Identification of Viruses in Oral Malignency and Chronic Periodontitis Patients with and Without Smoking : Microbiological Study

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

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

DOI: http://dx.doi.org/10.13005/bbra/1659

(Received: 09 February 2015; accepted: 08 March 2015)

Aim

Detection of HSV (type 1) in patients of Oral malignancy and chronic periodontitis and to study the relationship between viruses and clinical severity of periodontal diseases.

Objectives

1. To detect the presence of Herpes simplex virus type 1 (HSV-1) in healthy oral malignancy and patients with periodontitis
2. Study the relationship between viruses and clinical severity of periodontal disease.

MATERIAL AND METHODS

45 subjects were to be taken in the study from Out patient Department of periodontics.
Groups were divided into 3 groups
Group 1 n=15 subjects Saliva were to be obtained from chronic periodontitis patients
Group 2 n=15 subjects Saliva were to be obtained from chronic periodontitis & oral malignancy patients with smoking
Group 3 n=15 subjects saliva were to be obtained from oral malignancy patients without smoking

DNA extraction was done from these samples and a POLYMERASE CHAIN REACTION (PCR) method was used to detect the viral DNA of viruses. Polymerase chain reaction (PCR) offers a rapid and relatively inexpensive method of identifying viral nucleic acids in clinical specimens[5].

Clinical Examination

Age
Sex
habits
past medical
dental history
clinical examination was performed on four surfaces of all the teeth.

Clinical parameters

1. Plaque Index (Silness and Loe, 1965)
2. Probing depth
3. Clinical attachment loss (CAL)

Samples

Saliva from periodontitis patients and Oral malignancy patients presenting to the Department of periodontics, thaimoogambigaiDental College & Hospital was collected

DNA extraction

SALIVA

The saliva samples in PBS were microcentrifuged at 8,000rpm for 5minutes, supernatant discarded and the pellet was resuspended in 100µl of lysis broth (10 mmol/L Tris-HCl, 1.0 mmol/L EDTA, 1.0% Triton X-100, pH 8.0)(WU et al., 2006)[4]. The lysis broth was further vortexed and kept in water bath at 100oC for 5

* To whom all correspondence should be addressed.

minutes, cooled and centrifuged at 4000 rpm for 2 minutes and the supernatant was stored at -20oC till PCR assay which was used as template for the PCR reaction[10].

Gel electrophoresis for detection of amplico

The PCR product was to be detected by 1.5% Agarose gel electrophoresis.

REAGENTS REQUIRED[14]

1. Preparation of TBE Buffer (1x)
   - 490 ml of double distilled water
   - 10 ml of 50 x TBE Buffer
2. Ethidium bromide
   - Ethidium bromide - 10 mg
   - Distilled water - 1 ml

Statistical analysis to be done to correlate the prevalence of virus and clinical parameters

Thus my study aims to prove presence of Herpes simplex virus type 1 (HSV-1) in the pathogenesis of periodontitis and oral malignancy

In addition[7], to prove HSV-1 can reside in periodontal deep pockets and increase clinical severity of disease

METHODOLOGY

RESULTS

- Out of 25 subgingival plaque samples of CP patients only four samples were positive for HSV-1.
- Out of 25 subgingival plaque samples of malignant patients 9 samples were shown positive for HSV-1

PCR protocol

Reagents	volume in µl
10 X PCR Buffer	5.0 µl
dNTP 0.25Mm **)**	1.0 µl
Forward PrimerI(25 picomol**))**	1.0 µl
Reverse PrimerI(25 picomol**))**	1.0 µl
Taq polymerase with $MgCl_2$ 2.5 mmol	1.0 U
Sterile Millipore water	31.0 µl
Template	10 µl
	50.0 µl

40 µl of the PCR reaction mix was pipetted into micro centrifuge tubes, 10 ìl of the template DNA of CP patients and healthy subjects was added and mixed thoroughly. The micro centrifuge tubes were placed in a thermocycler (eppendorf) and cycling conditions were set[8]

DISCUSSION

- Herpes simplex virus type 1 (HSV-1),) has role in pathogenesis of human periodontitis, because viruses can infect a variety of inflammatory cells.[1] **Slots etal 2000 (J.P)**
- In addition, HSV can reside in periodontal pockets, periodontal abscesses and saliva of immunocompetent patient[15]

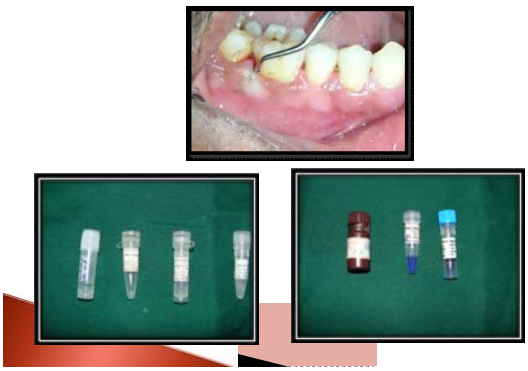
PCR – Polymerase chain reaction & visualization of products

PCR Thermocycling Condition

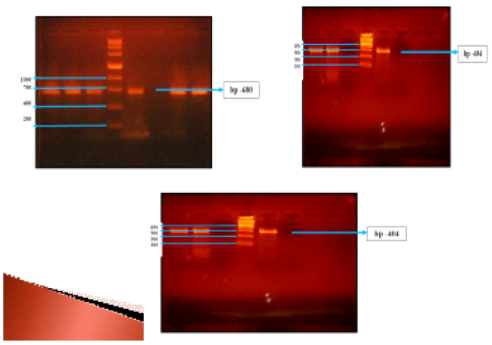
Denaturation	- 94C / 30 secs	
Annealing	- 55oC / 30 secs	40 cycles
Extension	- 72oC / 40secs	

Greenberg et al 2004 (J.C.P)

- The result of our study is fairly similar to



PCR detection of HSV



Parra *et al* 1996 with respect to HSV prevalence in malignant Group than C.P

´ **Parra *et al*** have demonstrated that human viruses may occur in malignant lesions with relatively high prevalence.

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

Lax AJ 2005

´ Several virus induce cell proliferation and DNA 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.

Coussens LM, Werb Z 2002

´ Several virus causes chronic infections and produce toxins that disturb the cell cycle leading to altered cell growth.

Littman AJ, White E 2004

´ Possible mechanism is the metabolism of potentially carcinogenic substances by the bacteria[17].

Pöschl G, Seitz HK 2004

´ Several infections cause intracellular accumulation of the pathogen, leading to

Species specific Primers used for PCR assay

Virus	Primer pairs (5'-3'),	Amplicon length
Herpes simplex virus 1(HSV^{-1})	CGGCCGTGTGACACTATCG CTCGTAAAATGGCCCCTCC	72bp

suppression of apoptosis primarily through modulation of the expression.

Nougayrede JP et al 2005

Summary

The present findings suggest that herpes virus infection could constitute an important role in etiopathogenesis of CP. However, further studies in a larger sample size, are needed to determine the association of these human viruses in destructive periodontal disease.

Future prospects

´ A screening test for oral cancer based on salivary counts of these 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 tumour self antigens.

´ Cancer vaccines although promising in treatment and prevention of certain cancer recurrence, there are present significant challenges in determining the most effective viral strains, addressing safety issues and the problem of overcoming the peripheral T cell tolerance against tumour self- antigens3.

Yet to be answered

´ Is it the viral infections that initiate cancer, or is it the pre-existing cancer that lowers

the host's immunity facilitating secondary microbial colonization ?.

´ Can the highly site – specific colonization of certain virus be of any value in its diagnosis or treatment ?.

´ Could attenuated bacteria be used in vaccines to modulate host's immunity against cancer ?

This calls for further exploration on this subject, which would clear our understanding of the role of the viral detection[2], 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–