PREVALENCE OF ORAL CANCER AND ASSOCIATION OF RISK FACTORS WITH TREATMENT OUTCOME STATUS OF ORAL SQUAMOUS CELL CARCINOMA IN KELANTAN: A RETROSPECTIVE STUDY

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by

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AAO-HNS	Academy of Otolaryngology – Head and Neck Surgery
ALDH2	Aldehyde Dehydrogenase 2
ALT	Alternative Lengthening of Telomerase
AOR	Adjusted Odds Ratio
APC	Annual Percentage Change
ASR	Age-Standardized Risk
ASIR	Age-Standardization Incidence Rate
BMI	Body Mass Index
CDK	Cyclin-Dependent Kinase
CHEMO-RT	Chemo-Radiotherapy
CI	Confidence Interval
CR	Crude Incidence Rate
CT	Chemotherapy
CTLA-4	Cytotoxic T Lymphocyte Associated Antigen 4
EC	Endothelial Cell
ECM	Extracellular Matrix
EGFR	Endothelial Growth Factor Receptor
FDA	Food and Drug Administration
GLOBOCON	Global Burden of Cancer Study
HPV	Human Papilloma Virus
HUSM	Hospital Universiti Sains Malaysia
IARC	International Agency for Research on Cancer
ICD	International Classification of Disease for Oncology
INHANCE	International Head and Neck Cancer Epidemiology
LAOC	Locally Advanced Oral Cancer
LOH	Loss of Heterozygosity

M1	Mortality Stage 1
MACH-NC	Meta-Analysis of Chemotherapy in Head and Neck Cancer
MAP	Mitogen-Activated Protein
MDT	Multidisciplinary Team
MMP	Matrix Metalloproteinase
MSI	Microsatellite Instability
MTOR	Mammalian Target of Rapamycin
NACHRS	Nicotinic Acetyl-Choline Receptors
NCCN	National Comprehensive Cancer Network
NHMS	National Health and Morbidity Survey
NICE	National Institute for Health and Care Excellence
NNN	N-Nitrosonornicotine
OR	Odds Ratio
OSCC	Oral Squamous Cell Carcinoma
OSR	Overall Survival Rate
PDGF	Platelet-Derived Growth Factor
PTEN	Phosphatase and Tensin Homologue
RB	Retinoblastoma
RIOG	Radiation Therapy Oncology Group
ROC	Receiver Operation Characteristics
RT	Radiotherapy
SCCHN	Squamous Cell Carcinoma of Head and Neck
SEER	Surveillance, Epidemiology and End Results
SLT	Smokeless Tobacco
STAT-3	Signal Transducer and Activator of Transcription 3
TERT	Telomerase Reverse Transcriptase
TGF	Transforming Growth Factor
TKI	Tyrosine Kinase Inhibitor

TSG	Tumor Suppressor Gene
UICC	Union for the International Cancer Control
UK	United Kingdom
UPA	Urokinase Plasminogen Activator
US	Unites States
USM	Universiti Sains Malaysia
VEGF	Vascular Endothelial Growth Factor
VIF	Variance Inflation Factor
WHO-IARC	World Health Organization – International Agency for Research on Cancer
WHO	World Health Organization

KELAZIMAN KANSER MULUT DAN PENGLIBATAN FAKTOR-FAKTOR RISIKO DAN STATUS HASIL RAWATAN KARSINOMA SEL SQUAMOUS ORAL DI KELANTAN : KAJIAN RETROSPEKTIF

ABSTRAK

Kanser adalah pertumbuhan sel yang tidak terkawal yang disebabkan ketidakseimbangan antara apoptosis dan pembahagian sel. Kanser oral adalah salah satu daripada kanser yang paling lazim di seluruh dunia dan menurut Pertubuhan Kesihatan Sedunia, pada tahun 2018, 199,560 daripada 665,093 pesakit kanser oral yang baru didiagnosis telah meninggal dunia. Kanser oral berpunca daripada mutasi DNA yang boleh menjejaskan pelbagai gen dan mempunyai etiologi pelbagai faktor, iatu merokok tembakau yang paling ketara, pengambilan alkohol, mengunyah sirih, kecenderungan genetik dan jangkitan HPV. Karsinoma sel squamous oral mewakili 90% daripada jumlah kanser mulut. Lidah dianggap sebagai lokasi intra-oral yang paling lazim terlibat dengan kanser mulut. Lelaki dan individu yang berumur (> 55 tahun) lebih cenderung mengalami kanser mulut. Di Malaysia, 327 daripada 667 pesakit kanser mulut yang baru didiagnosis meninggal dunia pada tahun 2018. Radiasi dan pembedahan adalah satu-satunya cara rawatan yang berkesan untuk karsinoma mulut. Kemoterapi sendiri bukan terapi pemulihan; Walau bagaimanapun, ia boleh meningkatkan hasil jika digunakan dalam kombinasi radiasi untuk kanser yang sudah merebak di lokasi berhampiran. Dalam kajian retrospektif ini (2000 - 2018), data mengenai faktor sosio-demografi, klinik-patologi dan hasil rawatan yang berkaitan dengan kanser mulut telah dikumpulkan dari arkib rekod perubatan Hospital Universiti Sains Malaysia. Daripada 301 pesakit kanser mulut,

majoriti pesakit mempunyai karsinoma sel squamous oral (n = 211). Umur nilai purata adalah 55 tahun. Pesakit lelaki (n = 189, 62.79%) adalah lebih daripada pesakit wanita (n = 112, 37.21%). Majoriti daripada mereka adalah bukan perokok (n = 173, 57.47%), bukan peminum alkohol (n = 251, 83.38%) dan bukan pengunyah sirih (n = 291, 96.67%) Melayu (n = 207, 68.77%). Lokasi yang paling kerap terlibat dengan kanser mulut ialah lidah (n = 107). 86.71% pesakit menerima rawatan dan 208 daripada 301 pesakit terselamat, manakala 93 pesakit kanser mulut meninggal dunia. Penggunaan alkohol, pembentangan / diagnosis lewat, pengkelasan histologi yang tidak dibezakan dan kes yang tidak dirawat dikaitkan dengan peningkatan risiko kematian. Faktor lain termasuk umur, etnik, betel quid mengunyah, sejarah keluarga tumor lepas, faktor predisposisi HPV dan tapak tumor mempunyai persamaan yang tidak signifikan dengan kadar kematian OSCC. Kesimpulannya, faktor risiko berpotensi yang berkaitan dengan kanser mulut wujud di kalangan penduduk Kelantan.

PREVALENCE OF ORAL CANCER AND ASSOCIATION OF RISK FACTORS WITH TREATMENT OUTCOME STATUS OF ORAL SQUAMOUS CELL CARCINOMA IN KELANTAN: A RETROSPECTIVE

ABSTRACT

Cancer is an unchecked growth of cells due to an imbalance between apoptosis and cell division. Oral cancer is one of the most prevalent cancers worldwide and according to the World Health Organization in 2018, 199,560 out of 665,093 newly diagnosed oral cancer patients died. Oral cancer results from mutations in the DNA that can affect different genes and has multifactorial etiology, most significantly tobacco smoking, alcohol intake, betel quid chewing, genetic predisposition and HPV infection. Oral squamous cell carcinoma (OSCC) represents 90% of the total oral cancers. Tongue is considered as the most commonly involved intra-oral site for oral cancers. Males and old individuals (> 55 years) are more likely to be encountered with oral cancers. In Malaysia, 327 out of 667 newly diagnosed oral cancer patients died in 2018. Radiation and surgery are the only reliable methods of treatment for early and locally advanced carcinoma of the mouth. Chemotherapy alone is not a remedial therapy; however, it may improve results if used in combination with radiation for locally advanced diseases. In this retrospective study (2000 – 2018), data regarding socio-demographic, clinico-pathological factors and treatment outcome associated with oral cancer was gathered from the archives of medical records of Hospital Universiti Sains Malaysia. Out of 301 oral cancer patients, the majority of the patients had OSCC (n=211). The mean age was 55 years. Male patients (n=189, 62.79%) were

more than female patients (n=112, 37.21%). Majority of them were non-smoker (n=173, 57.47%), non-alcohol consumer (n=251, 83.38%) and non-betel quid chewer (n=291, 96.67%) Malay (n=207, 68.77%). The most commonly involved site by oral cancer was tongue (n=107). 86.71% patients received treatment and 208 out of 301 patients survived, whereas 93 oral cancer patients died. Alcohol consumption, late presentation/diagnosis, poorly differentiated histological grading and untreated cases were associated with an increased risk of mortality. Other factors including age, ethnicity, betel quid chewing, past family history of tumor, HPV predisposing factors and tumor site had a non-significant association with the mortality rate of OSCC. In conclusion, potential risk factors associated with oral cancer do exist in Kelantanese population.

CHAPTER 1.

INTRODUCTION

1.1 Background of the study

Quoted from the inaugural speech of Prof. Wilfried Schilli from the University of Freiburg, Germany, in 1989 (Das and Nagpal, 2002):

'According to the World Health Organization (WHO) agreement of 1973, oral cancer is a malignant neoplasm in the 8 anatomic regions of the oral cavity. Thus, oral cancer refers to cancerous tumours of the upper respiratory and alimentary tracts and in the near vicinity, whose draining lymphatic vessels are all located in the neck. Since the treatment of malignant disease which has spread to the lymphatic system entails treating the lymphatics and the primary tumour as one entity, the therapeutic plan must include the neck region. Thus, the definition of oral cancer also applies to so called neck and head tumors'.

Oral cancer is one of the most prevalent cancers worldwide and it attributed as the leading cause of death in certain geographical regions such as South-Central Asia (Gupta et al., 2016). Oral squamous cell carcinoma (OSCC) represents 90% of the total oral cancers (Massano et al., 2006). Tongue is considered as the most commonly involved intra-oral site for oral cancers (Moore et al., 2000b). Posterior-lateral border and ventral surfaces of the tongue are the most frequently involved sites in tongue cancers followed by floor of the mouth. Relatively less common intra-oral sites are gingiva, hard palate, buccal and labial mucosa (Zini et al., 2010).

Sometimes, they are preceded by precancerous lesions including leukoplakia and erythroplakia (Neville and Day, 2002). Males and older individuals (> 55 years) are more likely to be encountered with oral cancers (Kanasi et al., 2016).

Globally, approximately 300,000 new cases of oral cancers are diagnosed annually. Developing countries account for around 75% of all oral cancers (De Souza et al., 2016). There is a significant local variation regarding the incidence of oral cancers. In south east Asia, oral cancers account for almost half of all malignancies, having betel quid chewing and tobacco use as the major contributing factors. A hike in the prevalence of oral cancers among young adults has been a cause of special concern. Over the past 30 years, the incidence of tongue cancers has increased up to 60% in adults who are aged under 40 years (Boffetta et al., 2008). Variations have been recognized at molecular level and the clinic-pathological behavior in alcohol-associated and tobacco-smoking oral cancers in Japan, United States (US), France, United Kingdom as well as tobacco-chewing oral carcinomas in south east Asia (Paterson et al., 1996). In Malaysia, the Indian race was detected to have higher risk of oral cancers than Malays and Chinese (Zain, 2001).

The habits of alcohol consumption, betel-quid chewing, and tobacco smoking are established cultural risk factors associated with pre-malignant oral lesions and oral cancers (Goldenberg et al., 2004). In some regions of the world, variations are observed due to different types of ethnicities in a population. Such inter-ethnic differences might be due to genetic predisposition of various ethnicities towards an escalated risk of pre-malignant oral lesions and oral cancers (Zain, 2001).

Human papilloma virus (HPV) is known to be a risk factor for head and neck cancers. In the US, the percentage of HPV-associated OSCC has risen from 20% to more than 70% (Sathish et al., 2014).

The successful treatment outcome of the oral cancers depends on the appropriate management of both the loco-regional lymphatics and the primary site. The site of the primary tumour occurrence, the tumour size (T stage), nodal metastasis (N stage) and extra-capsular spread of the primary tumour dictates the treatment modality – chemotherapy (CT), radiotherapy (RT), surgery or a combination (Sankaranarayanan et al., 2015).

The site of oral cancer determines its prognosis. Loco-regional involvement of lymph nodes signifies the most vital prognostic factors. The five-year survival rate declines to 20% from 40% with the involvement of lymph nodes (Bagan et al., 2010). Development of second primary cancer, poorly differentiated and large sized tumours are associated with decreased survival rate. Hence, prevention and early detection of oral cancers remain crucial (Petersen, 2009; Rivera, 2015).

1.2 Justification of the study

In Malaysia, oral cancer is the 19th most common cancer which cannot be considered among the top "killer" malignancies when compared with other cancers such as ovarian and breast cancers. However, the concerns related to its burden of death cannot be brushed aside. The studies of risk factors, treatment modalities and outcome of oral cancer are not only of research interest, but also of clinical importance. Head and neck surgery alone or in combination with radiotherapy and chemotherapy procedures are performed to achieve good results. More importantly, in order to offer better awareness, prevention and treatment options, it is better for the people to avoid the associated risk factors. So, this study will be beneficial for awareness of people about the associated risk factors and treatment outcomes of the oral cancers, eventually for prevention of cancers. Therefore, it is imperative to determine the prevalence of oral cancers and associated risk factors which influence treatment outcome status of patients suffering from oral cancer.

1.3 Objectives of the study

1.3.1 General objective

To determine the prevalence and association of risk factors with treatment outcome status of oral cancer in patients who attended Hospital Universiti Sains Malaysia from January 2000 to December 2018.

1.3.2 Specific Objective

i. To determine the prevalence and association of socio-demographic as well as clinico-pathological risk factors with oral cancer.

ii. To determine the prevalence and association of socio-demographic as well as clinico-pathological risk factors with oral squamous cell carcinoma.

iii. To determine the association of socio-demographic and clinico-pathological factors with treatment outcome status of oral squamous cell carcinoma.

1.4 Research questions

i. What is the prevalence and association of risk factors with treatment outcome status of oral cancer patients who attended Hospital Universiti Sains Malaysia from January 2000 to December 2018?

1.5 Null hypothesis

i. Age, gender, ethnicity, betel quid chewing and genetic predisposition are not the risk factors associated with development of oral cancer in Kelantanese population.

ii. Tobacco smoking, alcohol drinking, and human papilloma virus infection are associated with development of oral cancer in Kelantanese population.

iii. Tumor site, histological grading, TNM classification and TNM grading of tumor as well as treatment status are associated with the mortality rates of oral cancer in Kelantanese population.

CHAPTER 2.

LITERATURE REVIEW

2.1 Cancer

Cancer is an unchecked growth of cells due to an imbalance between apoptosis and cell division (Ponder, 2001). According to WHO (World Health Organization, 2009), cancer is a disease caused by inherited and somatic mutations in genes known as tumour suppressor genes (TSG) and oncogenes.

Under normal circumstances, old and damaged cells are replaced by new cells. However, during cancer, the cell's DNA is damaged or altered causing mutations, thus influencing growth of cell. A tumour can be categorized either as malignant or benign. A malignant tumour has the ability to spread to nearby tissues (local invasion) as well as other parts of the body (metastasis). A benign tumour is localized and does not spread to other parts of the body (Lichtenstein, 2005).

According to the American National Cancer Institute (National Cancer Institute, 2009), cancer can be classified into five primary types including central nervous system cancers, sarcomas, leukaemias, carcinomas, myelomas and lymphomas. Cancers of central nervous system arise from tissues of spinal cord and brain. Sarcomas arise from the supportive tissues which constitute bone, cartilage, blood vessels, fat, connective tissues and muscle. Leukaemia is an immature blood cells' cancer that arises from the bone marrow and has the possibility to accumulate in abundant quantity in the bloodstream. Carcinoma is the most common type of cancer that arises from the cells that cover external (Sen *et al.*) and internal surfaces, i.e. mouth, lung, breast and colon.

Myeloma and lymphoma arise from cells and lymph nodes present in the human immune systems. Table 1 shows a brief history of oral cancer through the ages.

Table 2.1 Oral cancer through the ages timeline (Petrosyan *et al.*, 2019)

Year	**Significant discovery**
3000 BC	First recorded description of cancer (breast cancer)
1550 BC	Oral cancer described
460 BC	Terms ‘carcinoma’ and ‘cancer’ created by Hippocrates
30 AD	A. Celsus surgically excised cancer of face and lip
200	Humoral theory of cancer aetiology popularised by Galen
1215	Surgery prohibited by the Catholic Church
1478	First printed edition of A. Celsus’ ‘De Medicina’ in Florence, Italy
1653	Lymphatic system discovered
1664	First glossectomy performed by P. Marchetti
1740	First cancer hospital in Rheims, France
1761	Tobacco snuff causes cancer
1858	R. Virchow published ‘Die Cellularpathologie’

1885	H. T. Butlin's 'Diseases of the tongue' F. Jawdyński performed first neck dissection for a metastatic tongue cancer
1888	F. Jawdyński performed first neck dissection for a metastatic tongue cancer
1891	W. Halstead advised radical resection en – bloc lymphatics in breast cancer cases
1895	X-rays discovered by Roentgen
1898	The Curies discovered Radium
1902	Polya and von Navratil describe lymphatic drainage from oral anatomical sites
1906	G. W. Crile's landmark neck dissection paper
1951	1450 cases of radical neck dissections by the Hayes Martin group
1962	O. Suarez describes the functional neck dissection
1965	V. Y. Bakamjian repopularises the deltopectoral flap
1979	S. Ariyan introduced the pedicled pectoralis major myocutaneous flap
1981	G.F Yang published success with radial forearm free flap

1989	D. Hidalgo reconstructs 10 mandibular defects with fibula free flaps
1991	Committee for Head and Neck Surgery and Oncology of the American Academy of Otolaryngology - Head and Neck Surgery (AAO-HNS) standardised neck dissection
2002	Update on neck dissection terminology by the American Head and Neck Society
2017	Sentinel node biopsy supported by National Institute for Health and Care Excellence (NICE)

2.2 Hallmarks of cancer

Over the past 30 years, hundreds of cancer-associated genes have been identified. Some, for instance TP53, are commonly mutated; others, including ABL, are only mutated in certain leukemias. Each cancer gene has a defined function whose dysregulation contributes to the initiation or progression of malignancy. Therefore, it is better to consider cancer-related genes in the context of several fundamental alterations in cell physiology, the so-called distinctive signs of cancer, i.e. hallmarks of cancer, which collectively dictate the malignant phenotype (Hanahan and Weinberg, 2011):

iv. Evading growth suppressors

v. Avoiding immune destruction

vi. Enabling replicative immortality

vii. Tumour promoting inflammation

viii. Activating invasion and metastasis

ix. Inducing angiogenesis

x. Genome instability and mutation

xi. Resisting cell death

xii. Deregulating cellular energetics

xiii. Sustaining proliferative signaling

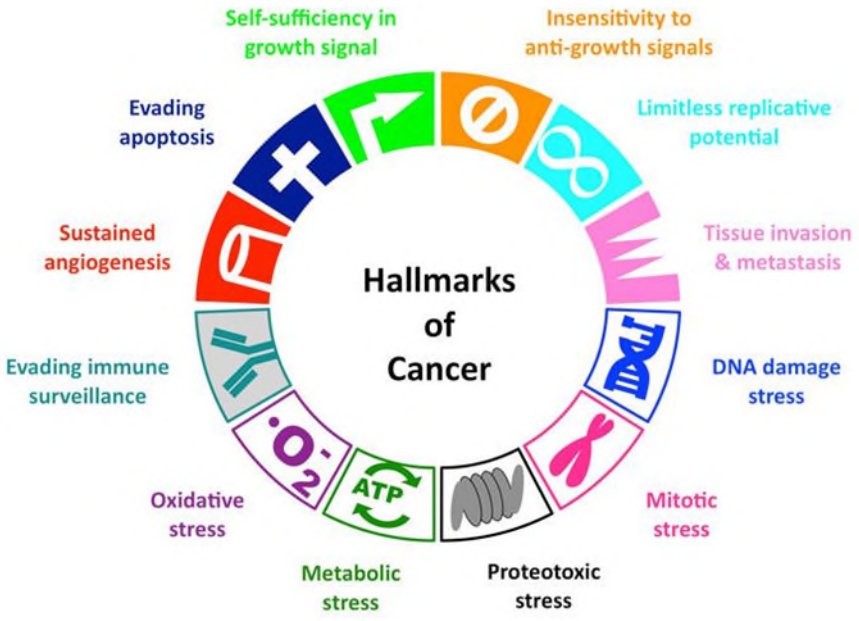

Figure 2.1 Hallmarks of cancer (Hanahan and Weinberg, 2011).

2.3 Cancer and cell cycle

Every cell is programmed to multiply and die. This ordered but complex program is controlled by the centre of the cell “nucleus” which contains chromosomes containing many genes made up of DNA (Kastan and Bartek, 2004). Sometimes, some of these genes undergo a change. The nucleus then sends out abnormal orders and the cell goes wrong. It multiplies uncontrollably and takes on a life of its own. Each new cell produced contains the same defect. The cells proliferate chaotically and form a tumour (Evan and Vousden, 2001). This process may be short, but is often long. 10-30 years may separate the birth of a first abnormal cell from the appearance of a tumour of about $1cm^3$ (Massagué, 2004).

Cancer was considered to initiate when the rate at which cells grow exceeds the rate at which cells die, so that cells divide at an uncontrollable speed (Foster, 2008). Now, it is accepted that cancer is described more accurately as the product of a defect within the regulation of the cell cycle, so that wounded or mutated cells that normally die can progress through the cell cycle, acquiring mutations (Saiki *et al.*, 2017). Mutations mostly occur in proto-oncogenes (Her2/neu, Ras, c-Myc) and TSGs (p53 and Rb).

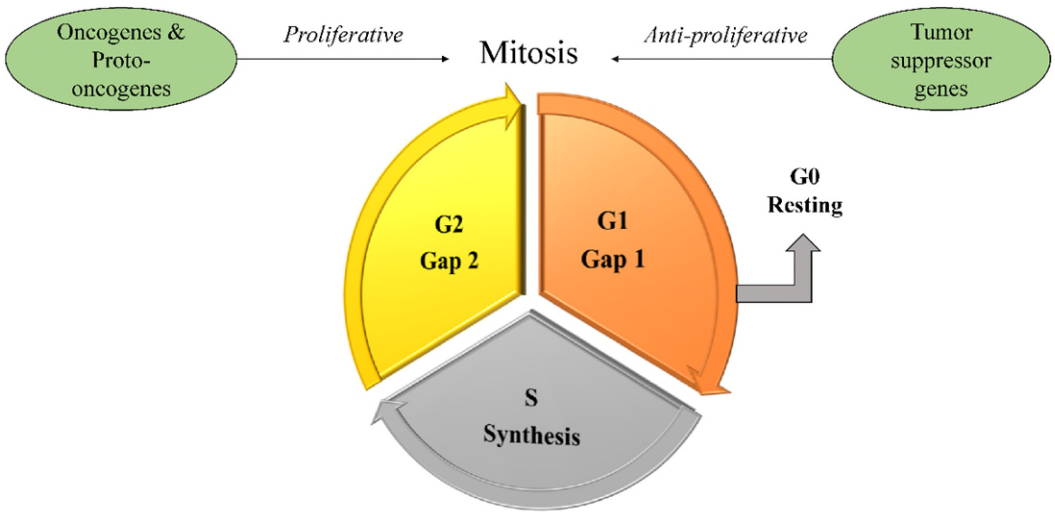

Figure 2.2 Genes affecting cell cycle control (with acknowledgement to Scully C, Warnakulasuriya S. Cancer of the mouth for the dental team. Comprehending the condition, causes, controversies, control and consequences (Kalavrezos, 2015).

One of the main proteins that regulate on a very high level is p53 (nicknamed as the “Guardian of the Genome”) (Efeyan and Serrano, 2007). It is so important, actually, that Science magazine called it the “molecule of the year” in 1993 (Culotta and Koshland Jr, 1993). p53 will bind DNA directly to produce proteins that block the progression of the cell cycle (Chen, 2016a). One of those proteins include p21. p21 will function to inhibit cyclin-dependent kinase (CDK). So, the CDK will not be able to activate DNA replication or activate mitosis (Karimian *et al.*, 2016). Retinoblastoma (RB) is another protein that is associated with the function of p53 and these proteins are considered as TSGs, so RB is a protein that is produced from a TSG, just like p53 (Giacinti and Giordano, 2006). If they are defected, or if they have a mutation in them that causes loss of function, cancer arises. More than 50% of tumours have a defect in p53. RB got its name because a defect in RB would lead to a tumour of the eye known as retinoblastoma, which is why these two genes

are considered as TSGs (Golabchi *et al.*, 2018). p21 is very unusual in that it does not actually lead to cancer when it is defected. Instead, it has been reported that mice that are without p21 have the ability to regenerate their limbs (Arthur and Heber-Katz, 2011).

2.4 The Hayflick limit

In 1961, Leonard Hayflick and his colleague Paul Moorhead discovered that normal cultured human cells have limited ability of division, after which they cease to grow, start to enlarge, engaging in new mechanism that has been called replicative senescence (Hayflick and Moorhead, 1961). At that time, it was totally unexpected because the research community strictly believed that such cells were immortal. In order to support the idea that normal human cells are mortal, Hayflick and Moorhead simultaneously cultured separate populations of male and female human fibroblasts. Today, it is known that this withdrawal from the cell cycle after a definite number of cell divisions (replicative senescence) is triggered because of shortened telomeres (Maciejowski and de Lange, 2017).

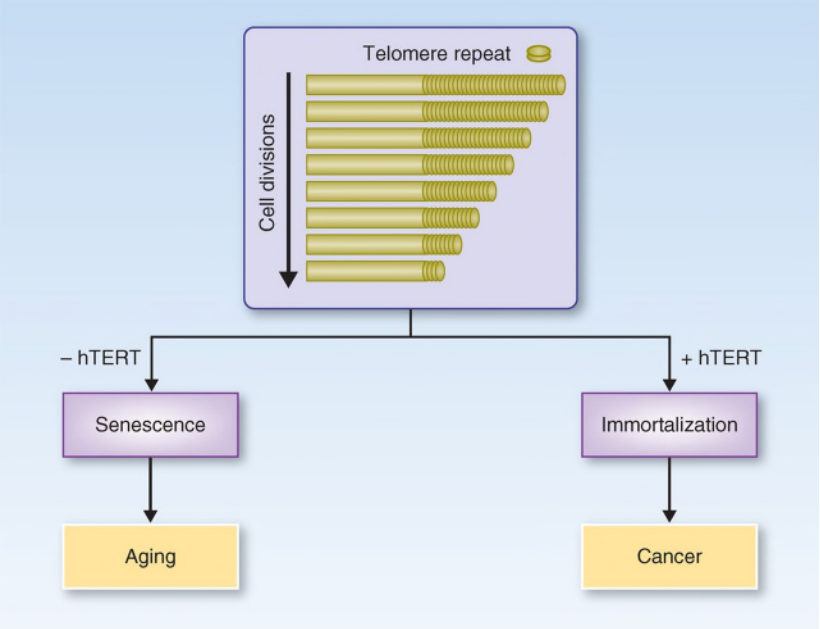

Figure 2.3 All somatic normal human cells display progressive telomere shortening with increased cell divisions. In the absence of a mechanism to maintain telomeres, cells eventually undergo replicative senescence (aging). Ectopically expressing just, the catalytic subunit telomerase reverse transcriptase (TERT) of the telomerase holoenzyme complex is sufficient to maintain telomere length and immortalize normal cells. Although normal cells with or without telomerase activity are not transformed, in the background of additional oncogenic changes, normal cells not only upregulate or reactivate telomerase but can become fully malignant (Shay and Wright, 2011).

2.5 Telomeres and telomerase

Telomerase is a ribonucleoprotein enzyme which was first discovered in Tetrahymena (Greider and Blackburn, 1985). Recently, telomerase has been identified in a line of immortal human cancer cells (Shay and Wright, 2019). The mechanism of action of telomerase is a repetitive duplication of the template domain, which involves an elongation phase during which the deoxyribonucleotides are added in sequence to the 3 'end of the telomere, ensued by a slower "translocation" phase, during which the relative

position of telomerase and telomere advances one repetition, hence positioning the enzyme for another elongation phase (Leão *et al.*, 2018).

Cancer cells have developed the ability to overcome senescence via mechanisms that can maintain telomere length (for instance telomerase expression), which allows tumour cells to grow indefinitely, a biomarker of almost every human malignancy (Lasry and Ben-Neriah, 2015). Telomerase, which is detected in approximately 90% of all malignant tumours (Shay, 2016), might predict poor or favourable outcome (Wu *et al.*, 2016), which makes telomerase a very attractive biomarker and a target for making mechanism-based cancer diagnostics, therapeutics and prognostics (Platella *et al.*, 2017).

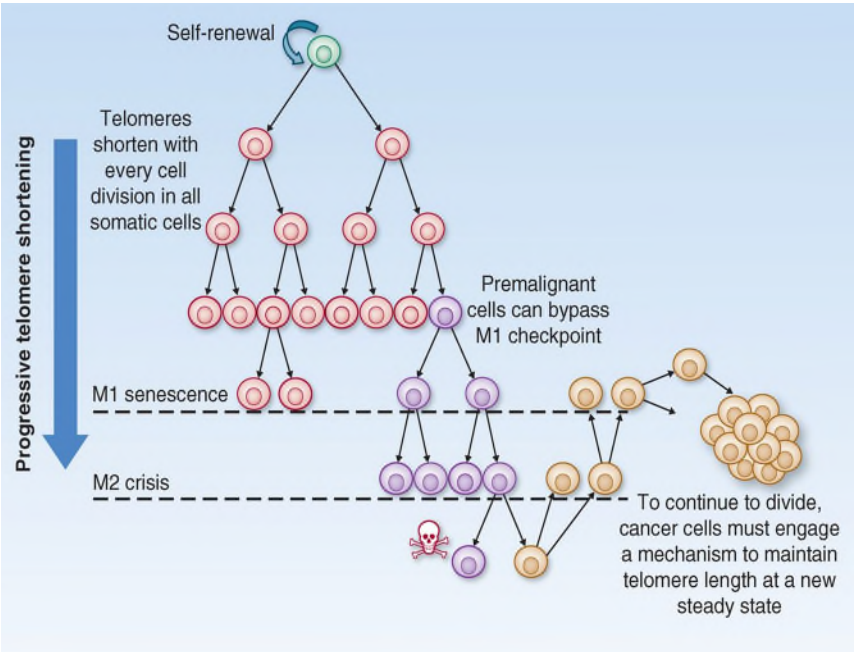

Figure 2.4 With increasing cell divisions, telomeres progressively shorten. Even in stem cells that self-renew, there is a gradual shortening of telomeres. After a finite number of cell doublings, eventually the cells have sufficient short telomeres that they undergo a growth arrest called senescence or the mortality stage 1 (M1). This has also been termed the Hayflick limit. Premalignant cells that have obtained a number of oncogenic changes can bypass M1 and enter into an extended lifespan period. This has

been termed the extended lifespan period, but eventually these cells also slowdown in proliferation and enter a period called crisis. In crisis, there is a balance between cell growth and apoptosis, and the vast majority of the cell population dies. A rare cell can upregulate telomerase or the much rarer alternative lengthening of telomerase (ALT) pathway and continue to proliferate. The hallmark of cells escaping crisis is, almost universally, stable but short telomere lengths and telomerase activity (Shay, 2016).

2.6 Molecular events in oral carcinogenesis

Carcinogenesis is a multi-stage process of aggregation of gene defects that are responsible for determining the characteristic traits of the cancer (Lichtenstein, 2005). It includes initiation, promotion, progression and malignant transformation (Tomasetti and Vogelstein, 2015). The development of cancer requires multiple genetic alterations affected by genetic predisposition and vulnerability to environmental carcinogens such as tobacco smoking, alcohol consumption, viral infection and chronic inflammation (Leemans *et al.*, 2018).

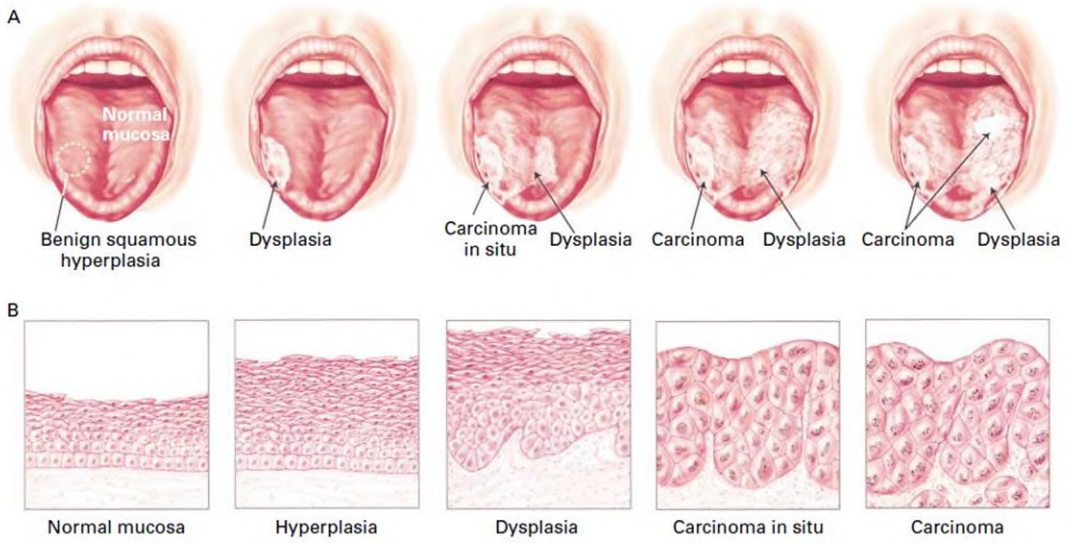

Figure 2.5 Clinical and Molecular Progression of Oral Cancer. (A) A typical clinical presentation of oral cancer. Benign squamous hyperplasia can often appear similar to normal mucosa. Novel molecular approaches have yielded considerable understanding of the field-cancerization hypothesis. (B) Normal-appearing mucosa already harbours early genetic changes (Farah *et al.*, 2014).

2.6.1 Oncogenes and proto-oncogenes

Genes whose protein products are considered important for signalling normal cell growth and whose mutation or overexpression lead to uncontrolled cell growth and to tumorigenesis are termed as "oncogenes" (Pierotti *et al.*, 2016). The first oncogenes were identified through the study of retroviruses (Mahalingam *et al.*, 2002). Based on the biochemical and functional properties of protein products of proto-oncogenes (normal counter parts of oncogenes), oncogenes can be categorized in to five groups. These are (Bateman *et al.*) growth factors, (2) growth factor receptors, (Benowitz *et al.*) transcription factors, (4) signal transducers, and (5) others, such as apoptosis regulators (Pierotti *et al.*, 2016).

Epidermal growth factor receptor (EGFR) and its ligands have been extensively studied in OSCC (Jia *et al.*, 2016; Kakei *et al.*, 2017; Liu *et al.*, 2019; Minabe *et al.*, 2019; Zeng *et al.*, 2000). Transforming growth factor (TGF-α) mRNA was found at levels 5 times higher in 95% of histologically normal tissues of OSCC patients and at levels 5 times higher in 88% of tumours than in normal mucosa (Das and Nagpal, 2002). EGFR mRNA increased 28 times in 92% of histologically normal tissues in cancer patients and increased 70 times in 91% of tumours than in normal mucosa (Grandis and Tweardy, 1993). Overexpression and amplification of c-myc/N-myc have been detected in 20-40% of oral cancers (Alrani *et al.*, 2016; Saranath *et al.*, 1989; Sharma *et al.*, 2017). The amplification of K-ras / N-ras, a point mutation in H-ras and the loss of the H-ras allele have been associated with oral cancer induced by tobacco chewing (Hoeben *et al.*, 2016; Krishna *et al.*, 2015; Prasad *et al.*, 2018; Saranath *et al.*, 1991; Xu *et al.*, 1998).

Several studies in western countries have also demonstrated an amplification of 11q13, which contains the oncogenes int-2, hst-1, cyclin D1 (prad-1 / bcl-1), in 30-50% of OSCC patients (Barros-Filho *et al.*, 2018; Blessmann *et al.*, 2013; Huang *et al.*, 2006; Huang *et al.*, 2002; Izzo *et al.*, 1998; Noorlag *et al.*, 2015; Ramos-García *et al.*, 2019; Ramos-García *et al.*, 2017; Reshmi *et al.*, 2007; Shuster *et al.*, 2000; van Kempen *et al.*, 2015), and these amplifications are linked with poor prognosis (Meredith *et al.*, 1995; Williams *et al.*, 1993). Recently, it has also been proposed that constitutive activation of Signal transducer and activator of transcription *3* (Stat-3) is an early event in oral carcinogenesis (Alkharusi *et al.*, 2019; Chen *et al.*, 2019).

2.6.2 Tumor suppressor genes

By acting as transducers of negative growth signals, TSGs are involved in regulation of cell cycle including cell cycle arrest and programmed cell death (apoptosis) (Marshall, 1991; Weinberg, 1991). The most vital TSG that is located on chromosome 17p13.1 is the p53 gene (Ho and Lane, 2018). It has been named as "Guardian of the Genome", since having its important role in maintaining genome stability, cell cycle progression, DNA repair, cell differentiation, and apoptosis (Chen, 2016b). Several mechanisms, such as point mutations, deletion and binding to cellular and viral proteins, may lead to inactivation of p53 gene (Joerger and Fersht, 2016). Mutation of p53 gene in 25-70% of oral cancers has been reported in several studies (Ali *et al.*, 2017; Langdon and Partridge, 1992; Shah *et al.*, 2011; Sinevici and O'sullivan, 2016; Yeh *et al.*, 2003).

In 20% of oral cancer (Patel *et al.*, 2018) and 22% of oral leukoplakia cases (Fonseca-Silva *et al.*, 2016), the loss of the heterozygosity of the p53 allele has been reported. In oral cancers, a rearrangement in the 5' region and in the coding of the p53 gene has been observed as well (Patnaik *et al.*, 1999).

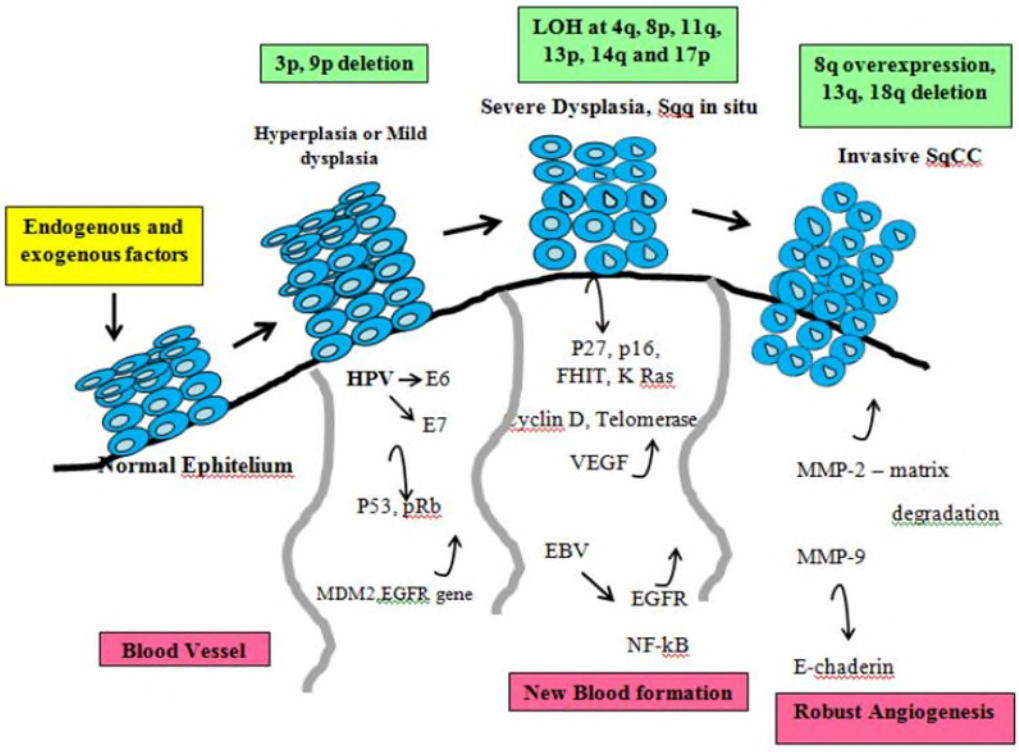

Figure 2.6 Possible oncogenes and tumor suppressor genes involved in oral cancer as the tumor progress from normal to squamous cell carcinoma (Knopf *et al.*, 2015).

2.6.3 Genomic instability

Genomic instability displays the predilection of the genome to achieve multiple alterations, for instance microsatellite instability (MSI) and loss of heterozygosity (LOH) in repetitive sequences, which may contribute to TSG inactivation (Ram *et al.*, 2011). Frequent LOH on chromosomes 3p, 9p, 13q and 17p has been reported as an early event in OSCC (Graveland *et al.*, 2011) and related with the transformation of pre-malignant oral lesions to OSCC (Zhang *et al.*, 2012).

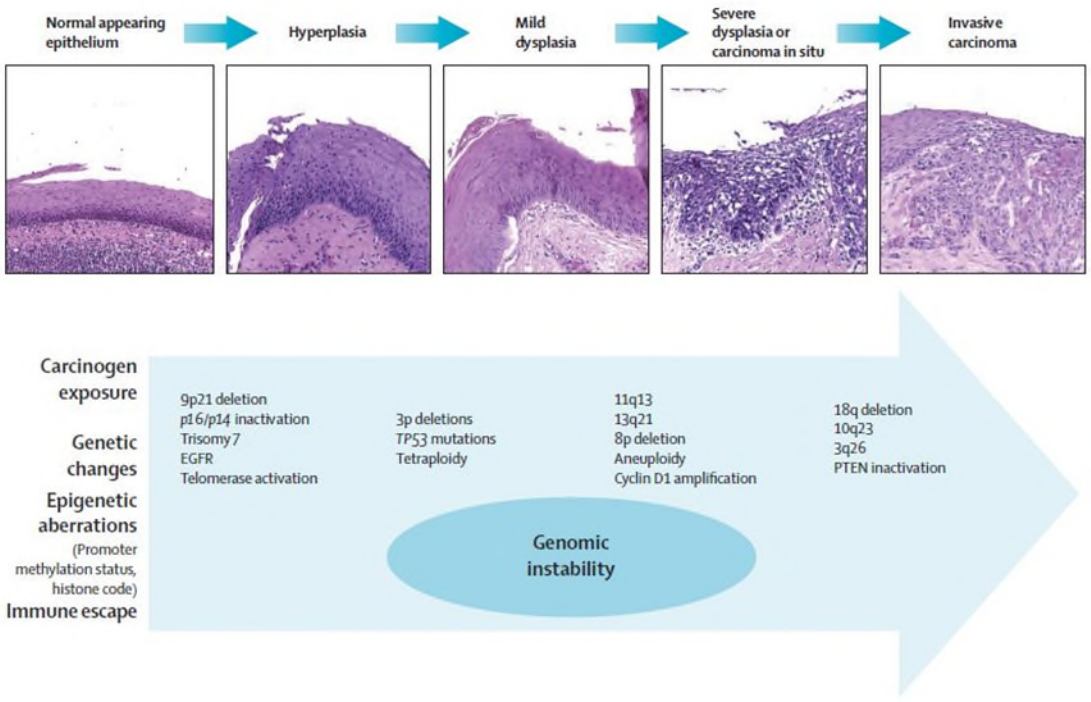

Figure 2.7 Presentation of phenotypical progression and accumulated molecular alterations in head and neck carcinogenesis. EGFR=epidermal growth factor receptor. PTEN=phosphatase and tensin homologue (Feldman *et al.*, 2016).

2.6.4 Immortalization by telomerase activity

As the loss of telomeres occurs during cell divisions, the chromosomal ends are no longer secured, leading to the fusion of chromosomes and karyotypic irregularities which ultimately result in cell death. The ribonucleoprotein enzyme telomerase is inactive in normal cells but active in most (90%) of the human cancers (Sinevici and O'sullivan, 2016). Several studies have reported high telomerase activity in oral cancers such as OSCC (Benhamou *et al.*, 2016; Boscolo-Rizzo *et al.*, 2016; Li *et al.*, 2011; Miyazaki *et al.*, 2015; Samadi *et al.*, 2018; Zhao *et al.*, 2015).

2.6.5 Angiogenesis

As the tumour grows, it will eventually reach a size where it requires additional vasculature to sustain continued growth. To achieve this, the tumour cells excrete certain proteins to stimulate blood vessel growth into and around the tumour, a process called angiogenesis (Hasina and Lingen, 2001). Vascular endothelial growth factor (VEGF) affects the endothelial cells that lines the blood vessels in a number of ways; (Bateman *et al.*) It can cause them to proliferate by activating the extracellular kinases and mitogen-activated protein (MAP) kinase signal transduction pathway (2) It can induce proteins that breakdown the basement membrane to allow the endothelial cells to migrate and invade. These proteins include matrix metalloproteinases (MMPs), urokinase plasminogen activator (uPA) and its receptor uPAR as well as tissue plasminogen activator (Benowitz *et al.*) It makes vessels more permeable allowing molecules and fluids to leak out. When MMP is secreted into the extracellular space, it degrades the extracellular matrix to allow proangiogenic factors to reach the vasculature. With the extracellular matrix degraded, pro-angiogenic factors including VEGF can reach receptors on the endothelial cells of the blood vessels surrounding the tumour. Thus, stimulating the angiogenic signal in the vessel. VEGF also helps the new endothelial cells survive by upregulating inhibitors of apoptosis. VEGF also activates the endothelial cells to express the proteins necessary to allow the new blood vessels to form. The end result is the growth of new blood vessels into the tumour. Because of this growth, additional nourishment can be delivered to the tumour. This angiogenesis facilitates further growth of tumour.

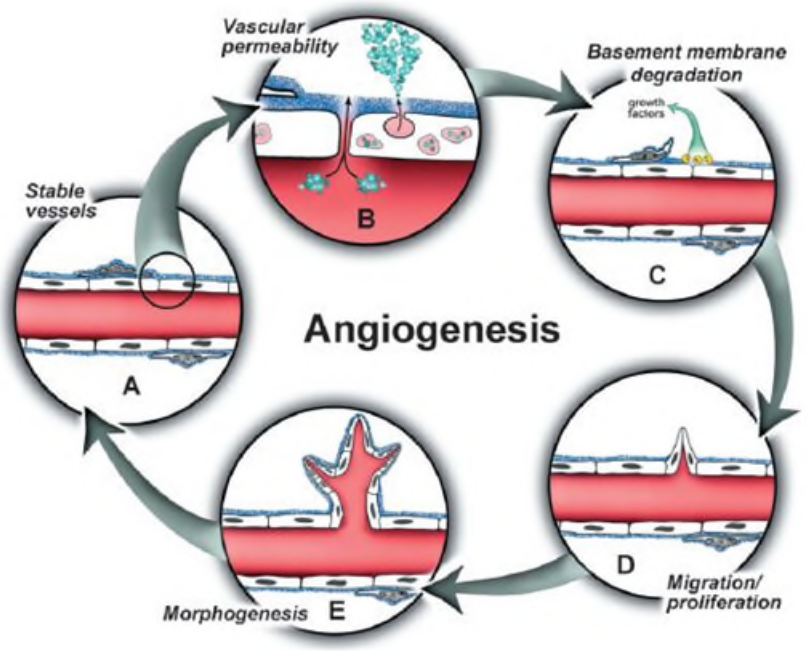

Figure 2.8 The angiogenic cascade. During the process of angiogenesis, stable vessels (a) undergo a vascular permeability increase, which allows extravasation of plasma proteins (b). Degradation of the ECM by MMPs relieves pericyte-EC contacts and liberates ECM-sequestered growth factors (c). ECs then proliferate and migrate to their final destination (d) and assemble as lumen-bearing cords (e). ECM, extracellular matrix; MMPs, matrix metalloproteases; EC, endothelial cell (Bryan and d'Amore, 2007).

2.7 Clinical features of oral cancer

2.7.1 Symptoms of oral cancer

Pain is a usual symptom in patients of oral carcinoma which accounts for 30-40% of their chief complaints (Cuffari *et al.*, 2006). Although, pain is a chief symptom, it usually happens only when a lesion has reached a considerable size. Since, the early carcinomas are asymptomatic, they often go unnoticed (Scully and Bagan, 2009). Other symptoms include bleeding, breathing problems, dysphagia, ear pain, paresthesia, problems using prosthesis, speech difficulty, teeth mobility and trismus (Haya-Fernández *et al.*, 2004).

2.7.2 Clinical presentation

2.7.2(a) Initial stages

The clinical presentation of an early lesion is usually an erythroleukoplastic lesion (Mashberg *et al.*, 1989). It is well demarcated and contains red or red and white patches with a slight roughness (Figure). On palpation, the elasticity of the soft tissue alters to a harder sensation. The lesion is often asymptomatic, however there might be some discomfort (Bagan *et al.*, 2010).