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

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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.

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, thaimoogambigai Dental 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). The lysis broth was further vortexed and kept in water bath at 100°C for 5
minutes, cooled and centrifuged at 4000 rpm for 2 minutes and the supernatant was stored at -20°C till PCR assay which was used as template for the PCR reaction\textsuperscript{10}.

**Gel electrophoresis for detection of amplico**

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

**REAGENTS REQUIRED**\textsuperscript{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\textsuperscript{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**

<table>
<thead>
<tr>
<th>Reagents</th>
<th>volume in µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 X PCR Buffer</td>
<td>5.0 µl</td>
</tr>
<tr>
<td>dNTP 0.25Mm</td>
<td>1.0 µl</td>
</tr>
<tr>
<td>Forward Primer(25 picomol)</td>
<td>1.0 µl</td>
</tr>
<tr>
<td>Reverse Primer(25 picomol)</td>
<td>1.0 µl</td>
</tr>
<tr>
<td>Taq polymerase with MgCl\textsubscript{2} 2.5 mmol</td>
<td>1.0 U</td>
</tr>
<tr>
<td>Sterile Millipore water</td>
<td>31.0 µl</td>
</tr>
<tr>
<td>Template</td>
<td>10 µl</td>
</tr>
<tr>
<td></td>
<td>50.0 µl</td>
</tr>
</tbody>
</table>

40 µl of the PCR reaction mix was pipetted into micro centrifuge tubes, 10 µl 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\textsuperscript{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.\textsuperscript{1} **Slots et al 2000 (J.P)**

In addition, HSV can reside in periodontal pockets, periodontal abscesses and saliva of immunocompetent patient\textsuperscript{15}

**PCR – Polymerase chain reaction & visualization of products**

**PCR Thermocycling Condition**

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

**Greenberg et al 2004 (J.C.P)**

The result of our study is fairly similar to
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. The regulation of these signaling factors is central to the development or inhibition of tumor 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.

Pöschl G, Seitz HK 2004

Several infections cause intracellular accumulation of the pathogen, leading to 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- antigens.

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 detection2, not only in prevention or early diagnosis of oral cancers but also in providing an effective treatment and improving the survival.

REFERENCES


8. Krasse B: The proportional distribution of Streptococcus salivarius and other streptococci in various parts of the mouth. Odont Rev 1954, 5: 203-211


