. Jiang M, Gao Y, Yang T, Zhu X, Chen J. Cyclin Y, a novel membrane-associated cyclin, interacts with PFTK1. FEBS Lett. 2009;583(13):2171–8. 23. Leung W, Ching A, Cha

31. Yang T, Chen JY. Identification and cellular localization of human PFTAIRE1. Gene. 2001 Apr 18;267(2):165-72.

32. Shu F, Lv S, Qin Y, Ma X, Wang X, Peng X, Luo Y, Xu BE, Sun X, Wu J. Functional characterization of human PFTK1 as a cyclin-dependent kinase. Proceedings of the National Academy of Sciences. 2007 May 29;104(22):9248-53.

33. Pang EY, Bai AH, To KF, Sy SM, Wong NL, Lai PB, Squire JA, Wong N. Identification of PFTAIRE protein kinase 1, a novel cell division cycle-2 related gene, in the motile phenotype of hepatocellular carcinoma cells. Hepatology. 2007 Aug;46(2):436-45.29PFTK1 regulates cell proliferation, migration and invasion in epithelial ovarian cancer

34. Yang HJ, Wang L, Wang M, Ma SP, Cheng BF, Li ZC, Feng ZW. Serine/threonine-protein kinase PFTK1 modulates oligodendrocyte differentiation via PI3K/AKT pathway. Journal of Molecular Neuroscience. 2015 Apr;55(4):977-84.31Y. Gao, M. Jiang, T. Yang, J. Ni, J. Chen, Cell Res. 16 (2006) 539–547.

35. Shu F, Lv S, Qin Y, Ma X, Wang X, Peng X, Luo Y, Xu BE, Sun X, Wu J. Functional characterization of human PFTK1 as a cyclin-dependent kinase. Proceedings of the National Academy of Sciences. 2007 May 29;104(22):9248-53.33[11] D. Liu, S. Guest, R.L. Finley Jr., Fly 4 (2010) 278–282.

36. Li S, Song W, Jiang M, Zeng L, Zhu X, Chen J. Phosphorylation of cyclin Y by CDK14 induces its ubiquitination and degradation. FEBS letters. 2014 May 29;588(11):1989-96..

37. Sun T, Co NN, Wong N. PFTK1 interacts with cyclin Y to activate non-canonical Wnt signaling in hepatocellular carcinoma. Biochemical and biophysical research communications. 2014 Jun 20;449(1):163-8.

38. Li S, Jiang M, Wang W, Chen J. 14-3-3 binding to cyclin Y contributes to cyclin Y/CDK14 association. Acta Biochim Biophys Sin. 2014 Apr 1;46(4):299-304.

39. D. Liu, R.L. Finley Jr., Cyclin Y is a novel conserved cyclin essential for development in drosophila, Genetics 184 (2010) 1025–1035

40. M. Jiang, Y. Gao, T. Yang, X. Zhu, J. Chen, Cyclin Y, a novel membrane-associated cyclin, interacts with PFTK1, FEBS Lett. 583 (2009) 2171–2178.

41. G. Davidson, J. Shen, Y.L. Huang, Y. Su, E. Karaulanov, K. Bartscherer, C. Hassler, P. Stannek, M. Boutros, C. Niehrs, Cell cycle control of wnt receptor activation, Dev. Cell. 17 (2009) 788–799.

42. X. He, M. Semenov, K. Tamai, X. Zeng, LDL receptor-related proteins 5 and 6 in wnt/beta-catenin signaling: arrows point the way, Development 131 (2004) 1663–1677.

43. K. Tamai, M. Semenov, Y. Kato, R. Spokony, C. Liu, Y. Katsuyama, F. Hess, J.P. Saint-Jeannet, X. He, LDL-receptor-related proteins in wnt signal transduction, Nature 407 (2000) 530–535.

44. X. Zeng, H. Huang, K. Tamai, X. Zhang, Y. Harada, C. Yokota, K. Almeida, J. Wang, B. Doble, J. Woodgett, A. Wynshaw-Boris, J.C. Hsieh, X. He, Initiation

of wnt signaling: control of wnt coreceptor Lrp6 phosphorylation/activation via frizzled, dishevelled and axin functions, Development 135 (2008) 367–375.

45. T.P. Rao, M. Kuhl, An updated overview on wnt signaling pathways: a prelude for more, Circ. Res. 106 (2010) 1798–1806.

46. C.Y. Logan, R. Nusse, The wnt signaling pathway in development and disease, Annu. Rev. Cell Dev. Biol. 20 (2004) 781–810.

47. H. Clevers, Wnt/beta-catenin signaling in development and disease, Cell 127 (2006) 469–480.

48. K. Schlessinger, A. Hall, N. Tolwinski, Wnt signaling pathways meet rho GTPases, Genes Dev. 23 (2009) 265–277.

49. M. Katoh, M. Katoh, WNT signaling pathway and stem cell signaling network, Clin. Cancer Res. 13 (2007) 4042–4045.

50. Sun T, Co NN, Wong N. PFTK1 interacts with cyclin Y to activate non-canonical Wnt signaling in hepatocellular carcinoma. Biochemical and biophysical research communications. 2014 Jun 20;449(1):163-8. J. Behrens, Control of beta-catenin signaling in tumor development, Ann. N. Y. Acad. Sci. 910 (2000) 21–33.

51. 50 H.H. Luu, R. Zhang, R.C. Haydon, E. Rayburn, Q. Kang, W. Si, J.K. Park, H. Wang, Y. Peng, W. Jiang, T.C. He, Wnt/beta-catenin signaling pathway as a novel cancer drug target, Curr. Cancer Drug Targets 4 (2004) 653–671.

52. 51 T. Pukrop, F. Klemm, T. Hagemann, D. Gradl, M. Schulz, S. Siemes, L. Trumper, C. Binder, Wnt 5a signaling is critical for macrophage-induced invasion of breast cancer cell lines, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 5454–5459.

53. 52 J. Dejmek, A. Dejmek, A. Safholm, A. Sjolander, T. Andersson, Wnt-5a protein expression in primary dukes B colon cancers identifies a subgroup of patients with good prognosis, Cancer Res. 65 (2005) 9142–9146.

54. 53 C.L. Huang, D. Liu, J. Nakano, S. Ishikawa, K. Kontani, H. Yokomise, M. Ueno, Wnt5a expression is associated with the tumor proliferation and the stromal vascular endothelial growth factor–an expression in non-small-cell lung cancer, J. Clin. Oncol. 23 (2005) 8765–8773.

55. M. Kurayoshi, N. Oue, H. Yamamoto, M. Kishida, A. Inoue, T. Asahara, W. Yasui, A. Kikuchi, Expression of wnt-5a is correlated with aggressiveness of gastric cancer by stimulating cell migration and invasion, Cancer Res. 66 (2006) 10439–10448

56. M.J. Smalley, N. Signoret, D. Robertson, A. Tilley, A. Hann, K. Ewan, Y. Ding, H. Paterson, T.C. Dale, Dishevelled (dvl-2) activates canonical wnt signalling in the absence of cytoplasmic puncta, J. Cell. Sci. 118 (2005) 5279–5289.

57. K.A. Wharton Jr., G. Zimmermann, R. Rousset, M.P. Scott, Vertebrate proteins related to drosophila naked cuticle bind dishevelled and antagonize wnt signaling, Dev. Biol. 234 (2001) 93–106.

58. D. Yan, J.B. Wallingford, T.Q. Sun, A.M. Nelson, C. Sakanaka, C. Reinhard, R.M. Harland, W.J. Fantl, L.T. Williams, Cell autonomous regulation of multiple dishevelled-dependent pathways by mammalian nkd, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 3802–3807.

59. M. Tada, M.L. Concha, C.P. Heisenberg, Non-canonical wnt signalling and regulation of gastrulation movements, Semin. Cell Dev. Biol. 13 (2002) 251–260.

60. K. Asanuma, E. Yanagida-Asanuma, C. Faul, Y. Tomino, K. Kim, P. Mundel, Synaptopodin orchestrates actin organization and cell motility via regulation of RhoA signalling, Nat. Cell Biol. 8 (2006) 485–491.

61. C.H. Chan, S.W. Lee, C.F. Li, J. Wang, W.L. Yang, C.Y. Wu, J. Wu, K.I. Nakayama, H.Y. Kang, H.Y. Huang, M.C. Hung, P.P. Pandolfi, H.K. Lin, Deciphering the transcriptional complex critical for RhoA gene expression and cancer metastasis, Nat. Cell Biol. 12 (2010) 457–467.

62. P. Gulhati, K.A. Bowen, J. Liu, P.D. Stevens, P.G. Rychahou, M. Chen, E.Y. Lee, H.L. Weiss, K.L. O'Connor, T. Gao, B.M. Evers, MTORC1 and mTORC2 regulate EMT, motility, and metastasis of colorectal cancer via RhoA and Rac1 signaling pathways, Cancer Res. 71 (2011) 3246–3256.

63. Wang C, Huang H, Huang Z, Wang A, Chen X, Huang L, Zhou X, Liu X. Tumor budding correlates with poor prognosis and epithelial-mesenchymal transition in tongue squamous cell carcinoma. Journal of oral pathology & medicine. 2011 Aug;40(7):545-51.

64. Angadi PV, Patil PV, Angadi V, Mane D, Shekar S, Hallikerimath S, Kale AD, Kardesai SG. Immunoexpression of epithelial mesenchymal transition proteins E-cadherin, β-catenin, and N-cadherin in oral squamous cell carcinoma. International journal of surgical pathology. 2016 Dec;24(8):696-703.

65. Fact sheet of lip and oral cavity cancers. Global Cancer. 2020, March 30.

66. Cristaldi M, Mauceri R, Di Fede O, Giuliana G, Campisi G, Panzarella V. Salivary Biomarkers for Oral Squamous Cell Carcinoma Diagnosis and Follow-Up: Current Status and Perspectives. Front Physiol. 2019;10:1476.

67. Rivera C, Venegas B. Histological and molecular aspects of oral squamous cell carcinoma (Review). Oncol Lett. 2014;8(1):7-11.

68. Giovannacci I, Vescovi P, Manfredi M, Meleti M. Non-invasive visual tools for diagnosis of oral cancer and dysplasia: A systematic review. Medicina oral, patologia oral y cirugia bucal. 2016;21(3):e305.

69. Ferrari E, Pezzi ME, Cassi D, Pertinhez TA, Spisni A, Meleti M. Salivary Cytokines as Biomarkers for Oral Squamous Cell Carcinoma: A Systematic Review. International journal of molecular sciences. 2021;22(13):6795

70. Almangush A, Bello IO, Coletta RD, Mäkitie AA, Mäkinen LK, Kauppila JH, et al. For early-stage oral tongue cancer, depth of invasion and worst pattern of invasion are the strongest pathological predictors for locoregional recurrence and mortality. Virchows Archiv. 2015;467(1):39-46

71. Zangeneh Motlagh M, Tamimi A, Golroo R, Hossein-Khannazer N, Aminishakib P, Mahdavi N, Hassan M, Vosough M. Oral squamous cell carcinoma, novel methods for early diagnosis and treatment. Modern Medical Laboratory Journal. 2022 Jan 10;5(1):14-26.

72. Hoda SA, Cheng E. Robbins Basic Pathology. American Journal of Clinical Pathology. 2017;148(6):557.

73. Henry NL, Hayes DF. Cancer biomarkers. Molecular Oncology. 2012;6(2):140-6.

74. Rivera C, Oliveira AK, Costa RAP, De Rossi T, Leme AFP. Prognostic biomarkers in oral squamous cell carcinoma: a systematic review. Oral oncology. 2017;72:38-47.

75. Bai Y, Sha J, Kanno T. The Role of Carcinogenesis-Related Biomarkers in the Wnt Pathway and Their Effects on Epithelial– Mesenchymal Transition (EMT) in Oral Squamous Cell Carcinoma. Cancers. 2020;12(3).

76. Li Q, Sun M, Wang M, Feng M, Yang F, Li L, Zhao J, Chang C, Dong H, Xie T, Chen J. Dysregulation of Wnt/β-catenin signaling by protein kinases in hepatocellular carcinoma and its therapeutic application. Cancer Science. 2021 May;112(5):1695-706.

77. Zhu J, Liu C, Liu F, Wang Y, Zhu M. Knockdown of PFTAIRE protein kinase 1 (PFTK1) inhibits proliferation, invasion, and EMT in colon cancer cells. Oncology research. 2016;24(3):137.

78. dos Santos Pereira J, de Vasconcelos Carvalho M, Henriques ÁC, de Queiroz Camara TH, da Costa Miguel MC, de Almeida Freitas R. Epidemiology and correlation of the clinicopathological features in oral epithelial dysplasia: analysis of 173 cases. Annals of Diagnostic Pathology. 2011 Apr 1;15(2):98-102.

79. Babu C, Pereira T, Shetty S, Shrikant G, Anjali A, Vidhale R. Epidemiological trends of oral squamous cell carcinoma-An institutional study. Muller Journal of Medical Sciences and Research. 2021 Jan 1;12(1):1-.

ANNEXURE I

ETHICAL CLEARANCE

Research and Ethics Committee
KLE V K INSTITUTE OF DENTAL SCIENCES
KLE University
Accredited 'A' Grade by NAAC Placed in Category 'A' by MHRD (GoI)
Nehru Nagar, Belagavi - 590 010, Karnataka State
☎: 0831-2470362
FAX: 0831-2470640
Web: http://www.kledental-bgm.edu.in
E-mail:principal@kledental-bgm.edu.in

Sl. No. : 1464

CERTIFICATE

This is to Certify that the synopsis titled

Evaluation of Immunoexpression of CDK14 in normal oral mucosa, oral epithelial dysplasia and Oral Squamous cell carcinoma. *Submitted by*

Dr. _____ *P. G. Student /*

Staff, Guided by _____ *from Department of*

Oral and Maxillofacial Pathology & Oral Microbiology *has been critically evaluated by committee members and granted ethical clearance to conduct the above mentioned study*

Date : 5/5/21

Member Secretary
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

Chairman
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

ANNEXURE II

WAIVER FORM

Department of Oral & Maxillofacial Pathology & Oral Microbiology

" EVALUATION OF IMMUNOEXPRESSION OF CDK14 IN NORMAL ORAL MUCOSA, ORAL EPITHELIAL DYSPLASIA AND ORAL SQUAMOUS CELL CARCINOMA "

Waiver of informed consent form

It is not feasible to obtain individual informed consent of donors of specimens used in the study. However, I assure that confidentiality of the participant information will be ensured and no identifying information related to the study participants will be disclosed in any report/publication arising from the study

Dr.

Post graduate

Department of Oral & Maxillofacial Pathology and

Oral Microbiology

Guide:

Dr.

Reader

Department of Oral & Maxillofacial Pathology and

Oral Microbiology

ANNEXURE III

BIOSTATISTICS CERTIFICATE

KLE V.K. Institute of Dental Sciences
(A Constituent unit of KLE Academy of Higher Education & Research
Deemed-to-be-University u/s 3 of the UGC Act, 1956)
Nehru Nagar, Belagavi-590 010 INDIA
Re-Accredited 'A' grade by NAAC (2nd Cycle) & Placed in Category 'A' by MHRD (GoI)
0831-2470362
FAX: 0831-2470640
Web: http://www.kledental-bgm.edu.in
E-mail: principal@kledental-bgm.edu.in

Biostatistics Clearance Certificate

This is to certify that the Biostatistics aspect of the Dissertation / Research work , **Post Graduate Student**, under the guidance of **Dr. Reader, Department of Oral and Maxillofacial Pathology and Oral Microbiology**, entitled **"Evaluation of Immunoexpression of CDK14 in Normal Oral Mucosa, Oral Epithelial Dysplasia and Oral squamous cell carcinoma "** has been done under my guidance and considered satisfactory.

Place: Belagavi
Date: 12.12.2022

Name & Signature of Biostatistician
Dr. S.B. Javali
Sr. Asso. Prof. in Statistics
Dept. of Com. Medicine
USM KLE IMP, Belagavi

ANNEXURE IV

PREPARATION OF APES (3-AMINO PROPYL TRIETOXYSALINE)

COATED GLASS SLIDES

1. Clean dried glass were dipped in 1 % APES in acetone.
2. Slides were drained and dipped in acetone
3. Slide were drained again and dipped in dipped in distilled water.
4. Slides were then placed in a rack and allowed to dry.
5. Slides can then be stored and used as required.

ANNEXURE V

HEMATOXYLIN AND EOSIN STAINING TECHNIQUE

(REGRESSIVE)

1. Sections were deparaffinized by warming on slide warmer for 10 min and passed through Xylene I and Xylene II for 10 min each.
2. Slides were passes through 90% and 70% alcohol for 5 min each.
3. Slides were rehydrated by keeping in running water for 10 min.
4. Slides were dipped in Harris hematoxylin for 3 minutes 30 seconds.
5. Slides were kept in water wash for 2-3 min.
6. Slides were differentiated by 1 dip in 1% acid alcohol
7. Slides were kept in water wash for 10 min.
8. Bluing was done by keeping the slides in lithium carbonate for 5-8 min and then water wash for 10 min
9. Slides were stained with eosin 10 sec.
10. Slides were passed through increasing grades of alcohol, 70% and 90% for 5 sec each.
11. Slides were dried, cleared in xylene and mounted.

ANNEXURE VI

Clinical details and Record no. of Normal Oral mucosa

S.NO.	OP NO.	AGE	SEX
1	3848	30	M
2	3850	26	F
3	1803	17	F
4	6231	54	F
5	6305	24	M
6	3846	45	M
7	6473	45	F
8	6264	38	F
9	1711	27	F
10	6270	20	F
11	3496	55	M
12	3262 A	21	M
13	4228	56	M
14	6234	53	F
15	6276	27	F
16	3262 B	43	F
17	3861	42	M
18	4509	19	F
19	6466	22	M
20	6258	25	F
21	6448	19	M
22	3703	60	F
23	3849	35	M
24	6464	29	F
25	6446	31	F
26	6291	24	F
27	6322	22	F
28	3858	26	M
29	4181 B	56	M
30	6364	32	F
31	6257	24	M
32	6331	50	F
33	4472	80	F
34	6305	45	M
35	4470	39	F
36	3703	60	F
37	5383 A	40	F
38	1766	50	F
39	6236	28	M
40	1715	25	M

ANNEXURE VII

Clinical details and Record no. of Oral Epithelial Dysplasia

S.NO.	OP NO.	AGE	GENDER
1	5505	45	F
2	490	40	M
3	5789	75	M
4	1896	32	M
5	5421	66	M
6	2061	43	M
7	416	22	F
8	6013	50	M
9	3513	39	F
10	4835	58	M
11	6049	42	M
12	4341	58	F
13	3894	65	M
14	6039	42	M
15	6275	43	M
16	3328	68	M
17	4098	54	M
18	3633	44	M
19	2373	58	M
20	5446	61	M
21	6211	73	M
22	3431	32	M
23	3129	40	M
24	4352	70	M
25	3401	55	M
26	6123	45	M
27	1326	70	F
28	5856	63	F
29	4398	62	M
30	6447	38	M
31	5121	62	M
32	6421	61	F
33	5619	74	M
34	4727	57	M
35	2641	56	M
36	3163A	49	M
37	3701B	62	F
38	3211	65	F
39	5519	38	M
40	5856	63	F

ANNEXURE VIII:

Clinical details, histopathological diagnosis and Record no. of OSCC cases

S.NO.	OP NO.	AGE	GENDER	HABIT	SITE	HISTOPATHOLOGICAL DIAGNOSIS
1	5454	66	M	1	1	1
2	5692	60	M	1	1	1
3	6239	60	M	1	7	1
4	4951	30	M	1	3	1
5	4906	45	M	1	1	1
6	3885	45	M	1	1	1
7	4152	37	M	1	6	2
8	5440	48	M	1	1	1
9	6213	55	M	1	1	1
10	6138	43	M	1	2	1
11	4820	34	M	1	4	1
12	5109	42	M	1	3	1
13	3828	52	M	1	4	2
14	5045	76	M	1	2	1
15	5601	68	M	1	1	1
16	4706	30	M	2	3	1
17	4373	46	M	2	4	1
18	5036	40	M	1	5	1
19	5420	41	M	1	5	1
20	4179	55	M	1	2	2
21	5250	51	M	1	1	3
22	6048	38	M	2	7	3
23	4072	50	M	1	4	2
24	5494	42	M	1	1	2
25	5375	45	M	1	1	1
26	4910	40	M	1	4	1
27	3716	47	M	1	4	1
28	4694	43	M	1	1	2
29	5518	43	M	1	3	1
30	6084	51	F	1	6	1
31	5913	44	M	2	2	3
32	5009	42	M	2	4	1
33	4632	57	M	2	1	2
34	3743	74	M	1	1	3
35	4814	57	M	1	1	1
36	5696	41	M	1	1	2
37	5341	65	M	1	1	2
38	4706	30	M	1	4	1
39	5375	45	M	1	1	2
40	3621	58	F	1	6	1

Habit: Chewing-1, Non chewing- 2
Site: Buccal mucosa-1, tongue-2, lip-3, Alveolus-4,Palate-5, GBS-6, RMT-7
Histopathological diagnosis: WDSCC-1, MDSCC-3,PDSCC-3

ANNEXURE IX :

Histopathogical parameters & scoring of OSCC case

TB	DOI	POI	STROMA	INFLAMMATION	LVI	PNI	MARGINS	LYMPHNODE METASTASIS
0	3	3	1	1	0	0	0	0
1	3	3	2	1	0	0	1	0
1	3	2	3	2	1	0	0	1
0	3	2	1	1	0	0	0	0
1	3	4	1	1	0	0	1	0
0	3	1	1	1	0	0	0	0
1	3	2	1	2	0	1	0	0
0	4	3	1	1	1	0	0	0
0	2	1	2	2	0	0	0	1
1	3	3	1	1	0	0	0	1
1	3	3	3	3	0	0	0	0
1	3	3	1	1	1	0	0	0
0	3	2	1	1	0	0	0	0
0	3	3	3	3	0	0	0	0
0	3	2	1	2	0	0	0	1
1	3	3	1	1	0	0	0	1
0	3	3	1	1	0	0	0	0
0	3	4	1	1	0	0	1	0
0	3	2	1	1	0	0	0	1
1	3	3	1	1	0	0	0	1
0	3	2	1	1	0	0	0	1
1	3	4	1	1	0	0	0	1
0	3	2	1	1	0	0	0	1
0	3	2	1	1	0	0	0	0
0	3	4	1	1	0	0	0	0
0	2	1	1	1	0	0	0	1
0	2	2	2	2	0	0	0	0
0	3	1	1	1	0	0	0	0
1	3	4	3	2	1	0	0	1
0	2	2	3	2	0	0	0	0
0	3	4	1	1	0	1	0	1
0	3	4	3	2	0	0	0	0
0	3	2	1	1	0	0	0	0
0	3	4	1	1	0	0	0	0
0	3	4	3	2	0	0	0	0
1	3	2	1	1	1	0	1	1
1	3	4	3	2	1	0	0	1
1	3	3	1	1	0	0	0	1
0	3	4	1	1	0	0	0	0
0	3	2	1	1	0	0	0	0

TB: Tumor budding, POI: Pattern of invasive front, DOI: Depth of invasion, Type of connective tissue stroma, Degree of inflammation, Lymphovascular invasion, Perineural invasion