EXPRESSION OF CYTOKERATIN 17 (CK17) IN ORAL SQUAMOUS CELL CARCINOMA AND NORMAL MUCOSA

Dissertation submitted to

THE TAMILNADU Dr.M.G.R. MEDICAL UNIVERSITY

In partial fulfilment for the Degree of

MASTER OF DENTAL SURGERY

BRANCH VI

ORAL PATHOLOGY AND MICROBIOLOGY

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CERTIFICATE

This is to certify that this dissertation titled "**EXPRESSION OF CYTOKERATIN 17 (CK17) IN ORAL SQUAMOUS CELL CARCINOMA AND NORMAL MUCOSA**" is a bonafide dissertation performed by **Dr M.PAVITHRA** under our guidance during the post graduate period 2019-2022

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ABSTRACT

BACKGROUND:

The most common cancer worldwide is head and neck cancer. Oral cancer is the 11th most common cancer in the world. Oral squamous cell carcinoma (OSCC) is the second most common cancer reported in India. OSCC is the most common malignancy and represents more than 90% of all head and neck cancers[3]. More than 640,000 new cases are reported worldwide annually, with high rate of morbidity and mortality of >50%. According to recent Globacon – 2018 data 130,000 new cases of OSCC are detected every year in India. The reason for such high prevalence in India is primarily tobacco consumed in form of gutka, snuff, betel quid and misri[2]. Cytokeratins (CKs) are a group of Intermediate Filament proteins in the epithelium comprising a heterodimer of an acidic and a basic keratin commonly called keratin pair. According to Moll et al, there are 19 subclasses of CK, which are classified based on their molecular weights. CKs are place specific and may change when growth rate rises or when degree of differentiation is altered pathologically[29]. Among the Cytokeratins, CK 17 immunoexpression increases as the grade of epithelial dysplasia advances. CK17 expression increase the cell mobility and migration, indicating that it may lead to architectural alterations in dysplastic epithelia and carcinoma[84]. In many studies, compared to normal, CK 17 is overexpressed in squamous cell carcinoma of oral cavity, cervix, larynx, oesophagus and lungs. The present study is designed with an aim to correlate Cytokeratin 17 expression by immunohistochemistry with histological grade of Oral Squamous Cell Carcinoma which predicts aggressiveness of the tumor and thus helps in early diagnosis and treatment.

AIM:

To study the expression of Cytokeratin 17 in normal oral mucosa and different grades of Oral Squamous Cell Carcinoma.

OBJECTIVE:

- To evaluate expression of Cytokeratin 17 on formalin fixed paraffin-embedded tissue sections of normal oral mucosa using immunohistochemistry.
- To evaluate expression of Cytokeratin 17 on formalin fixed paraffin-embedded tissue sections of Oral Squamous Cell Carcinoma using immunohistochemistry.
- To compare the expression of Cytokeratin 17 on formalin fixed paraffin-embedded tissue sections of normal oral mucosa and Oral Squamous Cell Carcinoma using immunochemistry.

MATERIALS AND METHODS:

In this study, the population comprised of 55 (N=55) formalin fixed, paraffin embedded tissue specimens. The samples were divided into 4 groups namely: Group I (oral normal mucosa), Group II (well differentiated OSCC), Group III (moderately differentiated OSCC) and Group IV (poorly differentiated OSCC). CK17 expression was done using immunohistochemistry and the sections were evaluated for tissue localization, cellular localization, nature and intensity of the staining. $P< 0.05$ was considered significant.

RESULTS:

Overall 98% of the samples showed with CK 17 expression in the superficial epithelium. A gradual decrease in intensity of staining of suprabasal layer was observed with increase in grades of OSCC. Interestingly, in our study, all the cases expressed in both suprabasal and basal layer having the staining intensity 100% diffuse. While comparing grades of OSCC, 67% of the poorly differentiated OSCC samples showed complete loss of CK 17 staining in malignant tumor islands and 73% of well differentiated OSCC samples showed intense staining of central keratotic foci of keratin pearls.

CONCLUSION:

Based on the data obtained, CK17 has a potential diagnostic marker of OSCC that are under high risk for malignant transformation.

KEY WORDS:

Oral Squamous Cell Carcinoma, immunohistochemistry, CK17, diffuse, intense, suprabasal, basal layers

CONTENTS

INTRODUCTION

The most common cancer worldwide is head and neck cancer. Oral cancer is the 11th most common cancer in the world. Oral squamous cell carcinoma (OSCC) is the second most common cancer reported in India. OSCC is the most common malignancy and represents more than 90% of all head and neck cancers[1].

The latest global estimate showed a incidence of 600,000 new cases and 300,000 deaths from this disease. OSCC shows a 5-year survival rate of 30% and a mortality rate of 50%. The most important prognostic factor for OSCC is the presence of lymph node metastases, resulting in a 50% reduction in survival rates. The primary etiologic factors of OSCC are alcohol and tobacco use[2].

OSCC arises by malignant change in oral mucosa and is graded into well-differentiated, moderately, and poorly differentiated. Well-differentiated SCC closely resembles normal squamous mucosa whereas moderately differentiated SCC displays nuclear pleomorphism, mitoses (including atypical forms), and usually less keratinization. In poorly differentiated SCC, immature cells predominate, with numerous typical and atypical mitoses, minimal keratinization, and sometimes [necrosis13].

Keratins belong to the superfamily of intermediate filament proteins. Based on the gene substructure and nucleotide sequence homology, keratins are divided into two groups: 28 type I acidic and 26 type II basic proteins. Type I and type II keratins form heterodimers for assembly of the 10-nm filaments which provide structural support for maintaining cellular integrity[29].

Over the last decades, much progress has been made in understanding the molecular alteration that lead to oncogenic transformation, accompanied by an extensive search for biomarkers that predict the behavior of cancer.

Cytokeratin 17 (K17, CK17), being a component of cytoskeleton protein with a low molecular weight of 48.1 kDa and 432 residues, is an acid-type I cytokeratin with an isoelectric pH of 4.7. The CK17 gene (KRT17) has seven introns and eight exons covering approximately 5.14 Kbp on 17q12-q21[55].

In addition, CK17 is expressed at the same time as CK6, with which it forms heterodimers, although it can also combine with CK5, CK8 and CK16. Cytokeratin 17 expression may also change after premalignant and malignant transformations. CK17 protein is overexpressed in cancerous tissues compared with normal tissues in cervical, laryngeal, esophageal, and lung carcinomas. However, CK17 expression has not been well studied in OSCC. Moreover, a paucity of material is available on a correlation between the expression and the differentiation in OSCC[21].

The main function of CK17 is involved in the formation and maintenance of various skin appendages, specifically in determining shape and orientation of hair. CK17 is also considered as a marker of basal cell differentiation in complex epithelia and recognizes cervical stem cells. Additionally, CK17 regulates the protein synthesis and cell growth in injured stratified epithelia by binding to a signaling molecule[78].

Immunohistochemistry is most commonly used to study CK17 expression. This study aims to evaluate and compare the expression of CK17 in formalin fixed and paraffin - embedded tissues of well differentiated, moderately differentiated and poorly differentiated OSCC and normal mucosa.

AIM AND OBJECTIVES

AIM:

To study the expression of Cytokeratin 17 (CK 17) in normal oral mucosa and different grades of OSCC

OBJECTIVES:

- To evaluate the expression of cytokeratin 17 on formalin fixed paraffin-embedded tissue sections of normal oral mucosa using immunohistochemistry (IHC)
- To evaluate the expression of cytokeratin 17 on formalin fixed paraffin-embedded tissue sections of Well Differentiated Oral Squamous Cell Carcinoma (WDOSCC) using IHC.
- To evaluate the expression of cytokeratin 17 on formalin fixed paraffin-embedded tissue sections of Moderately Differentiated Oral Squamous Cell Carcinoma (MDOSCC) using IHC.
- To evaluate the expression of cytokeratin 17 on formalin fixed paraffin-embedded tissue sections of Poorly Differentiated Oral Squamous Cell Carcinoma (PDOSCC) using IHC
- To compare the expression of cytokeratin 17 on formalin fixed paraffin-embedded tissue sections of normal oral mucosa and OSCC using IHC

HYPOTHESIS: (NULL)

There is no difference in the expression of cytokeratin 17 between normal oral mucosa and OSCC and between different grades of OSCC.

MATERIALS AND METHODS

STUDY DESIGN:

This retrospective study was done to evaluate the CK17 expression in Oral Squamous Cell Carcinoma and normal mucosa.

STUDY GROUPS:

- Group I – Normal inflamed tissues from buccal mucosa (n=10)
- Group II – Archival tissues from Well differentiated OSCC (n=15)
- Group III – Archival tissues from Moderately differentiated OSCC (n=15)
- Group IV – Archival tissues from Poorly differentiated OSCC (n=15)
- **Immunohistochemistry Control :** Normal human skin tissue

STUDY SETTING:

This study was done at Ragas Dental College and Hospital and approved by the Institutional Ethics Committee of Ragas Dental College and Hospital, Chennai (ANNEXURE II).

ARMAMENTARIUM USED:

- Microtome
- Autoclave
- Hot air oven
- Slide warmer
- Coplin jars
- Measuring jar
- Weighing machine
- APES (3 amino propyl triethoxysilane) coated slides
- Slide box
- Micro-pipettes
- Toothed forceps
- Electronic timer
- Beakers
- Rectangular steel tray with glass rods
- Sterile gauze
- Cover slips
- Light microscope

REAGENTS USED:

1) Xylene
2) Absolute alcohol (Isopropyl alcohol)
3) Harris Hematoxylin
4) 1% acid alcohol

5) Eosin

6) APES

7) 1 N sodium hydroxide

8) 1 N Hydrochloric acid

9) Citrate buffer

10) 3% Hydrogen peroxide

11) PBS buffered Saline

12) Distilled water

13) Ammonium hydroxide

ANTIBODIES USED:

Primary antibody: Catalogue number: ab233912

Mouse monoclonal (SPM 560) to Cytokeratin 17 (abcam)

HSN code: 38220090

Unit: 100µg (1:100 dilution)

Secondary antibody: Mouse and Rabbit specific

HRP/DAB IHC detection kit – micropolymer (abcam)

PROCEDURE:

1) A detailed case history including patient's age, gender, past medical and dental history, history of drug intake, deleterious habits and trauma was taken from records for control and study group.

2) Tissue samples of normal mucosa, well differentiated OSCC, moderately differentiated OSCC and poorly differentiated OSCC were taken from the archival blocks.

3) From the Formalin Fixed Paraffin Embedded tissues, 5 micron thick sections were cut and used for routine Hematoxylin and Eosin (H&E) staining and Immunohistochemical (IHC) staining.

4) Positive control for CK17 was a section of human skin tissue.

APES (3 Amino propyl tri ethoxy silane) COATING:

Slides first dipped in couplin jar containing acetone for 2 minutes

↓

Dipped in APES for 5 minutes

↓

Dipped in two changes of distilled water for 2 minutes each

↓

Slides left to dry

IMMUNOHISTOCHEMICAL STAINING OF CK17:

After the slides were dried, tissue sections of 5 micron thickness were made in a rotary manual microtome. The ribbons of tissue section were transferred onto the APES coated slides from the tissue float bath such that two tissue bits come on to each slide with a gap in between. One of the tissue sections towards the frosted end of the slide was labelled negative and the tissue section away from the frosted side is the positive. The slides with tissue sections were treated with three changes of

xylene to remove paraffin wax. They were put in descending grades of alcohol and then rehydrated with water. Circles were drawn using a diamond marker around the tissues, so that the antibodies added later are restricted to the circle. The slides were transferred to citrate buffer of pH 6 and autoclaved for antigen retrieval at 15 lbs pressure for 15 minutes at 121^0C . Slides were then treated with hydrogen peroxide for 10 minutes to quench endogenous peroxidase activity of cells that would result in non-specific staining. Then, the slides were dipped in PBS buffered saline with for 5 minutes. The slides were wiped carefully without touching the tissue section. The sections were incubated at room temperature with mouse monoclonal primary CK17 antibody (abcam). Primary antibody was detected using goat anti-rabbir HRP/DAB IHC Detection system (abcam). After thorough washing with PBS buffered saline at pH 7.0, sections were treated with mouse specifying reagent, for 1hour at room temperature followed by incubation with Goat anti-rabbit HRP-conjugate for 1 hour at room temperature. After three washes with PBS, substrate DAB was applied to the sections for 12 min in the dark. Slides were then washed in distilled water to remove excess chromogen and counterstained with hematoxylin, dehydrated with ethanol and xylene and mounted permanently with DPX. The slides were then observed under the Light Microscope (LM).

H & E STAINING:

The slides were deparaffinized in xylene and hydrated through grades of alcoholto water. The sections on the slides were flooded with Harris Hematoxylin for 5 minutes. The slides were washed in running tap water for 5 minutes. The slides were differentiated in 1% acid alcohol for 5 minutes. The slides were washed well in running tap water for 5 minutes. The tissue sections on the slides were then stained in eosin for 30 seconds. The slides were washed in running tap water for 1 minute. The

slides were then dehydrated through alcohol, cleared, mounted and viewed under light microscope and tumors were graded into varying histologic gradings of malignancy as well, moderately and poorly differentiated.

POSITIVE AND NEGATIVE CONTROL:

Section of human skin tissue that was previously known to be positive for CK17 was used as positive control. Negative control sections were processed by omitting primary antibody[81].

STEPS INVOLVED:

1. APES coated slides with 2 paraffin embedded tissue placed in warming table.
2. Placed in xylene twice (5 minutes each)
3. Placed in 100% isopropanol (5 minutes)
4. Placed in 90% isopropanol (5 minutes)
5. Placed in 70% isopropanol (5 minutes)
6. Washed in distilled water (2 minutes each)
7. Keep in citrate buffer buffer at pH 6 autoclave for 15 min for antigen retrieval
8. Cooling of solution done for 25 minutes
9. Slides were transferred to distilled water.
10. Placed in hydrogen peroxide (7 minutes)
11. Washed in PBS buffer saline (2-3 minutes)
12. Primary antibody added and incubated (30 minutes)
13. Washed in PBS buffer saline (2-3 minutes)
14. Mouse specifying reagent added and incubated (12 minutes)
15. Washed in PBS buffer (2-3 minutes)
16. Goat anti-rabbit HRP added and incubated (12 minutes)
17. Washed slides in PBS buffer (2-3 minutes)

18. DAB added and incubated in an enclosed in hydrated container (2 minutes)
19. Washed in PBS buffer (2-3 minutes)
20. Stained with Harris Hematoxylin (20 seconds)
21. Washed in tap water
22. Placed in 70% alcohol (1 minute)
23. Placed in 100% alcohol (1 minute)
24. Placed in xylene (1 dip)
25. Slides to be mounted using DPX
26. Slides to be observed under the LM and graded

CRITERIA FOR EVALUATION OF STAINING:

Evaluation of H & E sections:

Tumors were graded as well, moderate and poorly differentiated tumors.

Evaluation for IHC:

Yellow brown staining indicated the positive Cytokeratin – 17 expression. Cells with distinct yellow to brown staining particles in the cytoplasm were considered as the positive cells.

Distribution of staining:

- Focal – examined only a small part of the tissue sample.
- Diffuse – examined throughout the tissue sample.

Localization of staining:

Epithelial cells that exhibited yellow to brown cytoplasmic staining were counted as positive for expression of CK 17. The sections were initially scored at low power. For sections that showed heterogenous staining, the predominant pattern of staining in basal, suprabasal and both basal and suprabasal cell layer was taken into account for scoring. The staining intensity was analysed in the study groups.

Intensity of staining:

Each case was graded as (-) nil or absence of stain, (+) mild, (++) moderate and (+++) intense staining, based on the intensity of staining taken up by the tissue as observed by two blinded observers independently with respect to positive control. Observer was a geneticist who has more than 15 years experience in the field of immunohistochemistry. Each case was evaluated by observer with respect to positive control.

STATISTICAL ANALYSIS:

Data entry was done in Microsoft Excel and the statistical analysis was carried out using SPSS version 22.0. The Chi-Square Test was utilized to find out the association of age, gender distribution, site of biopsy, habits, localisation of expression, expression of CK17, staining intensity in basal layer, suprabasal layer and both basal and suprabasal layer and the connective tissue between the four groups. A p value of <0.05 was considered as statistical significance.

ORAL SQUAMOUS CELL CARCINOMA:

OSCC is a malignant condition of the tissues lining the oral cavity (oral mucosal epithelium) that can arise at any location within the anatomical confines of the oral cavity, which is capable of local, regional and distant spread. Tissues that may be involved as the site of origin include the labial and buccal mucosa, the anterior two-thirds of the tongue, the retromolar pad, the floor of the mouth, the gingiva and the palate[1].

The most common cancer in the head and neck is oral squamous cell carcinoma (OSCC). OSCC is caused by DNA mutation and there is an increased risk of acquiring the mutations on exposure to a range of physical, chemical or microbial mutagens[2].

Warnakulasuriya S in 2009 reported that OSCC accounts for more than 90% of all head and neck cancers. India is rated as the country with the highest incidence of OSCC in the world, although some recent reports have ranked Sri Lanka and Pakistan as the highest. The ratio of men and women diagnosed with oral cancer has been declining for decades, and for oral cancer it is 1.5:1 and for oropharyngeal cancers it is around 2.8:1 in men and women. The risk of oral cancer increases with age[3].

Markopoulos AK in 2012 observed that despite the advances of therapeutic approaches, percentages of morbidity and mortality of OSCC have not improved significantly during the last 30 years. Rate of Morbidity and Mortality in males is 6.6/100,000 and 3.1/100,000 respectively, while in females it is 2.9/100,000 and 1.4/100,000. Additionally, the incidence of OSCC is increasing among young white individuals of age 18 to 44 years, particularly among white women. The percentage of 5-year survival for patients with OSCC varies from 40-50%. Regardless of the easy access of oral cavity for clinical examination, OSCC is usually diagnosed in advanced stages. Most

common reasons are the initial wrong diagnosis and the lack of knowledge from the patient and ignorance from the patient or from the attending physician[4].

GLOBOCAN 2018 estimated 18.1 million new cases of cancer and 9.6 million deaths from cancer in 2018, which was 14.1 and 8.2 million respectively in 2012. Amongst all the cancers, Head and Neck Squamous cell carcinoma (HNSCC) presents with 600,000 cases worldwide, with 40–50% mortality annually and the burden is estimated to almost double in developing countries by 2030. Most of these tumors arise from the epithelial cells of oral cavity (OC), oropharynx, larynx or hypopharynx. Male to female ratio was 3.26:1. Mean age was 51.35 ± 14.39 years and 55.35 ± 8.87 years in males and females, respectively. The most common site of occurrence was buccal mucosa and gingivo-buccal sulcus (GBS). Most of the cases (66.32%) were well-differentiated OSCC. In most of the cases (66.32%) the diagnosis was made within 2-6 months of onset of symptoms[5]

Oral squamous cell carcinoma (OSCC) has the highest prevalence in the HNSCC group that has shown to be 11th and 18th most common cancer worldwide as per 2012 and 2018 data respectively. This overall global decrease in the prevalence of OSCC is attributed to the reduced chewing habits and geographic heterogeneity, however, it is still the most common cancer in South Asia, South Central Asia as well as the Pacific Islands (Papua New Guinea, with the highest incidence rate worldwide in both sexes). In 2018, India alone had estimated 120,000 new patients diagnosed, of which about 72,000 patients died. Taiwan presents with the world's highest incidence rates of oral cancer which accounted for 8% of all new cancers diagnosed and 6.3% of all cancer deaths in 2014. In the subcontinent, this cancer ranks first in Bangladeshi and Pakistani males. The total number of the patients included 65 males

(44.8%) and 80 females (55.2%) whose age ranged from 23 to 80 years (mean ± standard deviation; 52.86 ± 13.18 years). An incidence was highest in 40-45 and 60-65 age group[6].

ETIOLOGY AND RISK FACTORS OF OSCC:

Major risk factors for oral cancer are cigarette smoking and alcohol misuse. Among Asian populations, regular use of betel quid (with or without added tobacco) increases oral cancer risks. Dentists should be aware of some emerging risk factors for oral, and particularly oropharyngeal cancer such as the role of the human papillomavirus infection (HPV). Decrease in risk could be achieved by encouraging high fruit and vegetable consumption. Some controversies related to the aetiology of this disease also need clarification. As interventions should be based on good scientific evidence it is important to take into consideration reported controversies. Clearing some myths on factors considered non-relevant to this cancer is important so that information provided to the patients and the public is not misleading and paves the way to plan strategies for prevention summarized in figure 1.[7(Warnakulasuriya)].

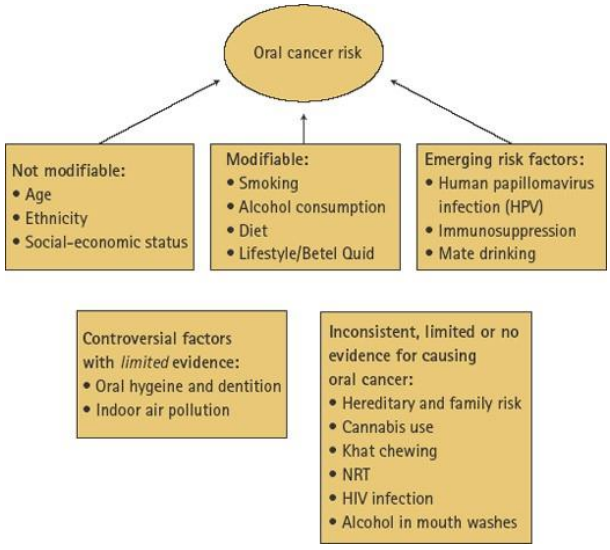

Figure 1: Factors influencing risk of oral cancer and those with no scientific evidence[7]

Madani AH, Dikshit M and Bhaduri D in between February 2005 and September 2006 conducted a case-control study of 350 cases and 350 controls, over a period of 19 months, among Indian population to investigate the association of tobacco and poly-ingredient oral dip products with oral cancer. They reported that the frequency of smoking, smokeless and oral dip products which were significantly higher than the controls. Among smoking types, *bidi*, of smokeless types, chewing tobacco and *mishiri*, and of oral dip products, consumption of *gutkha* and *supari* indicated strong association with oral cancer upon adjustment. This study provides strong evidence that *gutkha*, *supari*, arecanut, chewing tobacco (tobacco flakes), *bidi* smoking and *mishiri* (tobacco powder, which applied as a tooth and gum cleaner) are highly associated with incidence of oral cancer[8].

Goldstein BY, Chang SC and Hashibe M et al in 2010 evaluated human carcinogenic evidence related to oral and pharyngeal cancer risk based on cohort and case-control studies published from 1988 to 2009. A large body of evidence from epidemiological studies of different designs and conducted in different populations have consistently supported that alcohol consumption is strongly associated with an increase in risk of oral and pharyngeal cancer. The relative risks are 3.2–9.2 for more than 60 grams/day (or more than 4 drinks/day) when adjusted for tobacco smoking and other potential confounders. A strong dose-response relationship on intensity of alcohol use is reported in most of the studies. However, no apparent association is observed for the duration of alcohol use. Compared with current drinkers, a decreased risk is associated with alcohol cessation for about 10–15 years. Similar associations have been observed among non-smokers in over 20 studies. Generally, the dominant type of alcohol consumption in each population is associated with the greatest increased risk. A large number of studies on joint exposure of alcohol and tobacco consumption demonstrate a more than multiplicative synergistic effect[9].

Subapriya R, Thangavelu A and Mathavan B et al in 2007 conducted a case–control study in 388 cases of OSCC in Chidambaram, a small town in Southern India, to evaluate the relative importance of different risk factors, including tobacco, alcohol, diet and other lifestyle factors, on OSCC. This case–control study evaluated risk factors involved in oral cancer development. This study confirmed that tobacco habits, alcohol drinking and dietary practices are the strongest causes of oral cancer[10].

Singh MP, Misra S and Rathanaswamy SP et al from January 2010 to December 2012 conducted a retrospectively studied 479 cases of histopathologically confirmed oral carcinoma to analyze the etiological factors. His finding reveals that tobacco consumption is the main etiological factor for the development of carcinoma of the oral cavity. The majority of cases are reported at an advanced stage of the disease which increases the burden of disease and worsens the prognosis. This is the most worrisome observation made in this study. Smokeless tobacco consumed in India is one of the most common forms of tobacco abuse and is the leading cause of cancer in India especially of the buccal mucosa and alveolus. There is need to spread awareness about this tobacco-related cancer and immediate consultation on suspicion of cancer[11].

Addala L, Pentapati CK and Thavanati PR et al in 2012 conducted a cross-sectional study in patients with histologically confirmed diagnosis of squamous cell carcinoma of the head and neck in two hospital-based cancer registries in Andhra Pradesh. They described that majority of the subjects were in the age range of 40-69 years with a significant male preponderance in all the age groups. The most common habit was the combination of smoking, alcohol, and chewing in both males and females (20.1 and 35.1%, respectively). Tongue and buccal mucosa were the most common sites of cancer in both males (26.8 and 12.8%, respectively) and females (22.9 and 19.8%, respectively). Tongue was the

commonest site of cancer occurrence with respect to all the habits except for chewing tobacco where buccal mucosa was the most common site. Males were more likely to be diagnosed in stage 3 (37.6%) and 4 (20.6%), while females were diagnosed in stage 1 (36.3%) and 2 (32.7%)[12].

Gupta B and Johnson NW in 2014 carried out a meta-analysis to evaluate the association of smokeless tobacco and betel quid without tobacco with incidence of oral cancer in South Asia and the Pacific. Meta-analysis of fifteen case–control studies (4,553 cases; 8,632 controls) and four cohort studies (15,342) showed that chewing smokeless tobacco is significantly and independently associated with an increased risk of squamous-cell carcinoma of the oral cavity. Furthermore, meta-analysis of fifteen case control studies (4,648 cases; 7,847 controls) when stratified on the basis of gender, demonstrated a positive relationship between betel quid without tobacco and incidence of oral cancer. This is presumably due to the carcinogenicity of areca nut[13].

Chewing of areca nut alone was a predisposing habit by itself. Other risk factors for oral cancers include diet, BMI, oral hygiene, and viral infections. The most commonly implicated viruses in oral cancer transformation have been the human papilloma virus (HPV), herpes group viruses, adenoviruses, and the hepatitis C viruses. Of these, HPV and herpes have been the most thoroughly studied and are now considered to be the most likely ‘‘synergistic viruses’’ involved in human oral cancer. The herpes viruses most often linked to oral cancer are the Epstein-Barr virus (EBV), human herpes virus (HHV)-8 and cytomegalovirus (CMV)[14].

Kumar SA, Indu S and Gautami D et al in 2020 reported that majority of oral cancer cases are linked to specific exposure to lifestyle behaviors and individual predisposition. The most important reasons for oral cancer are heavy alcohol intake, tobacco

usage (Smoked and smokeless), high risk human papilloma virus (HPV) infection and poor oral hygiene. There are also other factors, such as diet any micronutrient deficiencies, that can balance the risk of developing oral cancer with environmental factors, genetic factors, etc.[15].

PATHOGENESIS:

Pindborg et al in 1977 defined OSCC as "a malignant epithelial neoplasm exhibiting squamous differentiation as characterized by the formation of keratin and/or the presence of intercellular bridges"[16].

Williams HK in 2000 discussed in his review that Oral Squamous Carcinogenesis is a multistep process in which multiple genetic events occur that alter the normal functions of oncogenes and tumor suppressor genes. This can result in increased production of growth factors or numbers of cell surface receptors, enhanced intracellular messenger signaling, and/or increased production of transcription factors. In combination with the loss of tumor suppressor activity, this leads to a cell phenotype capable of increased cell proliferation, with loss of cell cohesion, and the ability to infiltrate local tissue and spread to distant sites[17].

The development of Oral Squamous Cell Carcinoma is a multistep process requiring the accumulation of multiple genetic alterations, influenced by a patient's genetic predisposition as well as by environmental influences, including tobacco, alcohol, chronic inflammation, and viral infection. He also concluded that tumorigenic genetic alterations consist of two major types. First, tumor suppressor genes which promote tumor development when inactivated. Second, oncogenes which promote tumor development when activated. Tumor suppressor genes can be inactivated through genetic events such as mutation, loss of heterozygosity, deletion, or by epigenetic modifications such as DNA methylation or chromatin remodeling. Oncogenes can be activated through overexpression due to gene

amplification, increased transcription, or changes in structure due to mutations that lead to increased transforming activity[17].

Ram H, Sarkar J and Kumar H et al in 2011 in their review report stated that oral carcinogenesis like any other cancer is a progressive disease and normal epithelium passes through various stages of dysplasia to invasive cancer. Although all types of carcinomas are seen in the oral cavity, the most common form of Oral Cancer is squamous cell carcinoma. Use of genetic and proteomic approach in recent years have revealed the molecular pathological picture of OSCC. There is ongoing active search to identify genetic alterations in oncogenes or tumour suppressor genes, role of genomic instability and epigenetic modifications and to generate a gene expression profile in oral oncogenesis. Understanding the genetic changes and gene expression patterns are keys to the understanding of molecular pathogenesis of OSCC. Though, there are some significant leads achieved, the complete understanding of molecular pathology of OSCC and its association with causative agent will require additional intensive research[18].

The development and progression of human cancers is a multistep process of genetic alterations. OSCC arises as a result of various molecular events that develop from the combined influences of an individual's genetic predisposition and exposure to environmental carcinogens. Chronic exposure to carcinogens such as tobacco, alcohol, oncogenic viruses, and ionizing radiation can damage individual genes as well as larger portions of the genetic material including chromosomes. The genetic alterations include activation or amplification of oncogenes that promote cell survival and proliferation, as well as, inactivation of tumor suppressor genes (TSGs). From these alterations, tumor cells acquire autonomous self-sufficient growth and evade growth inhibitory signals and lead to uncontrolled tumor growth. Tumor cells also escape apoptosis and then replicate infinitely

through the immortalization process by telomere lengthening. As OSCCs grow, invade, and metastasize, new blood vessel formation is critical. OSCCs, like most tumors are able to create a blood supply by stimulating endothelial cell proliferation and new blood vessel formation. During oral carcinogenesis, there is selective disruption of this process, such that proangiogenic factors predominate. This angiogenesis is an essential part of solid tumor formation. The subsequent progression of OSCC includes tissue invasion and metastasis. Invasion of adjacent normal tissue requires the loss of cellular adhesion molecules such as integrin and E-cadherins, to allow cancer cells to leave their primary site[19].

Feller LL, Khammissa RR and Kramer BB et al in 2013 analysed the pathobiology of oral squamous cell carcinoma in relation to fields of precancerised oral epithelium and concluded that most OSCCs develop in fields of precancerized epithelium in which there is clonal expansion of phenotypically normal but genetically, altered keratinocytes. These genetically unstable precancerous keratinocytes manifest aneuploidy, gain or loss of chromosomal material, or alterations in the sequences of nucleotides. The genomic instability favors further acquisition of genetic alterations leading to growth superiority or inferiority of the affected cells. The genetically advantaged cells may ultimately acquire a cancerous phenotype[20].

Rivera C and Venegas B in 2014 describe oral carcinogenesis as a multistage process, which simultaneously involves precancerous lesions, invasion and metastasis. Degradation of the cell cycle and the proliferation of malignant cells (figure 2) results in the loss of control mechanisms that ensure the normal function of tissues[21].

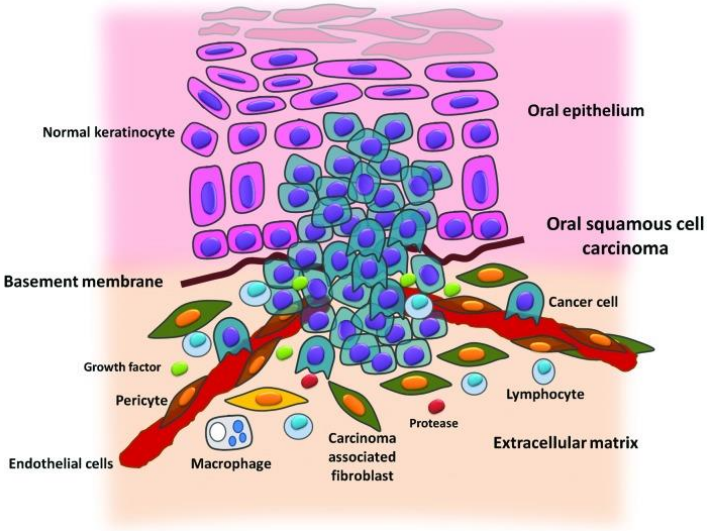

Figure 2: In the tumoral microenvironment (TME), different stromal cells, as well as tumor cells were observed, including vascular and lymphatic endothelial cells, and pericyte support fibroblast innate and adaptive immune cells. Furthermore, the TME contained no cellular components, including the extracellular matrix, growth factors, proteases, protease inhibitors or other signaling molecules that are significant in the reactions of the stroma in the TME[21].

Alhammad ZA in 2018 reviewed the literature on various concepts regarding the pathogenesis, prevalence, risk factors, clinical features, and treatment of OSCC were identified in 25 articles from PubMed which were included in the final systematic review. He concluded that OSCC is considered to be the most common neoplasm in the oral cavity. High prevalence of OSCC was seen in comparison to other lesions, and recognizable risk factors. OSCC is a complicated multi-step process that involves sophisticated genetic changes and gene expression patterns. On the other hand, it also appears that we are still lagging behind when it comes to fully understanding the exact pathogenesis and pathophysiology of OSCC. Therefore OSCC can be present with complexly with respect to different clinical features and different of occurence[22].

HALLMARKS OF CANCER:

Hanahan D and Weinberg RA in 2011 proposed that the following "10 hallmarks of cancer" (figure 3) are pivotal in tumor progression:

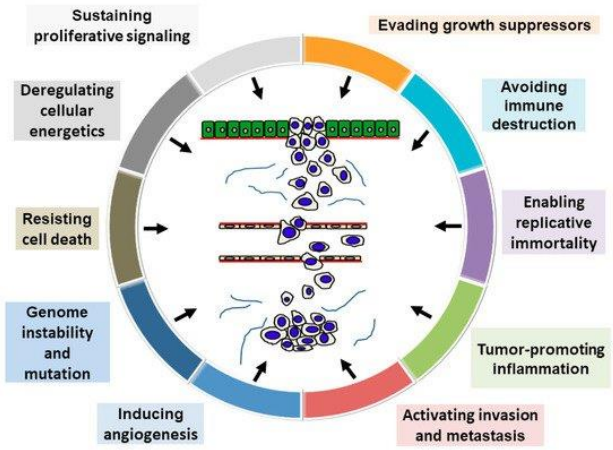

Figure 3: Schematic representation of the hallmarks of cancer. This schema presents the 10 hallmark capabilities as follows: sustained proliferative signals, evasion of growth suppressors, resistance to cell death, replicative immortality, induction of angiogenesis, activation of invasion and metastasis, avoidance of immune destruction, deregulation of cellular energetics, genome instability and mutation, and tumor-promoting inflammation[23].

MOLECULAR MODEL OF ORAL CARCINOGENESIS:

In normal oral mucosa, various tightly controlled excitatory and inhibitory pathways regulate oral epithelial cell biology such as cell division, differentiation, and cell death (apoptosis). An extracellular ligand like growth factor (a protein) binds with a specific cell surface receptor. The receptor-ligand complex generates excitatory or inhibitory signals sent through intracellular and nuclear messengers that can either alter cell function by changing the effect of proteins. Carcinogenesis is a complex, multi-step process in which genetic events within signal transduction pathways are subverted/altered resulting to cell's enhanced

ability for proliferation, uncontrolled apoptosis or growth by local invasion or metastasizing to distant sites[24].

Jain A in 2020 described the histologic progression of oral carcinogenesis from hyperplasia to dysplasia, followed by severe dysplasia and eventual invasion and metastases (figure 4) and reported that they are believed to reflect the accumulation of changes in the p or q arm of chromosomes 3, 4, 8, 9, 11, 13, and 17 (figure 5)**.** Genetic alterations occurring during the carcinogenesis may present in the form of point mutations, amplifications, rearrangements, and deletions[25].

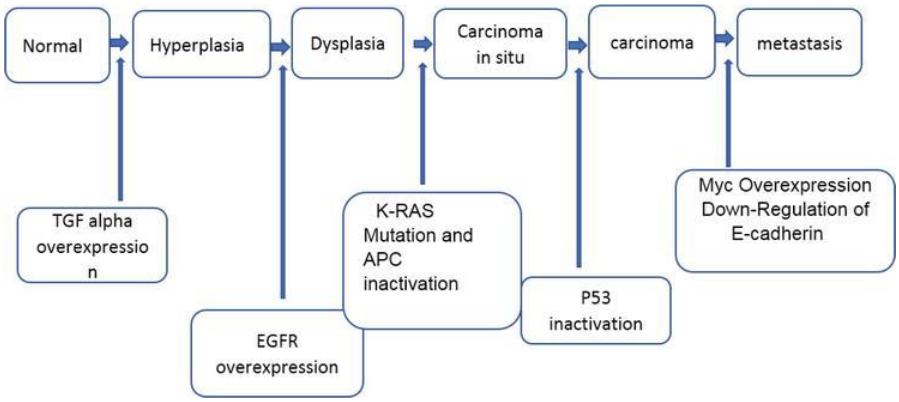

Figure 4: Oral cancer progression model. The histopathologic progression of normal oral mucosa from hyperplasia to malignancy and metastasis appears driven by interplay of activation of oncogenes in early cellular transformation and inactivation of tumor suppressor genes closer to the initiation of malignancy and metastasis[25].

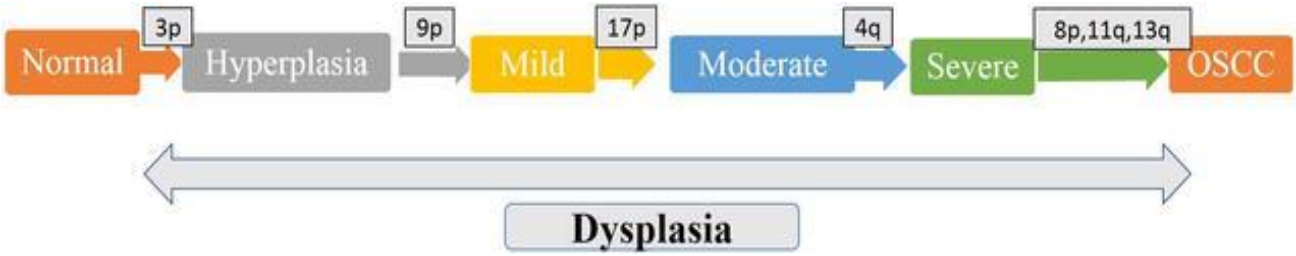

Figure 5: Molecular model of oral carcinogenesis. The diagram shows the genetic progression from dysplasia to oral squamous cell carcinoma (OSCC), through changes in the p or q arm of chromosomes 3, 4, 8, 9, 11, 13, and 17[25].

Pena-Oyarzun D, Reyes M and Hernández-Cáceres MP et al in 2020 in his review, reported that the normal epithelium, composed of epithelial cells known as keratinocytes, is located over a basement membrane that separates the epithelium from the connective tissue, which is composed of fibroblasts, immune cells and vessels. Exposure to carcinogens derived from the risk factors of the top right panel (figure 6) generate a potentially malignant lesion, characterized by an altered cellular morphology that starts affecting the inner layers of the epithelium close to the basement membrane, progressing toward the outer layers of the epithelium. Continuous exposure to carcinogens leads to OSCC development, a phenomenon that alters all the epithelial cell layers both genetically and morphologically. Interplay between connective tissue cells and OSCC cells is also observed, which assists OSCC growth and metastasis[26].

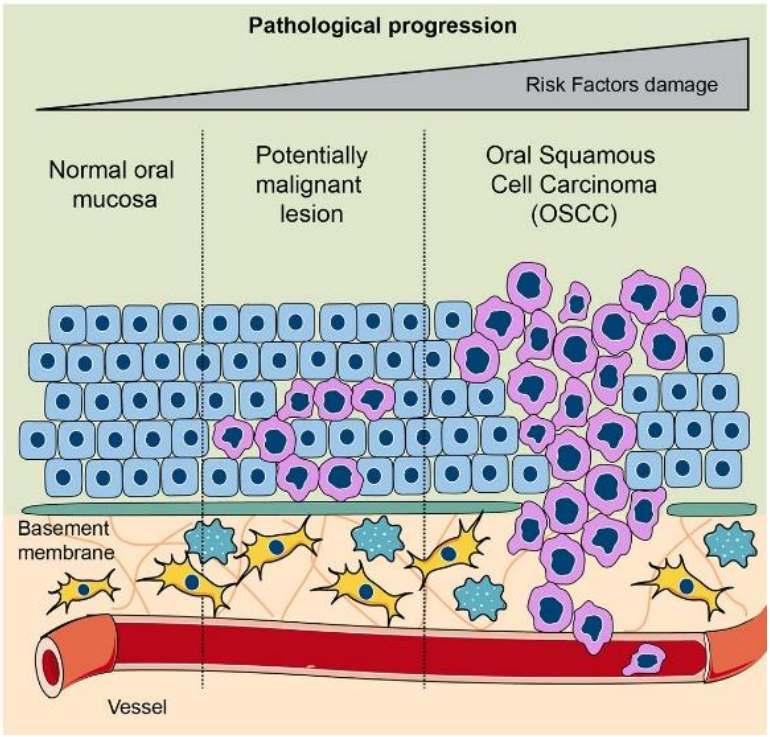

Figure 6: The pathological progression after diagnosis of OSCC[26].

KERATIN:

William T Astbury and Francis Crick contributed to the structure of keratin, following which Sun and Green popularized the monoclonal keratin antibodies. The identification of types I and II subunits in keratin and requirement of both these types to constitute a stable keratin assembly was noted by Fuchs and co-workers. All these developments were followed by extensive research in biology and pathology using monoclonal antibodies with the discovery of Epidermolysis bullosa simplex (EBS) as the first disease of IF[27].

The word "keratin" first appears in the literature around 1850 to describe the material that made up hard tiss ues such as animal horns and hooves (keratin comes from the Greek "*kera"* meaning horn). At that time, keratins intrigued scientists because they did not behave like other proteins. In particular, normal methods for dissolving proteins were ineffective for solubilizing keratin. Although methods such as

burning and grinding had been known for some time, researchers were more interested in dissolving hair and horns in order to make better products. The resolution to the insolubility problem came in 1905 with the issue of a United States patent to John Hoffmeier that described a process for extracting keratins from animal horns using lime[28].

Moll R, Franke WW and Schiller DL et al in 1982 and **Fraser RD, MacRae TP and Parry DA et al** in 1986 were the first among the researchers to document the term "keratin" originally referred to the broad category of insoluble proteins that associate with intermediate filaments (IFs) and form the bulk of cytoplasm in epithelial cells and epidermal appendageal structures (*i.e.*, hair, wool, horns, hooves and nails). Subsequent research of these structural proteins led to the classification of mammalian keratins into two distinct groups based on their structure, function and regulation[29,30].

1. "Hard" keratins form ordered arrays of intermediate filaments embedded in a matrix of cystine-rich proteins and contribute to the tough structure of epidermal appendages. In hard keratins, intensive concentration of sulfur occurs through the amino acids cysteine and methionine. Hard keratin is found in hair and nails

2. "Soft" keratins preferentially form loosely-packed bundles of cytoplasmic IFs and endow mechanical resilience to epithelial cells. Soft keratins in the Stratum corneum are cross-linked by intermolecular disulfide bonds. Soft keratin is found in the epidermis of the skin[29,30].

Schweizer J, **Bowden PE and Coulombe PA et al** in 2006 developed a new consensus nomenclature for hard and soft keratins to accommodate the functional genes and pseudogenes for the full complement of human keratins. This system classifies the 54 functional keratin genes as either epithelial or hair keratins. The structural subunits of both epithelial and hair keratins are two chains of differing molecular weight and composition

(designated types I and II) that each contain non-helical end-terminal domains and a highly-conserved, central alpha-helical domain. The type I (acidic) and type II (neutral-basic) keratin chains interact to form heterodimers, which in turn further polymerize to form 10-nm intermediate filaments. Although hard and soft keratins have closely related secondary structures, distinct differences in amino acid sequences contribute to measurable differences between the filamentous structures[31].

NEW HUMAN KERATIN NOMENCLATURE:

Schweizer J, Bowden PE and Coulombe PA et al in 2006 structured the new nomenclature system (figure 7), the 54 human keratins and their genes are divided into three categories: (1) epithelial keratins/genes, (2) hair keratins/genes, and (3) keratin pseudogenes.

Keratin types	Type I		Type II	
	New name	Former name	New name	Former name
Epithelial keratins	K9	K9	K1	K1
	K10	K10	K2	K2
	K12	K12	K3	K3
	K13	K13	K4	K4
	K14	K14	K5	K5
	K15	K15	K6a	K6a
	K16	K16	K6b	K6b
	K17	K17	K6c	K6e/h
	K18	K18	K7	K7
	K19	K19	K8	K8
	K20	K20	K76	K2p
	K23[a]	K23	K77	K1b
	K24[a]	K24	K78[a]	K5b
			K79[a]	K61
			K80[a]	Kb20
Hair follicle specific epithelial keratins (root sheath)	K25	K25irs1	K71	K6irs1
	K26	K25irs2	K72	K6irs2
	K27	K25irs3	K73	K6irs3
	K28	K25irs4	K74	K6irs4
			K75	K6hf
Hair keratins	K31	Ha1	K81	Hb1
	K32	Ha2	K82	Hb2
	K33a	Ha3 I	K83	Hb3
	K33b	Ha3 II	K84	Hb4
	K34	Ha4	K85	Hb5
	K35	Ha5	K86	Hb6
	K36	Ha6		
	K37	Ha7		
	K38	Ha8		
	K39	Ka35		
	K40	Ka36		

[a]Expression pattern still unknown, only gene information available

Figure 7: The new human keratin nomenclature[31]

CLASSIFICATION OF KERATIN:

Keratins are defined as intermediate filament forming proteins with specific physicochemical properties produced in any vertebrate epithelia. They are multigene family of proteins constituting 85% of the total cellular protein in the cornified cells of the epidermis and encoded by a family of approximately 30 proteins. Different types of keratins are distinguished according to various characteristics such as physicochemical properties or according to cells and tissues that produce certain keratin. The keratins are broadly divided into four (figure 8):

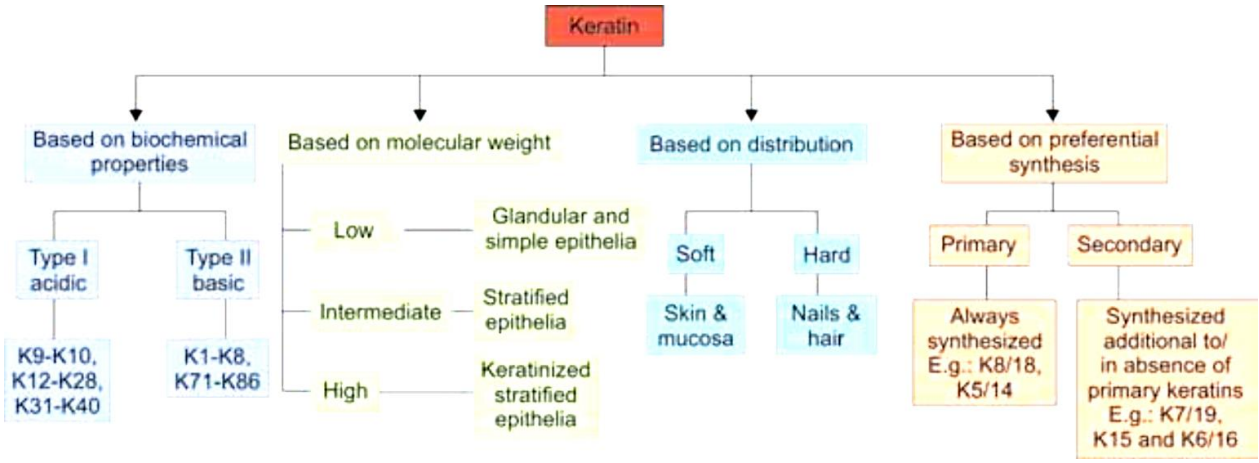

Figure 8: Classification of keratin[32]

FUNCTIONS OF KERATIN:

The primary function of keratins is to impart mechanical strength to cells and maintain the cell shape and tissue integrity. Furthermore, genetic and molecular analyses revealed that point mutations in highly conserved amino or carboxyl terminal ends of the rod domains of keratin led to autoimmune skin blistering diseases like epidermolysis bullosa simplex (mutations in keratin 5/14 genes), epidermolytic hyperkeratosis (mutations in keratin 1/10 genes), and epidermolytic palmoplantar keratoderma (mutations in the keratin 9 gene). The studies conducted over the past two decades to understand the multiple

functions of keratins have indicated that they modulate processes such as osmolarity and apoptosis and regulate protein synthesis. In addition, various experimental evidence in recent years has revealed many more complex functions of keratins, such as intracellular organelle transport, intracellular communication, cell - cell contact, translation control, proliferation, differentiation, various stress responses, cell signaling and malignant transformation. This diversity of epithelial functions may answer why distinct keratins genes are evolved (figure 9)[33].

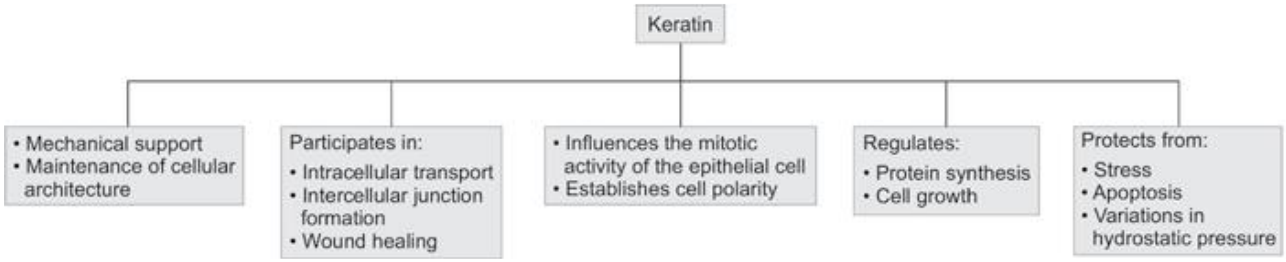

Figure 9: Functions of keratin[33]

CYTOKERATIN:

Schweizer J, **Bowden PE and Coulombe PA et al** in 2006 classified the 54 different human keratins can be further divided into three subcategories: (I) epithelial keratins/genes, (II) hair keratins/ genes, and (III) keratin pseudogenes. The 37 different epithelial keratins are expressed in epithelia that line internal body cavities, and a certain epithelial cell type can be categorized by the specific pattern of its keratin components. Epithelial keratins constitute the cytoskeletal network of intermediate filaments (IF). Following the recent nomenclature that adheres to the guidelines issued by the Human and Mouse Genome Nomenclature Committees, the commonly known cytokeratins should simply be called keratin. Thus, these proteins represent highly interesting markers for identification and classification of carcinoma cells. Based on their amino acid composition epithelial keratins are categorized as either type I or type II IF proteins. The human type I epithelial keratins

encompass K9–K28, while K1–K8 and K71–K80 cover the twenty human type II epithelial keratins[31].

CYTOKERATIN STRUCTURE

Irvine AD and McLean WHI in 1999 reported that keratins form type I and type II heterodimers, whereas type III intermediate filaments such as desmin and vimentin form homopolymers. The formation of heterodimers where compatible type I and II chains are aligned in parallel and in exact axial register is the first step in keratin intermediate filament assembly. Two heterodimers associate, forming tetramer units that may be aligned in an antiparallel manner[34].

The keratins vary in size between 40–70 kDa and are divided into two groups based on molecular weight: the smaller or low molecular weight acidic type I (40–64 kDa, with PI: 4.7–6.1) and the larger or high molecular weight neutral-basic type II (52– 70 kDa, with PI: 5.4–8.4) subgroups of IF proteins[35].

Strnad P, Usachov V and Debes C et al in 2011 found that glycine is the most abundant residue in cytokeratins. The heads and/or tails of epidermal keratins are glycine and phenylalanine rich but alanine poor, whereas parallel domains of hair keratins are abundant in prolines, and those of simple-type epithelial keratins are enriched in acidic and/or basic residues. Cysteines and histidines, which are infrequent keratin amino acids, are involved in de novo mutations that are markedly overrepresented in keratins. Hence, keratins have evolutionarily conserved and domain-selectively enriched amino acids including glycine and phenylalanine (epidermal), cysteine and proline (hair), and basic and acidic (simple-type epithelial), which reflect unique functions related to structural flexibility, rigidity and solubility, respectively[36].

Rao RS, Patil S and Ganavi BS in 2014 describe that filament assembly (figure 10) begins by parallel association of a type I chain with its type II counterpart to form a paired dimer. Two such paired dimers associate in an antiparallel fashion to form a staggered tetramer. Two tetramers pack together laterally to form the protofilament.

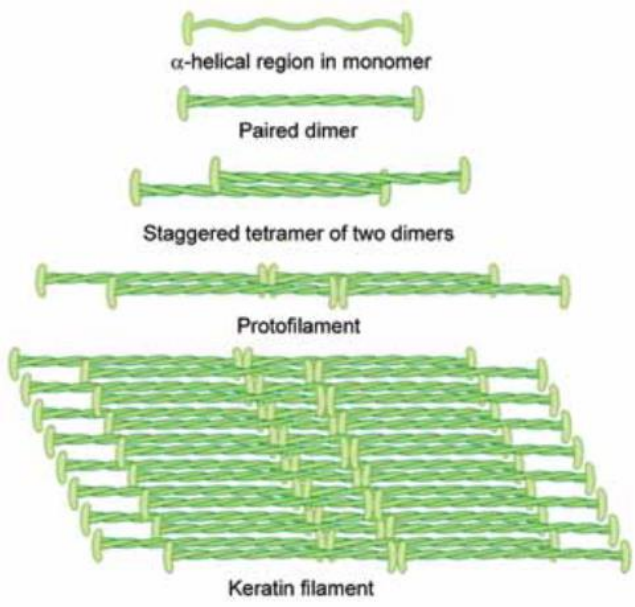

Figure 10: Assembly of keratin filament[32]

Eight such protofilaments are twisted into a rope which forms the keratin filament. Each individual keratin filament therefore has a cross section of 32 individual α helical coils. Strong lateral hydrophobic interactions stabilize the polypeptide chains. Keratin filaments are subsequently bundled and assembled into macromolecular networks that radiate throughout the cytoplasm. All keratin molecules contain a central rod domain of 310 aminoacids with α-helical conformation. This central core is made up of four subdomains separated by three nonhelical linker sequences (L1, L2 and L3). Diversity among keratin filaments resides in nonhelical extensions at the amino and carboxy terminals (H, V and E end domains). Further, there are two highly conserved helix boundary sequence motives on each rod, called helix initiation peptide (HIP) in the 1A

domain and the helix termination peptide (HTP) at the end of helix. Any mutations in these regions, lead to more severe disease phenotypes than the other regions[32].

Toivola DM, Boor P and Alam C in 2015 summarizes that the keratin proteins consist of the non-helical N-terminal head- and C-terminal tail-domains as well as the central helical rod-domain.The four a-helical segments (1A, 1B, 2A and 2B) of the rod domain are interconnected through the linker domains L1, L12 and L2. Most disease-causing mutations found in keratins of stratified epithelia occur at the mutation 'hotspots' (red arrows) (figure 11). The mutations responsible for the most severe phenotypes (e.g. Epidermolysis bullosa simplex Dowling-Meara) are located around the helix initiation and termination motifs at the periphery of domains 1A and 2B (in orange), or in the L12 linker domain (e.g. for Epidermolysis bullosa simplex Weber-Cockayne type). Red arrows with asterisks (*) indicate mutation hotspots for K4/K13 and K3/12 (i.e. non-epidermal stratified keratins), which also lie at the endpieces of the rod domain and cause diseases. In contrast to mutations in keratins of stratified epithelia, simple epithelial disease-predisposing keratin variants (blue arrows indicate the most prominent variants) are typically located at the more variable head or tail regions. Posttranslational modifications, such as phosphorylation, often also occur at the head/tail regions. Keratin variants in these regions can therefore interfere with the modifications, for example, inhibition of K8 S74 and S432 phosphorylation by neighboring G62C or G434S variants, respectively[37].

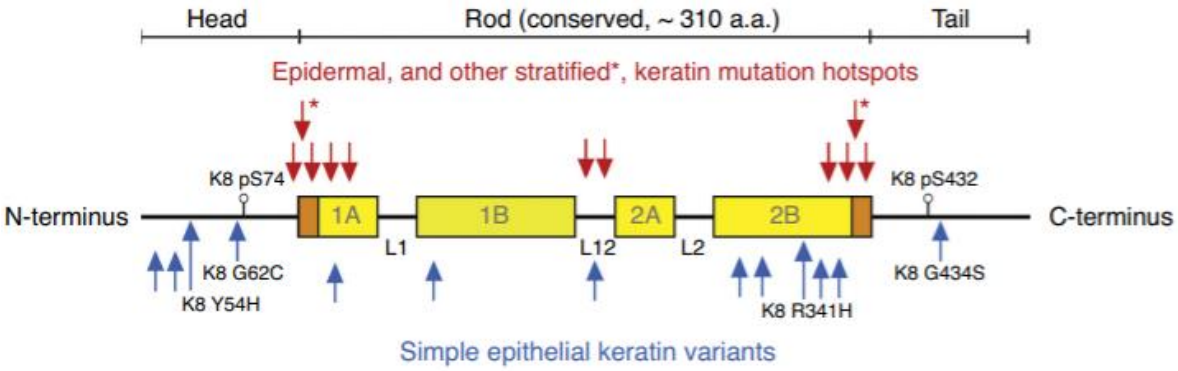

Figure 11: Keratin structure and mutation hotspots[37]

KERATINIZATION PATTERNS IN ORAL EPITHELIA

Oral epithelia demonstrate one of the 2 patterns of epithelial maturation (figure 12)[38].

1. Keratinization—mucosa matures by formation of surface layer of keratin.

   a. Orthokeratinization—refers to the absence of nuclei in the surface layer of squames on maturation.

   b. Parakeratinization—refers to the retention of pyknotic nuclei in the surface layer of squames on maturation.

2. Nonkeratinization—refers to maturation with absence of keratin layer. Hence the surface cells retain their nuclei with sparse keratin filaments in the cytoplasm.

Meeting the functional demands, gingiva demonstrates both types of epithelia-keratinized (e.g. Attached and free gingiva) and nonkeratinized (e.g. Sulcular and junctional epithelia).

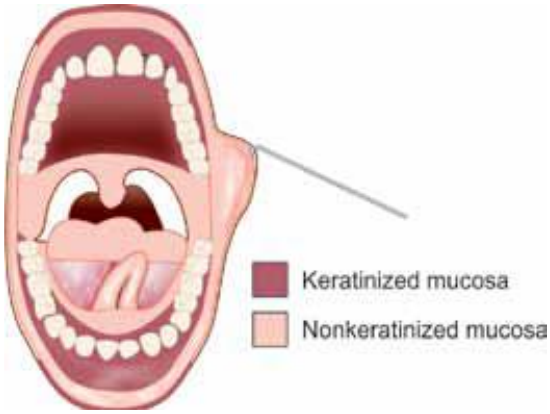

Figure 12: Keratinization in oral mucosa[38]

The following terms denote pathologic states:

• Keratosis: When keratinization occurs in a normally nonkeratinized tissue, it is referred to as keratosis.

• Parakeratosis: When normally keratinizing tissue, such as epidermis, becomes parakeratinized, it is referred to as parakeratosis[38].

FACTORS INFLUENCING EPITHELIAL DIFFERENTIATION:

Differentiation within the oral epithelia shows region-specific patterns of expression of the keratin proteins as well as associated proteins. In general, the basal cell layer in all regions shows similar keratin expression, while the suprabasal cell layers express a specific set of markers, indicating commitment to a pattern of differentiation[39].

Many extracellular factors influence expression of the genes for these proteins. One important factor is retinol (vitamin A), which is now known to exert its effect on gene expression by a group of nuclear receptor proteins similar to the steroid hormone and thyroid hormone receptors. Some region-specific gene expression may be mediated through the differential expression of these receptors in the different regions of the oral cavity and skin[40].

Shapiro SS, Seiberg M and Cole CA in 2013 report about vitamin A and its derivatives in experimental photocarcinogenesis. He reported that deficiency of vitamin A leads to squamous metaplasia and epithelial keratinization whereas, excess vitamin A inhibits keratinization. Also, high calcium concentrations are necessary for stratification and desmosome assembly. Also, he stated that the discovery of vitamin A in the 1920s paved the way for its use in the treatment of skin conditions such as acne, psoriasis, and photodamage. Retinoids also inhibit tumor formation and skin cancer development in experimental systems and in humans[41]

CYTOKERATIN DISTRIBUTION IN NORMAL ORAL EPITHELIA:

Rao RS, Patil S and Ganavi BS in 2014 show that cytokeratin distribution is highly specific and varies with site, type of epithelium and extent of differentiation. Hence keratin expression is a sensitive and specific marker for assessment of differentiation in epithelial cells. Cytokeratin distribution in normal oral epithelia is represented in (figure 13). Further, the regional specificity of keratin expression may be attributed to intrinsic specialization of regional keratinocyte stem cells. CK7, 8, 18, 19 are the markers for simple epithelia and merkel cells. Hyperproliferative epithelia are known to express CK6, 16[32].

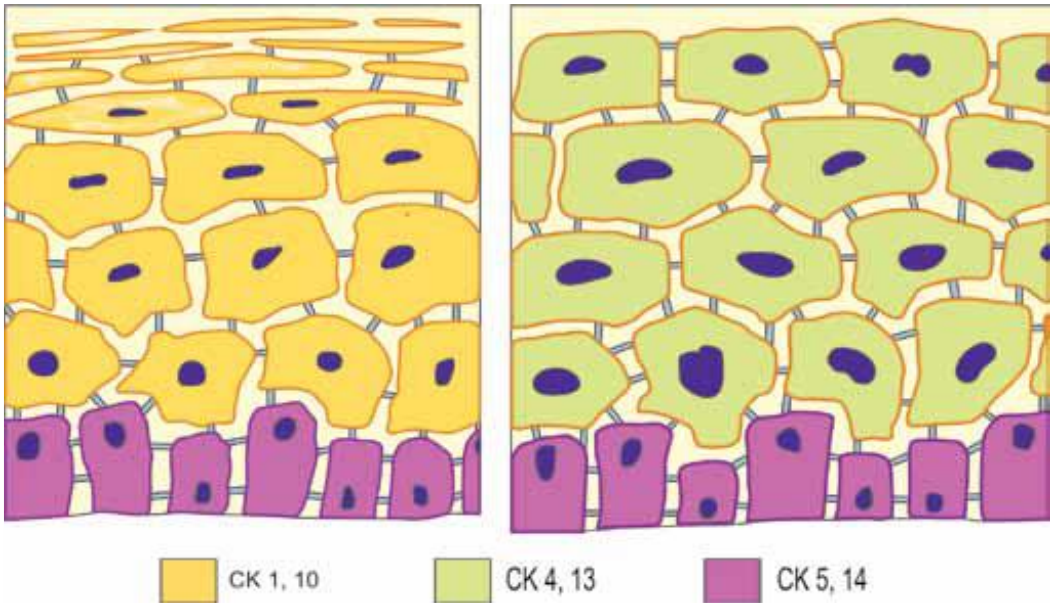

Figure 13: Normal cytokeratin distribution in oral mucosa: (A) Keratinized epithelia, (B) non-keratinized epithelia[32]

Cytokeratins are the basic structural proteins of epithelial cells. They are abundant in oral cavity, salivary gland epithelia and are expressed during odontogenesis (figure 14). Also, cytokeratins are the leading biomarkers in diagnostic pathology[32].

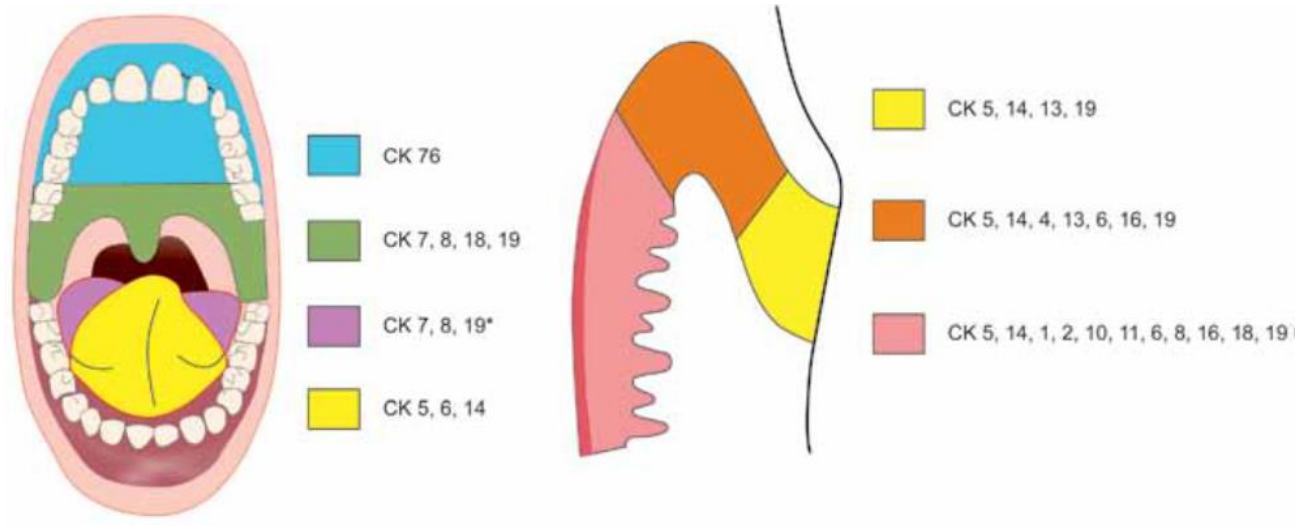

*Figure 14: (A) Regional variation in keratin distribution in normal oral mucosa, (B) cytokeratin distribution patterns in normal gingiva. *Expressed by taste buds*[32]

Cytokeratin Distribution in Salivary Epithelia and Odontogenic Tissues:

Dardick I in 1996 classified cytokeratins distribution in salivary epithelia as CK 14 in myoepithelial cells and basal cells (ductal nonluminal cells)**,** CK 18, 19 in epithelium elements of salivary gland**,** CK 7, 8, 18, 19 in luminal duct cells and CK 8, 18 in epithelium of striated and intercalated ducts[42]

Crivelini MM, de Araújo VC, de Sousa SO and de Araújo NS in 2003 classified cytokeratins distribution in odontogenic tissues as CK 7, 13, 14, 19 in enamel organ, CK 14 in most cells of enamel organ (Odontogenic epithelial marker), CK 7 in stellate reticulum and HERS, CK 19 in preameloblasts and secretory ameloblasts (secretory differentiation) and CK 5 and CK 19 in cell rests of Malassez. He also reported that the typical intermediate filament of odontogenic epithelium is CK14, observed in the dental lamina, the reduced enamel epithelium and in almost all cells of the enamel organ except for preameloblasts and secreting ameloblasts[43].

CYTOKERATIN AS MARKERS OF CELL PROLIFERATION AND DIFFERENTIATION:

ROLE OF CYTOKERATINS IN CELL PROLIFERATION

Paramio JM, Segrelles C, Ruiz S and Jorcano JL in 2001 showed that keratin K10 function as a negative modulator of cell cycle progression involves changes in the phosphoinositide 3-kinase (PI-3K) signal transduction pathway. Physical interaction of K10 with Akt (protein kinase B [PKB]) and atypical PKCζ causes sequestration of these kinases within the cytoskeleton and inhibits their intracellular translocation. As a consequence, the expression of K10 impairs the activation of PKB and PKCζ. They also demonstrate that this

inhibition impedes pRb phosphorylation and reduces the expression of cyclins D1 and E. Functional and biochemical data also demonstrate that the interaction between K10 and these kinases involves the non-α-helical amino domain of K10 (NTerm). Together, these results suggest new and essential roles for the keratins as modulators of specific signal transduction pathways[44].

Alam H, Sehgal L and Kundu ST et al in 2011 demonstrated a significant reduction in proliferation in HaCaT and in an oral squamous cell carcinoma (OSCC)-derived cell line AW13516 cells in response to downregulation of the K5/14 pair. They attributed the reduction in cell proliferation to delay in cell cycle progression. These observations together suggest that CK 5 and CK 14 play an important role in regulating cell proliferation in the basal cells of the stratified epidermis via the PI3K/Akt pathway[45].

Mikami T, Cheng J and Maruyama S et al in 2011 conducted a study to evaluate differential expressions for keratin (K) subtypes 13 and 17 in oral borderline malignancies in 67 surgical specimens of the oral mucosa for their immunohistochemical profiles. From those specimens, 173 foci of epithelial dysplasia, 152 foci of carcinoma in situ (CIS), and 82 foci of squamous cell carcinoma (SCC) were selected according to our diagnostic criteria, along with 20 areas of normal epithelia. This study results indicate that expressions of K17 and K13 are reciprocal in oral epithelial lesions and that the K17 emergence is related to malignancies[46].

Mikami T, Maruyama S and Abe T in 2015 also 160 samples: 20 of well differentiated SCC, 10 of moderately-differentiated SCC, 36 of CIS, 74 of epithelial dysplasia, and 20 of normal epithelium[49]. Both studies proved that KRT17 was expressed in tumor regions in OSCC specimens including carcinoma in situ but not non-tumor regions, such as normal and dysplasia, and regulated not only OSCC cell growth but also nuclear–

cytoplasmic translocation of 14-3-3 sigma in OSCC cells. KRT17 is highly expressed in OSCCs with high frequencies and that KRT17 promotes tumor cell growth through the anti-apoptotic function. They also demonstrated that KRT17 expression is regulated by GLI-1 or GLI-2[47].

Khanom R, Nguyen CT and Kayamori K et al in 2016 showed that KRT17-knockout HSC-3 cells, an OSCC cell line, had decreased proliferation capabilities in vivo. In addition, the KRT17 expression was correlated with the proliferation of HSC-3 cells in vitro. To our knowledge, the function of KRT17 was firstly reported to regulate cell growth through binding to the adaptor protein 14-3-3 sigma, leading to the activation of Akt/mTOR (mammalian target of rapamycin) signaling using KRT17-null mouse embryo keratinocytic regeneration models[48].

Yang F, **Fan X and Cui T et al** in 2017 showed that Nrf-2 transcription factor regulates the expression of K6, K16 and K17 in psoriasis. Nrf-2 promoted the expression of K6, K16 and K17 by binding to the ARE domain located in the promoter of these genes. In mice with imiquimod-induced psoriasis-like dermatitis, topical application of Nrf-2 small-interfering RNA alleviated the epidermal hyperplasia with reduced expression of these keratins, suggesting that Nrf-2 is responsible for an increase in the expression of these keratins[49].

So, the evidence from the above studies proves that keratin filaments are not only important for structural support but also play an important role in cell proliferation, wound repair, protein synthesis, and epithelial cell growth in a context-dependent manner.

ROLE OF CYTOKERATINS IN CELL DIFFERENTIATION

Casanova ML, Bravo A and Martínez-Palacio J et al in 2004 proved that fillagrin, a marker of cell differentiation, was expressed in the granular cells of the epidermis in wild-

type mice. However, in transgenic mice, the fillagrin was also seen in the dysplastic hair follicles. Staining for loricrin and involucrin, major precursors of the cornified epithelium, was increased in the dysplastic hair follicles of transgenic mice. Hyperplastic epithelium bordering also demonstrated an increase in the loricrin expression in transgenic mice. Furthermore, there was an aberrant expression of K6 in the dysplastic hair follicles, without any increase in their proliferative rate, suggesting that they follow an alternative differentiation pathway. Thus, the expression of K8 in skin abrogates the differentiation status of the epidermal and follicular cells. Thus, keratins play an important role in cell proliferation and differentiation. At the molecular level, the signaling molecules controlling the expression of keratins and their regulation are the areas still being explored[50].

Alam H, Sehgal L and Kundu ST et al in 2011 showed that upon the downregulation of K14 in HaCaT (derived from human adult skin) and AW13516 cells (derived from human squamous cell carcinoma of tongue), there was an increase in the cell differentiation markers such as involucrin and K1. Notch-1, a key modulator of the squamous cell differentiation process, was also found to be elevated in K14 knockdown cells, both at the surface as well as at the nuclear level. An increase in the activated Notch-1, that is the Notch-1 intracellular domain (NICD), was also observed. These results suggest that K14 downregulation leads to an increase in NICD which further modulates the levels of differentiation markers such as involucrin and K1. Thus, it was concluded that K14 is a negative regulator of cell differentiation[45].

Dmello C, Srivastava SS and Tiwari R et al in 2019 showed the role of K16 and K10 in modulating cell proliferation/differentiation. He suggests that K10 has a role to play in cell differentiation. Altered composition of the suprabasal IF in K10-/ - increases the differentiation of epidermal stem cells towards sebocyte lineage. In another study, chimeric

protein, which consists of the K14 rod domain fused to the K10 head and tail domains (K1014chim), was expressed in the basal sheath of hair follicles. Interestingly, K10 end domain did not have any effect on basal cell proliferation in vivo. The mutant mice demonstrated increased susceptibility to benign tumor formation when subjected to the chemical carcinogenesis protocol. The authors further found that the increase in tumor burden was due to a decrease in resistance to apoptosis. K10-expressing HaCaT cells appear to be partially protected from chemically induced apoptosis. Thus, the function of K10 is to inhibit apoptosis for the timely differentiation of keratinocytes. K10 also inhibits basal cell proliferation and induces differentiation of keratinocytes (figure 15)[51].

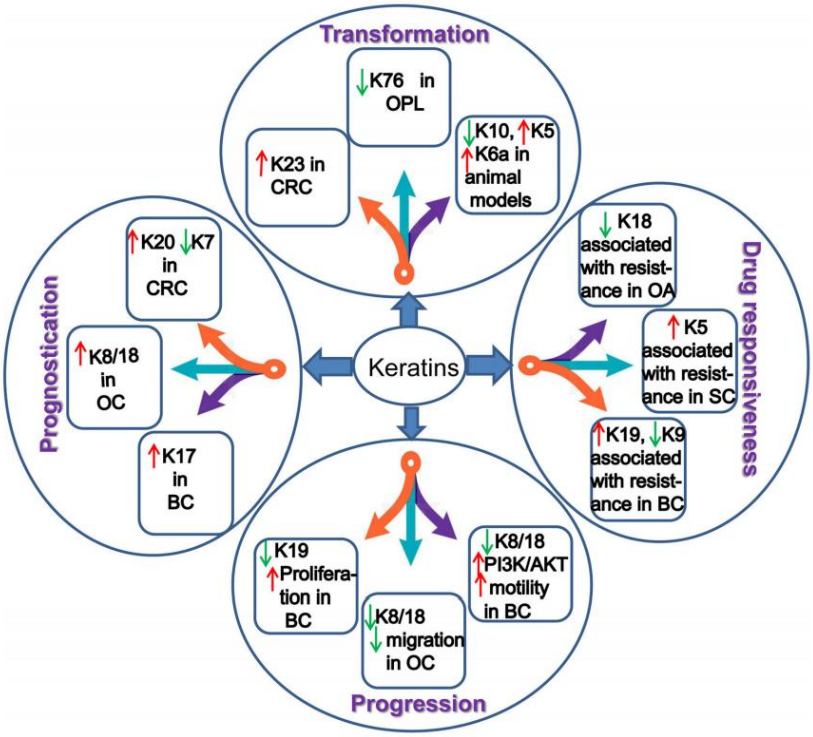

Figure 15: Schematic displaying the role of keratins in cancers with respect to prognostication, transformation, progression, and drug responsiveness. (Keys: OPL: oral premalignant lesions; CRC: colorectal cancers; BC, breast cancers; OA, ovarian adenocarcinomas; SC, serous ovarian cancers.)[46].

ABERRANT EXPRESSION OF CYTOKERATIN:

Toivola DM, Boor P and Alam C in 2015 represented this picture (figure 16) as Inherited keratin alterations cause or associate with human diseases.

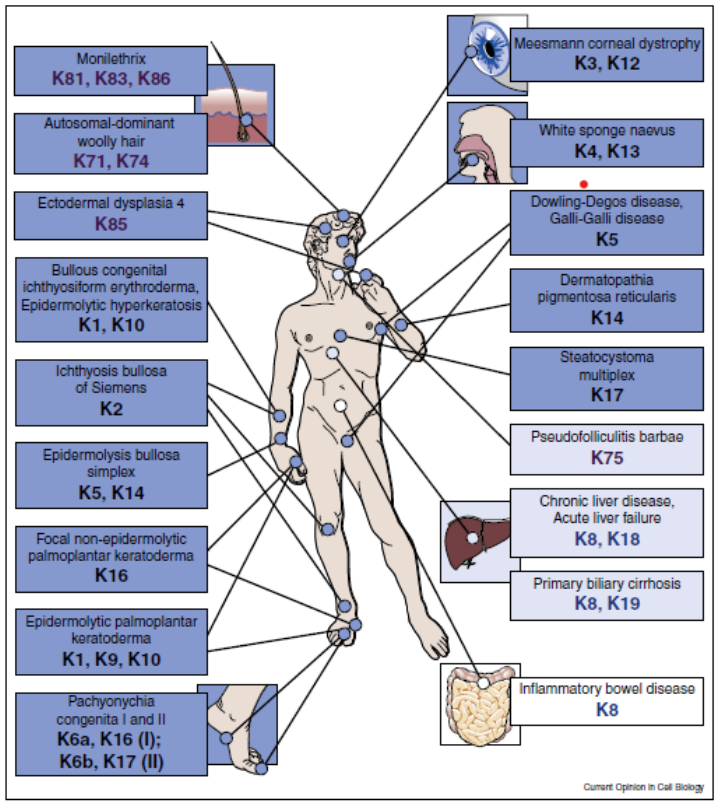

Figure 16: The schematic summarizes human diseases linked to inherited keratin changes together with the affected body areas. Diseases caused by a mutation are shown in dark blue, while disorders where keratin variants represent a risk factor are highlighted in light blue. Diseases where a link still remains to be convincingly demonstrated are shown in white. Simple keratins are shown in blue font, stratified keratins in black font, and hair keratins in purple font[52].

ABERRANT EXPRESSION OF CYTOKERATIN IN CANCER:

Given the characteristic cell type-, differentiation- and functional status-dependent keratin expression patterns in epithelial cells, the availability of specific keratin antibodies, and the fact that epithelial tumors largely maintain the features of specific keratin expression associated with the respective cell type of origin, keratins have long and extensively been used as immunohistochemical markers in diagnostic tumor pathology (figure 17)[53].

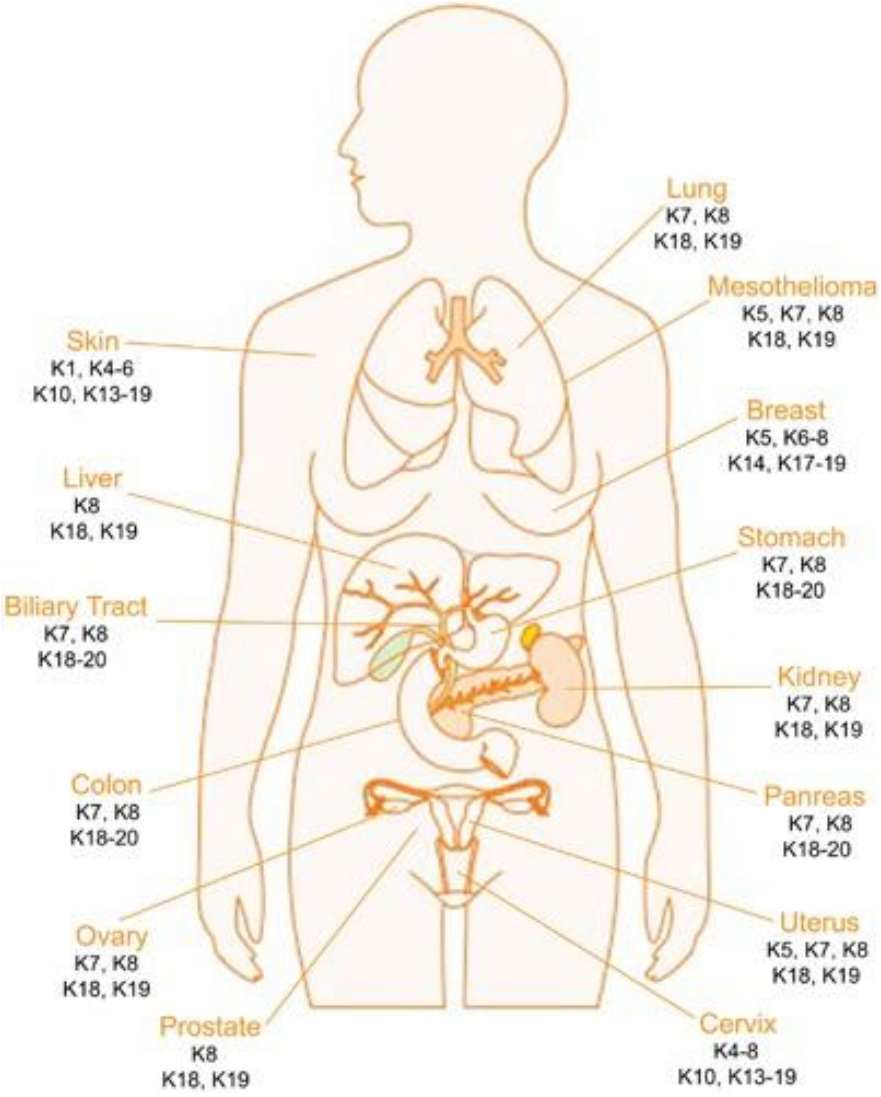

Figure 17: Keratin expression in human cancer: Keratins are normally expressed in a cell type-, differentiation- and functional status-dependent manner, and epithelial cancers largely maintain the characteristics of keratin expression associated with their respective

cell type of origin, so keratins have long been recognized as diagnostic markers in tumor pathology. Examples of keratins commonly used in the diagnosis of human epithelial malignancies are presented in this figure[53].

CYTOKERATIN 17:

Keratin, type I, Cytokeratin 17 is a protein that in humans is encoded by the *KRT17* gene. Keratin 17 is a type I cytokeratin. It is found in nail beds, hair follicles, sebaceous glands, and other epidermal appendages. Mutations in the gene encoding this protein lead to PC-K17 (previously known as Jackson-Lawler) type pachyonychia congenita and steatocystoma multiplex[54].

K17 has a MW of 48 kDa in both humans and mice and an isoelectric pH of 5.1. The amino acid sequences of both orthologous keratins are very similar: 88% in the head domain, 96% in the rod domain and 97% in the tail domain. This great similarity in all three domains is unusual among orthologue keratins and points to the possibility that K17 is subject to a strong stabilizing selection, at least partly because it is involved in wound-healing and in developmental processes (e.g. the formation of ectodermal placodes for hair, glands, thymus and teeth, as well as for the formation of modified skin of the palms and soles)[55].

Moll R et al in 1982 described that components 14-l7 are small and acidic keratins occurring, in different combinations, in epidermis and cultured keratinocytes, hair follicles and many noncornified stratified epithelia as well as in trachea and some glands. Of these, cytokeratins 14, 16 and 17 are closely related, as recognized by peptide maps. CK17 was initially described within the pilosebaceous unit and basal cell carcinomas, and was considered to be a purely follicular keratin[29].

Proby CM et al in 1993 examined keratin expression in benign warts from various cutaneous and mucosal sites along with dysplastic warts and squamous cell carcinomas using a panel of monospecific antibodies to epithelial keratins. He found that CK17 expression in skin SCC, and observed a CK17 diffuse and peripheral staining pattern in the basal and suprabasal cells of invasive SCC cases. Keratin 17 was found suprabasaly in hyperproliferative lesions, including benign warts, but marked basal and suprabasal expression was seen increasingly in malignantly transformed epidermis. These findings were not specific to immunosuppression, as shown by identical findings in control OSCC from nonimmunosuppressed individuals. Keratin 17 expression may prove prognostically helpful when assessing dysplasia in epidermal tumors[56].

Komine M et al in 1996 analyzed lesional samples of inflammatory diseases using immunofluorescence, transfected keratinocytes with K17 gene promoter DNAs in the presence of various cytokines, and followed nuclear translocation of STAT1 in keratinocytes using specific antibodies. He found K17 in small amounts in the cells of the simple epithelium of the seminal vesicular gland and epididymis, in basal cells of transitional and pseudostratified epithelia, in myoepithelial cells of secretory units of exocrine serous glands, and in injured human interfollicular epidermis. In the interfollicular epidermis, K17 is expressed in the suprabasal cells only when the epidermis is injured and, in this case, it is induced by interferon-γ that is secreted by invading T-lymphocytes. K17 promotes hair follicle growth by attenuating the pro-apoptotic signal tumor necrosis factor-α. K17 is expressed at the same time as K6, with which it forms heterodimers, although it can also combine with K5 and K8[57].

Yamamoto O et al in 1999 investigated immunostaining patterns of 10 different anticytokeratin (CK) antibodies and several other markers in these neoplasms, comparing them with the patterns in normal adult and fetal skin. He noted that CK17 is typically

expressed in the suprabasal cells of the outer root sheath (ORS) of the hair follicle, the sebaceous duct, the suprabasal cells of the sebaceous gland, the basal cells of sweat glands and a few epidermal basal cells at sites of entry of the acrosyringium. CK17 protein expression is induced in activated keratinocytes in the suprabasal layers of the epidermis, despite the fact that the normal epidermis does not positively stain for CK17. CK17 expression has been reported in cultured wild-type epidermis and under hyperproliferative conditions, including psoriasis, warts and wound healing, and is thought to reflect a hyperproliferative cell state[58].

McGowan KM et al in 1999 inactivated the *K17* locus in mouse via gene targeting and homologous recombination to describe the peculiar hair phenotype that arises in a genetic strain-dependent fashion in *K17* null mice, and implications for *K17* function and role in disease. He stated that onset of type I keratin 17 (K17) synthesis marks the adoption of an appendageal fate within embryonic ectoderm, and its expression persists in specific cell types within mature hair, glands, and nail. His findings reveals that K17 null mice develop severe alopecia during the first week postbirth, correlating with hair fragility, alterations in follicular histology, and apoptosis in matrix cells. These alterations are incompletely penetrant and normalize starting with the first postnatal cycle. Absence of a hair phenotype correlates with a genetic strain-dependent compensation by related keratins, including K16. So, CK17 is considered to be an early marker of keratinocyte activation following injury, and is expressed in migrating epithelial cells[59].

In addition, **Lu S et al in** 1999 conducted a study in 120 biopsies of benign (verruca vulgaris and keratoacanthoma), premalignant (actinic keratosis and extragenital Bowen's disease) and malignant (squamous cell carcinoma) skin lesions using immunohistochemical expression of cell-cycle proteins p53, p21 (WAF-1), PCNA and Ki-67. He observed CK17

staining in the basal cells of complex epithelial groups, including glandular epithelium containing a myoepithelial component, and transitional and pseudostratified epithelia[60].

Ikeda K et al in 2008 examined the immunohistochemical staining of p16, CK8, and CK17 in 134 cervical tissues obtained by punch biopsy. He found a direct correlation between the degree of immunohistochemical staining for CK17 and increasing grade of cervical intraepithelial neoplasia and SCC among 139 cervical lesions**.** Also, lack of diffuse expression of CK17 in the aerodigestive tract and cutaneous SCCs of the basaloid subtype has been shown. These disparate findings could reflect alternate tissue distribution patterns for the expression of basal keratins. He also observed that premalignant and malignant cells of the cervix exhibited CK17 expression, while wild-type ectocervical epithelial cells did not[61].

CYTOKERATIN 17 IN NORMAL EPITHELIUM:

Mikami et al in 2011 conducted a study on "oral borderline malignancies" including OED and OSCC to assess the differential expressions for CK 13 and CK 17. They also included normal oral mucosal biopsies in their study. In normal oral epithelium, none of the biopsies showed CK 17 positivity (0%) in contrast to definite (100%) CK 13 positivity. Same pattern was observed in case of mild to moderate dysplasia. In comparison to this, the cases of severe dysplasia and invasive OSCC, definite (100%) CK 17 positivity was observed[48].

Kitamura R et al in 2012 examined in 105 patients with OSCC and 108 patients with leukoplakia using immunohistochemical expression of CK13 and CK17. The over-expression levels of CK17 mRNA were analyzed by real-time RT-PCR in 5 OSCC cell lines (HSC-2, HSC-3, SAS, SQUU-A, SQUU-B). He proved that CK17 was not completely expressed in normal oral epithelium. CK17 was predominantly expressed in the cellular cytoplasm. In the cancer nest, CK17 was expressed in the inner layers and not expressed in

the outer layers. In well-differentiated OSCC, CK17 was strongly expressed in the majority of tumor cells. In moderately differentiated OSCC, CK17 was weakly expressed in the majority of tumor cells. In poorly differentiated OSCC, CK17 was absent in the majority of tumor cells but expressed in a few of tumor cells[62].

Kiani MN, Asif M and Ansari FM et al in 2020 retrieved 170 cases from record files of Histopathology Department, to conduct a cross sectional study at Armed Forces Institute of Pathology, Rawalpindi, over a period of one year from June 2018 to June 2019. In comparison, out of 170 cases, 133 (78.2%) cases showed positive expression of cytokeratin 17 whereas 37 (21.8%) cases showed negative expression of cytokeratin 17. He also stated that the expression of CK17 is reported in malignant tissues compared to normal tissues[63].

ABERRANT EXPRESSION OF CYTOKERATIN17 IN DISEASES:

Wetzels RH et al in 1992 conducted a study in 30 SCC, 14 adenocarcinoma, 6 small cell lung carcinoma and 6 carcinoid using immunohistochemistry to correlate between the presence of type VII collagen and the basal cell keratins 14 and 17, and a negative correlation between these components and keratin 18. They concluded that the carcinogenesis mechanism of SCC is very complex, and a wider range of CKs is expressed in SCC. CK17 may play an important role in the diagnosis of SCC, since several studies have reported that the over-expression of CK17 could be detected in malignant tissues compared to normal tissues in squamous cell carcinoma of lung[64]**.**

Takahashi H et al in 1995 conducted a study in twenty-two specimens of oesophageal carcinomas and adjacent histologically normal oesophagus obtained from 22 patients who underwent surgical treatment using immunohistochemistry to clarify the keratin staining patterns of invasive carcinoma of the oesophagus. They concluded that the characteristic

profile of squamous cell carcinoma was a strong and diffuse expression of keratin 14 and 16, strong but localized expression of keratin 17, and loss of keratin 13 expression. They also suggested that neoplastic epithelial cells showed different keratin reactivity and distribution compared to normal oesophageal epithelium. In addition, histologically normal epithelium, dysplasia and carcinoma-in-situ adjacent to or overlying carcinoma expressed keratin 14[65].

McGowan KM et al in 1999 cloned the mouse K17 gene and investigated its expression during skin development. They demonstrated that ectopic lef-1 expression induces K17 protein in the skin of adult transgenic mice. The pattern of K17 gene expression during development has direct implications for the morphogenesis of skin epithelia, and points to the existence of a molecular relationship between development and wound repair[66].

Van De Rijn M et al in 2002 conducted a study to evaluate the expression of cytokeratins 17 and 5 which identifies a group of breast carcinomas with poor clinical outcome using the recently developed technique of tissue microarrays (TMA) in a retrospective immunohistochemistry evaluation of 611 breast tumor samples. They found that expression of cytokeratin 17 and/or cytokeratin 5/6 in tumor cells was associated with a poor clinical outcome. Moreover, multivariate analysis showed that in node-negative breast carcinoma, expression of these cytokeratins was a prognostic factor independent of tumor size and tumor grade[67].

McGowan KM et al in 2002 conducted a study to address the issue of *K17* function in vivo, they inactivated the *K17* locus in mouse via gene targeting and homologous recombination. They concluded that K17 null mice develop severe alopecia during the first week postbirth, correlating with hair fragility, alterations in follicular histology, and apoptosis in matrix cells. These alterations are incompletely penetrant and normalize starting

with the first postnatal cycle. Absence of a hair phenotype correlates with a genetic strain-dependent compensation by related keratins, including K16. These findings revealed a crucial role for K17 in the structural integrity of the first hair produced and the survival of hair-producing cells. Given that identical inherited mutations in this gene can cause either pachyonychia congenita or steatocystoma multiplex, the features of this mouse model suggest that this clinical heterogeneity arises from a cell type-specific, genetically determined compensation by related keratins[59].

Luo A et al in 2004 conducted a study to identify genes that are differentially expressed in human esophageal squamous cell carcinoma (ESCC)**.** They developed a cDNA microarray representing 34 176 clones to analyse gene expression profiles in ESCC A total of 77 genes (including 31 novel genes) were downregulated, and 15 genes (including one novel gene) were upregulated in cancer tissues compared with their normal counterparts. Immunohistochemistry and Northern blot analysis were carried out to verify the cDNA microarray results. They concluded that genes involved in squamous cell differentiation were coordinately downregulated, including annexin I, small proline-rich proteins (SPRRs), calcium-binding S100 proteins (S100A8, S100A9), transglutaminase (TGM3), cytokeratins (KRT4, KRT13), gut-enriched Krupple-like factor (GKLF) and cystatin A. Interestingly, most of the downregulated genes encoded Ca(2+)-binding or modulating proteins that constitute the cell envelope (CE). Moreover, genes associated with invasion or proliferation were upregulated, including genes such as fibronectin, secreted protein acidic and rich in cystein (SPARC), cathepsin B and KRT17. Functional analysis of the alteration in the expression of GKLF suggested that GKLF might be able to regulate the expression of SPRR1A, SPRR2A and KRT4 in ESCC[68].

Carrilho C et al in 2004 conducted a study to clarify the usefulness of studying the expression of keratins 8, 10, 13, and 17 for diagnostic purposes in human cervix carcinomas.

Forty-four invasive squamous carcinomas, 10 cervical intraepithelial neoplasia grade III (CIN III), and 10 reference cervix were examined immunohistochemically with monoclonal antibodies. They concluded that expression of keratins 8 and 17 and loss of keratins 10 and 13 are good markers of malignant transformation in human cervix. Keratin expression patterns, namely expression of keratin 10, can be useful for subtyping and grading squamous cell carcinomas of the cervix[69].

Martens JE et al in 2004 identified the stem cell population of the cervical epithelium by monoclonal antibodies against p63, a homologue of the tumor suppressor gene p53 and cytokeratin 17 (CK17). CK17 was expressed in mild, moderate, severe dysplasia of uterine cervix**.** Thus it is a marker of the premalignant lesion and it is important to predict the malignant transformation of cervical epithelium. Nevertheless, CK17 is expressed in a portion of dysplastic leukoplakias and thus not enough to diagnose individually premalignant lesion. They concluded that p63 expression consistently in the nuclei of reserve cells, hyperplasia of the cells and the basal layer of the ectocervical epithelium, while CK17 only stained endocervical reserve cells and reserve cell hyperplasia. So, both p63 and CK 17 are suitable markers for cervical stem cell identification. Both markers, therefore, qualify for the identification of the HPV target cell. Therefore, combination of CK17 and CK13 might be a useful marker to diagnose premalignant lesion with high potential of transformation. In addition, over-expression of CK17 and absence of CK13 might be associated with malignant transformation[70].

Cohen-Kerem R et al in 2004 conducted a study to assess cytokeratin-17 (CK17) as an immunohistochemical marker for squamous cell carcinoma of the larynx, in their study they stained 63 tissue samples from 63 consecutive patients who were believed or suspected to have squamous cell carcinoma of the larynx for CK17 and analyzed them by computerized histomorphometry. They concluded that CK 17 is a highly sensitive and specific

immunohistochemical marker for premalignant and malignant transformation in the larynx. The levels of CK17 in polyps, dysplasia, and normal epithelium proximal to a tumor were similar; however, their biological behaviors vary greatly[71].

Ikeda K et al in 2008 conducted a study to determine immunohistochemical expression profiles for CK8 and CK17 in 134 uterine cervical specimens obtained by punch biopsy. They concluded that CK8 and CK17, markers of cervical reserve cells, are valuable markers for the diagnosis of CIN, and CK8 and CK17 immunostaining is correlated with increasing lesion grade of CIN. They also showed that CK8 and CK17 staining profiles in p16-positive cases of CIN were similar to those in all cases, indicating that CK8 and CK17 expression is independent of p16 expression[61].

ABERRANT EXPRESSION OF CYTOKERATIN 17 IN OSCC:

Kawahara E et al in 1995 studied invasion-related adhesion events in vitro using three squamous carcinoma cell lines (HSC-3), poorly differentiated type; OSC-19, well-differentiated type; and KB cells, undifferentiated type). They proved that combined with the ability of muscle invasion and cervical lymph node metastasis in vivo, SQUU-B resembles to HSC-3 closely, because HSC-3 also has the ability. Therefore, SQUU-B might have feature of poorly differentiated OSCC compared to SQUU-A[72].

Morifuji M et al in 2000 conducted a study in two human tongue squamous cell carcinoma cell lines, SQUU-A and SQUU-B, which were established from the same patient. They proved that CK17 mRNA quantity was significantly higher in SQUU-A than in SQUU-B, though both are derived from well-differentiated OSCC. The in vitro formation of intermediate filaments is prominently observed in SQUU-A compared to SQUU-B. Furthermore, piling up of SQUU-A cells was partially seen, whereas SQUU-B cells developed multiple layers throughout[73].

Ohkura S et al in 2005 conducted a study 21 OSCC and 11 leukoplakia tissues (one hyperplasia and one mild, five moderate and four severe dysplasias) To identify differentially expressed genes during the development of oral malignancy, differential display, northern blotting, reverse transcription–polymerase chain reaction (RT–PCR) and immunohistochemical analyses were undertaken. He concluded that significantly higher levels of keratin (Ker)-14 and -17 mRNAs, combined with lower levels of Ker-4, Ker-13 and transglutaminase 3 (TG-3) transcripts, were observed in OSCC and severely dysplastic tissues, whereas this expression profile was reversed in hyperplasia and in mild to moderate dysplasia. The expression of Ker-4 and Ker-13 was elevated in density-arrested OSCC cell lines (Ca9-22, HSC-2, -3 and -4) but the expression of Ker-17 mRNA was elevated in these cells, regardless of the growth. So, quantity of CK17 mRNA in HSC-2 derived from well-differentiated OSCC is significantly higher than that in HSC-3 and SAS derived from poorly differentiated OSCC. This conflicts with the detection of CK17 mRNA in OSCC cell lines by real-time RT-PCR despite their differentiation[74].

Toyoshimha T et al in 2008 conducted a study in 10 OSCC and 5 normal mucosal samples, the expression patterns of 31 CK genes were examined by cDNA microarray in order to identify CKs with most pronounced over-expression. The results were verified for CK 17, CK 19, and CK 20 in addition to 46 OSCC samples by relative quantification (RQ) using SYBR green real-time reverse transcriptase polymerase chain reaction (RT qPCR). A correlation of the CK expressions with the tumor classification was carried out. They proved that CK17 might be the suitable marker of OSCC out of CK family[75].

Ye H et al in 2008 conducted a study in 53 primary OSCCs and 22 matching normal tissues based on genome-wide transcriptomic profiles. Furthermore, up-regulation of CK17 mRNA is observed by microarray analyses in OSCC compared to normal oral epithelium**.** In the view of these reports, CK17 is markedly expressed in malignant tissue and thus could

be a diagnostic marker of OSCC. The results of CK17 indicate the significant expression in well-differentiated OSCC and the decreasing expression in moderately and poorly differentiated OSCC[76].

Toyoshimha T et al in 2009 conducted a study in fifty-two pairs of OSCC cells and normal oral mucosal cells were obtained by brush biopsy from OSCC patients to determine the detection of cytokeratin (CK) mRNA in oral squamous cell carcinoma (OSCC) cells and to evaluate the CK relevance for OSCC diagnosis in a brush biopsy test. mRNA was extracted from cell pellets for real-time quantitative reverse transcriptase polymerase chain reaction (RT-qPCR). The over-expression levels of CK 17, CK 19 and CK 20 mRNA in OSCC cells were examined by SYBR green real-time RT-qPCR. He proved that brush biopsy properly serves for detection of CK mRNA using real-time RT-qPCR. He also proved that elevated expression of CK17 in patients with well-differentiated OSCC[77].

Wei KJ et al in 2009 conducted a study to determine the cytokeratin 17 (CK17) expression in oral squamous cell carcinoma (OSCC) both in vitro and in vivo. They analyzed in vitro cellular carcinogenesis model of OSCC (including a line of human immortalized oral epithelia cells (HIOECs), a line of cancerous HB96 cells and another kind of cells (HB56 cells) at the early stage of carcinogenesis was performed to identify differentially expressed proteins. CK17 was further validated in vitro (cellular carcinogenesis model and other three OSCC lines) and in vivo (tissues from six healthy persons and 30 primary OSCC patients) by Western blotting and immunohistochemistry respectively. They concluded that increased CK17 expression may play an important role in carcinogenesis of OSCC. But the number of cases was too small for a statistical evaluation, since only 30 OSCCs were examined in the study. Therefore, CK17 could be a precise candidate for a diagnostic marker of well-differentiated OSCC[78].

Kitamura R et al in 2012 conducted a study in 105 patients with OSCC and 108 patients with leukoplakia to confirm the expression profile of cytokeratin (CK)17 in comparison with that of CK13 in oral squamous cell carcinoma (OSCC) and leukoplakia and to clarify an association of CK17 with the OSCC differentiation. The over-expression levels of CK17 mRNA were analyzed by real-time RT-PCR in 5 OSCC cell lines (HSC-2, HSC-3, SAS, SQUU-A, SQUU-B). They concluded that CK17 expression could be associated with the differentiation and the malignancy of OSCC. A combination pattern of CK17/CK13 might be a suitable marker of malignant transformation[68].

Mashhadiabba F et al in 2017 conducted a study on three OSCC cell lines with a minimum of 45 passages. Cells isolated from three patients with OSCC were cultured and passaged. Expression of CK8 and CK17 epithelial markers was assessed using reverse-transcription polymerase chain reaction. He concluded that CK8 and CK17 expressed in superficial and basal layers using RTPCR and also confirmed that these markers can be used as a epithelial specific markers with more specificity in malignancies[79].

Regenbogen E et al in 2018 conducted a study using immunohistochemical staining for K17 of oral, oropharyngeal, and laryngeal SCCs, and normal oropharyngeal mucosa. The HPV status was determined using polymerase chain reaction (PCR). They concluded that elevated expression of keratin 17 in oropharyngeal squamous cell carcinoma significantly associated with overall decreased patient survival by using immunohistochemical staining technique[80].

Sanguansin S in 2021 conducted a study using immunohistochemical staining of CK17 was overexpressed in OL with dysplasia and OSCC. A gradual increase of CK17 expression from normal oral mucosa to OL without dysplasia, OL with dysplasia, and OSCC was also demonstrated. These results suggest that CK17 plays a pivotal function in the early and late

stages of oral carcinogenesis and is involved in the development of premalignant lesions and malignant transformation[81].

ABERRANT EXPRESSION OF CYTOKERATIN 17 IN PREMALIGNANCY:

Ranganathan K, Kavitha R, Sawant SS and Vaidya MM in 2006 conducted a study in 50 OSF cases, 10 normal and 10 OC cases were stained with pancytokeratin (PanCK), high molecular weight cytokeratin (HMWCK), CKs 18, 14, 8, 5, 4 and 1 by immunohistochemistry. Study showed a remarkable difference in the CK staining was seen in between normal mucosal tissue, OSF, and OSCC. The OSF showed an increased intensity of staining for panCK and high molecular weight CK, aberrant expression of CK8 and low expression of CKs 5 and 14. It has been shown that changes in the underlying connective tissue are reflected in the adjacent epithelium and also result in alterations in CK expression. CK expression has been shown to be altered under various pathological conditions like psoriasis, gingivitis, and hyperkeratosis[82].

Lalli A, Tilakaratne WM and Ariyawardana A et al in 2008 defined the keratin expression profile, by immunohistochemistry and quantitative image analysis, using a panel of 22 anti-keratin monoclonal antibodies on 28 OSF samples. He observed an increase of K1 and K10 in the suprabasal layers, induction of K6 in the basal layer and complete loss of K19 in the epithelium. Furthermore, there was increased K17 expression in the suprabasal layers, which correlated with disease severity. In a subset of the most severe OSF cases (14%), K17 expression was completely lost in the basal layer which might define them to be at most risk to undergo malignant transformation. There was no detectable expression of K8, K18, K7 and K9 and the expression of K4, K13, K14, K15 and K16 did not change in OSF[83].

Mikami T et al in 2011 conducted a study to evaluate differential expressions for keratin (K) subtypes 13 and 17 in oral borderline malignancies in 67 surgical specimens of

the oral mucosa for their immunohistochemical profiles. From those specimens, 173 foci of epithelial dysplasia, 152 foci of carcinoma in situ (CIS), and 82 foci of squamous cell carcinoma (SCC) were selected according to our diagnostic criteria, along with 20 areas of normal epithelia. The results indicate the strong expression of CK17 in mildly, moderately, and severely dysplastic leukoplakia. This conflicts with the absence of CK17 in mildly and moderately dysplastic leukoplakia, but no severely dysplastic leukoplakia was examined in the study[46].

RESULTS

SAMPLE CHARACTERISTICS:

The study population comprised of 55 cases taken from the archival blocks. They were categorized into four groups. Group I (n=10) comprised of Normal Mucosa samples, Group II (n=15) comprised of Well Differentiated Oral Squamous Cell Carcinoma (WDOSCC), Group III (n=15) comprised of Moderately Differentiated Oral Squamous Cell Carcinoma (MDOSCC) and Group IV (n=15) comprised of Poorly Differentiated Oral Squamous Cell Carcinoma (PDOSCC). All the samples were analyzed for immunohistochemical expression of CK17.

DISTRIBUTION OF AGE AMONG THE STUDY GROUPS (TABLE 1 & GRAPH 1):

The age of patients were divided into 4 groups: <25 years, 26 - 50 years, 51 – 75 years and above 75 years. Group I consisted of 4 (40%) cases in <25 years, 5(50%) cases in 26 - 50 years, 1(10%) case in 51 – 75 years. Group II consisted of 5(33.3%) cases in 26 - 50 years, 8 (53.3%) cases in 51 – 75 years and 2 (13.3%) cases above 75 years. Group III consisted of 8(53.3%) cases in 26 – 50 years and 7(46.7%) cases above 51 - 75 years. A significant difference was found with respect to age in the study groups (**p=0.001***)

DISTRIBUTION OF GENDER AMONG THE STUDY GROUPS (TABLE 2 & GRAPH 2):

In group I, 5(50%) cases were males and 5(50%) cases were females. In group II, 10(66.7%) cases were males and 5(33.3%) cases were females. In group III, 13(86.7%) cases were males and 2(13.3%) cases were females. In group IV, 8(53.3%) cases were males and 7(46.7%) cases were females. However, no significant difference was found with respect to gender in the study groups (p=0.17*)

DISTRIBUTION OF HABITS AMONG THE STUDY GROUPS (TABLE 3 & GRAPH 3):

Based on the prevalence of habits in the study groups, they were categorized in to six groups. They were those with

1. No habits
2. Habit

In group I (normal mucosa), 10 (100%) cases had no habit history. In group II, 15 (100%) cases had habit history. In group III, only 1(6.3%) case had no habit history which was a non-habit associated moderately differentiated Oral Squamous Cell Carcinoma, whereas, 14(93.3%) cases had the habit history. In group IV, only 1(6.3%) case had no habit history which was a non-habit associated poorly differentiated Oral Squamous Cell Carcinoma, whereas, 14(93.3%) cases had the habit history. A significant difference was found with respect to habits in the study groups (**p=0.000***)

DISTRIBUTION OF SITE OF BIOPSY AMONG THE STUDY GROUPS (TABLE 4 & GRAPH 4):

In group I of 10(100%) cases, the site of biopsy of 1(10%) case was retromolar area, 3(30%) cases were gingiva, 4(40%) cases were buccal mucosa and 2(20%) cases were alveolar mucosa. In group II, 1(6.7%) case was retromolar area, 4(26.7%) cases were gingiva, 5(33.3%) cases were from buccal mucosa, 3(20%) cases were from tongue, 1(6.7%) case was from floor of the mouth as from alveolar mucosa. In group III, 2(13.3%) cases were from gingiva, 8(53.3%) cases were from the buccal mucosa, 2(13.3%) cases were from tongue and 3(20.3%) cases were from palate. In group IV, 5(33.3%) cases were from gingiva, 1(6.7%) case was from floor of the mouth, 3(20%) cases were from tongue, 5(33.3%) cases were from buccal mucosa,

and 1(6.7%) case was from left mandibular angle. A statistically significant difference was found with respect to site of biopsy among the study groups (p=0.000). However, no significant difference was found with respect to site of biopsy in the study groups (p=0.253*).

COMPARISON OF LOCALIZATION OF CYTOPLASMIC EXPRESSION OF CK17 BETWEEN THE STUDY GROUPS (TABLE 5 & GRAPH 5):

On comparing the distribution of expression between the study groups, in group I, 3(30%) cases showed focal expression and 7(70%) cases showed diffuse expression. In group II, 6(40%) cases showed focal expression and 9(60%) cases showed diffuse expression. In group III, 4(26.7%) cases showed focal expression and 11(73.3%) cases showed diffuse expression. In group IV, 1(6.7%) case showed no expression, 3(20%) cases showed focal expression, 11(73.3%) cases showed diffuse expression. However, no significant difference was found with respect to CK17 expression between the study groups (p=0.677*).

COMPARISON OF DISTRIBUTION OF CYTOPLASMIC EXPRESSION OF CK17 BETWEEN THE STUDY GROUPS (TABLE 6 &GRAPH 6)

All the cases enrolled in the study showed CK17 staining except 1 case which was poorly differentiated Oral Squamous Cell Carcinoma. In group I, group II and group III and group IV, CK17 staining was present in all of the 10(100%) cases, 15(100%) cases, 15(100%) cases and 14(93.3%) cases respectively. The following parameters were used to evaluate CK17 in the study groups:

- Distribution of expression and
- Staining intensity

On comparing the distribution of expression between the study groups, in group I, 6(60%) cases showed positive expression in basal layer, 10(100%) cases

showed positive expression in suprabasal layer and 9(90%) cases showed positive expression in keratin layer. In group II, 5(33.3%) cases showed positive expression in basal layer, 15(100%) cases showed positive expression in suprabasal layer and 15(100%) cases showed positive expression in keratin layer. In group III, 2(13.3%) cases showed positive expression in basal layer, 15(100%) cases showed positive expression in suprabasal layer and 15(100%) cases showed positive expression in keratin layer. In group IV, 1(6.7%) case showed no expression, 7(46.7%) cases showed positive expression in basal layer, 14(93.3%) cases showed positive expression in suprabasal layer and 14(93.3%) cases showed positive expression in keratin layer. However, no significant difference was found with respect to CK17 expression between the study groups (p=0.351*).

COMPARISON OF DISTRIBUTION OF STAINING INTENSITY OF CYTOPLASMIC CK17 BETWEEN THE STUDY GROUPS IN BASAL LAYER (TABLE 7 & GRAPH 7):

On comparing the staining intensity between the study groups in basal layer, in group I, 4(40%) cases showed no expression, 2(20%) cases showed mild expression, 2(20%) cases showed moderate expression and 2(20%) cases showed intense expression. In group II, 10(66.7%) cases showed no expression, 4(26.7%) cases showed mild expression and 1(6.7%) case showed moderate expression. In group III, 13(86.7%) cases showed no expression, 2(13.3%) cases showed mild expression. In group IV, 8(53.3%) cases showed no expression, 4(26.7%) cases showed mild expression, 3(20%) cases showed moderate expression. However, no significant difference was found with respect to CK17 expression between the study groups (p=0.067*).

COMPARISON OF DISTRIBUTION OF STAINING INTENSITY OF CYTOPLASMIC CK17 BETWEEN THE STUDY GROUPS IN SUPRABASAL LAYER (TABLE 8 & GRAPH 8):

On comparing the staining intensity between the study groups in suprabasal layer, in group I, 3(30%) cases showed mild expression, 2(20%) cases showed moderate expression and 5(50%) cases showed intense expression. In group II, 3(20 %) cases showed mild expression, 5(33.3%) cases showed moderate expression and 7(46.7%) cases showed intense expression. In group III, 5(33.3%) cases showed mild expression, 6 (40%) cases showed moderate expression and 4(26.7%) cases showed intense expression. In group IV, 1(6.7%) case showed no expression, 5(33.3%) cases showed mild expression, 6(40%) cases showed moderate expression and 3(20%) cases showed intense expression. However, no significant difference was found with respect to CK17 expression in suprabasal layer between the study groups (p=0.671*).

COMPARISON OF LOCALIZATION IN DIFFUSE OF EXPRESSION OF CK17 BETWEEN FOUR GROUPS (N=38) (TABLE IX and GRAPH IX):

When a combined analysis of CK17 expression was studied in both the basal and suprabasal layer, in group I, 6 (85.7%) cases, in group II 4(26.7%) cases showed mild expression, 6 (40%) cases showed moderate expression and 5(33.3%) cases showed intense expression. In group IV, 10(66.7%) cases showed no expression, 3(20%) cases showed mild expression, 2(13.3%) cases showed moderate expression and 3(20%) cases showed intense expression. There were significant difference found with respect to CK17 expression in suprabasal layer between the study groups (p=0.00).

INTENSITY OF CYTOPLASMIC EXPRESSION OF CK17 IN THE TUMOR ISLANDS BETWEEN GRADES OF CARCINOMA (TABLE X and GRAPH X):

In group II, 3(20 %) cases showed mild expression, 1(6.7%) case showed moderate expression and 11(73.3%) cases showed intense expression. In group III, 4(26.7%) cases showed mild expression, 6 (40%) cases showed moderate expression and 5(33.3%) cases showed intense expression. In group IV, 10(66.7%) cases showed no expression, 3(20%) cases showed mild expression, 2(13.3%) cases showed moderate expression and 3(20%) cases showed intense expression. There were significant difference found with respect to CK17 expression in suprabasal layer between the study groups (p=0.00).

TABLES AND GRAPHS

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