TITLE: Periodontitis, oral hygiene habits and risk of upper aero-digestive tract cancers: a case-control study in Maharashtra, India

Running Title: Periodontitis and risk of upper aero-digestive tract cancers

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Statement of Clinical Relevance:

If further studies confirm the linkage between periodontitis and oral hygiene habits and upper aero-digestive tract (UADT) cancers, it may have implications for modifications of present

preventive strategies for UADT cancer where current predominant focus on tobacco and alcohol may need to be modified in form of promotion of oral health measures required to control periodontitis.

ABSTRACT

Aim

Chronic destructive periodontitis, a cause of systemic inflammation, affects some 10-15 per cent of adults across the globe, with severity of disease increasing with age. The aim of the study was to explore a relationship between periodontitis and oral hygiene habits and upper aero-digestive tract (UADT) cancers.

Materials and Methods

A case-control study was conducted with 240 UADT cancer cases and 240 controls matched by gender and age (≥5 years) from two different hospitals in Pune, India. In-person interviews, and intra-oral examinations were conducted for all subjects.

Results

Severe periodontitis and more than five missing teeth were associated with a significant risk of UDAT cancers (adjusted OR=2.25; 95% CI:1.12-4.91 and adjusted OR=3.28; 95% CI: 1.95-5.49). Amongst the self-reported oral hygiene habits, dental check-ups only at time of pain was associated with an elevated risk for UADT cancers (adjusted OR=4.12; 95%CI: 2.63-6.47). Topical application of mishri on gums and tooth brushing frequency less than once daily were also associated with elevated risk (adjusted OR=3.06; 95%CI: 1.75-5.35 and adjusted OR 2.09; 95%CI: 1.27-3.45 respectively). Furthermore, the habit of ever-chewing tobacco was associated with elevated risk of severe periodontitis.

Conclusions

Severe periodontitis is associated with elevated risk for UADT cancers and chewing tobacco strengthens this association in this population.

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Introduction

Upper aero-digestive tract cancers which consists of the following subsites: lip, oral cavity, oropharynx, hypopharynx, larynx and oesophagus, together contribute to be the ninth most common cancer site globally, accounting for 1,277,815 cases and 866,729 deaths per annum.[1,2] These cancers usually present at a later stage, and progress rapidly, resulting in death within a few years of initial diagnosis, as achieving local control at the cancer site is extremely difficult with late presentation.[3-5] Exposure to tobacco, areca nut and alcohol, remain the major risk factors, along with persistent infection by "high risk" genotypes of human papillomavirus (HPV) in a substantial subset of cases. Emerging risk factors include chronic oral infections, especially periodontitis, poor oral hygiene and inadequate personal and professional dental care. Consumption of fruits and cruciferous vegetables are established protective factors against UADT cancers.[3] Malignant neoplasms of the nasopharynx and of salivary glands are excluded in our description of UADT neoplasms as these have different biology and risk factors.[6, 7]

Periodontitis (chronic and destructive of the periodontium) is characterised by chronic inflammation of both the soft tissues of the attachment apparatus and of alveolar bone.[8] It is caused by the metabolic activity of the total microbiota of the subgingival biofilm.[9-11] In addition, periodontal pockets may act as reservoirs for HPV, and Human herpesviruses including cytomegalovirus and Epstein-Barr virus, which have been associated with oral cancer, the commonest UADT cancer site.[12, 13] High levels of inflammatory markers associated with carcinogenesis, in particular IL1-ß and TNF-α, have also been measured in the lesions of chronic periodontitis.[14]

The tissue characteristics relating cancer to inflammation include presence of inflammatory cells and inflammatory mediators (e.g. cytokines and prostaglandins) in tumour tissue as well as tissue remodelling and angiogenesis in tumours which is similar to the changes observed in chronic inflammatory response and tissue repair.[15] It is estimated that up to 20% of all malignant neoplasms are initiated by infection/inflammation.[16] Chronic inflammation poses an increased risk for malignant transformation of affected epithelia.[14] Multiple examples of this possible aetiology include: oral lichen planus, which may transform into malignancy in 0.4-5.3% cases.[17] Similarly, Barrett's oesophagus, another chronic inflammatory condition, is a precancerous condition for adenocarcinomas of oesophagus. [18]

Although the microbiome (viral, bacterial, and fungal) inhabiting the oral cavity also differs between healthy individuals and Subjects with cancer, a causal association between oral microbial infections and UADT cancers is yet to be firmly established, though it is being increasingly explored.[19] Whilst there is no uniformity between individual species of the oral bacteriome associated with oral squamous cell carcinoma, there is a common pro-inflammatory metabolic outcome, which one of us has recently characterised as a "passenger turned driver" scenario, in which the presence of an invasive neoplasm encourages a proinflammatory and procarcinogenic flora.[18, 20]

With increasing evidence supporting the association between chronic infection/inflammation and cancer, a potential link between periodontitis and UADT cancers seems probable. This is the first study from India designed to study the plausible association of presence of periodontitis and oral hygiene habits with UADT cancers.

Materials & methods

Study design and recruitment of study participants

The sample for this case-control study enlisted subjects from Pune Maharashtra, India, attending any of the two multidisciplinary hospitals during June 2014 – May 2015.[21] Further details of identification, selection and recruitment of study participants has been described elsewhere.[21] All the study subjects from either of the two hospitals with histopathologically confirmed diagnoses of squamous cell carcinomas of UADT were recruited. The cancer subsites were in accordance with WHO international standard for reporting diseases and health conditions.[22] These cases were identified from the indoor or
or outpatient clinics and the treatment or surgery registers maintained in the medical, surgical and radiation oncology departments and wards. Thereby, cases were a total of 240 UADT cancer patients. Similarly, an equal number of controls, frequency matched to cases by gender and age (±5-years), were also identified and approached as indoor/outdoor patients from various hospital departments. Subjects were not enrolled as controls in the study if they were diagnosed with a disease related to tobacco and alcohol or cancer of lung/liver or with a previous history of UADT cancers.

Potential cases and controls were not included in the study if they were significantly unable to communicate due to their state of physical or mental health in addition to their inability to give an oral consent. Recording an oral consent of the subjects before the initiation of study procedures was mandatory. Interviews and clinical examinations performed in the study had a prior approval from Griffith University Human Research Ethics Committee (Reference No: DOH/10/14/HREC).

Exposure assessment/Data collection

Various methods were used for data collection including access to hospital records, in-person interviews and a complete intra-oral examination. Hospital records were used to gain insight on date of clinical diagnosis of cancer, basis of diagnosis, histologic type, site and stage of cancer. Furthermore, medical history and demographic information of the subjects was also retrieved from the medical records. In-person interviews were conducted to record patient's self-reported details, by adopting a standardized closed-ended questionnaire supplemented with some recall and memory probing aids like life grid tool which helps to minimise recall bias in a case-control study.[23] The questionnaire was based on the multiple questionnaires used in the past health surveys and case-control studies in India.[24-27]

Subjects' self-reported data before the diagnosis of their cancer or disease embraced their sociodemographic profile, oral hygiene habits (bleeding gums, number of times teeth were cleaned, type of instrument and substance used for cleaning and number of times of dental check-ups), in addition to lifetime history of behavioural risk factors (chewing and smoking tobacco, consumption of areca nut, and alcohol drinking). Furthermore, co-morbidity status and family history of any cancer was also documented.

Intra-oral examinations at the study sites were performed by the primary author (BG, a qualified dental surgeon) to file the number of missing teeth and periodontal status. Risk factors were categorized into 'never', 'yes' and 'yes only in the past'. Limited number of subjects in the latter category, resulted in a combination of 'yes' and 'yes only in the past' into one category, leading to a binary variable of 'ever (yes)' and 'never'. Study participants who self-reported as users of smoked bidis or cigarettes at least once a day for a minimum duration of six months before the confirmatory diagnosis of cancer were defined as ever smokers. Likewise, ever tobacco chewers were defined as those who self-reported as having

chewed any form of tobacco including mishri for at least once a day for a minimum duration of six months prior to the date of diagnosis. There were negligible independent users of areca nut/betel quid in this population. Those who consumed alcoholic beverages at least once a week for a minimum of six months were defined as ever alcohol drinkers. Study participants who had denied themselves or self-reported as rare users of smoked or chewing tobacco and alcohol in their lifetime were recorded as never users of the respective habits.

Basic periodontal examination

For assessment of full-mouth periodontal clinical examination, the Basic Periodontal Examination (BPE), screening tool was applied.[28] The advantage of using BPE is that it can achieve the task of differentiating periodontally healthy from periodontally diseased subjects usually in two-three minutes.[28] The mouth excluding third molars was divided into six sextants: upper right (17 to 14), upper anterior (13 to 23), upper left (24 to 27), lower right (47 to 44), lower anterior (43 to 33) and lower left (34 to 37). The highest score for each sextant was recorded. For a sextant to be measurable, it had to contain at least two teeth . In case of presence of only one tooth in a sextant, the score for that tooth was included in the recording for the adjoining sextant.[28]

Periodontal status was evaluated using a World Health Organization periodontal probe. The probe has a "ball end" measuring 0.5 mm in diameter, and a black band from 3.5 to 5.5 mm depth of the instrument. While carrying out the periodontal examinations, a light probing force of 20-25 grams was exercised. The probe was "walked around" the sulcus/pockets in each sextant, and the highest score recorded. As soon as a code 4 was identified in a sextant, the examination shifted directly on to the next sextant. If a code 4 was not detected, then all sites were examined to ensure that the highest score in the sextant was recorded.[26] Scoring code of 0 indicates pockets <3.5 mm, no calculus/overhangs, no bleeding on probing (black band entirely visible), code 1 indicates pockets <3.5mm, no calculus/overhangs, bleeding on probing (black band entirely visible), code 2 indicates pockets <3.5mm, supra or subgingival calculus/overhangs (black band entirely visible), code 3 indicates probing depth 3.5--5.5mm (black band partially visible, indicating pocket of 4--5 mm), code 4 indicates: probing depth >5.5mm, (black band disappears, indicating a pocket of 6 mm or more), * indiactes furcation involvement.[28]

Statistical analysis

Descriptive statistics were used to describe and compare the study population. Adjusted odds ratios (OR) for UADT cancers were estimated by statistically carrying out an unconditional logistic regression model. Additionally, effect modification was also conducted. Variables like age (years), education, family income per month, smoking and chewing tobacco and drinking alcohol habits (never/ever) were considered as confounders and were adjusted for in the analysis. Possible effect modification by the tobacco chewing (never/ever) was also examined, since tobacco chewing has been identified as a potential effect modifier in some research studies.[29, 30] Two-sided p-values<0.05 were considered statistically significant. All the statistical analyses were performed using the Statistical Package for Social Sciences Version for Windows—SPSS Inc., Chicago, IL, USA).

Results

The total sample size of the study was 480, with 67% males and 32.9% females. The distribution of socioeconomic status and the medical and family history of UADT cancers among the cases and controls are depicted in Table 1. Chewing tobacco was the most common behavioural habit reported among the cases as compared to controls (p<0.001). The co-morbidity status and the family history of any type of cancer did not show any significant differences among the cases and controls. Table 2 illustrates that nearly 78% of the cases presented with histopathological diagnosis of lip and oral cavity cancer. Forty-five percent of the UADT cancer cases were diagnosed at Stage II.

Table 3 describes OR and their corresponding CIs for periodontal status and oral hygiene habits among UADT cancer cases and controls. Significant risk for UADT cancers was observed with severe periodontitis (adjusted OR= 2.35; 95% CI: 1.12-4.91) in comparison with no, mild and moderate periodontitis. More than five missing teeth also showed an elevated risk for UDAT cancers (adjusted OR= 3.28; 95% CI: 1.95-5.49). Among the self-reported oral hygiene habits, dental check-ups only at the time of pain showed the highest risk associated with UADT cancers (adjusted OR=4.12; 95% CI: 2.63-6.47). Topical application of mishri to clean the mouth was also associated with an increased risk (adjusted OR=3.06; 95% CI:1.75-5.35). Among the UADT cancer cases and controls, ever tobacco chewers were associated with an increased risk of severe periodontitis (p<0.001) (Table 4).

The innovative findings of this study, are the increased risk of UADT cancers with severe periodontitis and with presence of poor oral hygiene habits in comparison no, mild, moderate periodontitis and good oral hygiene habits.

Discussion

Our study found inverse association with periodontitis, poor oral hygiene measures and chewing tobacco with incidence of UADT cancers. Though use of tobacco remains the most significant risk factor for these cancers, primary control based on this knowledge is accepted universally. Importantly, chronic inflammatory disorders are gaining attention as independent risk factors for these cancers.[31, 32] If additional risk factors contributing to an increased risk for incidence of UADT cancers could be identified, this should aid in improving screening and early detection, as well as public health messages for primary prevention.

Chronic periodontitis causes continuous release of inflammatory mediators into surrounding tissues, the bloodstream, and into oral fluid. Mediators such as TNF-α and IL1-ß are characteristic of early carcinogenesis.[6, 33] The reduction of risk of certain cancers by non-steroidal anti-inflammatory drugs further demonstrates the association of cancer and chronic inflammation.[14]

Biologically, the association between periodontitis and malignant transformation of tissues in oral cavity, oropharynx and oesophagus can be possibly explained by chronic inflammation, presence of bacteria and viruses as well as periodontal pockets acting as a reservoir for possible carcinogenic agents like HPV, cytomegalovirus and Epstein-Barr virus which have been associated with oral cancer.[12, 34] The mechanisms by which bacterial infections may cause cancer are not yet clear. Bacteria may induce cellular proliferation, inhibit apoptosis, interfere with cellular signalling mechanisms, and act as tumour promoters. Also, there are specific oral bacteria, like Streptococcus anginosus and Treponema Denticola, which have been linked with various upper gastrointestinal malignancies including oesophagus.[35, 36] There is emerging evidence that oral health (tooth loss, poor oral hygiene, and possibly periodontal disease) is linked to oesophageal cancer risk as the oral microbiota shape the oesophageal microbiome, due to migration of oral bacteria to the oesophagus and therefore may contribute to oesophageal malignant transformation.[37-39]

Our study aimed to determine the risk of UADT cancers associated with periodontitis and its severity. Although periodontitis emerged as a biologically plausible risk indicator for UADT cancers, the association was determined by the severity of the periodontitis and not merely by its presence. Severe periodontitis remained a potential independent risk indicator for UADT cancers, even after adjusting for tobacco use and alcohol consumption, whereas mild and moderate periodontitis did not pose a significant risk after similar adjustment. The strength of association was strongest amongst concurrent tobacco chewers. This is consistent with previous studies.[40-43] Even in absence of tobacco use habits, periodontitis has been shown to be a significant risk factors for various cancers.[44] Since periodontitis generates proinflammatory cytokines which induce continuous cell proliferation, including of local keratinocytes, the chances of replication errors and of erroneous DNA repair are increased.[14, 20, 45, 46]

Interviewer and self-reported oral hygiene variables associated with increased risk of UADT cancers included tooth-brushing frequency less than once daily, having more than five missing teeth, topical application of mishri for cleaning the mouth and dental check-ups only when in pain. The process of preparing mishri involves roasting and baking tobacco, until it gets converted to homogenous black powder. It is one of the commonly used substance for cleaning teeth and gums with fingers in Maharashtra.[47] Chewing tobacco strengthened the association between UADT cancers and periodontitis, whereas gender and age did not have any significant association.

Regular dental check-ups were associated with a lower incidence of UADT cancers. Such individuals maintain better oral hygiene. Also, regular dental visits give an opportunity to dentists to screen for any suspicious oral lesions. A recent systematic review and meta-analysis exploring the relationship between past dental visits and incidence of head and neck cancers confirms this protective effect.[48]

We found a positive association and an increased trend of risk between toothbrushing less than once daily as well as topical application of mishri on gums with incidence of UADT cancers. Less frequent cleaning of mouth and teeth allows increased load of biofilm, with ecological shifts towards more pathogenic metabolism.[49]Conversely, good oral hygiene may improve periodontal status and reduce the risk of UADT cancers.[50]

The merit of our study is that it used full mouth examination as well as interviewer-based questionnaires to assess periodontal status. Parameters considered in other studies[5, 51, 52] may not be as reliable as tooth loss may not be related to periodontal disease, and periodontal indices as well as self-assessment questionnaires may underestimate the prevalence of periodontitis status. Nevertheless, this study has some limitations as well: since this is a hospital based case-control study, it is subjected to selection and recall bias, it was also not possible to blind the interviewers to case/control status during intra-oral examinations. The presence of cancer itself adversely affects oral hygiene, so it is hard to determine which came first. We did not assess radiological features of oral health and disease. Lastly, due to limited sample size of UADT cancer cases, it was not possible to analyse cancer sub-sites in relation to hypothesized risk factors.

Conclusions

Our case-control study results suggest that severe periodontitis is possibly an independent risk factor for UADT cancers and chewing tobacco strengthens this association in this population in Maharashtra, India. As severe chronic periodontitis is common, regular monitoring and early treatment of this may contribute to prevention of UADT cancers, and to early diagnosis and management thereof. Further, prospective clinical and confirmatory basic science studies are required to fully understand the relationship, and the mechanisms involved. There are considerable public health implications.

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