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Detection of Human Cytomegalovirus (Hcmv) Epstein Barr Virus (Ebv) and Herpes Simplex Virus (Hsv) in Periodontal Disease and Effect of Scaling and Root Planing (Srp) on the Presence of These Viruses.

KEYWORDS: human cytomegalovirus, EBV, herpes virus 1, polymerase chain reaction, scaling and root planing, periodontal disease.

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ABSTRACT *Aim: To evaluate the presence of human cytomegalovirus (HCMV), Epstein- Barr virus (EBV) and herpes simplex virus type 1 (HSV-1) in subjects with chronic periodontitis and periodontally healthy subjects and effect of scaling and root planing (SRP) on the presence of these viruses.*

Materials and methods: At baseline in the test group, subgingival plaque samples were collected from the periodontal pocket and in the control group, plaque samples were collected from the gingival sulcus. In the test group after the baseline plaque sampling was done, scaling and root planing was performed. Plaque was collected after 8 weeks of the SRP again. Hot STAURT multiplex PCR method was used to detect the viruses in the plaque sample.

Results: Overall the presence of HSV-1 in the test group was statistically high at base line compared to the control group. There was reduction in HSV-1, HCMV and EBV after SRP in the test group compared to the baseline.

Conclusion: Herpes viruses were found in higher frequency in chronic periodontitis patients than in healthy subjects. SRP reduced the percentage of these viruses after 8 weeks.

Introduction: Periodontal disease is a microbial infection involving a variety of microbes that trigger inflammation, loss of connective tissue attachment and alveolar bone around the teeth. The primary etiologic factor of periodontitis is bacterial plaque. In fact the propensity of periodontitis to proceed with periods of exacerbation and remission could suggest that the presence of other organisms contributes to the disease.[1] Various studies have shown that human viruses, especially human cytomegalovirus (HCMV), Epstein-Barr virus (EBV) and herpes simplex virus (HSV-1) seem to play a part in the pathogenesis of periodontal disease.[2,3,4] The hypothesis of a correlation between HCMV and EBV infection and the pathogenesis and progression of aggressive periodontitis has been proposed by various studies.[5,6,7] Periodontal destruction may be associated with the coexistence of periodontal herpes viruses, especially HCMV, EBV and periodontopathic bacteria. The herpes viral infection can stimulate the release of cytokines and chemokines from inflammatory and non inflammatory cells and impair the periodontal immune defense, resulting in more virulent resident bacteria.[8] Till date several techniques have been employed for the detection of the viruses, which include culture methods, DNA-DNA hybridization technique and various types of polymerase chain reactions (PCR) such as Hot STAURT PCR, multiplex PCR, nested PCR, reverse transcriptase and real time PCR.[9, 10, 11, 12]

PCR is a rapid, accurate and sensitive technique for the detection of bacterial and viral DNA sequences. Scaling and root planing resulted in reduction of periodontal pathogens [13,14,15,16] The aim of this study was to examine the presence of human cytomegalovirus (HCMV), Epstein-Barr virus (EBV) and herpes simplex virus (HSV) in subjects with healthy periodontium and in subjects having chronic periodontitis and to examine the effect of SRP on these viruses in chronic periodontitis subjects.

Methods: It is a pilot study where in subjects with severe chronic periodontitis having pocket depth ≥7 mm with good general health, no history of periodontal therapy in past 6 months were included in the study. Smokers**,** pregnant women and lactating mothers, subjects not compliant with the terms of the study, subjects who were on antimicrobial drugs in the previous six months were excluded from the study. Informed consent was taken from all the subjects and the approval from the ethical committee was taken. Subjects having healthy periodontium and no signs of gingival inflammation and no probing pocket depth were sixteen. Ten subjects having chronic periodontitis were selected. There were 16 periodontal pockets measuring ≥ 7mm in these subjects. Two groups were made for the study having 16 sites each. All clinical parameters were assessed in test group at baseline and 8 weeks after SRP. The clinical parameters which were measured by UNC 15 probe were, Probing pocket depth (PPD), Relative attachment level[17] Plaque index,[18] and Gingival index.[18]

The viruses HCMV, EBV and HSV-1 were detected by Hot STAURT multiplex PCR*. In the test group, supra gingival plaque was gently removed with sterile cotton pellets and the sample site was isolated with cotton rolls to avoid contamination with the saliva and blood. Sterile periodontal curette was inserted into the bottom of the pocket and the plaque was removed by single stroke and collected into the vial containing phosphate buffered saline, in the same way plaque sample was collected from the gingival sulcus in the control group.[6]

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The primers[⚙] which were used in the study are as follows[19]

HSV-1 5'- F; 5'- CGTACCTGCGGCTCGTGAA

R; 5'-AGCAGGGTGCTCGTGTATGGGC

HCMV- F; 5'-ACGTGTTACTGGCGGAGTCG-3'

R; 5'-TTGAGTTGTGGCCAGACTGAG-3'

EBV-F; 5'-AGCACTGGCCAGCTCATATC-3'

R; 5'TTGACGTCATGCCAAGGCAA-3' 38

DNA extraction procedure from plaque sample: Plaque was transferred to the tube containing "TRIS-EDTA (T.E) buffer". It was centrifuged at 50,000 rpm for 2 minutes, supernatant was discarded. A fresh 200 micro liter T.E. buffer was added and then centrifuged for 3-4 minutes. Above procedure was repeated 3-4 times with fresh T.E. buffer, supernatant was discarded and 500 micro liter lysis buffer 1 was added and centrifuged at 5,000 rpm, supernatant was discarded and 50 micro liter lysis buffer 2 and 5 micro liter proteinase – K was added. It was kept in water bath over night then kept in boiling water bath for 10 minutes at 90^0C. DNA was stored at -20^0C. The tube containing DNA was taken and allowed to thaw. Then the master mix was prepared.

The contents of the master mix; For one reaction; Water – 14.25µL, dNTP mix - 2 µL, Enzymes – 0.25 µL, Primer- 0.5 µL. To a fresh tube, 22 µL of master mix was added and 3 µL of the DNA sample was added and it was inserted into the tube into the PCR cycler.

[⚙]biosera

Statistical evaluation: The collected data was entered in the excel format. It was subjected to statistical analysis using SPSS software. The statistical tests applied were as below

1. Fisher exact test
2. Mann Whitney U test
3. Kruskal-Wallis test

For all statistical tests $p<0.05$ was considered statistically significant.

Results: Decrease in plaque index and gingival index scores from baseline to 8 weeks in the test group which was statistically significant**.** There was a significant decrease in the probing pocket depth and gain in the attachment levels from baseline to 8 weeks in the test group**.**

Overall the presence of HSV-1 in the test group was significantly high compared to the control group at baseline. **(Table 1 & Graph1)** Presence of HCMV & EBV was high in the test group compared to the control group at baseline which was not statistically significant **(Table 1& Graph1)** there was reduction in HSV-1, HCMV and EBV after SRP in the test group compared to the baseline. Only HSV-1 reduction was statistically significant. **(Table 2 Graph 2.)**

Discussion: Viruses were found in patients with aggressive periodontitis and chronic periodontitis.[1]Viral DNA had been detected in gingival tissue, gingival crevicular fluid (GCF) and subgingival plaque from periodontally diseased sites.[20] In particular, DNA from herpesviruses such as HCMV and EBV had been detected in high percentages of subgingival plaque samples from periodontitis patients. Periodontally healthy individuals had very low prevalence of such viruses. [21, 22]

In this study the viruses were isolated from subgingival plaque sample as all the microorganisms are colonized in the subgingival plaque. It is the simplest method of collection and less time consuming. In the test group, plaque sample was taken at baseline and 8 weeks after SRP as there will be recolonization and reestablishment of subgingival micro biota within 8 weeks after SRP.[23] The subgingival plaque was collected from pockets having ≥ 7mm as the HCMV, EBV and HSV were detected with higher frequency in deep than in shallow periodontal pockets.[24] Full mouth plaque index and gingival index were taken to ascertain the patient compliance towards periodontal therapy. All patients had shown improvements in the indices scores after SRP compared to baseline which could be attributed to constant reinforcement of oral hygiene instruction and Hawthorne effect as given by Knowles et al., in 1979 and Ramfjord et al.,1987.[25] There was significant reduction in the probing depth and gain in relative attachment level which was in accordance to the study done by Shibata Y 1989 where in there was decrease in the probing depth and gain in attachment level after SRP.[26,27] Patients with chronic periodontitis showed higher percentage of HSV-1 (81.25 %), HCMV (50%), and EBV (37.50 %) than patient having healthy periodontium who had HSV-1 (31.25 %), HCMV (31.25%), and EBV (25 %). The lesser percentage of viruses in healthy sites may be because of the presence of virus in latent form.[20] The frequency of viruses in periodontal disease increases as these viruses may get reactivated. Contreras et al., 1999 studied the presence of herpes viruses in adult periodontitis patients. He showed the higher percentage of HCMV than EBV and HSV by using nested PCR method.[3] Li Jane Ling et al., found the higher prevalence of HCMV than HSV and EBV-1 which was determined by nested PCR method.[24] Higher percentage of HCMV was found by Kamma JJ et al., when compared with EBV-1 and HSV.[28] Our study showed higher percentage of HSV-1 than HCMV and EBV, the variation could be due to demographic and geographic difference.

EBV and HCMV were rarely found in chronic periodontitis patients by Dolphus R et al., by using real time PCR.[1] Similar results were found by Nibali L et al., in plaque samples of chronic periodontitis, generalized aggressive periodontitis and localized aggressive periodontitis.[29] Our study showed similar results as the study done by Imbronito et al., who found higher percentage of HSV-1 than HCMV in subjects with generalized aggressive periodontitis, chronic periodontitis and gingivitis by nested PCR method.[30] These studies suggest that Herpes viruses were found in higher frequency in chronic periodontitis patients than in healthy subjects.

The variation in the results of the above mentioned studies and our study might be due to the differences in the methodology, method of collection of samples, the type of PCR used and the periodontal disease activity at the time of sample collection. Nested PCR which was used in most of the previous studies is very sensitive and susceptible to contamination and may give rise to false positive results (Burkardt, 2000).[20] The multiplex Hot STAURT PCR which was used in our study is a qualitative PCR method with a detection range of 50-100 subgingival viruses. The

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main advantage of multiplex PCR is that large scale amplification of viral DNA can be done with minimum cost and time (Shin C et al., 2003).[31]

The mixed herpes viral infections might promote subgingival colonization of pathogenic organisms by a multiplicity of mechanisms. A gingival herpes viral infection may impair the local host defense by infecting and altering the function of polymorphonuclear leukocytes, [32, 33] lymphocytes[34, 35] and macrophages.[36, 37] A virally induced neutrophil dysfunction may serve to potentiate the overgrowth and virulence of P. gingivalis and other members of the subgingival microbiota.[4] In addition the herpesvirus infection can lyse or affect oral epithelial cells. The disruption of the protective epithelial barriers of the periodontium may facilitate access of periodontopathic bacteria to deeper tissues of the periodontium and create additional sites for bacterial binding.[38, 39] The frequency of viruses was higher in active than in inactive sites (Ling L et al.).[13] Virus-infected periodontal sites tend to exhibit more breakdown than herpes virus free sites and a herpes viral active infection was associated with an elevated risk of progressive periodontal disease.[14]

After SRP, there was reduction in the HSV-1, HCMV and EBV score compared to the baseline score in chronic periodontitis group which was significantly higher in the HSV-1. This indicates that SRP was effective in reducing the amount of viral load. The above results are in accordance to the study done by Grenier et al 2009 who found reduction in the scores of HCMV, EBV and HSV after SRP, [26] this suggests that periodontal therapy was effective in reducing the viral load.

Our study showed the presence of viruses in healthy periodontium and chronic periodontitis. The percentage of viruses was more in subjects with chronic periodontitis. SRP was effective to reduce the viral load after 8 weeks in chronic periodontitis subjects. Very few studies are done where in nonsurgical periodontal therapy is used to reduce the viral load, and viruses were detected by Hot STAURT PCR, where in most of the studies viruses were detected by nested PCR.

Future studies are required with more sample size to identify the oral sites of herpes viral latency and characterization of viral gene expression during latency, to determine whether herpes viral replication, host immune response or direct effect of herpes viruses on immune cells act as determinants of periodontal disease.

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TABLES

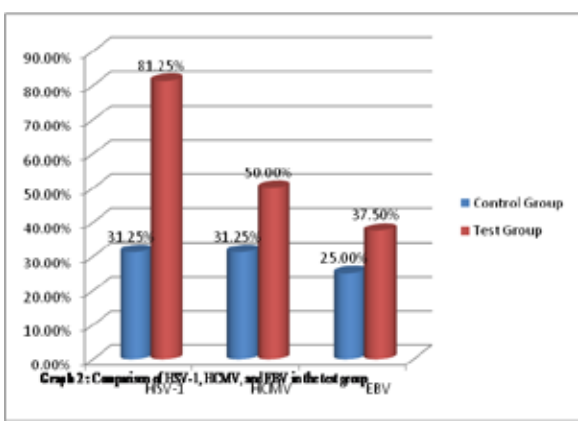
Table 1: Comparison of HSV-1, HCMV & EBV-1 in the test and the control group at baseline

Viruses	Group	Negative	%	Positive	%	Total
HSV-1	Test	3	18.75	13	81.25	16
	Control	11	68.75	5	31.25	16
	Total	14	43.75	18	56.25	32
	Fisher exact test	p=.00567				
	Z-value	-2.4121				
	p-value	0.0159				S
HCMV	Test	8	50.00	8	50.00	16
	Control	11	68.75	5	31.25	16
	Total	19	59.38	13	40.63	32
	Fisher exact test	p=.23				
	Z-value	-0.90				
	p-value	0.36				NS
EBV-1	Test	10	62.5	6	37.50	16
	Control	12	75.00	4	25.00	16
	Total	22	68.75	10	31.25	32
	Fisher exact test	p=.35				
	Z-value	-0.60				
	p-value	0.54				NS

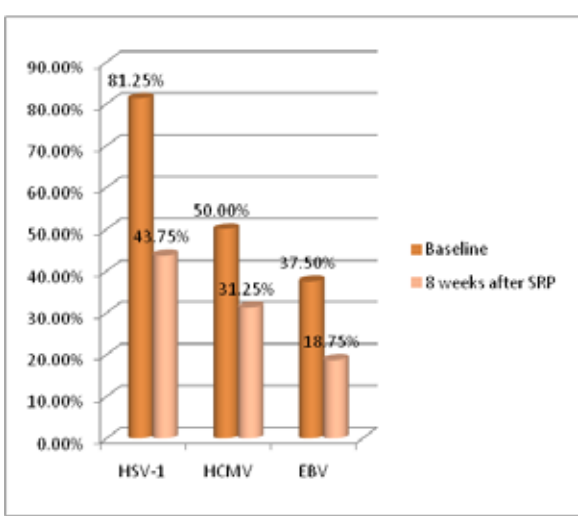
Table 2: Comparison of HSV-1, HCMV, and EBV in the test group.

Viruses	Time	Mean	SD	T-value	Z-value	p-level	Signi
HSV-1	BL-8weeks	0.81-0.44	0.40-0.51	0.00	2.20	0.02	S
HCMV	BL-8weeks	0.50-0.31	0.52-0.48	3.00	1.21	0.22	NS
EBV	BL-8weeks	0.38-0.19	0.50-0.40	0.00	1.60	0.10	NS

Graph 1: Comparison of the HSV-1, HCMV, and EBV in the test and control at baseline.



Graph 2 : Comparison of HSV-1, HCMV, and EBV in the test group



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REFERENCE

1. Dawson DR, Wang C, Danaher RJ, et al., Real time polymerase chain reaction to determine the prevalence and copy number of Epstein-Barr virus and cytomegalovirus in subgingival plaque at individual healthy and periodontal disease sites. J Periodontol 2009; 80: 1133-1140. | 2. Contreras A, Slots JJ. Herpesviruses in human periodontal disease. J Periodontal Res 2000; 35: 3-16. | 3.Contreras A, Umeda M, Chen C, Bakker I, Morrison JL, Slots J. Relationship between Herpesviruses and Adult periodontitis and periodontopathic bacteria. J Periodontol 1999; 70: 478-484. | 4. Slots J. Herpesviruses in periodontal diseases. Periodontology 2000; 2005; 38: 33-62. | 5. Kamma JJ, Slots J. Herpesviral bacterial interactions in aggressive periodontitis. J Clin Periodontol 2003; 30: 420-426. | 6.Kubar A, Saygun I, Yapar M, ozedmir A, Slots J. Real-time PCR quantification of cytomegalovirus in aggressive periodontitis lesions using Taqman technology. J Periodontal Res 2004; 39: 81-86. | 7. Saygun I, Yapar M, Ozdemir A, Kubar A, Slots J. Human cytomegalovirus and Epstein-Barr virus type 1 in periodontal abscesses. Oral Microbiol Immunol 2004 ; 19: 83-87. | 8. Imbronito AV, Okuda OS, Freitas N, Moreira RF, Nunes F D. Detection of herpesviruses and periodontal pathogens in subgingival plaque of patients with chronic periodontitis, generalized aggressive periodontitis or gingivitis. J Periodontol 2008; 79: 2313-2321. | 9.Savitt ED, Strzempko MN, Vaccaro KK, Peros WJ,French CK. Comparison of culture methods and DNA probe analysis for the detection of Actiniobacillus actinomycetomcomitans, Bacteroides gingivalis and Bacteroides intermedius on subgingival plaque samples. J Periodontol 1987 ; 59 : 431-438. | 10.Shelburne CE, Sandberg GP, Binsfeld CA, Wolff LF, Curry RA. Monoclonal antibodies to lipopolysaccharide of four oral bacteria associated with periodontal disease. J Periodontol Res 1993; 28: 1-9. | 11.French CK, Savitt ED, Simon SL, Eklund SM, Chen MC, Klotz LC. DNA probe | detection of periodontal pathogens. Oral microbiology and Immunology 1986 ; 1 : 58-62. | 12.Loesche WJ, Syed SA, Stoll J, Trypsin like activity in subgingival plaque: a Diagnostic | marker for spirochetes and periodontal disease. J Periodontol 1987 ; 58 : 266-273. 13.Newmann MG, Takei HH, Klokkevold PR, Carranza FA Jr., Carranza's Clinical | Periodontology,10thedition.St Louis, Missouri: Saunders, Elsevier Inc; 2006. P.749. | 14.Renvert S, Wikstorm M, Dahlen G, Slots J, Egelberg J. Effect of debridement on The elimination of Actinobacillus actinomycetomcomitans and Bacteroids gingivalis from periodontal pockets. J Clin Periodontol 1990; 17: 345-350. | 15.Baehni P, Thilo B, Chapuis B, Pernet D. Effects of ultrasonic and sonic scalers on dental plaque microflora in vitro and in vivo. J Clin periodontol 1992; 19: 455- 459. | 16.Darby IB, Hodge PJ, Riggo MP, Kinane DF. Clinical and microbiological effect of scaling and root planing in smoker and non smoker chronic and aggressive periodontitis patients. J Clin Periodontol 2005; 32: 200-206. | 17.Philstrom BL. Measurement of attachment levels in clinical trials: Probing | methods. J Clin Periodont 1992; 63: 1072-1077. | 18.Dr. Soben Peter. Essentials of preventive and community dentistry, 3st edition; | New Delhi: Arya publishing house ; 2000. p.148. | 19.Shin C et al. Detection and typing of HSV-1, HSV-2, CMV and EBV by | Quadruplex PCR Yonsei Medical Journal 2003; 44:1001-1007. | 20.Cappuyns I, Gugerli P, Mombelli A. Viruses in periodontal disease – a review Oral diseases 2005 ; 11 : 219-229 | 21. Saygun I, Ozedmir A, Kurtis B, Yapar M, Kubar A. Detection of Human Viruses in patients with Chronic Periodontitis and the relationship between Viruses and Clinical parameters. J Periodontol 2002 ; 73 : 1437 – 1443. | 22.Yapar M, Saygun I, Ozdemir A, Kubar A, Sachin S. Prevalence of Human herpesviruses | in patients with aggressive periodontitis. J Periodontol 2003 ; 74 ; 1634-1640. | 23.Magnusson I, Lindhe J, Yoneyama B, Lijenberg B. Recolonization of subgingival | microbiota following scaling in deep pockets J clin Periodontol 2005 ; 11 : 193-200. | 24.Ling L, Hi C, Wu C, Chen Y, Hung S. Association between Human cytomegaloviruses and the severity of periodontitis J Periodontol 2004 ; 75 : 1479-1485. | 25. Knowles JW. Results of periodontal treatment related to pocket depth and attachment level J Periodontol 1979 ; 50 : 225-233. | 26. Grenier G, Gagnog G, Grenier D. Detection of herpetic viruses in gingival crevicular fluid of patients suffering from periodontal diseases: Prevalence and effect of treatment. Oral microbiology and immunology 2009; 24: 506-509. | 27.Shibata Y, Tani M, Hara Y, Kato I. Effects of scaling and root planing on clinical parameters and bacterial flora in periodontal pockets. 2. Correlation between decrease of probing pocket depth and pretreatment status. Nippon Shishubyo Gakki Kaishi 1989; 3: 905-913. | 28.Kamma JJ, Contreras A, Slots J. Herpes viruses and periodontopathic bacteria in early onset periodontitis J Clin Periodontol 2001 ; 28 : 879-885. | 29.Nibali L et al Low prevalence of subgingival viruses in periodontitis patients J Clin Periodontol 2009 ; 36 : 928-932. | 30.Imbronito A, Okudo O, Freitas N, Lotufa R, Nunes F. Detection of Epstein –Barr virus and human cytomegalovirus in blood and oral samples: Comparison of three sampling methods. J Oral Sci 2008 ; 50 : 25-31. | 31.Shin C et al. Detection and typing of HSV-1, HSV-2, CMV and EBV by Quadruplex PCR Yonsei Medical Journal 2003 ; 44 : 1001-1007. | 32.Schooley TR, Densen HD, Felsentein D, Hirch MS, Henle E, Weitzman S. Antineutrophil antibodies in infectious mononucleosis. Am J Med 1984 ; 76 : 85-90. | 33.Tyler KL, Fields BN. Pathogenesis of viral infection in fields BN, Knife DM, Howley PM, eds. Fields Virology, 3rd edition. Philadelphia: Lippincott- Raven Publishers; 1996 : 173-218. | 34.Tosato G, Magrath I, Koski N, dolley N, Blaese M. Activation of suppressor –T cells during Epatein- Barr virus induced infectious mononucleosis. N Engl J Med 1979 ; 301 : 1133-1137. | 35.Confer DL, Vercellot Gm, Kotasek D, Goodman JL, Ochoa A, Jacob HS. Herpes simplex virus infected cells disarm killer lymphocytes. Proc Natl AcadSci 1990 ; 87 : 3609-3613. | 36. Bale Jf, O Neil ME, Grenier T. The interaction of murine cytomegalovirus with murine neutrophils; Effect on migratory and phagocytic activities. J Leukoc Biol 1985 ; 38 : 723-734. | 37.Winston DJ, Stevens P, Lin CH, Gale RP. Cytomegalovirus inhibitis luminal-dependent chemiluminiscence of phagocytizing polymorphonuclear granulocytes. Clin Res 1981 ; 29 : 398. | 38. Klein Bs, Dollete FR, Yolken RH. The role of respiratory syncytial virus and other pathogens in acute otitis media. J Pediatric 1982 ; 101 : 16-20 | 39. Watanabe M, Ihara T, Kamiya H, Sakurai M. increased expression of adhesion molecules on fibroblast infected with cytomegalovirus. Microbiol Immunol 1995; 39: 129-133 |