BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, April 2015. Vol. **12**(1), 263-267

Microbiological Assessment in Plaque Samples of Patients with Oral Cancer with or without Smoking

V. Shankar Ram, Lakshmi Narayanan, Umasudhakar, Thamizh Chelvan and Parthiban

Thai Moogambigai Dental College, Goldengeorge Nagar, Chennai - 600107, India.

DOI: http://dx.doi.org/10.13005/bbra/1661

(Received: 15 February 2015; accepted: 10 March 2015)

Oral cancer is the sixth most common malignancy worldwide and ranked third of all cancers in india.90% of cancers in oral cavity are Squamous cell carcinomas originating from the oral mucosa. Majority of case of oral cancer can be related to tobacco use and tobacco smoking.Other risk factors include viral,bacterial infections, poor oral hygiene and candidalinfections.Association of bacteria with oral tumours and its relationship of smoking with oral bacterial flora is of increasing interest.Most important etiological factor of oral squamous cell carcinoma is smoking.Smoking also being a risk factor for chronic periodontitisRecently it has been reported that patients with oral SCC (OSCC) tend to possess significantly raised concentrations of certain specific pathological bacteria in saliva. This research article we will see the prevalence of micro-organisms from the plaque in patients with oral malignancy with or without smoking

Key words : OSCC (oral squamous cell carcinoma),Tobacco smoking, Subgingival plaque.

Oral cancer is the sixth most common malignancy worldwide and ranked third of all cancers in india.. 90% of these lesions are oral squamous cell carcinomas [1].Major risk factors for oral cancers in are use of tobacco and alcohol, which account for 75 to 80% of all oral cancers[2]although tobacco is a well-recognized risk factor for oscc, the public is generally unaware poororal hygiene synergizes with tobacco. those who both smoke and poor oral hygiene have 15 times the risk of developing oral cancer [3]. Thus oral microbiota also play a role in carcinogenesis[4]Studies have reported that certain common oral bacteria are elevated on or in oral cancer lesions and their associated lymph nodes [5]. although increased colonization of facultative oral streptococci have been reported most often [6], anaerobic prevotella, veillonella, porphyromonasand capnocytophaga species were also elevated [7]. currently, studies are examining whether bacteria may be incidentally or causally associatedwith oral cancer.

Oral cancer and bacterial colonization

The reason for these shifts in bacterial colonization of cancer lesions is unclear. Mechanistic studies of bacterial attachment provide some insights, however. Research has repeatedly shown that oral bacteria demonstrate

* To whom all correspondence should be addressed.

specific tropisms toward different biological surfaces in the oral cavity such as the teeth, mucosa, and other bacteria [8]. The non-shedding surfaces of the teeth offer a far different habitat than the continually shedding surfaces of the oral mucosa. Due to the repeated shedding of epithelial cells, there is less time for a complex biofilm to develop on soft tissue surfaces; thus, a premium is placed on potent mechanisms of adhesion. The differences in bacterial tropisms for specific oral sites suggest that different intra-oral surfaces and bacterial species have different receptors and adhesion molecules that dictate the colonization of different oral surfaces.[9]

It is now recognized that bacteria bind to and colonize mucosal surfaces in a highly selective manner via a "lock- and key" mechanism. Adhesins on bacteria bind specifically to complementary receptors on the mucosal surfaces of the host. These adhesins differ from species to species leading to specificity in attachment to different surfaces. Studies have shown that even within genera, colonization patterns of individual species may differ markedly [10]. Streptococcus salivarius, for example, preferentially colonized the oral soft tissues and saliva compared to the teeth, while the reverse was true of Streptococcus sanguis.

Cancer has been referred to as a molecular disease of cell membrane glycoconjugates,[11]. Certain glycoconjugates serve as receptors for specific bacteria and recent reports support the notion that shifts in the colonization of different

Group A: Identification Of Microorganisms In Patients Witout Malignancy &With Smoking

S.No		*Capnocytophaga gingivalis*	*Prevotella melaninogenica*	*Streptococcus mitis*	Helicobacter pylori	Porphyromonas gingivalis
01.	Subject 1	+ ve	+ ve	- ve	- ve	- ve
02.	Subject 2	+ ve	+ ve	+ ve	- ve	- ve
03.	Subject 3	+ ve	+ ve	+ ve	- ve	- ve
04.	Subject 4	+ ve	+ ve	- ve	- ve	- ve
05.	Subject 5	+ ve	+ ve	- ve	- ve	- ve
06.	Subject 6	+ ve	+ ve	+ ve	- ve	- ve

Group B: Identification Of Microorganisms In Patients Witout Malignancy &With Smoking

S.No		*Capnocytophaga gingivalis*	*Prevotella melaninogenica*	*Streptococcus mitis*	Helicobacter pylori	Porphyromonas gingivalis
01.	Subject 1	+ ve	- ve	- ve	+ ve	+ ve
02.	Subject 2	- ve	- ve	- ve	- ve	- ve
03.	Subject 3	+ ve	- ve	- ve	+ ve	- ve
04.	Subject 4	+ ve	- ve	- ve	+ ve	+ ve
05.	Subject 5	- ve	- ve	- ve	+ ve	+ ve
06.	Subject 6	+ ve	- ve	- ve	- ve	+ ve

cancer cells are associated with observed changes in cell surface receptors [12].Though separate role of tobacco in oscc is known theRole of tobacco in association with oral microbiota changes leading to shift in cancer cells is unknown

Thus this study aims to isolate micro-organisms from the subgingival plaque in patients with oral malignancy with or without smoking

MATERIALS AND METHODS

OSCC patients were taken from adyar cancer institute Chennai, Group A total of 6 subjects diagnosed with OSCC via biopsy were recruited .Inclusion criteria required that subjects be 18 years or older and immunocompetent, with a primary untreated OSCC and smokers. Exclusion criteria included systemic conditions associated with immune dysfunction (e.g., diabetes), previous chemotherapy or radiation, an inability to properly consent, and/or lesions that could not be sampled due to discomfort, anatomic location or that did not affect the surface oral epithelium.GROUP B. six OSCC-free subjects were recruited from the out patient department of periodontics. All subjects were 18 years or older, and immunocompetent ,but current smokers. Another group C six OSCC-free subjects were also recruited from the out patient department of periodontics. All subjects were 18 years or older, and immunocompetent ,andnon smokersExclusion criteria included: antibiotic therapy within the previous 3 months, pregnancy or lactation, systemic conditions associated with immune dysfunction (e.g., diabetes), previous chemotherapy or radiation and the presence of any oral mucosal lesions. Ethical clearance for the study obtained from Dr.mgruniversity ,maduravoyal.chennai

Microbiological assessment

The subgingivalplaque samples were taken from each group with help of sterile curettes and were stored and sent to the laboratory in the transport medium.The microbiological assessment was carried out in Department of Microbiology in A.C.S. Medical College.with help of culture medium

RESULTS and DISCUSSION

Results from this investigation demonstrated that oral cancer subjects had elevated counts of C. gingivalis, P. melaninogenica and S. mitis in samplescompared to OSCC-free subjects.with or without smoking

The reason for this finding is unclear.explanation may relate to the altered cell surface receptors observed in cancer cells [13]. It seems reasonable that alterations in tumor cell receptors could change the adhesion of certain species of bacteria. And Several bacteria whichcauses chronic infections and produce toxins that disturb the cell cycle leading to altered cell growth[14]They induce cell proliferation and DNA

Group C: Identification Of Microorganisms In Patients Without Malignancy &Without Smoking

S.No		*Capnocytophaga gingivalis*	*Prevotella melaninogenica*	*Streptococcus mitis*	Helicobacter pylori	Porphyromonas gingivalis
01.	Subject 1	- ve	- ve	- ve	+ ve	+ ve
02.	Subject 2	- ve	- ve	- ve	- ve	- ve
03.	Subject 3	- ve	+ve	- ve	- ve	- ve
04.	Subject 4	- ve	- ve	- ve	- ve	+ ve
05.	Subject 5	- ve	- ve	- ve	- ve	- ve
06.	Subject 6	- ve	- ve	- ve	- ve	- ve

replication through activation of mitogen activated kinase (MAPK) pathways which increases the incidence of cell transformation and rate of tumour development through increased rate of genetic mutation. [15]

Many pathogenic bacteria causing chronic infection with intracellular access subvert host cell signaling pathways , enhancing the survival of pathogen. The regulation of these signaling factors is central to the development or tumour formation[16]

Possible role of smoking is it enhances metabolism of potentially carcinogenic substances by the bacteria.[17]

CONCLUSION

Results of the present study suggest that high counts of C. gingivalis, P. melaninogenica and S. mitis in plaque samples may be diagnostic indicators of OSCC. And These findings indicate that the presence of an OSCC has a more powerful effect on the oral microbiota with smoking .compared to the non malignant group with or without smoking.Further exploration on this subject, which would clear our understanding of the role of the microbial detection, not only in prevention or early diagnosis of oral cancers but also in providing an effective treatment and improving the survival.

Future prospects

A screening test for oral cancer based on salivary counts of bacterial species is appealing

Some investigators have recently come up with designing new treatments that stimulate the immune system through attenuated bacterial vaccines to recognize and target lesion by safe and effective delivery of plasmids encoding tumourself antigens.

Cancer vaccines although promising in treatment and prevention of certain cancer recurrence, present significant challenges in determining the most effective bacterial strains, addressing safety issues and the problem of overcoming the peripheral T cell tolerance against tumour self- antigens.

This calls for further exploration on this subject, which would clear our understanding of the role of the microbial detection, not only in prevention or early diagnosis of oral cancers but also in providing an effective treatment and improving the survival.

REFERENCES

1. SEER: Cancer Statistics Review. US Department of Health and Human Services, Public Health Service, National Institutes of Health, Bethesda, MD; 2002.
2. Blot WJ, McLaughlin JK, Winn DM, Austin DF, Greenberg RS, Preston-Martin S, Bernstein L, Schoenberg JB, Stemhagen A, Fraumeni JF Jr: Smoking and drinking in relation to oral and pharyngeal cancer. Cancer Res 1988, 48:3282-3287
3. Mignogna MD, Fedele S, Russo LL: The World Cancer Report and the burden of oral cancer. *Eur J Cancer Prev* 2004, **13**:139-142.
4. Rice DH, Weimert TA: Altered bacterial flora and clinical course with intraoral cancer. *Laryngoscope* 1978, **88**: 1861-1863
5. Crean S-J, Nair SP, Fardy M, Wilson M, Spratt D: Identification of bacterial DNA using PCR cloning within oral squamous cell carcinomas [abstract]. *J Dent Res* 2002, **81**:A364
6. Liljemark WF, Gibbons RJ: Proportional distribution and relative adherence of Streptococcus mitis on various surfaces in the human oral cavity. *Infect Immun* 1972, **6**: 852-859
7. Sakamoto H, Naito H, Ohta Y, Tanakna R, Maeda N, Sasaki J, Nord CE: Isolation of bacteria from cervical lymph nodes in patients with oral cancer. *Archives of Oral Biology* 1999, 44:789-793
8. Krasse B: The proportional distribution of Streptococcus salivarius and other streptococci in various parts of the mouth. *Odont Revy* 1954, **5**: 203-211
9. VanHoute J, Gibbons RJ, Pulkkinen AJ: Adherence as an ecological determinant for streptococci in the human mouth. *Arch Oral Biol* 1971, **16**:1131-1141
10. Crean S-J, Nair SP, Fardy M, Wilson M, Spratt D: Identification of bacterial DNA using PCR cloning within oral squamous cell carcinomas [abstract]. *J Dent Res* 2002, **81**:A364
11. Neeser JR, Grafstrom RC, Woltz A, Brassart D, Fryder V, Guggenheim B: A 23 kDa membrane glycoprotein bearing NeuNAc alpha 2-3Gal beta 1-3GalNAc O-linked carbohydrate chains acts as a receptor for Streptococcus sanguis OMZ 9 on human buccal epithelial cells. *Glycobiology* 1995, **5**: 97-104
12. Tieng V, Le Bouguenec C, du Merle L, Bertheau

P, Desreumaux P, Janin A, Charron D, Toubert A: Binding of Escherichia coli adhesinAfaE to CD55 triggers cell-surface expression of the MHC class I-related molecule MICA. *ProcNatlAcadSci* 2002, **99**: 2977-2982

13. Williams P, Chaudhry Y, Goodfellow IG, Billington J, Powell R, Spiller OB, Evans DJ, Lea S: Mapping CD55 function. The structure of two pathogen-binding domains at 1.7 A. *J BiolChem* 2003, **278**:10691-10696

14. Littman AJ, White E, Jackson LA, Thornquist MD, Gaydos CA, Goodman GE, et al. Chlamydia pneumoniae infection and risk of lung cancer. *Cancer Epidemiol Biomarkers Prev.* 2004;**13**: 1624–30.

15. Coussens LM, Werb Z. Inflammation and cancer. *Nature*. 2002; **420**: 860–7

16. Lax AJ. Bacterial toxins and cancer – case to answer? *Nat Rev Microbiol.* 2005; **3**: 343–9

17. Pöschl G, Seitz HK. Alcohol and cancer.*J Alcohol.* 2004;**39**: 155–