Association between periodontal health and oral cancer in a sample of subjects from India

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PREFACE

This thesis conforms to the guidelines and requirements of a Master's thesis at McGill University. This thesis is divided into five chapters. Chapter one, the introduction, provides the background and rationale of the thesis. Chapter two reviews the literature on oral cancer epidemiology highlighting the importance of periodontal disease as a risk factor for oral cancer and the potential biological plausibility which explain these associations. Chapter three provides the aim, objectives and hypotheses of the thesis. Chapter four explains the methodology used in this thesis. Chapter five presents the results and finally chapter six discusses the findings and their importance for public health. A full reference list is provided at the end of the thesis.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ICD…….International Classification of Diseases

HIV……..Human immunodeficiency virus

AIDS……...Acquired immunodeficiency syndrome

USA……United States of America

DNA……Deoxyribonucleic Acid

HPV……Human papilloma virus

ENT……Ear, nose, and throat (Otolaryngology)

BBC……...British Birth Cohort

IARC……..International Agency for Research on Cancer

SPSS…….Statistical Package for the Social Sciences

DMFT…….Decayed, Missing and Filled Teeth

OR……..Odds Ratio

CI…….Confidence interval

ABSTRACT

Introduction: Oral cancer is among the most common cancers in the world. Its major risk factors are smoking, alcohol consumption, and HPV infections; and in India betel quid chewing, beedi smoking, and smokeless tobacco consumption. India has the highest incidence of oral cancer in the world, with more than 69,000 new cases reported each year. Although recent studies have shown an association between poor periodontal heath and oral cancer, no studies have assessed this association in the Indian population. **Objectives:** To estimate the extent to which poor periodontal health is a risk factor for oral cancer and to examine how much of this association is explained by smoking status of the subject. Furthermore, we investigate whether this association varies by anatomical sub-sites within the oral cavity. **Methodology**: The data for this analysis was drawn from a hospital-based case-control study– HeNCe Life (Head and Neck Cancer Life course) study -Indian site. Two hundred and ninety three newly diagnosed squamous cell carcinoma cases in the mouth at stages I-IV and 314 non-cancer control subjects were recruited from Government Dental and Medical Colleges. Data pertaining to behavioural habits, socioeconomic position, oral health, diet and family environment were collected using a standardized questionnaire and a life grid technique. Descriptive and logistic regression analyses were performed. **Results**: After adjusting for the effects of covariates

including sex, age, behavioural habits, socioeconomic position and oral hygiene, preliminary analysis showed that periodontal health was associated with oral cancer risk. Males subjects with severe periodontal disease had an increased risk of oral cancer (odds ratios (OR) =2.53; 95% confidence intervals (CI):1.15–5.56) when compared to subjects with normal periodontium. This association was only apparent among those subjects who were smokers (OR=2.74; 95% CI: 1.16 – 6.47) and in sub-site gum (OR=3.35, 95% CI: 1.39-8.03). **Discussion:** Our results suggest an association between periodontal health and oral cancer risk. The association seems to be stronger among smokers and in sub-site gum.

ABRÉGÉ

Introduction: Le cancer de la bouche est l'un des cancers les plus répandus dans le monde. Ses principaux facteurs de risque sont le tabagisme, la consommation d'alcool, et les infections au VPH ; et en Inde le chiquage de bétel, la consommation de beedi à fumer, et de tabac sans fumée. L'Inde a la plus forte incidence de cancer de la bouche dans le monde, avec plus de 69000 nouveaux cassignalés chaque année. Bien que des études récentes aient montré une association entre une mauvaise santé parodontale et le cancer de la bouche, aucune étude n'a évalué cette association dans la population indienne. **Objectifs:** Estimer la mesure dans la quelle une mauvaise santé parodontale est un facteur de risque indépendant de cancer de la bouche et examiner dans quelle mesure cette association s'explique parle statut tabagique du sujet. En outre, nous explorons si cette association varie selon le sous-site anatomique de la cavité buccale. **Méthodologie:** Les données pour cette analyse ont été tirées d'une étude cas-témoin réalisée en milieu hospitalier - L'étude HeNCe Life (*Head and Neck Cancer Life course study*) – site indien. Deux cent quatre-vingt-treize cas nouvellement diagnostiqués de carcinome épidermoïde de la bouche aux stades I-IV et 314 sujets témoins non cancéreux ont été recrutés dans les Collèges publics de soins dentaires et médicaux. Les données relatives aux habitudes de comportement, à la position socio-économique, la santé buccodentaire,

l'alimentation et l'environnement familial ont été recueillies à l'aide d'un questionnaire standardisé et technique de grille de vie. Des analyses descriptives et de régression logistique ont été effectuées. **Résultats:** Après ajustement pour les effets de covariables, y compris le sexe, l'âge, les habitudes de comportement, la position socio-économique et de l'hygiène buccodentaire, l'analyse préliminaire a montré que la santé parodontale était associée à un risque de cancer de la bouche. Les sujets masculins atteints de maladie parodontale sévère avaient un risque accru de cancer de la bouche (rapports de cotes (*odds ratios*, RC) = 2,53 ; intervalle de confiance à 95% (IC) :1.15-5.56) par rapport aux sujets ayant un parodonte normal. Cette association était seulement apparente parmi les sujets qui étaient fumeurs (RC = 2,74 ; IC 95% : 1,16-6,47) et au sous-site gencive (RC = 3,35 ; IC 95% : 1,39-8,03). **Discussion:** Nos résultats suggèrent une association entre la santé parodontale et le risque de cancer de la bouche. Cette association semble plus forte parmi les fumeurs et au sous-site des gencives.

Chapter1: Introduction

1.1 Brief background and rationale

The oldest documented evidence of cancer incidence was reported in 3000 BC, since then it has become a major public health problem across the globe[1]. Despite advancements in cancer research, its incidence and mortality continues to increase. According to Globocan, cancer incidence worldwide has increased from 10.9 to 12.7 million and mortality from 6.7 to 7.6 million between 2002 and 2008[2, 3]. Cancers of the oropharyngeal region represent a weighty element of the total global cancer burden[4].

The incidence of cancers that affect the oral cavity varies considerably across the globe. Oral cancer is an important public health issue faced by many less developed countries, especially in the southern and eastern parts of Asia[5]. Among south Asian countries, India and Pakistan top the chart with highest oral cancer incidence. In India, oral cancer incidence accounts for 30-44% of all cancers[6]. The high incidence of oral cancer is attributed to behavioural habits such as betel quid chewing and beedi smoking[6, 7]. Other major risk factors associated with this chronic disease are cigarette smoking, alcohol consumption; diet low in fruits and vegetables, certain viruses and bacteria[8].

Recent literature shows that 16.1% of all cancers can be attributed to chronic infectious agents, and this figure is higher among less developed countries[9]. Chronic periodontal infections are the result of an accumulation of pathogenic bacteria, which is further linked to poor oral hygiene. Recent advancements in the research on pathogenic oral bacteria and its causal pathway to oral cancer show an association between chronic periodontal disease and oral cancer risk [10, 11]. Detection of H.pylori and certain viruses such as HPV in oral cavity further support the hypothesis that infections may cause cancer [12, 13]. However, most of the studies relating to chronic periodontal infections and oral cancer have concentrated on western populations and, among Indian populations the role of such variables has been overshadowed by the importance of tobacco, pan chewing and alcohol[14]. Even though there are studies that assessed the poor periodontal health and oral cancer risk, most of them have used oral hygiene measures and missing teeth as exposure variables, or were limited by the use of non-dentists as oral cavity examiners. In addition, the adjustments for other major risk factors were constrained by the use of traditional data collection methods [8, 15].

Based on the evidence presented above, the association of poor periodontal health and increased risk of oral cancer need to be examined on Indian population with the use of better data collection methods.

Chapter 2: Literature Review

2.1 Introduction

The following literature review is divided into 5 sections, covering substantial evidence on topics related to the present study. Section 2.2 provides an overview of oral cancer epidemiology; section 2.3 presents the definition, epidemiology of periodontal diseases and a brief review of the associations between periodontal diseases, plaque and missing teeth, and oral cancer risk. Section 2.4 presents a general review of the known oral cancer risk factors. This is followed by section 2.5, which provides a general summary and conclusions of the review.

2.2 Oral cancer: definition and epidemiology

2.2.1 Oral cancer definition

Due to the intricate nature of the oral cavity and the difficulty in locating the site within this cavity where a cancer originates, defining oral cancer has been a challenging task for both clinicians and researchers[16]. According to the International Classification of Diseases (ICD-10), oral cancer is defined as a subclass of neoplasms that arises from different parts of the oral cavity such as the lips, tongue, gums, floor of mouth, palate, and cheek mucosa[17].

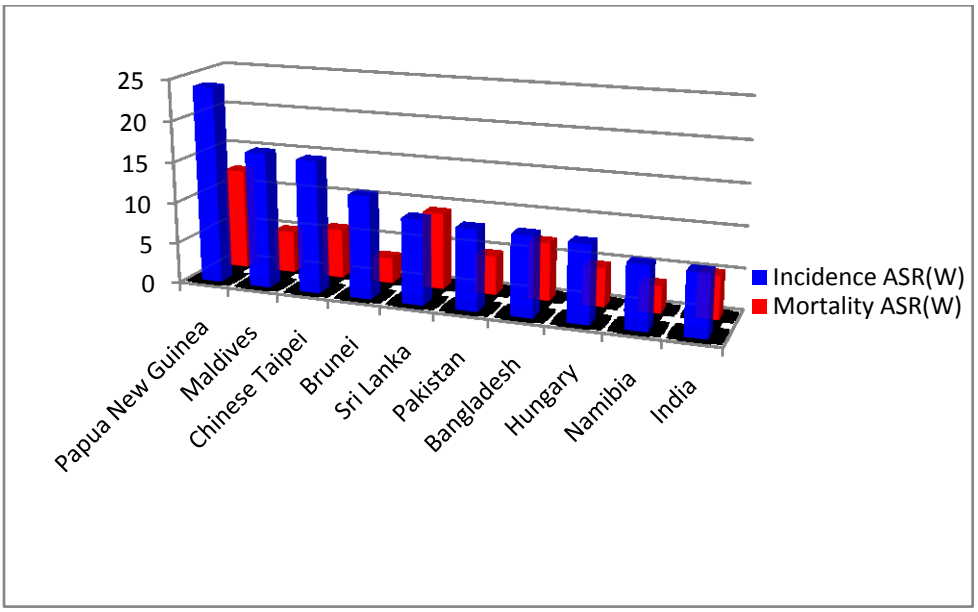
2.2.2 Epidemiology

Cancer is an uncontrolled division of abnormal cells that can occur in all-multicellular animals. In human populations, the occurrence of cancer types and sites varies between different geographic locations and ethnic groups[1]. Cancers that affect the oral cavity constitute a major health problem in developing countries and to some extent in developed countries[18]. Globally 65% of oral cancer cases are reported from developing countries while 35% arise from developed countries. They represent the 16th most common cancer with an annual incidence of 263,020 and mortality over 127,654 [19]. Oral cancer is more frequent in men, as it is the 10th most common cancer among men, whereas it the17thmost common among women. Among males, the incidence of oral cancer increases with age, peaking between the 5th and 7th decades of life[19]. Age standardized incidence and mortality rates per 100,000 are 6.9 and 2.3, and 2.4 and 0.6 among males and females, respectively. In spite of important advancements in diagnostic tools, treatment, and prevention efforts, mortality from this cancer continues to be high; it is higher than for cancers such as those of the cervix, larynx, testes, Hodgkin's lymphoma, and malignant melanoma [2, 19, 20]. Histologically, 90-95% of diagnosed oral cancer cases are squamous cell carcinomas. Overall, 35.2% of these cancers occur in the tongue, however site

prevalence differs between countries due to differences in habits. In India, the buccal mucosa is the most common site of oral cancer[1, 21].

Globally, the highest oral cancer rates are found in Melanesia, South-Central Asia, and Central and Eastern Europe[2]. A comparison of countries with the highest oral cancer incidence and mortality rates is shown in Figure1. Between South-Central Asian countries, oral cancers rank as the third most common type of cancer, and India has the highest number of this cancer, accounting for 35% of all cases reported from developing countries[2, 8, 22]. The incidence of oral cancer varies significantly in different regions of the word. In India, it accounts for 30-40% of all cancers, with an annual incidence of over 69,000[6, 22]. It represents the 2ndand 4thmost common cancer among Indian men and women, respectively. The age standardized incidence and mortality rate per 100,000 population is of 9.8 and 6.8, and 5.2 and 3.6 among males and females, respectively[6, 19]. The highest oral cancer incidence in India is reported in the country's southern states[23].

Figure1: Countries with the highest oral cancer incidence and mortality (Age standardized rates)



2.3 Risk factors for oral cancer

The variations observed in the incidence of oral cancer across the globe can be attributed to its multifaceted etiological nature. This disease develops from a combination of extrinsic and intrinsic factors acting over a long period of time, which promotes multiple molecular changes resulting in carcinogenesis[1, 24, 25].

The major risk factors related to oral cancer are tobacco use in any form, alcohol consumption, and betel quid chewing among Asian populations. In addition, the human papilloma virus, diets low in vegetables, vitamins and fruits, socio demographic factors (e.g., age, sex, socio-economic position), genetics, have also been associated with an increased risk of oral cancer. In addition,

certain bacteria and viruses, and oral health status and oral health behaviour play a role in the development of oral cancer [6, 12-14]. According to the recent evidence, non-smokers are a rapidly growing population of oral cancer patients, which suggests a pattern shift in the cause of this cancer. Tezal et al. have shown an association between alveolar bone loss, a common sign of periodontal diseases, and an increased risk of cancer in the tongue[10] as well as other head and neck squamous cell carcinomas[10, 11]; each millimeter of alveolar bone loss was associated with a 5.23 times higher risk of tongue cancer[10]. Among head and neck sub-sites, the association was stronger in the oral cavity compared to the oropharynx and larynx [10, 11]. These findings suggest an association between chronic periodontal diseases, which results from bacterial colonization, and the risk of oral cancer. The presence of bacteria and viruses in the oral cavity is, in turn, linked to poor periodontal heath and oral hygiene. The accumulation of dental plaque acts as a reservoir for many bacteria, resulting in the development of periodontal diseases. These inflammations are chronic in nature and, over time, the toxins and by-products released can lead to the development of oral cancer[10]. This thesis will examine periodontal diseases as a risk factor for oral cancer development.

2.3.1 Periodontal diseases: Definition and epidemiology

The oral cavity is home to different types of micro flora, which varies from individual to individual in composition and amount. Critical changes in the oral environment resulting from poor oral hygiene lead to a reorganization of the composition of the microbial community. As a consequence, inflammation will support more anaerobic bacteria in the gingival crevice, increasing the risk of chronic periodontal disease[26].

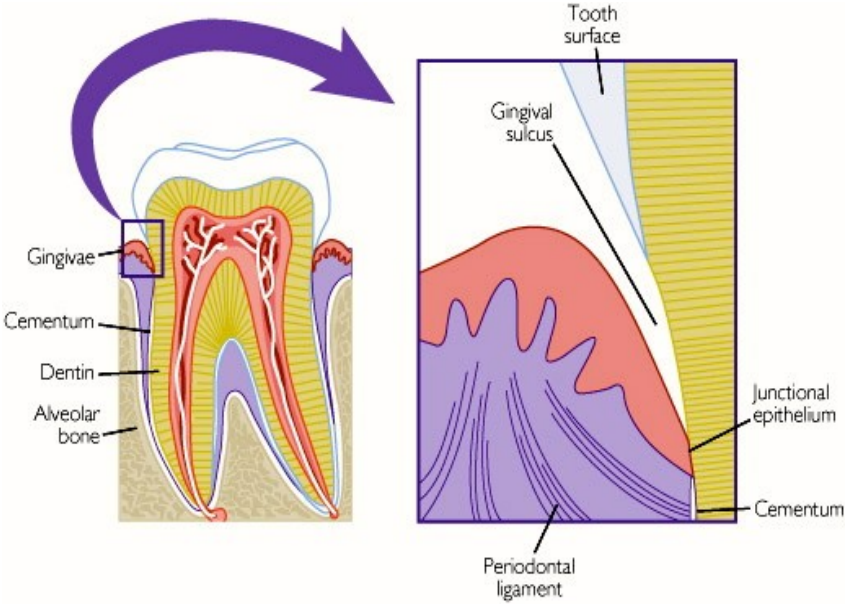
2.3.1.1 Definition and components of the periodontium

The periodontium consists of the gingiva, alveolar mucosa, periodontal ligament, cementum, and alveolar bone Figure2 [27]. The gingiva firmly attaches to the underlying bone, and its main function is to protect the underlying structure. The periodontal ligament is the connective tissue that connects the tooth to the bone. Microscopically, the gingival epithelium consists of stratum basale, stratum spinosum, stratum granulosum, and stratum corneum. The periodontium also contains other microscopic cells such as fibroblasts, mast cells, and immunologic cells[28].

The periodontium is susceptible to changes caused by chronic inflammatory reactions to the accumulation of anaerobic bacteria on the teeth. These chronic inflammatory reactions lead to irreversible destruction of tissues (e.g., bone and periodontal ligament) surrounding the teeth [28, 29]. Although

chronic infectious disease starts very slowly and symptoms are very mild at the initial stage, numerous changes occur in the periodontium as the disease progresses. Major changes that occur are an alteration in normal gum colour, gum bleeding, gum swelling, gingival recession and tooth lengthening, periodontal pockets and alveolar bone loss[28].

Figure2: Tooth supporting structures: gingiva, periodontal ligament, cementum, and alveolar bone



2.3.1.2 Gingival inflammation

The presence or absence of gingival inflammation is one of the major indicators of diseased gingiva. Gingival inflammation can be assessed by looking for signs of inflammation such as colour change, presence of marked redness, swelling, bleeding and purulent exudates. Initial signs of gingival inflammation are redness and swelling, which usually appear together[30].

The normal colour of the gingiva is generally defined as a coral pink colour resulting from the underlying vascular supply, the thickness and amount of keratinization of the epithelium and the presence of pigmentation[28]. Gingival colour varies among individuals and among different ethnic groups. Usually it is lighter in colour among people with a fair complexion than among those with a darker skin colour. A study among dark-skinned people showed a colour variation in healthy oral tissues from very light pale pink to purplish black, and 72% of the all examined cases showed some degree of melanogenous pigmentation. The amount of pigmentation observed varied in different parts of the oral cavity[31]. In a diseased gingiva, the colour may vary from slight redness to marked redness depending on the severity and extent of the disease.

2.3.1.3 Gingival recession

Gingival recession is the most common sign of chronic periodontal disease. In a fully erupted tooth, the gingiva is positioned at the cement-enamel junction, and its main function is to support the tooth structure[28]. Gingival recession is characterized by the apical displacement of the gingival margin from the cement-enamel junction. This attachment position may differ in certain conditions that can be either normal or a disease state. It varies according to age, as well as anatomical, physiological, and pathological factors[32]. Gingival recession is more frequent in men than in women. Malpositioned teeth and

trauma from brushing are the most common etiologic factors for localized recession[33, 34]. In addition to abnormal tooth position, anatomical factors that influence the gingival recession are lack of alveolar bone, fenestration and dehiscence, tooth shape and abnormal eruption. In addition, physiologically orthodontic tooth movements can lead to abnormal attachment[35]. All this usually leads to localized recessions.

Generalized recession is usually a result of periodontal diseases. The inflammation of the periodontium resulting from chronic periodontal infections produces proinflammatory cytokines (e.g., interleukin-1, tumour necrosis factor (TNF)) that lead to periodontal bone resorption and attachment loss. Attachment loss is the damage to the periodontal ligament and other tooth supporting structures resulting from chronic periodontal disease[28, 36]. The extent of recession depends on the severity of the disease. Smoking is associated with gingival recession; smokers have a significantly greater mean recession than non-smokers, and the severity of recession is related to the number of cigarettes smoked per day[37].

2.3.1.4 Pocket formation and alveolar bone loss

As described above, the periodontal tissues in the periodontium are susceptible to changes caused by chronic inflammatory reactions. These chronic inflammatory reactions lead to pathological deepening of the gingival sulcus (the

space or groove between a tooth and the surrounding gingival tissue), see Figure2 and the destruction of supporting periodontal tissues results in a shifting of the sulcus bottom apically (i.e., a deepening of the space). This process results in the formation of periodontal pockets, which contain microorganisms and their by-products, as well as other debris. Pocket depth is assessed using a periodontal probe[28]. The depth of the pocket and the amount of bone loss are major signs of chronic periodontal disease and determine the severity of the disease. Bone and attachment loss result from the combined action of factors such as interleukin-1, tumour necrosis factor-alpha, and antigen stimulated lymphocytes. The degree of alveolar bone loss depends on the amount of inflammatory infiltrate present[28]. A study conducted in Sri Lanka reported 0.2-0.3 mm of bone loss per year on facial and proximal surfaces[38].

2.3.1.5. Epidemiology of chronic periodontal infections

Chronic periodontal infections are the second most common oral disease; it affects approximately 90% of the world population. The disease starts as its mildest form, gingivitis, which occurs in response to bacterial accumulation. If untreated, the disease extends to deep tissues, leading to the loss of supporting connective tissue and alveolar bone[29, 39]. Poor oral hygiene measures are considered as a major contributor to the development of chronic periodontal infections[39]. Estimations of the global extent of periodontal disease have

changed with the evolution in measurement methodologies, starting with the introduction of the Community Periodontal Index of Treatment Needs (CPITN) developed in late 1970s, which was later renamed as the Community Periodontal Index (CPI). Since the introduction of these indices, studies have relied on periodontal pocketing to assess periodontal disease. However, studies subsequently started to use attachment loss along with pocket depth measurement to improve data accuracy[40].

Gingivitis, the initial stage of periodontal disease, may eventually progress to periodontitis if untreated. Different forms of this disease are aggressive, chronic, and necrotizing periodontitis. Currently, periodontal disease is one of the major global oral health problems[28]. According to American Surgeon General report, 80% of the adult American population suffers from gum disease[41]. A recent report by the World Health Organization shows that 5-20% of the adult population worldwide has severe periodontitis and that it is the main cause of tooth loss in both the industrialized and industrializing world[42, 43]. The prevalence of periodontal disease increases with age and it is estimated that 4 million Americans aged above 35 have some form of the disease[29]. A 15-year longitudinal study demonstrated a rapid progression of periodontitis among 8% of the sample, while 81% showed moderate progression, and 11% showed no progression beyond gingivitis[38]. Microorganisms present in the dental plaque

are considered as the major factor responsible for the development of periodontal disease; other contributing factors include age, education, genetics, tobacco and alcohol consumption, HIV and AIDS, osteoporosis, and diabetes[44].

Periodontal disease is the most prevalent oral disease in India and it affects more than 50% of the country's population[45]. To put these data in context, India is the second most populated country in the world, with 72% of its population living in rural areas. Oral health care is limited by a low dentist: population ratio (1 dentist to 200,000 populations) and a low literacy rate in the country's rural areas. More severe signs of periodontal disease were found among older age groups, and males are more likely to develop periodontal disease than females[46, 47].

2.3.2 Associations between periodontal disease, plaque and oral cancer

Increasing evidence suggests that infectious agents and chronic inflammatory diseases are associated with different types of cancers[48]. Almost all forms of periodontal diseases are chronic in nature, caused by pathogenic bacteria present in the overgrowth of dental plaque; recently, studies have suggested an association between bacterial infections and carcinogenesis [10, 29, 49]. A case-control study reported a 5.23-fold increase in the risk of tongue cancer among people with chronic periodontitis, independent of other risk

factors[10]. Another case-control study reported a similar observation between base of tongue cancer and periodontitis[13]. A prospective cohort study among American health professionals showed a significant association between periodontal disease and lung, kidney and pancreas cancer. This study did not report a statistically significance between periodontal disease and cancer in the upper aerodigestive tract. However, this may be due to the small number of cancer cases for these analyses[50]. A review article that assessed the relation between periodontal diseases, tooth loss and cancer suggested a two-fold higher risk among individuals with extensive tooth loss[51]. Fatal cancer occurrence has also been positively associated with chronic periodontal diseases[52].

Studies have also shown an association between microorganisms involved in the aetiology of periodontal diseases and oral cancer[53, 54]. For example, in a national health and nutrition survey among a non-institutionalized U.S.A. population, serum immunoglobulin G (IgG) antibody for P.gingivalis was found to be higher among orodigestive cancer (Cancer of lip, oral cavity, pharynx, esophagus, stomach, pancreas, liver, colon, rectum and anus) patients with cancer in the oral pharyngeal cancer. In addition, periodontitis was associated with an increased risk of orodigestive cancer mortality, and the risk increased with increases in the severity of periodontal disease[55]. Several studies have shown a positive association between chronic periodontal disease and cancer,

however the exact mechanism by which these chronic diseases lead to cancer is unknown, which could be a reason why some studies have treated this association as spurious.

2.3.3 Poor oral hygiene, missing teeth, and their relation to oral cancer

Poor oral hygiene is correlated with the risk of oral cancer [8, 15, 56]. Regular oral hygiene measures can prevent periodontal disease, tooth loss and dental caries. Conversely, poor oral hygiene can lead to an accumulation of pathogenic bacteria, which produces carcinogen precursors[57]. Studies have used different variables as markers of poor oral hygiene (poor oral health): missing teeth, low frequency of brushing, decayed teeth, and prophylactic dental visits are among them[58]. An Italian case-control study has shown that poor general oral conditions were 4.5 fold more frequent among cases compared to control subjects[59]. Another case-control study from central Europe and Latin America reported that poor condition of the mouth, lack of toothbrush use and daily mouthwash use increased the incidence of cancer in the upper aero-digestive tract (head and neck cancer) [60]. A significant association has been shown between low frequency of teeth cleaning and infrequent visits to a dentist and the risk of oral cancer [58, 61, 62]. A seven fold-increased risk of oral cancer was reported among subjects who performed less frequent teeth cleaning in a study from South India [15]. A matched case-control study from Western New

York showed an increased risk of oral cancer among subjects with poor oral hygiene, however, this effect was small compared to the risk attributable to cigarette smoking and alcohol consumption[63].

Although several studies have examined the association between missing teeth and different types of cancers, the results are inconclusive. While a few have reported positive associations, others have failed to show any association [56, 57, 64-66]. A study from Poland has shown a positive association between number of missing teeth and oral cancer; the risk of oral cancer was increased up to 9.8 fold for the group with the highest number of missing teeth compared to the group with the lowest number of missing teeth[61]. Another study from China reported a 5.3 and 7.3 times higher risk of oral cancer among men and women, respectively, with more than 15 missing teeth compared to those who had lost none[56]. A study from Japan showed a positive relationship between missing teeth and the risk of gastric cancer; a smaller number of teeth led to insufficient chewing and shorter mealtime, which exerted a digestive burden on the stomach that promoted the development of cancer[67]. A study that assessed tooth loss and its relation to cancer among an Asian population showed that individuals with a higher than the age-specific median number of tooth loss had a 35% higher risk of death by an upper gastrointestinal track cancer and that this higher risk was equal among smokers and non-smokers[68].

2.3.4 Viral causes for chronic periodontal disease and its synergy with bacteria in oral cancer

The role of bacteria in the development of periodontal disease is well understood, but studies have recently demonstrated that viruses also play a role in chronic periodontal infections. Recent studies have established an associations of certain viruses such as the human immunodeficiency virus (HIV), the herpes virus, the Epstein-Barr virus and the cytomegalovirus with periodontal disease[29, 69]. The presence of these viruses and bacteria contributes to the initiation of chronic periodontal disease, and together they may also exert a synergistic effect in causing oral cancer[69]. The presence of certain viruses (human papilloma virus, cytomegalovirus, and Epstein-Barr virus) in periodontal pockets supports this hypothesis[70, 71]. However, the molecular mechanism by which bacterial infections and the synergistic effect lead to cancer is not characterized completely[69].

Saliva acts as a medium for communication between viruses, inflammatory mediators and different carcinogens, and also acts as a vehicle to transport carcinogens to different sites[72]. Viral load is excessive in the saliva of those with periodontal disease, and significantly reduced after periodontal treatment[73]. Herpes simplex virus type1, type2, and adenovirus type2 were detected in 66% of oral squamous cell carcinoma biopsy specimens and 33% of

normal specimens. The role of bacteria and viruses in chronic periodontal disease development provides a conceivable link supporting the hypothesis of a synergistic effect of bacteria and viruses in oral cancer development.

2.4 Known risk factors associated with oral cancer

Other major risk factors related to oral cancer are tobacco use in any form, alcohol consumption, and betel quid chewing. In addition, the human papilloma virus, a diet low in vegetables, vitamins and fruits, socio-demographic factors (e.g., socio-economic position, age, sex) and genetics also play a role in the development of oral cancer. The following sections will give a brief description of further factors associated with the risk of oral cancer.

2.4.1 Tobacco

The tobacco plant is believed to originate from North and South America. About 6,000 BC, American Indians began using tobacco in medical and religious practices[74]. Soon commercial exchanges with different parts of world brought tobacco beyond America. In India, in the late 16th or early 17th century, Portuguese traders introduced tobacco, and thereafter it became a cultural practice to use it in different forms, which broadly fall under the categories of smoking tobacco and smokeless tobacco[74, 75]. Today tobacco use in various forms is an integral part of Indian culture and tradition, and it is offered in various religious ceremonies[76]. Tobacco usage is significantly increasing among Indian

youths and it is estimated that over 54% of children under the age of 15 use tobacco in one form or the other. The main motivating factors for this increase were tobacco use by family members or friends (65.6%) and use of tobacco among television and movie actors (26.4)[77]. Presently, tobacco is considered as a major risk factor for oral cancer; more than 60 chemicals in tobacco have been identified as potential carcinogens, and smokeless tobacco contains around 28 carcinogens[78, 79]. The main carcinogens are non-volatile alkaloid-derived tobacco-specific N-nitrosamines (TSNA) and N-nitrosamine acids.

2.4.1.1 Smoked tobacco

2.4.1.1.1 Cigarette smoking

Cigarette smoking is associated with an increased risk of oral cancer [61, 80, 81]. This increase in risk is related to the frequency of smoking, its duration, cumulative consumption and the type of cigarette smoked (e.g., filtered, non-filtered, hand-rolled). Studies have demonstrated that the use of non-filtered cigarettes compared to filtered cigarettes increases the risk of oral cancer, and the risk also increases with a longer duration or a higher frequency of smoking[61, 81]. Site-specific analysis in a study on head and neck cancer demonstrated a higher risk of laryngeal cancer in cigarette smokers who were never drinkers[81]. Studies from India have shown similar results [6, 8, 18].

2.4.1.1.2 Beedi smoking

Beedi is an Indian form of tobacco smoking, commonly practiced among people of south Asian countries. Indeed, it is believed that beedi was first produced in the east coast region of India. As beedi is very low in cost, it is more widely consumed among people of lower socioeconomic status, which gave it its nickname of “poor man’s cigarette”[82]. It is made by hand rolling about 0.2 grams of dried and crushed tobacco in a tendu leaf[7]. It has been documented that, compared to a regular cigarette, beedi exposes the smoker to three times the amount of nicotine and carbon monoxide, and roughly five times the amount of tar. Beedi smoke also contains volatile phenols and carcinogenic hydrocarbons benz(a)anthracine, benz(a)pyrene, and radioactive uranium[7, 8, 82-84]. Because of the hard consistency and filter less nature of beedi, the smokers require more frequent and deeper puffs, resulting in higher intakes of carbon monoxide, nicotine and tar, which produce greater carcinogenic effects compared to those of cigarette smoking. The overall toxic level of beedi smoke components is markedly higher when compared to cigarettes[7].

It is estimated that 53% of the tobacco smoked in India is in the form of beedi[82]. A meta-analysis of studies from south Asian countries has shown that oral cancer risk increased 3.1-fold among beedi smokers compared to non-

smokers, and this risk increased with a longer duration of the smoking habit and a higher number of beedi smoked per day[7, 85].

2.4.1.1.3 Cigar, pipe and other forms of smoking

Nicotine contents in the particulate matter of cigar and pipe smoke are similar to that of cigarette smoke, however the benzo(a)pyrene and phenol concentration is two to three times higher than that found in cigarette smoke tar[86]. An increased risk of oral cancer has been reported among cigar and pipe smokers independent of cigarette smoking. The risk increased with an increase in the number of cigars or pipes smoked per week[80].

2.4.1.2. Smokeless tobacco

Smokeless tobacco is used in different forms in various parts of the world and it has been associated with oral cancer for decades. Loose leaves (a form of chewing tobacco), moist snuff, and dry snuff are the most common types of smokeless tobacco used orally. Unlike smoked tobacco, smokeless tobacco is not burned, but it exerts its effects by direct mucosal contact, particularly in the oral cavity and pharynx[87]. The risk of oral cancer associated with smokeless tobacco depends on the type of tobacco product used, the procedure used in its manufacturing and the population that consumes it [88, 89]. Studies from the western world have reported a higher oral cancer risk ratio ranging between 4and 15 for dry snuff users[88]. Common smokeless tobacco products in south Asia

are betel quid/pan masala, gutkha, snuff, red tooth powder, khaini, mawa, and gul. Studies may have omitted to include data from this region because of the difficulties involved in assessing the risk of such a wide range of products[88, 90].

In India, individual farmers and small-scale companies process smokeless tobacco with no control over fermentation and curing, and as it is combined with many other products such as betel leaf, areca nut, and slaked lime, it loses its homogenous property, leading to an increased concentration of carcinogenic compounds[89]. In India, over 80% of oral cancer cases are attributed to betel quid chewing, smoking and alcohol consumption[23]. The male to female incidence ratio of oral cancer in India is approximately 1:1, whereas most areas with a high oral cancer risk have a ratio ranging between 3:1 and 10:1. The high incidence of oral cancer among both Indian women and men is attributed to pan chewing, a common habit among both sexes[23].

2.4.2 Betel quid or pan masala chewing

Betel quid or pan masala is a mixture of areca nut, slaked lime, tobacco and spices wrapped in betel leaf (leaves of the piper betel vine)[91]. Betel quid chewing is an ancient custom practiced in India for more than 2,000 years. The introduction of tobacco by Portuguese traders in the late 16th or early 17th century reinforced this practice[91, 92]. Today, betel quid chewing is a popular and socially accepted habit. A study from India showed a significant association of

daily frequency of pan tobacco chewing with oral cancer; males and females who chewed 10 or more times per day were at 15.07 and 13.69 times greater risk of developing oral cancer compared to those who did not chew[93]. A part from the betel leaf, all ingredients of betel quid have demonstrated carcinogenic properties. The following section will include a brief explanation of all ingredients of the betel quid and their carcinogenicity.

2.4.2.1 Betel leaf, areca nut, slaked lime and their association with oral cancer

The betel leaf is the leaf of the betel vine. It is the most common garnish chewed with the areca nut. In India, the betel leaf is used in various cultural and religious practices[94]. Betel leaves contain phenols, hydroxychavicol, eugenol, betel phenol and chavicol, vitamin C, and carotenes[95].

The areca nut is the seed found in the fruit of the areca catechu; it is one of the basic ingredients of the betel quid[96, 97]. The areca nut is chewed by 10-20% of the world population is some form[96]. The major chemical components of the areca nut are polyphenols and alkaloids, including arecoline; biochemical studies have identified at least six alkaloids[95]. Studies on animal models have confirmed the carcinogenic property of the areca nut[98]. A study among Taiwan areca nut users showed a relative risk of 58.4 for oral cancer[97]. Cytogenetic studies, which examine chromosomes and their relationship to human disease, have also confirmed the genomic damage caused by the areca nut and found a

significant increase in chromosomal aberrations in peripheral blood lymphocytes[99].

Slaked lime or calcium hydroxide is obtained by heating the covering of sea shells and is often used in the betel quid[95]. Studies have demonstrated the role of lime in pH change, the major determinant factor for the generation of reactive oxygen, which is considered the principal factor that causes DNA damage, leading to cancer development[100]. The use of lime causes an inflammation of cells, which leads to exposure of the basal stem cells to the mutagenic effects of different carcinogens present in the betel quid[101].

Several lines of evidence show that betel quid chewing is associated with an increased risk of oral cancer. The carcinogenic properties of the betel quid depend on the constituents used to form it. The ingredients used vary across cultures, hence the carcinogenicity may also vary among them[102].

2.4.3 Alcohol consumption

The consumption of alcohol has been consistently associated with oral cancer since the 1980's. This increase in risk from alcohol consumption depends on the type and amount of alcohol consumed[80]. Even though alcohol consumption has not shown a direct carcinogenic effect on the upper aerodigestive tract, enzymes such as alcohol dehydrogenase type3 metabolizes

ethanol to acetaldehyde; which is highly carcinogenic and has been identified as a tumour promoter[103, 104].

Indeed several studies have shown that heavy alcohol consumption and the use of hard liquors increase the risk of oral cancer[105]. An American study showed a 9-fold increased risk of oral cancer among those who consumed more than 30 drinks of hard liquors per week compared to those who consumed less than 14 drinks a week when controlling for tobacco smoking[80].

2.4.3.1 Combined effect of tobacco and alcohol

Different studies have shown strong evidence of an association between combined and individual alcohol and tobacco use and an increased risk of oral cancer [15, 80, 106, 107]. Over 70% of oral cancer cases in western countries are attributed to the combined effect of alcohol consumption and tobacco smoking, while in south Asian countries smoking is often replaced by betel quid chewing. The combined effect of alcohol and tobacco shows a multiplicative effect, in which the oral cancer risk increases with an increase in either habit[106]. Combining alcohol and tobacco increases the permeability of carcinogens found in tobacco, thereby increasing the effect of tobacco use[108]. A study from India has reported a 11.34-fold higher risk for oral cancer with the combined consumption of betel quid chewing, tobacco smoking and alcohol[15].

2.4.4 Socioeconomic status and risk of oral cancer

Socioeconomic status is associated with the risk of oral cancer; different studies have shown that being from a low socioeconomic status increases the risk of different chronic diseases[109]. The most widely used indicators of socioeconomic status are education, level of employment, occupation status, housing condition and income[110]. A study that assessed the relation between SES and the risk of oropharyngeal cancer using education, occupation status and percentage of time spent in employment as indicators, showed an inconsistent pattern of association with the different variables used[111].

2.4.5 Diet and risk of oral cancer

Diet is associated with different types of cancer. The ability of micronutrients to prevent cancer has been demonstrated in both animal model studies and intervention trials[24]. Several studies show a protective effect for oral cancer with the intake of fruits, vegetables, vitamin C, and carotenes. However, inconsistent results have been reported for vitamin B, E, foliate and iron intake[61, 103, 112]. Studies from India showed an 80% increase in the risk of oral cancer among those with a non-vegetarian diet, and a protective dose-response trend was observed with vegetarian foods such as fish, eggs, raw green vegetables, cruciferous vegetables, pulses and apples; these results were consistent with those from western countries[15, 23]. Even though the exact

mechanism has yet to be established, it is believed that foods rich in beta-carotene, vitamin C, and E with anti-oxidant properties can prevent damage at the DNA level, thereby preventing mutation[20, 112].

2.4.6 Viral causes for oral cancer

Human papilloma viruses(HPV) and the herpes simplex virus have been implicated in the development of oral cancer[113, 114]. Genital HPV is transmitted through sexual contact and strains of HPV responsible for oral cancer are usually transmitted by oral sex. Over 100 types of HPV have been identified and studies have demonstrated the presence of HPV-16 and HPV-18 viral DNA in oral tumours, however the role of the herpes simplex virus is yet to be delineated [24, 103, 113, 115-117]. Approximately 3% of oral cancer cases are attributed to HPV[118]. The presence of other risk factors in an HPV-infected lesion increases the risk of malignant transformation [119-121]. A study among Indian betel quid chewers showed HPV DNA in 74% of oral cancer lesions[122]. Another study in southern India demonstrated a 3.14-increased risk of oral cancer among men who practiced oral sex[23].

2.5. Summary and conclusions

Oral cancer is a chronic disease with multifactorial etiology, which can result from exposure to single or multiple etiological factors over a long period of time. In the above sections, we discussed several risk factors associated with

this disease. The role of chronic infections in the causal pathway that leads to the development of cancer is increasingly recognized. Being chronic in nature, periodontal inflammatory diseases that result from the build-up of dental plaque and the associated persistent immune response can play a major role in oral cancer development. Although India has a high incidence of oral cancer, few studies have looked into chronic inflammatory features of the gingiva, associated chronic periodontal infections, and the risk of oral cancer. This thesis aims to provide a better understanding of how poor periodontal health conditions are associated with the risk of oral cancer in an Indian sample.

Chapter 3 Aims, Objectives and Hypotheses

3.1 Aim

The aim of this study is to assess the association between periodontal health and the risk of oral cancer among a sample of Indian subjects.

3.2 Specific objectives

The objectives of this study are: -

A) To estimate the extent to which a subjective measure of periodontal status is associated with the risk of oral cancer.

B) To examine whether this association varies by smoking status of the subject.

C) To examine whether the association between a subjective measure of periodontal status and oral cancer differs by anatomical sub-site in the oral cavity (tongue and floor of mouth, gum, buccal mucosa, palate and tonsil).

3.3 Hypothesis

We hypothesize that, independent of other risk factors; poor periodontal status increases the risk of oral cancer among a sample of Indian subjects. Also, we hypothesize that this association will be stronger among subjects with smoking history and those who had oral cancer on the sub-site gum.

Chapter 4 Methodology

4.1 An overview of study design

In an epidemiologic case-control study, cases are all individuals or a random sample of subjects having the disease under investigation in the study base. The base is defined as the population that the researchers target. Controls are individuals who are free of disease selected from the same base as the cases, independently of exposure. In other words, the controls need to be derived from the base in such a way that they accurately reflect the exposure distribution in the study base, so that the distribution of exposure among them is the same as in the base. Thus, a comparison of differences in exposure between cases and controls will help to assess the potential risk and protective factors related to the disease under study.

The data for this study was collected at the Indian site of an international hospital based case-control study, The HeNCe Life study (Head and Neck Cancer Life course study). It uses a multidisciplinary approach to investigate the role of behavioural, genetic, viral, and social and psychosocial risk factors in the aetiology of cancers of the upper aerodigestive tract, commonly known as head and neck cancer (H&NC). Data were collected using a questionnaire-based interview and a life grid technique. In addition, a detailed oral cavity examination

was performed and biological samples for HPV and genetic analysis were obtained by qualified trained dentists.

4.2 Study population and study center

Oral cancer cases and control subjects were recruited from the Government Dental College and the Government Medical College and Hospital located in the state of Kerala, southwest India. Both hospitals serve nearly 40% of the population of Kerala spread across 6 districts of the Malabar region (Thrissur, Palakkad, Malappuram, Kozhikode, Wayanad, and Kannur). The study sample consists of 350 oral cancer cases and 371 controls recruited between September 2008 and March 2012.

4.2.1 General inclusion and exclusion criteria

To be eligible to participate in the study, subjects had to meet the following criteria: (i) to speak either English or Malayalam; (ii) to be born in Kerala; (iii) to live within a 150-kilometer radius of the hospital area; (iv) to have no previous history of any kind of cancer and no debilitating disease (e.g., HIV, AIDS); (v) To have no cognitive or mental disorders. Subjects with severe conditions such as chronic disabling conditions, mental disorders, and diseases of the central nervous system were also excluded from the study.

4.2.2 Case definition and selection

In a case-control study, precise criteria for the definition of cases are necessary in order to reduce the risk of weakening the case group with any non-cases, which would decrease the chances of explaining the real exposure difference. Oral cancer cases were defined as newly diagnosed and histopathologically confirmed oral squamous cell carcinoma cases at stages I-IV in the inner lips, tongue, lingual tonsils, gums, floor of mouth, hard palate, soft palate, uvula, cheek mucosa, vestibule of mouth, and retromolar area. Cases were recruited shortly after diagnosis from the oral pathology and oncology clinics, at the Government Dental and Medical Colleges and Hospital, respectively, Calicut, Kerala, India. Patients with a past history of cancer or patients who were being treated were not recruited as local or systemic treatment could interfere with biomarkers under study. In addition, prevalent cases were not considered to minimize the possibility of recall bias.

4.2.3 Control definition and selection

Control subjects were frequency matched to cases based on age (range 5 years) and sex. Subjects who met the study's inclusion and exclusion criteria were randomly selected from several outpatient clinics (dentistry, dermatology, gastroenterology, gynecology, ophthalmology, orthopedics, ENT, and nephrology) at the same hospitals as the cases. Controls were selected among

individuals without a history of cancer or pre-cancer lesions. Patients diagnosed with diseases related to tobacco and alcohol (e.g., chronic lung disease, cirrhosis) were not eligible. Efforts were made to ensure a good balance in the distribution of diseases among controls, with no single diagnostic group contributing more than 20% of all controls, to ensure a good representation of the risk base.

4.3. Data collection

4.3.1 Interviewer recruitment and training

Following an interview by the principal investigator, 3 qualified dentists were recruited and appointed as research assistants. They were trained by the principal investigator and study coordinator to understand the purpose of the study, and to perform all study procedures. A personal interviewer guide and a video describing all the steps in the study data collection were also provided to each research assistant (RA).The RA then performed an interview under the supervision of the international research coordinator. After minor adjustments, the pilot study was carried out to test the fieldwork procedures.

4.3.2 Recruitment of cases and controls

RAs collected information relevant for recruitment from patients attending the oral pathology and oncology clinics at the Government Dental and Medical Colleges and Hospital. After assessing their eligibility, patients with

histopathological diagnoses of oral cancer were invited to participate in the study. All patients read the description of the study and its procedures. In addition, the interviewers provided further explanation if necessary. For those who could not read, the interviewer read and explained in detail the study procedures. Consent forms were available in Malayalam (local language) and in English. In the presence of a witness, the study participants then signed the consent form. A copy of the form was retained at the site and one was given to subjects.

Control selection was based on a matching list prepared by the international coordination site located in Canada, and sent to the Indian site on a monthly basis. The list presented the number of controls to be recruited in each 5-year age and sex category, to establish adequate frequency matching with the cases already recruited. To ensure a good balance among the control clinics, the list also included the distribution of controls according to the clinic from which they should be recruited. Based on the matching list, RAs collected the medical appointment lists from the outpatient units of each control clinic and, after assessing for the inclusion and exclusion criteria, eligible subjects were randomly selected and approached. Informed consent procedures were the same as those used for cases.

4.3.3 Participation rate among cases and controls

The recruitment lasted for a span of 43 months, which began in September 2008 and ended in March 2012. A total of 426 eligible patients diagnosed with oral squamous cell carcinoma were approached and asked to participate in the study and 76 patients refused, representing a response rate of 82%. For control subjects, 865 eligible patients were invited to participate in this study, out of which 494 subjects refused to participate, leaving a total of 371 participants (response rate: 43%). Reasons for refusal varied among cases and controls; the majority of non-participating cases refused because of the unwillingness shown by the person who accompanied them, and a few patients mentioned the advanced stage of their disease. Among controls, the lengthy interview procedure was the main reason for non-participation.

Table1 displays the distribution of participating and non-participating cases and controls. Age and sex of the participating case and control subjects were similar. Non-participating cases were on average older (Mean=67.00, standard deviation (SD) ±15.59) compared to participating cases (Mean=60.77, SD ±11.24) and this difference was statistically significant ($p<0.000$). This is somehow expected as the main reason to refuse to participate in the study was ill health and advanced disease stage. However, we believe that this does not invalidate our results as the response rate among case subjects was very good

(82%). The difference among participating and non-participating cases regarding sex was negligible Table1. Regarding the control group, although we had a relatively low response rate, there were no significant differences in sex and age among participating and non-participating control subjects. The low participation rate among control subjects might have introduced a selection bias. The potential selection bias introduced by these differences will be presented in the discussion section.

Table1: Distribution of participating and non-participating cases and controls

	Participating cases	**Non-participating cases**	**P- values**
Age (Mean ± SD)	60.77±11.24	67.00±15.59	0.000
Sex			
Females	154(44.0)	36(47.4)	0.612
Males	196(56.0)	40(52.6)	
	Participating controls	**Non-participating controls**	**P-values**
Age (Mean ± SD)	60.53±11.68	61.55±11.22	0.195
Sex			
Females	168 (45.3)	205(41.5)	0.268
Males	203 (54.7)	289(58.5)	

4.3.4 Study instruments

4.3.4.1 Questionnaire

Following consent procedures, a route sheet was filled out for all the subjects. This sheet was used to record eligibility information on the subject and schedule the interview. RAs conducted a face-to-face interview with each subject, which lasted approximately 90 to 120 minutes. A questionnaire and a life

grid were used to collect information on an array of exposures at three stages of life: childhood (from birth to 16 years old), early adulthood (from 17 to 30 years old), and late adulthood (after 30 years old). Questions collected information on medical history, demographics, indicators of socioeconomic position (e.g., education, occupation, housing conditions, and residential environment), behavioural factors (e.g., smoking, chewing and drinking habits, diet, and oral health), family history of cancer, and psychosocial factors (e.g., subjects' parents' relationship, marital life, and social support). The study instrument was developed based on questions used in previous studies: British Civil Servants, Whitehall II, British Birth Cohort (BBC) 1946, BBC 1958, and IARC studies on upper aerodigestive tract cancers [123-125]. Before being used in the main study, the questionnaire was adapted to the Indian and local context, and translated into the local language Malayalam using the back translation method. The instrument was tested in two pilot studies in the target population and minor adjustments were made for the final version.

4.3.4.2 Life grid

The questionnaire was used interactively with a "life grid" throughout the interview. This technique, adapted from Blane et al. [126], servesas a memory tool to recollect past events with accuracy. Major events in the subject's life and cultural historical events are first identified and recorded. Subsequently, as

questionnaire data is collected (e.g., on employment, education, housing environment, and habits), the dates of personal and historical events in the subject's life are cross-referenced. Thus, the life grid helps the subject to recollect information more precisely by relating it to other past life events. Indeed, studies have highlighted the use of the life grid technique as a very effective method to collect retrospective data. For example, Berney&Blane say that "the life grid approach is extremely flexible and allows for the subject to determine the recall cues. The researcher can quickly identify those areas that assist the recall process whilst simultaneously developing rapport"[127]. In addition, the life grid has been shown to stimulate and organize the interviewees' memory[128].

4.4 Quality assurance and data management procedure

All the procedures in the project strictly followed the study protocol. An individual identification number was given to each subject in order to protect the subject's identity. After each interview, the RA verified the questionnaire and any discrepancy were clarified. To test the reliability of the collected information, re-interviews were conducted on 10% of the sample after 6-12 weeks. Log sheets were maintained separately for participating and non-participating cases and control subjects throughout the study.

The collected data was entered into a study database located on an online server using the specialized software 'FileMaker', and then the data was

exported for processing and analysis with the statistical software SPSS version 18. Before data analysis, data cleaning was completed and initial frequencies were performed.

4.5 Measures

4.5.1 Outcome variable

The outcome variable in our study was cancer of the oral cavity, which includes cancers in the inner side of the lips, tongue, lingual tonsils, gums, floor of mouth, hard palate, soft palate, uvula, buccal mucosa, vestibule of mouth, and retromolar area. The binary variable was coded into: (1) presence of the disease: cancer case, (0) absence of the disease: cancer-free control.

4.5.2 Main exposure variable

4.5.2.1 Periodontal status

Recent studies have shown an association between periodontal diseases and oral cancer. Periodontal status, the main exposure variable, was evaluated visually by qualified trained dentists at the oral pathology clinic of the Government Dental College. The dental exams were performed while the subjects were sitting down in a semi-supine position on a dental chair, using a halogen light and a mouth mirror. The dental examinations were performed in a standard order; starting from the upper right quadrant and finishing in the lower

right quadrant. First, the condition of each tooth was assessed and recorded as (0) sound, (1) cavity/decayed, (2) filled, or (3) missing tooth. Subsequently, the gingival tissues were evaluated. Using a dental mirror, both labial and lingual surfaces of all teeth were visually examined and the overall gingival status of the subject recorded. This included the presence or absence of gingival inflammation based on the alteration in the colour of the gingival tissue and on spontaneous bleeding. Variations in normal gingival colour (e.g., purplish black, reddish) and the intensity of pigmentation were also recorded. Similarly, the presence of debris, plaque, and calculus on tooth surfaces as well as tooth mobility were described. Moreover, the degree of gingival inflammation was recorded, from no sign of inflammation to severe gingival inflammation, the latter corresponding to the presence of marked redness and swelling. Finally, generalized and localized gingival recession was assessed visually by looking at the displacement of the soft tissue margin of the gingiva from the cement-enamel junction. Subsequently, this qualitative information was coded following standard criteria by one of the dentists (SH), based on three main indicators of periodontal diseases: presence of inflammation, recession, and reddish gingival colour. Each of these variables was coded into numeric values. Inflammation was coded as (0) no inflammation, (1) mild inflammation, (2) moderate inflammation, and (3) severe inflammation. Gingival recession was coded as (0) normal attachment and (1) generalized

gingival recession. Localized gingival recession might have occurred for reasons other than the presence of plaque or of a chronic infectious disease (e.g., crowding, trauma from occlusion). In order to address this issue and to avoid overestimating the disease condition, localized gingival recession was categorized as normal. Gingival colour other than normal variations is usually associated with inflammation; however for greater accuracy colour was recorded separately. This variable was coded as (0) normal colour (including all the normal variations) and (1) reddish gingiva (indicating a diseased condition).

The next step was to create a variable that combined the three assessments. The16 resulting combinations were grouped into 3categories: (1) normal gingiva, where the three indicators (colour, recession, inflammation) were considered as normal, (2) moderately compromised gingival health, this category comprised of all conditions other than normal gingiva, severe inflammation and generalized recession, and (3) severely compromised gingival health (includes all severe inflammatory conditions and all generalized gingival recession conditions). Table2 describes these combinations. It is important to notice that the last category, that is, severely compromised gingival health condition, included only severe inflammatory conditions and generalized gingival recession condition. This procedure reduced the chance of misclassification. This variable was used as the main exposure variable in the final analysis model.

Nevertheless, although we believe that the 3 categories variable depicted reasonably well the overall gingival status of the subjects; to further decrease potential misclassification we also created a dichotomous variable. This periodontal status variable had 2 categories: 1) normal gingival health and (2) compromised gingival health; the second category included only the generalized recession conditions, which is the best indicator of past history of periodontal diseases.

Finally, it is important to mention that to further minimize measurement error in this variable, all the steps in the coding process were discussed with the other dentists who participated in the study data collection. Similarly, the coding process was closely supervised by the study' principal investigators.

Table2: Description of the initial and final categories of the gingival health variable

Initial categories	**Combinations**			**Final categories**
	Colour	Recession	Inflammation	
Normal gingival health	**0**	**0**	**0**	Normal
Mildly compromised gingival health	**0**	**0**	**1**	Mildly compromised gingival health
	1	**0**	**1**	
	1	**0**	**0**	
	0	**0**	**2**	
	1	**0**	**2**	
Severely compromised gingival health	**0**	**0**	**3**	
	1	**0**	**3**	
	1	**1**	**0**	Severely compromised gingival health
	0	**1**	**0**	
	0	**1**	**1**	
	0	**1**	**3**	
	1	**1**	**3**	
	0	**1**	**2**	
	1	**1**	**2**	
	1	**1**	**1**	

4.5.3 Covariates

4.5.3.1 Poor dental hygiene

Poor dental hygiene and oral health status have been associated with an increased risk of cancer of the oral cavity[129]. In this study, the frequency of tooth brushing was used as an indicator of poor oral hygiene. Subjects were asked about the frequency of their oral hygiene behaviours and their answers were classified into the following categories: (0) never cleaned teeth, (1) less than once a week, (2) 1-2 times a week, (3) every other day, (4) once a day, and (5) twice or more a day. All the categories other than twice a day tooth brushing, which is considered a standard oral hygiene measure, were combined to form a new category indicating a lower frequency of cleaning. Thus the final variable included two categories: (1) brushing twice or more per day, and (2) brushing once a day or less.

4.5.3.2 DMFT index

Detailed DMFT observations were recorded for all teeth and values were assigned as: (0) sound tooth, (1) cavity/decayed tooth, (2) filled tooth, and (3) missing tooth. We included all the 32 teeth in this analysis (i.e., the 3rd molar on all the 4 quadrants was included). In this study, we first considered components of DMFT as 3 separate variables and we also conducted analyses with DMFT as a single variable. The total number of decayed, missing, and filled teeth was

counted for all the subjects, which resulted in 3 continuous variables. Then the DMFT index variable was created from the initial continuous variables. Decayed, missing, and filled components of the DMFT index were combined together by adding up the counts of the 3 components for each subject. This addition resulted in a continuous variable. We used both DMFT and missing teeth variables in our analysis.

4.5.3.3 Indicators of socioeconomic position

We used two indicators of socio-economic position in our analysis and they are described below.

4.5.3.3.1 Material deprivation index

Detailed information on housing condition tenure and amenities were collected over the 3 stages of life: childhood, early adulthood and late adulthood. A material deprivation index was created for each of these stages of life using 11 questions on housing tenure, house conditions(e.g., material used to construct the floor, roof and wall, drinking water source) and house amenities (e.g., clock, radio, car). Each of these items was coded as zero (high levels of material deprivation) and one (low levels of material deprivation) according to the presence or absence or the costs of the items (e.g., type of flooring: mud (low SEP) or ceramic (high SEP)). Following this coding, we created a continuous variable by adding the values of each question, so that the values of the new variable ranged from 0-11. Subsequently, using the median as the cut off point,

this continuous variable was categorized into high and low SEP. As mentioned previously, this procedure was performed for each period of life: childhood, early and late adulthood. Thus, our final indicator of life course material deprivation was created by permutation combination of the participant's level of deprivation in each period of life. These permutations produced 4 categories: 1) subjects who experienced high levels of material deprivation in all three stages of life, 2) subjects who experienced high levels of material deprivation in 2 stages and low levels of material deprivation in 1 stage of life, 3) subjects who experienced low levels of material deprivation in 2 stages and high levels of material deprivation in 1 stage of life, and 4) subjects who experienced low levels of material deprivation in all three stages of life. This variable was used in our final models.

4.5.3.3.2 Education

Information on subjects' educational background was collected as 5 dimensions. Whether or not subjects had attended school was recorded as (1) yes or (0) no; ability to read and write was recorded as (1) yes or (0) no; years of formal education was noted as a specific number of years successfully completed at school; the highest level of education was collected as 7 categories ranging from lower primary to post-graduate; and failed school years was recorded as 4 categories, from 0 to 3 times or more. Our study included subjects ranging in age from 24 to 89, and thus belonging to different birth cohorts.

Therefore, the level of education not only varied between subjects, the quality of education might also have changed over time, an influence usually referred to as a cohort effect[130]. Because pooling up all the data might introduce bias, in order to address this issue the continuous variable was converted into low and high levels of education. Based on evidence indicating that the education system drastically changed after 1950 throughout India, the year 1950 was used as a cutoff point to categorize subjects into cohorts[131]. As recruitment for this study was completed in 2012, subjects were divided into 2 groups based on their age: group 1 included subjects 62 years of age or older (i.e., those born in 1950 and earlier), and group 2 included subjects below the age of 62 (i.e., those born after 1950). For group 1, 4 years or more of education was considered as a high level of education, and below 4 years was considered as a low level of education. For group 2, an education of 8 years and above was considered as a high level of education and below 8 years was considered as a low level of education.

4.5.3.4 Tobacco smoking

Tobacco smoking is one of the major risk factors associated with oral cancer. Detailed data was collected separately for each of the different forms of tobacco smoking. Data for cigarette smoking included information about age at initiation of smoking and at cessation, as well as ages when changes in the amount or brand of cigarette smoked occurred, quantity of tobacco smoked

(recorded as number of cigarettes smoked in a day, a week or a month), brand of cigarette smoked, and type (filter tipped or non-filter tipped). Similar information was collected for beedi. However, type and brand was not relevant for beedi as it is always produced without a filter and a single brand is available.

Using the collected information, a new analysis variable known as pack-year was created to represent the intensity of tobacco smoked (cigarette and beedi) by a subject in his/her lifetime. A pack-year is equal to the consumption of 10 cigarettes (1 pack) per day for 1 year or 2 packs per day for half a year. The pack-year variable is calculated by multiplying the number of packs of cigarettes smoked per day by the number of years smoked[132]. For this calculation, initially the duration of smoking (years as a smoker) was calculated by subtracting the age at initiation of smoking from the age at cessation (or age at interview if the subject was a current smoker). Then the number of cigarettes or beedi smoked (per day, week, or year) was converted to the number of cigarettes or beedi smoked per day. The next step was to convert the number of cigarettes or beedi smoked per day to the number of packs smoked per day, which was done by dividing the total number of cigarettes or beedi smoked in a day by the number of cigarettes or beedi in a pack. In India, a standard cigarette pack contains 10 cigarettes, and a single beedi pack contains 20 beedi. Thus, the number of cigarettes smoked in a day was divided by 10 and the number of

beedi smoked in a day was divided by 20. Then the number of packs smoked in a day was multiplied by years as a smoker.

To better understand this complex yet standard variable in the health epidemiology literature, let us consider an example. Consider a man who started smoking at age 20 and stopped at the age of 50. The number of years as a smoker for this man will be 30. And if he smoked 10 cigarettes a day, the total packs of cigarettes smoked in a day will be 1. By multiplying packs per day by the number of years as a smoker, this person will have 30 pack-years, that is: 30 multiplied by 1 = 30. For subjects who have not smoked continuously or who have experienced changes in the amounts or brands smoked, the life span smoking of cigarettes or beedi is cumulative, therefore lifetime pack-years are calculated by adding up the pack-years in each smoking period.

Finally, pack-years for both cigarette and beedi smoked were classified into 3 categories (never smokers, light smokers, and heavy smokers) based on the distribution of cigarette and beedi use among control subjects. In the final analysis model, both cigarette and beedi smoking were used as a continuous variable.

4.5.3.5 Betel quid chewing

In India, betel quid chewing is one of the major etiologic factors associated with the risk of oral cancer. Betel quid consists of 4 major ingredients, which each

has a different carcinogenic property. In addition, use of these ingredients varies among individuals. The data collected on this variable included information relating to the age of initiation and cessation of chewing, the type of quid chewed collected as 9 categories (tobacco, betel quid with tobacco, betel quid without tobacco, areca nut with tobacco, areca nut without tobacco, pan masala, betel leaf, and others), the duration of chewing (the length of time one holds the quid in the mouth), and the quantity consumed (recorded per day, week, or month). We used the data collected to calculate the total number of minutes of chewing by a subject in his/her lifetime. This variable was subsequently converted to the total number of days of chewing per year in his/her life by dividing the total number of minutes of chewing by the total number of minutes in a day (1440 minutes). This variable was used as a continuous variable in the final analysis model.

4.5.3.6 Alcohol consumption

Data related to alcohol consumption was collected on type of beverage (toddy, wine, beer, hard liquor, and other), age at the start of alcohol consumption and age at which drinking stopped, unit of drinking (small glass, i.e. 50ml, medium glass 100ml, big glass 250ml, small bottle 330ml, bottle 700-750 ml), and quantity of drinks consumed (per day, week, or month). Based on the ethanol content of alcoholic drinks (5% for beer, 8.1-10% for toddy and wine, and 50% for hard liquor) available in Kerala, lifetime ethanol consumption by a

subject was calculated[133, 134]. In order to standardize the ethanol consumption to that of a standard alcoholic drink, the total ethanol consumption of each subject was divided by the ethanol content in a standard drink. In different parts of the world, a standard alcoholic drink varies in its ethanol content, and ranges from 13 to 28 grams[135]. In this study, we standardized the ethanol content by dividing the total ethanol consumption by 18. This standardized ethanol consumption amount was used to calculate the number of standard drinks consumed per year, which was achieved by dividing the total ethanol consumption by the total drinking period of a person. The number of drinks per week was calculated by dividing the standard drinks per year by 52. This weekly consumption variable was also considered as a continuous variable in the final analysis model.

4.5.3.7 Diet

This study collected detailed information on dietary habits from childhood and adult life. Information regarding subjects' adult dietary habits was collected from 2 years prior to their disease diagnosis. Data pertaining to the consumption of bananas, citrus fruits (e.g., orange, lemon), other fruits (e.g., apple, mango, papaya), vegetables (cabbage, cauliflower, tomatoes, carrots), spinach, meat (red meat, white meat, and fish), dairy products, and cereals were collected. Subjects were also asked how frequently they consumed fruits and vegetables in

a week. For this study, only consumption of fruits and vegetables was considered. Two continuous variables were created for analysis by adding up the frequencies of individual fruits and vegetables categories. These variables were then classified into two groups based on the consumption distribution of the control subjects. Fruit consumption was categorized as (1) 0-2 servings and (2) more than 2 servings per week, and vegetable consumption was categorized as (1) less than 13 servings and (2) 13 or more servings per week.

4.6. Statistical analysis

4.6.1 Descriptive statistics

In order to enable the presentation and interpretation of basic features of the collected data, descriptive statistics were performed. We calculated the mean and standard deviation of a continuous variable (e.g., age) to present the socio demographic characteristics among case and control subjects. For the categorical variables (e.g., sex, proxy, education, periodontal health, beedi smoking, cigarette smoking, paan chewing, alcohol consumption, DMFT index, tooth brushing frequency), we performed cross tabulations.

4.6.2 Logistic regression

The next step in this analysis was to explain the association between the main exposure variable (periodontal diseases) and the outcome variable (oral cancer). In order to do this, we performed a logistic regression analysis. Logistic

regression is regularly used in epidemiology to measure the association of a binary outcome variable to an explanatory variable.

As explained in section 4.5.2.1, we coded our main exposure variable periodontal diseases in two ways: 3 categories (normal, mild and severe periodontal diseases) and 2 categories (normal and mild together, and severe). All our models were tested using both variables but we present final results with the 3 category variable. The only exception is the site specific analysis where we used our main exposure variable as a binary variable. This procedure was performed to decrease the number of parameters in our models.

4.6.3 Building the logistic model

In order to evaluate the association between periodontal diseases and oral cancer, we conducted a logistic regression analysis. First, we evaluated the association between poor periodontal health, behavioural habit variables (cigarette and beedi smoking, paan chewing, alcohol consumption), education, and life course material deprivation, other oral health indicators (missing teeth and brushing frequency) fruit and vegetable consumption and oral cancer risk adjusting for age and sex. Then, based on AIC (Akaike information criterion), the model with the best fit, was identified. This model included age, education, material deprivation indicators, behavioural habits and oral health indicators. Adding diet into the equation reduced the fitness of the model. Furthermore, it did

not show a significant change in the results compared to the previous analysis, so we decided to remove this variable in further analysis. This model was used in all subsequent analysis. As the risk profile of males and females in our sample were very different, that is, very few females smoked either cigarette or beedi or drank alcohol, we carried out further analysis stratifying by sex.

Since smoking is an established risk factor for periodontal diseases, we decided to test for an interaction between periodontal diseases and smoking in the risk of oral cancer. Tests for interaction were based on the P (two-sided) of the likelihood ratio test for adding the interaction term (periodontal diseases by smoking status) to the model that included the main effect variable (periodontal diseases). A statistically significant interaction was observed. Thus, further analysis was stratified by smoking habits.

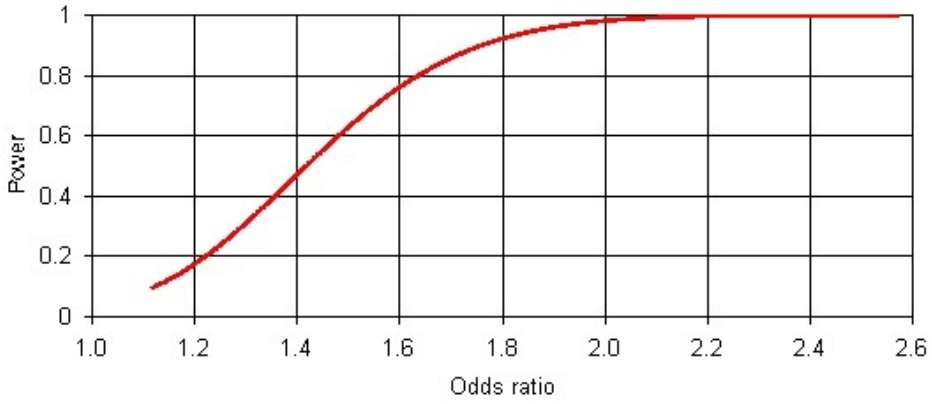
Finally, we assessed the difference in the risk profile for oral cancer among anatomical sub-sites. In order to do this, we performed a regression analysis with the same variables as in the previous model stratified according to the anatomical sub-sites in the oral cavity (tongue and floor of mouth, gum, buccal mucosa, palate and tonsil).

4.7 Sample size calculation

The collected data used for this calculation included 293 oral cancer patients (cases) and 314 non-oral cancer subjects (controls). We performed a

post-hoc power calculation using the probability of exposure among controls for our main exposure variable, gingival health. The proportion of controls with a severely compromized periodontal health conditionis 23.5%, therefore if the true odds ratio for severe periodontal disease in exposed subjects relative to unexposed subjects is 1.70, we can reject the null hupothesis that this odds ratio equals 1 with a probability (power) of 85.6% (Figure3). The type 1 error probability associated with this test is 0.05.

Figure3: Statistical power analyses based on the sample (n=640), for ORs and according to the proportion of exposure among controls for the main exposure variable.



4.8 Ethics

Prior to the start of data collection, the ethical committees of both participating hospitals, the Government Dental College and the Government

Medical College and Hospital, Calicut, Kerala, India, approved the study. The Institutional review board (IRB) of McGill University, Montreal, Canada, also approved this study.

Chapter 5 Results

5.1 Characteristics of the sample

In this hospital-based study, we recruited a total of 350 squamous cell carcinoma cases of the oral cavity and 371 matched controls. In this analysis, 114 subjects (57 (16.2%) cases and 57 (15.3%) controls) were excluded for various reasons. We excluded 81 subjects that were edentulous (37 cases and 43 controls) and excluded 1 case because the oral examination details were not available. In addition, 33 subjects (19 cases and 14 controls) were excluded because their socioeconomic measurements were not available.

5.2. Distribution of cases according to clinical and histological characteristics

The majority of cases (41.6%) reported to the clinic with lesions located on the buccal mucosa (ICD coding 'C06' other and unspecified parts of mouth). In 29.7% of the cases, the lesions were located on gums, followed by 16.4% on the base of the tongue and other unspecified parts of the tongue, 5.8% on the floor of the mouth, 4.8% on the palate and 1.7% on the tonsil.Table3 contains further details on the distribution of oral cancer cases according to anatomical sub-sites.

As the number of subjects in several of these categories was low for the site-specific analysis, we first combined the numbers for the tongue and the floor of mouth, as well as the palate and the tonsil. Our final case distribution

percentages are for the buccal mucosa (41.6%), gum (29.7 %), tongue and floor of mouth (22.2%), and the palate and tonsil (6.5%).

According to TNM clinical staging, 29.4% of the cases had a tumour size (T) between 2 and 4 centimeters, whereas 23.5% of the cases had T4 tumours that invaded the cortical bone and other deeper structures. Judging within the 'N' component (lymph node metastasis), 76.8% of the cases reported some sort of lymph node metastasis, with 65.2% of the cases reporting metastasis in a single ipsilateral lymph node. For the majority of these cases, diagnosis occurred during later TNM stages, 52.2% were diagnosed at stage III and 29.0% were diagnosed at stage IV. Table4 presents the distribution of oral cancer cases according to clinical characteristics.

Control subjects were randomly selected from several outpatient clinics of participating hospitals. Based on the causes of hospitalization (main diagnosis), they were grouped into 13 diagnostic categories which included the ICD 10 main diagnostic groups: A00-B99, C00-D48, E00-E90, G00-G99, H00-H59, H60-H95, J00-J99, K00-K93, L00-L99, M00-M99, N00-N99, R00-R99 and S00-T98. Diseases related to the digestive system (ICD10 - K00-K93) was the main diagnosis of 21.3% of the control subjects, whereas 19.4% of subjects had diseases relating to the genitourinary system (ICD10 - N00-N99) and 13.4% of subjects were diagnosed with diseases of the eye and adnexa (ICD10 - H00-

H59). Table5 describes the distribution of admission conditions amongst hospital controls.

Table3: Distribution of oral cancer cases according to anatomical sub-sites

ICD	Topographic site	N	%
C01- C02	Base of the tongue and Other unspecified parts of the tongue	48	16.4
C03	Gums	87	29.7
C04	Floor of mouth	17	5.8
C05	Palate	14	4.8
C06	Other & unspecified parts of mouth	122	41.6
C07	Tonsil	5	1.7

Table4: Distribution of oral cancer cases according to clinical characteristics

Variable	Categories	N	%
T classification	T1	61	20.8
	T2	86	29.4
	T3	77	26.3
	T4	69	23.5
N classification	N0	68	23.2
	N1	191	65.2
	N2	32	10.9
	N3	2	0.7
TMN stage	Stage I	29	9.9
	Stage II	26	8.9
	Stage III	153	52.2
	Stage IV	85	29.0

Table5: Distribution of admission conditions among hospital controls

ICD- Main diagnostic group	Description	N	%
Certain infectious and parasitic diseases (A00-B99)	Intestinal infectious diseases, Arthropod-borne viral fevers and viral haemorrhage, Mycoses, Pediculosis, acariasis and other infestations	12	3.8
Neoplasms (C00-D48)	Fibroid in uterus	5	1.6
Endocrine, nutritional and metabolic diseases(E00-E90)	Disorders of other endocrine glands, Metabolic disorders, Disorders of thyroid gland	3	1.0
Diseases of the nervous system (G00-	Nerve, nerve root and plexus disorders, Other disorders of the nervous system	3	1.0

G99)			
Diseases of the eye and adnexa (H00-H59)	Disorders of vitreous body and globe, Disorders of sclera, cornea, iris and ciliary body, Visual disturbances and blindness, Disorders of optic nerve and visual pathways, Disorders of ocular muscles, binocular movement, Disorders of lens, Glaucoma, Disorders of conjunctiva, Disorders of choroid and retina	42	13.4
Diseases of the ear and mastoid process (H60-H95)	Other disorders of ear, Diseases of middle ear and mastoid, Diseases of inner ear	20	6.4
Diseases of the respiratory system (J00-J99)	Acute upper respiratory infections, Other diseases of upper respiratory tract	13	4.1
Diseases of the digestive system (K00-K93)	Diseases of oral cavity, salivary glands and jaws, Other diseases of intestines, Diseases of oesophagus, stomach and duodenum, Hernia, Noninfective enteritis and colitis, Diseases of liver	67	21.3
Diseases of the skin and subcutaneous tissue (L00-L99)	Dermatitis and eczema, Other disorders of the skin and subcutaneous tissue, Urticaria and erythema, Disorders of skin appendages	28	8.9
Diseases of the musculoskeletal system and connective tissue (M00-M99)	Arthropathies, Soft tissue disorders, Dorsopathies	29	9.2
Diseases of the genitourinary system (N00-N99)	Other diseases of urinary system, Noninflammatory disorders of female genital tract, Glomerular diseases, Inflammatory diseases of female pelvic organs, Urolithiasis, Glomerular diseases, Renal tubulo-interstitial diseases, Renal failure, Diseases of male genital organs, Disorders of breast	61	19.4
Symptoms, signs and abnormal clinical and laboratory findings, not elsewhere classified (R00-R99)	Symptoms and signs involving the urinary system, Symptoms and signs involving cognition, perception, Symptoms and signs involving the digestive system, General symptoms and signs, Abnormal findings on examination of urine, without, Symptoms and signs involving the nervous and musculoskeletal systems	21	6.7
Injury, poisoning and certain other consequences of external causes (S00-T98)	Other and unspecified effects of external causes, Complications of surgical and medical care, Injuries to the head, Injuries to unspecified part of trunk, limb or body, Injuries to the knee and lower leg, Injuries to the elbow and forearm, Injuries to the wrist and hand	10	3.2

5.3. Distribution of cases and controls according to study variables

Table6 displays the sample characteristics according to sex amongst cases and control subjects. The distribution of ages was designed to be similar amongst cases and controls for both sexes. Among male cases, the subjects'

age varied from 35 to 87 years, while the age of male controls varied from 35 to 80. The age distribution was 32 to 85 and 33 to 88 among female cases and controls, respectively. The majority of subjects from both sexes were 60 years or older (56.4% of males and 59.2% of females). A major portion of the cases (66.1% of males and 93.0 % of females) had a low education status compared to the control subjects (44.7% of males and 53.5% of females). In addition, more female cases (18.0%) asked for assistance in answering the interview questions compared to 9.7% of male cases.

An overview of the distribution of behavioural factors is also presented in Table6. As expected, both the prevalence and the intensity of the betel quid (also known as paan) chewing habit were higher among cases of both sexes (64.3% vs.16.4% among males, 85.2% vs. 20.8% among females). In addition, cases from both sexes had a higher number of mean chewing days per year (604.36 vs. 71.86 among males, 768.17vs.148.25 among females). Other behavioural habits such as bidi smoking, cigarette smoking and alcohol consumption were more prevalent among male cases. The proportion of bidi smokers was 65.4% among male cases vs. 3.1% among female cases. The numbers for cigarette smoking showed 51.5% among male cases compared to 1.6% among female cases, while the numbers for alcohol consumption showed 52.2% among male cases compared to 0.8% among female cases.

Among males, cases had a higher proportion of subjects with a bidi smoking habit compared to controls (65.4% vs. 45.3%). Further observations of the cumulative and more comprehensive measurements of smoking habits in males revealed that the cases possessed a bidi-smoking habit (mean pack-years) of almost twice the intensity as the controls (15.53 vs. 7.56). A similar ratio was observed among males for alcohol habits. On average, the cases drank more than controls (52.1% vs. 33.6%). The mean number of drinks per week was also much higher among the cases compared to the controls. However, the habit of smoking cigarettes was more commonly found amongst the male controls compared to the cases (51.5% of cases vs. 62.4% of controls). We also found a marked difference in fruit and vegetable consumption between cases and controls in both sexes. On average, the cases consumed lower amounts of vegetables and fruits compared to the controls.

The distribution of indicators of oral health such as missing teeth and brushing frequency was also very different amongst the cases and controls of both sexes. The cases brushed their teeth less often (once a day) than controls, with 82.4% of males and 74.2% of females reporting this fact. Missing teeth were also more common amongst cases of both sexes. Similarly, the cases had more advanced periodontal diseases compared to controls (males 28.5% vs. 10%, females 28.9% vs. 11.1%).

5.4 Odds ratio of oral cancer according to periodontal status

Table7 displays the association between periodontal diseases and oral cancer risk according to sex. In both univariate and multivariate analyses, severe periodontal disease conditions were significantly associated with the risk of oral cancer among males and this association persisted after adjusting for confounders (age, sex, beedi and cigarette smoking, alcohol consumption, paan chewing, level of education, life course material deprivation, oral hygiene and missing teeth). Males who had severe periodontal diseases were more likely to have oral cancer compared to those who had normal periodontium (OR=2.53; 95% CI: 1.15–5.56).

Since smoking habits are a well-established risk factor for periodontal diseases, we tested for an interaction between smoking habits and periodontal diseases among males. The interaction was statistically significant (p=0.001), therefore our analysis was subsequently stratified by smoking status. Amongst male smokers, those with severe periodontal diseases had an increased risk of oral cancer compared to those with normal periodontium (OR=2.74; 95% CI: 1.16–6.47). The association between periodontal diseases and oral cancer amongst non-smokers was not apparent (OR=1.69; 95%CI: 0.21–13.33), however we had very few subjects in this group, as shown in Table8.

Table9 displays the association of oral cancer with periodontal diseases according to tumour site and sex. When the analysis was stratified for specific oral cancer sub-sites (including the tongue and floor of mouth, gum, buccal mucosa, palate and tonsil), males with gum cancer showed an increase association between periodontal disease and gum cancer than males with cancer in other sub-sites (OR=3.35; 95% CI: 1.39-8.03). However, we did not observe similar associations among women. Due to low numbers for some of the oral cancer sub-sites, the analysis was performed with dichotomous variables.

Table6:Characteristics of study subjects, Calicut India, 2008-2012

	Males				Females			
	Cases(N=165)		Controls (N=170)		Cases(N=128)		Controls(N=144)	
Variable	**N**	**%**	**N**	**%**	N	%	N	%
Age in years(Mean ± SD)	59.70±10.94		59.01±10.79		60.02±11.30		59.97±11.98	
Respondent type								
Use of proxy	16	(9.7)	2	(1.2)	23	(18.0)	8	(5.6)
No use of proxy	149	(90.3)	168	(98.8)	105	(82.0)	136	(94.4)
Education								
Low	109	(66.1)	76	(44.7)	119	(93.0)	77	(53.5)
High	56	(33.9)	94	(55.3)	9	(7.0)	67	(46.5)
Cigarette smoking pack-year (Mean ± SD)	9.91±19.47		16.71±30.79		0.02±0.17		0.79±9.29	
Never smoked	80	(48.5)	64	(37.6)	126	(98.4)	142	(98.6)
Moderate smoker	55	(33.3)	52	(30.6)	2	(1.6)	0	(0)
Heavy smoker	30	(18.2)	54	(31.8)	0	(0)	2	(1.4)
Bidi smoking pack-year (Mean ± SD)	15.53±20.97		7.56±16.44		0.12±0.97		0.10±0.88	
Never smoked	57	(34.5)	93	(54.7)	124	(96.9)	142	(98.6)
Moderate smoker	36	(21.8)	39	(22.9)	3	(2.3)	1	(0.7)
Heavy smoker	72	(43.6)	38	(22.4)	1	(0.8)	1	(0.7)
Paan chewing days per year (Mean ± SD)	604.36±955.58		71.86±272.66		768.17±994.39		148.25±438.75	
Never chewer	59	(35.8)	142	(83.5)	19	(14.8)	114	(79.2)
Moderate chewer	13	(7.9)	14	(8.2)	33	(25.8)	15	(10.4)
Heavy chewer	93	(56.4)	14	(8.2)	76	(59.4)	15	(10.4)
Alcohol consumption per week (Mean ± SD)	32.65±262.46		2.68±10.19		0.06±0.63		0.07±0.81	
Never drinker	79	(47.9)	113	(66.5)	127	(99.2)	142	(98.6)
Medium drinker	23	(13.9)	28	(16.5)	0	(0)	1	(0.7)
Heavy drinker	63	(38.2)	29	(17.1)	1	(0.8)	1	(0.7)

Vegetable consumption								
<=13 servings per week	95	(57.6)	91	(53.5)	80	(62.5)	73	(50.7)
>13servings per week	70	(42.4)	79	(46.5)	48	(37.5)	71	(49.3)
Fruit consumption								
0-2 servings per week	130	(78.8)	122	(71.8)	110	(85.9)	111	(77.1)
>2 servings per week	35	(21.2)	48	(28.2)	18	(14.1)	33	(22.9)
Missing teeth(Mean ± SD)	8.96±8.12		6.66±7.88		10.01±8.53		7.63±6.89	
Brushing frequency								
Once a day	136	(82.4)	85	(50.0)	95	(74.2)	71	(49.3)
2 or more per day	29	(17.6)	85	(50.0)	33	(25.8)	73	(50.7)
Periodontal health								
Normal	58	(35.2)	99	(58.2)	39	(30.5)	82	(56.9)
Mild disease	60	(36.4)	54	(31.8)	52	(40.6)	46	(31.9)
Severe disease	47	(28.5)	17	(10.0)	37	(28.9)	16	(11.1)

Table7: Association of oral cancer with periodontal diseases according to sex, Calicut India, 2008-2012

	Males (N=335)			Females (N=272)		
Variable	**N_{con}**	**N_{case}**	**OR(95%CI)**	**N_{con}**	**N_{case}**	**OR(95%CI)**
Total oral cancer cases	**170**	**165**		**144**	**128**	
Periodontal health						
Normal	99	58	Reference	82	39	Reference
Mild disease	54	60	1.26(0.69 – 2.31)	46	52	1.98(0.94 - 4.18)
Severe disease	17	47	2.53(1.15 – 5.56)	16	37	1.64(0.67 - 4.03)

Table8: Association of periodontal diseases and oral cancer among males, stratified by smoking status, Calicut India, 2008-2012

	Smokers (N=261)			Non-smokers (N=74)		
Variable	**N_{con}**	**N_{case}**	**OR(95%CI)**	**N_{con}**	**N_{case}**	**OR(95%CI)**
Periodontal health	**130**	**131**		**39**	**35**	
Normal	41	72	Reference	27	17	Reference
Mild disease	50	45	1.34(0.70 – 2.57)	9	10	0.76(0.13 – 4.31)
Severe disease	39	14	2.74(1.16 – 6.47)	3	8	1.69(0.21 – 13.33)

Table9: Association of oral cancer with periodontal diseases according to sex and tumour site, Calicut India, 2008-2012

	Males (N=360)			Females (N=280)		
Variable	**N_{con}**	**N_{case}**	**OR(95%CI)**	**N_{con}**	**N_{case}**	**OR(95%CI)**
Total oral cancer cases	**181**	**179**		**147**	**133**	
Periodontal health						
Normal	136	95	Reference	115	69	Reference
Severely compromised	45	84	1.93(1.14 – 3.28)	32	64	1.27(0.65- 2.48)
Tongue & FOM	**181**	**51**		**147**	**20**	
Periodontal health						
Normal	136	34	Reference	115	14	Reference
Severely compromised	45	17	1.39(0.64-3.02)	32	6	0.81(0.25-2.64)
Gums	**181**	**43**		**147**	**50**	
Periodontal health						
Normal	136	18	Reference	115	20	Reference
Severely compromised	45	25	3.35(1.39-8.03)	32	30	1.47(0.60-3.63)
Buccal mucosa	**181**	**70**		**147**	**57**	
Periodontal health						
Normal	136	37	Reference	115	30	Reference
Severely compromised	45	33	1.97(0.96-4.08)	32	27	1.11 (0.48-2.58)
Palate & Tonsil	**181**	**15**		**147**	**6**	
Periodontal health			--			--
Normal	136	6	--	115	5	--
Severely compromised	45	9	--	32	1	--

Chapter 6 Discussion

Although previous studies have been inconsistent, they have suggested an association between poor periodontal conditions and the risk of various cancers [11, 13, 51, 52, 136]. Our results show that - independent of other risk factors - poor periodontal health conditions increased the risk of oral cancer in males (OR=2.53; 95% CI: 1.15–5.56) but not in females (OR=1.64; 95% CI: 0.67-4.03). Additionally, a stratified analysis according to smoking status showed that severe periodontal diseases increased the risk of oral cancer, but only among smokers (OR=2.74; 95% CI:1.16 – 6.47). When we analyzed the data according to anatomical sub-site, males with severe periodontal diseases had an increased risk of cancer of the gum (OR=3.35; 95% CI: 1.39-8.03). We did not observe any similar associations in other sub-sites, nor did we observe any similar associations in females.

There are two possible interpretations of these findings. On the one hand, we can hypothesize that periodontal diseases are a risk factor for oral cancer. On the other hand, we can hypothesize that periodontal diseases simply capture an unmeasured aspect of an individual's smoking history and thereby induces spurious associations between periodontal diseases and the risk of oral cancer. However, our results show that periodontal disease was a risk factor for oral

cancer only among male smokers. This may indicate a synergistic effect that is similar to results of previous studies investigating these associations [137, 138].

In this study, histories of periodontal diseases were self-reported, and measurement errors due to inaccurate reporting could have occurred. However, the visual assessment was performed by trained dentists, which decreases the chance of misclassification. In an attempt to decrease the odds of misclassification, we only included subjects who had severe inflammation and generalized recession within the category of subjects with severe periodontal disease.

Another potential limitation, inherent to case-control designs in which exposures and outcomes are assessed concurrently, is the ability to establish whether our main exposure variable (periodontal disease) occurred before the outcome variable (oral cancer). It could be argued that periodontal disease occurrence was due to the presence of oral cancer. However, the positive associations observed with severe periodontal diseases, which include only cases with generalized recession, make this explanation less plausible, since generalized recession is a well-established marker of past history of periodontal diseases. The most commonly used clinical measures of periodontal diseases are (i) clinical attachment loss, which combines recession and probe depth, and it is highly correlated with alveolar bone loss[139-141] and; (ii) probing depth,

which does not reflect history of periodontitis accurately because it can be reduced with treatment[142, 143]. Indeed, clinical attachment loss, of which recession is a large part, represents reasonably well the history of periodontitis regardless of treatment [141, 144]. In addition, it is unlikely that reverse causality would explain these associations. Not only is the magnitude of the association between periodontal diseases and oral cancer high in the gingival cancer sub-site, but previous literature also suggests that inflammation plays an important role in oral cancer aetiology [145, 146]. Finally, this study's use of the life grid technique assisted in reducing measurement errors in the assessment of covariates included in this analysis.

The low response rate among patients approached as potential control subjects was an additional limitation of this study. However, the low response rate is unlikely to affect the validity of the study as the distribution of the main variables investigated in this study (e.g., periodontal health, socioeconomic status, smoking and paan chewing habits) was similar to those in previous studies[8, 147, 148]. Moreover, we did not observe any statistically significant differences in the ages and sexes of participating and non-participating control subjects.

Several mechanisms could potentially explain the associations observed in this study. Inflammation appears to play an important role in oral cancer

aetiology [149, 150], although the inflammatory mediators that lead to the development of oral cancer remain poorly defined. An association between periodontal diseases and systemic inflammation has been observed using biomarkers[151, 152].

Periodontal diseases are chronic infectious diseases resulting from a marked overgrowth of bacteria; certain viruses and a poor immune response to chronic infections play a role in the onset of advancing periodontal disease[40, 153]. It often progresses as a painless lesion resulting in the destruction of tooth-supporting structures, along with low-grade systemic inflammation and an elevated level of circulating inflammatory markers[152]. The inflammatory cells and a susceptible immune system contribute to the proliferation of cancer cells[145]. The process of how chronic infections and inflammations result in carcinogenesis is intricate. Initial evidence of the relationship between inflammation and cancer was reported decades ago, and as cancers became increasingly attributed to infections, scientists began to focus on explaining the complex initiation of cancer cells by inflammation. Recent literature has documented this hypothesis extensively [9, 145, 146].

The association of H. pylori with gastric cancer is well established, and a recent demonstration involving H. pylori, Prevotellamelaninogenica, Staphylococcus aureus, and other bacterial taxa - along with certain viruses such

as HPV - suggests that microorganisms and their products play a direct role in cancer causation amongst oral cancer patients[12, 13, 154]. Guided by previous studies, Tezal et al. developed a hypothetical model. Microorganisms and their products - such as endotoxins (Lipopolysaccharides complex associated with the Gram-negative pathogens), enzymes, and metabolic byproducts - are toxic in nature and they can directly obstruct the normal apoptosis process by causing metamorphosis in tumour suppressor genes and anti-oncogenes, or they can cause a variation in cellular signaling mechanisms. These changes are characterized as tumour promoters and play a direct role in the process of carcinogenesis [11, 155].

Carcinogenesis is a multistep process that involves initiation, promotion and progression. Each step in the process is governed by multiple factors and a single exposure to an initiator can result in cancer if it is followed by frequent exposure to a promoter [150, 156].

Different cells can accumulate somatic mutations as a result of exposure to trace amounts of carcinogens, while the presence of inflammation acts as a promoter[150]. The microorganisms and their products present in chronic periodontal inflammation can promote previously mutated cells, resulting in alterations in growth control and carcinogenesis. From this foundation, Tezal et al. proposed another indirect mechanism by which chronic infections stimulate

growth of tumors originated from epithelium by a method of initiation of surrounding inflammatory cells. Persistent infections within the host stimulate chronic inflammation and epithelial cells are therefore exposed to elements with carcinogenic properties. The microbes and their byproducts in the inflammation process activate granulocytes, macrophages, monocytes, lymphocytes, and mast cells, thereby producing an assorted collection of reactive oxygen species (peroxides and oxygen ions), reactive nitrogen species and membrane-perforating agents. Persistent aberration of these chemically reactive molecules can cause more damage to DNA in epithelial cells while the microorganisms and their byproducts can produce cytokines and chemokines, creating a favorable environment for proliferation, migration and inhibition of cell death[11, 48, 152, 157-159]. Different authors have discussed various hypotheses relating inflammation, innate immunity and cancer. While some of them are widely known, the molecular and cellular mechanisms in many of these hypothetical models are unexplained and require further extensive study.

6.1 Conclusion

To this date, only a limited number of studies have reported the association of periodontal diseases with oral cancer. Within the limitations of our own study, we observed a significant positive association between chronic periodontal diseases and an increased risk of oral cancer in an Indian population.

This association persisted despite tight controls for other major risk factors such as smoking and paan chewing. Considering the measurement limitations and the fact that we have no way of knowing the temporal details of this association, this connection should be further tested with a larger sample and the proper tools for measuring periodontal diseases. Several hypothetical models were proposed to justify the role of chronic infection and inflammation in the process of carcinogenesis. However, none of them could successfully explain the complexity of this association. Properly explaining these pathways and identifying the interplay of different microorganisms will help to develop improved methods of clinical intervention.

India is a developing country and over half of its population lives in rural communities, where access to health care and awareness of proper oral care are limited. This reveals the importance of implementing various primary preventive measures at a public level. The poor oral hygiene that leads to the accumulation of plaque and calculus is strongly correlated with periodontal diseases and the data suggests that over half of the participating subjects lacked proper oral hygiene measures. Proper health education, regular oral prophylaxis and the promotion of research will help to tackle oral cancer. A collective effort from the community and professional participation, along with progressive politics, can make this a reality.

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