Original Research Paper

University J Dent Scie 2021; Vol. 7, Issue 3

# Effect of Non-surgical Periodontal Therapy on Viruses- HSV, EBV and HCMV using Multiplex PCR in Chronic Periodontitis patients.

**Abstract:**

**Aim:** The aim of the study is to evaluate the comparative levels of HSV, EBV and HCMV before and after non-surgical periodontal therapy in patients with chronic generalized periodontitis using multiplex PCR.

**Material and Method:** Subgingival plaque samples were collected from 30 chosen patients with chronic generalized periodontitis, pre and post non-surgical periodontal therapy. Laboratory investigations were done for sub-gingival plaque levels of viruses, HSV, HCMV and EBV by Multiplex PCR techinique. Clinical parameters which had to be measured were Plaque index, Gingival index, probing pocket depth and clinical attachment level.

**Statistical analysis:** Descriptive analysis was presented in forms of mean and standard deviation. Paired t-test was employed to determine any difference in mean of clinical parameters between pre and post treatment among study population. $P < 0.05$ was considered statistically significant.

**Result:** Scaling and root planing resulted in a notable decrease in the level of HSV in chronic periodontitis from baseline to the follow-up of after 3 months with a statistical significance (p value 0.004).The measure of HCMV decreased to signify (p value 0.001),whereas EBV detected level decreased to give statistical significance of (p value 0.001). The severity of clinical parameters at baseline decreased after 3 months and showed a strong and positive statistical significance.

**Conclusion:** A frequency of viruses was predominantly present at baseline which was comparatively reduced after non-surgical periodontal therapy after 3 months. Hence, the non-surgical periodontal therapy proves out to be fruitful to reduce the virus in chronic periodontitis affected individuals.

**Key words:** HSV, EBV, HCMV, Multiplex PCR, Non Surgical Periodontal Therapy.

## Introduction:

This Latin phrase clearly describes that any need or problem encourages creative efforts to meet the need or solve the problem. This phenomenon has also reverberated in the quest for elucidating the pathogenesis behind chronic periodontitis. Since long time researchers have mulled over the etiology for chronic periodontitis. This led them to find the causative organisms of the disease. Initially only the bacteria like Treponema denticola, Porphyromonas gingivalis and Tannerella forsythia were linked with the malady and hence the term periodontal microbiology was recoined as periodontal bacteriology.

In line with this, periodontal diseases are chronic inflammatory diseases of the periodontal tissues leading to their destruction and have poly-microbial and multifactorial etiology.[1] Periodontitis, the most prevalent periodontal pathology, are a chronic disease orchestrated amongst the infectious organisms and humoral and cellular immune responses of the host.[2]

HSV (Herpes Simplex Virus) in periodontal pockets quantifies to show their positive association with increased intensity of periodontal diseases.[4] Also, various scientific evidences strengthened the link between the viruses and

[1]SHWETA RACHALWAR, [2]VEENA KALBURGI, [3]SAI SRI HARSHA NIMMALA, [4]ANUSHRI GUPTA, [5]YASH CHAKKARWAR, [6]AKHIL TRIVEDI

**Address for Correspondence:** Dr. Shweta Rachalwar

Email:

**Received :** 5 August, 2021, **Published :** 31 December, 2021

**How to cite this article:** Rachalwar, S., Kalburgi, V., Nimmala, S. S. H., Gupta, A., Chakkarwar, Y., & Trivedi, A. (2021). Effect of Non-surgical Periodontal Therapy on Viruses- HSV, EBV and HCMV using Multiplex PCR in Chronic Periodontitis Patients. UNIVERSITY JOURNAL OF DENTAL SCIENCES, 7(3)

Access this article online

Website: www.ujds.in

Quick Response Code

DOI: https://doi.org/10.21276/ujds.2021.7.3.4

periodontal diseases in which the herpes virus-bacterial theory of periodontal pathogenesis was propagated around for a decade.[2] Later a few researchers explained, the relationship of HSV and bacteria, so as to evaluate and improvise the understanding of pathogenesis.[9] The knowledge of Real-time PCR (Polymerized Chain Reaction), Nested PCR, Reverse-Transcription PCR and rarely used Multiplex PCR was harnessed to understand the role of viruses like HSV, HCMV (Human Cytomegalovirus) and EBV (Epstein Barr Virus) in aggressive periodontitis, chronic periodontitis and the association of viruses with the severity of the periodontal diseases.[3]

Multiplex PCR is the synchronous identification of numerous targets in a single reaction well, using a different pair of primers for an individual target. This technique is indispensable with couple of probes or even more which are easily distinguishable from each other and recognized simultaneously[19]. Following are the advantages of using multiplex PCR:

a. It yields immense detail with lesser sample,
b. Higher throughput,
c. Time saving,
d. More precision of data normalization,
e. Hardly any pipetting errors.

Multiplex PCR assays execute the minimum time and cost of optimization and validation, bringing into perspective numerous advantages of multiplex PCR. Also, very few studies have been conducted so far by employing multiplex PCR, the present study is being done with the hope of evaluating the comparative levels of HSV, HCMV and EBV before and after non-surgical periodontal therapy in chronic periodontitis patients using this investigation modality and also record the changes occurred in the clinical parameters such as oral hygiene index-simplified, plaque index, gingival index, clinical attachment loss and probing pocket depth.

## Material And Methodology:

### Study Population:

The study population was selected from patients attending the outpatient section of the Department of Periodontics, People's college of dental sciences and research center, Bhopal, India. Subgingival plaque samples were collected from 30 chosen patients with chronic generalized periodontitis.

### Inclision Criteria:

i) Patients having moderate to severe periodontitis.
ii) Patients with more than 20 teeth
iii) Patients who are systemically healthy
iv) Patients aged between 25-60 years.
v) Patients with probing pocket depth of $\geq$ 5 mm in more than 30% of the sites involved.

### Exclusion Criteria:

i) Patients who are medically compromised.
ii) Patients who have undergone periodontal therapy in last 6 months.
iii) Patients who are on antimicrobial therapy in previous 6 months.
iv) Smokers
v) Pregnant or lactating mothers
vi) Patients who are not willing to take part in the study.

### Parameters to be measured:

Clinical parameters which had to be measured pre and post non-surgical periodontal therapy were:

a. Plaque Index (Silness And Loe, 1964)
b. Gingival Index (Loe And Silness, 1963)
c. Probing Pocket Depth (PPD)
d. Clinical Attachment Level (CAL)

Parameters to be evaluated in the laboratory were the sub-gingival plaque levels of viruses, at the baseline and post non-surgical periodontal therapy.

a. Human Simplex Virus (HSV-1, HSV-2)
b. Epstein-Barr Virus (EBV)
c. Human Cytomegalovirus (HCMV)

### Study Design:

The nature and purpose of the study was explained to the patients and an informed consent was obtained from patients and clinical parameters were recorded like plaque index, gingival index, simplified oral hygiene index, probing pocket depth, clinical attachment level.

### Plaque Index :

After the tooth is dried and light properly focused, Shephard's hook dental explorer was placed on distal half of the tooth and moved towards the mesial half, distal, facial, mesial and lingual surfaces of the tooth were examined.[7]

### Gingival Index:

The gingival status of the patient was assessed to know the changes in colour, contour, consistency, position, surface texture, and bleeding on probing of the gingiva. A mouth mirror and blunt periodontal probe were used to assess the bleeding potentiality on a scale ranging from 0 to[3]. The tissues surrounding each tooth were divided into four gingival scoring units namely: disto-facial papilla, facial margin, mesio-facial papilla and the entire lingual margin.[7]

### Probing Pocket Depth (PPD):

Probing pocket depth measurement is the distance from the base of pocket to the gingival margin. The periodontal pocket is a soft tissue change and the only accurate method of detecting and measuring periodontal pockets is careful exploration with a William's graduated periodontal probe. The probe should be inserted parallel to the vertical/long axis of the tooth and walked circumferentially around each surface of each tooth to detect the areas of deepest pocketon distobuccal/ disto-labial, buccal/labial, mesio-buccal/ mesiolabial, disto-lingual, lingual/palatal and mesio-lingual sites.[7]

### Clinical Attachment Level (CAL):

The distance from the cement enamel junction to the base of the pocket during periodontal diagnostic probing is referred to as clinical attachment level. All teeth except third molars were probed using a William's calibrated periodontal probe.[7]

### Sub-Gingival Sample Collection:

Supra-gingival plaque was gently removed with hand scaling from sample sites which were air-dried and isolated with cotton rolls. Sub-gingival plaque was collected from the deepest pocket by sterile curette. The specimens were transferred in a 500µl of TE buffer suspension (10mM Tris-hydroxy-chloride, 1mM EDTA, pH 8) and mixed well vigorously on a vortex. The procedure was again followed after three months, post non-surgical periodontal therapy.

### Nucleic Acid Extraction:

Samples collected were stored at -20□C and centrifuged at 10,000 rpm for 5 mins, the next morning. TE buffer was used to wash the obtained precipitate three times. After the third wash, the supernatant was discarded and the precipitate was treated with 500µl lysis buffer (Tris HCl, Nonidet p-40,Tween 200 with 100µg/ml freshly prepared proteinase K) and kept at 60□C for 2 hours followed by 95□C for 10 mins and deep frozen at -70□C until amplification. Then all the samples are to be sent to the laboratory for the analysis.

### DNA Amplification by Multiplex PCR Technique:

In order to achieve maximum of multiplex PCR, a series of titrations of primer concentrations and deoxy nucleotide triphosphate (dNTP) levels were performed. Primers concentrations of 10, 25, 50 and 100 pmol from each primer pair were titrated simultaneously with dNTP (0.1, 0.2 and 0.3 mM concentrations of each of the dNTPs).

Amplification was performed with the PCR system (Corbett research palm cycler version 2.2, imported by J.H. BIO Innovations, Bangalore, India). Forty amplification cycles of 30s at 94°C, 40s at 60°C and 50s at 72°C were carried out in a 50-□l final volume containing 5□l of ×10 reaction buffer (Bangalore Genei, Bangalore, India), 0.2 mM concentrations of each dNTP, 10 pmol of each of the 12 primers, and 2.5 U of cloned Pfu DNA polymerase (Bangalore Genei). Five microliters of pertinent DNA sample was added to the reaction mixture. The samples were incubated for 15 min at 78°C to complete the extension of primers, after the last cycle. The molecular weight of the individual PCR product was 147 bp (HSV), 182 bp (EBV), and 256 bp (HCMV).

10□l of each amplified product was analyzed by agarose gel electrophoresis on 3.5% agarose (Sigma- Aldrich, Bangalore, India) containing 1□g of ethidium bromide/ml in 1X TBE buffer and was visualized in an UV transilluminator.

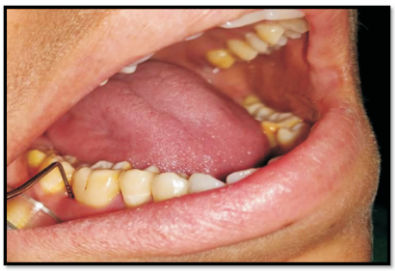


Figure 1: Pre-operative probing pocket depth

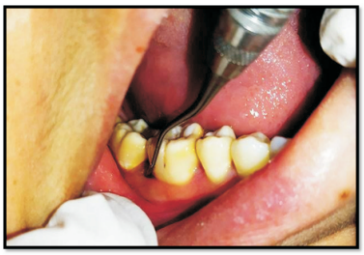


Figure 2: Collecting sub-gingival plaque sample using Gracey's curette

Figure 3: Collected sub-gingival plaque sample transferred to TE buffer solution (Tris-hydroxy chloride, EDTA)

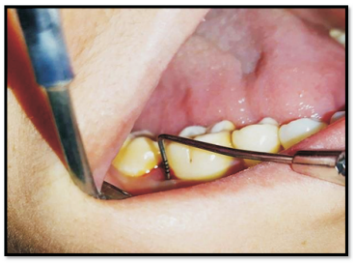


Figure 4: Post-operative probing pocket depth

## Results:

The present study entailed the levels of viruses HSV, HCMV and EBV along with clinical parameters in 30 patients at baseline and after 3 months of non-surgical periodontal therapy. The Institutional Ethics Committee (IEC) project code for the research is EC201806.

Data collected was entered into spreadsheets and analyzed using SPSS version 20.0 (IBM; Chicago). Descriptive analysis was presented in forms of mean and standard deviation. Paired t-test was employed to determine any difference in mean of clinical parameters between pre and post treatment among study population. $P < 0.05$ was considered statistically significant.

The count of demographics including age and gender are summarized in Table 1 and [2].

Table 1: Age distribution of study population (in years)

| Minimum | Maximum | Mean | Standard deviation |
|---|---|---|---|
| 25 | 51 | 36.40 | 7.11 |

Table 2: Gender distribution of study population

Prevalence of herpes virus DNA

Amongst the viruses evaluated, HSV virus showed a significant predominance with a greater frequency of 60%. Relatively less frequency followed by HCMV of 33.3% and EBV of 23.3% at baseline. [Table 3] [Graph 1,2,3,4]

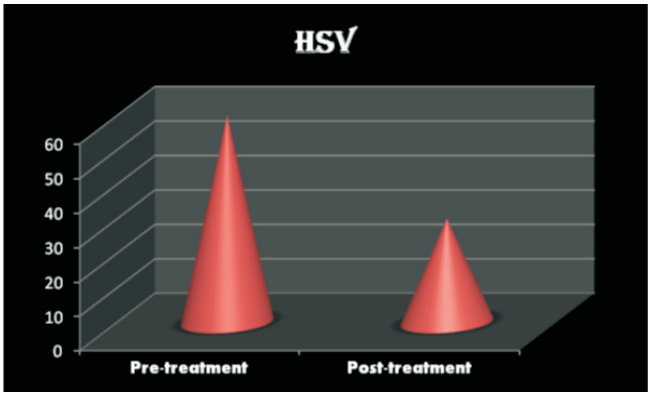
| Gender | Frequency (n = 30) | Percentage (%) |
|---|---|---|
| Male | 16 | 53. 3 |
| Female | 14 | 46.7 |
| Total | 30 | 100 |

Table 3: Virus assessment of study subjects pre – treatment

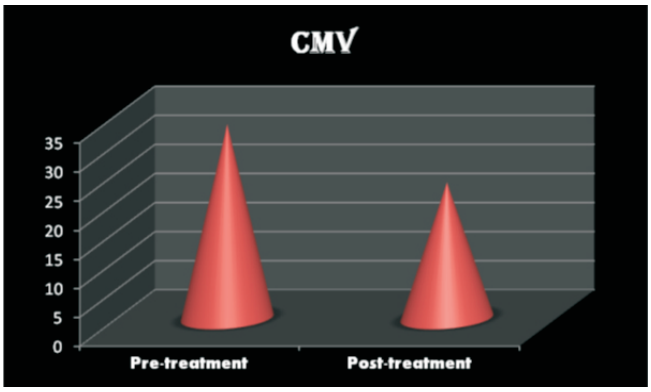
Note* = The percentage in the above table will be greater than 100% as the variables overlap between the groups. (Due to co-infection)

| Virus | Frequency ( n =30) | Percentage |
|---|---|---|
| HSV | 18 | 60.0 |
| HCMV | 10 | 33.3 |
| EBV | 7 | 23.3 |
| Co infection | 12 | 40.0 |

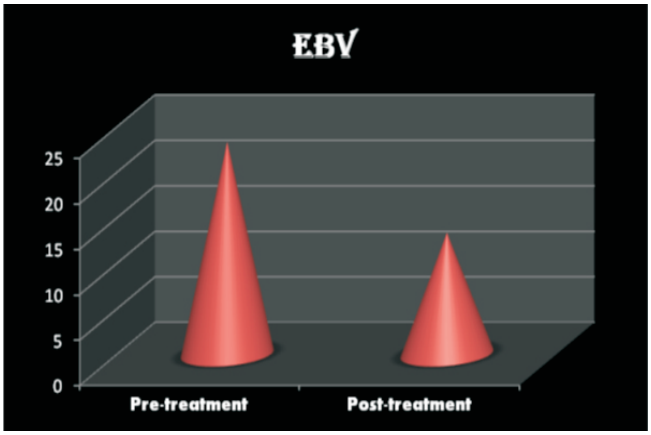
Graph 1: Percentage scores of HSV - Pre- treatment and Post-treatment



Graph 2: Percentage scores of CMV - Pre- treatment and Post-treatment



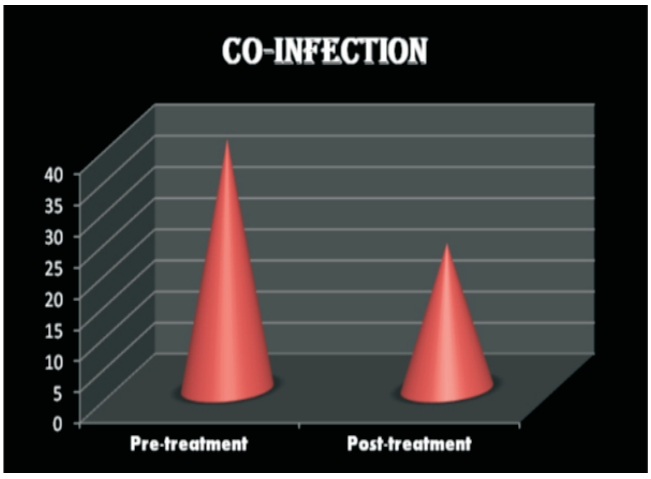

Graph 3: Percentage scores of EBV - Pre- treatment and Post-treatment




Graph 4: Percentage scores of Co-infection- Pre- treatment and Post-treatment

Relationship of detection of clinical parameters at baseline and after 3 months

The graph 5 revealed the severity of clinical parameters at baseline which decreased after 3 months and showed a strong and positive statistical significance [Table 4].




Graph 5. Effect of non-surgical periodontal therapy on clinical parameters

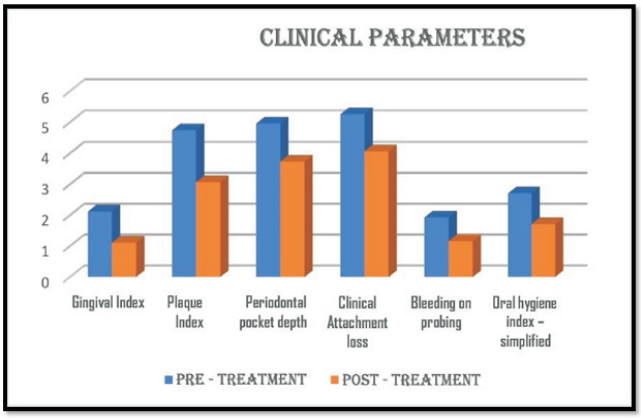



Table 4: Comparison of Clinical parameters measurements amongst study subjects pre and post periodontal treatment Scaling and root planing resulted in a notable decrease in the level of HSV in chronic periodontitis from baseline to the follow-up of after 3 months with a statistical significance (p value 0.004).The measure of HCMV decreased to signify (p value 0.001),whereas EBV detected level decreased to give statistical significance of (p value 0.001). [Table 5]

| Clinical parameters | Mean ± S.D Pre-treatment | Mean ± S.D Post-treatment | P value |
|---|---|---|---|
| Gingival Index | 2.120 ± 0.55 | 1.11 ± 0.59 | <0.001** |
| Plaque Index | 4.74 ± 2.29 | 3.07 ± 1.77 | <0.001** |
| Periodontal Pocket Depth | 4.96 ± 1.51 | 3.73 ± 0.90 | <0.001** |
| Clinical Attachment Loss | 5.26 ± 1.20 | 4.06 ± 1.04 | <0.001** |
| Bleeding on probing | 1.93 ± 1.14 | 1.16 ± 0.74 | <0.001** |
| Oral Hygiene Index Simplified | 2.71 ± 1.29 | 1.71 ± 0.98 | <0.001** |

Table 5: Comparison of pre-treatment versus post-treatment for viruses analzed

| Virus analyzed | Paired t-test | P value |
|---|---|---|
| HSV | 8.51 | 0.004 |
| HCMV | 18.26 | 0.001 |
| EBV | 15.16 | 0.001 |

## Discussion:

The ubiquity for HSV, HCMV and EBV has been detected for chronic periodontitis.[1] The present study embrace multiplex PCR technique for viral identification of different species. Primary requirements of the study were primers which pair specifically to each calculated organisms and gets engaged in a single-tube amplification process.[4] The first few rounds of thermal cycling have substantial effect on the overall sensitivity and specificity of PCR. Assuming efficient denaturation of the target, overall success of specific amplification depends on the rate at which primers anneal to their target and the rate at which annealed primers are extended along the desired sequence during the early, middle, and late cycles of the amplification. The extension rate of specific primer-target hybrids depends on the activity of the enzyme, availability of essential components such as deoxyribonucleoside triphosphates (dNTPs), and the nature of the target DNA. Thus, the majority of modifications to improve PCR performance have been directed towards the factors affecting annealing and/or extension rates.[19] PCR is a very sensitive technique than other conventional and basic microbiological techniques as it can detect micro-organisms in smaller plaque samples.[4]

The present study assessed and estimated the presence of viruses HSV, HCMV and EBV in chronic periodontitis patients at baseline and after 3 months of non-surgical periodontal therapy.[1] In total 30 patients were selected for the study who were systemically healthy and diagnosed with chronic periodontitis falling into the age group of 25-60 years with the probing pocket depth of $\geq$5mm. The plaque samples were collected from the deepest pocket site and were evaluated using multiplex PCR.[1]

On evaluation of the study, it revealed that the gender had no constraints on the study. On evaluating, the results obtained showed the prevalence of HSV significantly greater followed by HCMV and EBV. On further observation, there was a significant reduction of the viruses after phase-I non-surgical periodontal therapy.[1] Contreras et al.[11] concluded that HSV detection rates are higher in severe chronic periodontitis than mild or moderate chronic periodontitis. Similar findings were noted in the study of Grenier et al.[18] Parra & Slots10 also majorly supported the association between HSV present in subgingival infections with severity of periodontitis. However, Mauricio et al8 reported an uncertain role of HSV with the severity of chronic periodontitis patients. In the present study, the detected HSV is 60% in pre- treatment evaluation which has been dropped down to 30% in post-treatment evaluation. On statistical analysis of virus HSV, the p value was 0.004 which was statistically significant. On considering overall detection of clinical parameters such as Oral Hygiene Index- Simplified, plaque index, probing pocket depth, attachment loss and gingival index, a positive and a strong statistical significance ($p < 0.001$) was observed. Authors reported HSV as a principal etiological agent in their respective studies, 100% HSV by Bilichodmath et al.[4], 81.2% by Kolliyavar et al.[8], 40% by Imbronito et al.[9], which is at par with the present study.

HCMV has customarily been related with periodontal diseases. In progressive periodontal disease, reactivation of HCMV occurs as the virus tends to infect periodontal monocytes/macrophages and T-lymphocytes.[13]

This study includes 33.3% of HCMV in pre-treatment of periodontitis patients. Studies reported various prevalence rates of HCMV, 26.3% in Bilichodmath et al.[4] 12% in Das et al.[20] 59% in Chalabi et al.[17] 19.33% in Kazi et al. from the cases of chronic periodontitis. In the present study, comparative decrease in the level of HCMV was seen to 23.3%. One of the study findings have explained that latent HCMV infection is more frequent than active HCMV in human periodontitis.[1] On statistical analysis of virus HCMV, the p value was 0.001 which showed a positive and strong correlation with chronic periodontitis making it statistically significant. Parra & Slots10 and Contreras & Slots11 have demonstrated the presence of HCMV and EBV in 40-78% of chronic periodontitis patients whereas 90% in aggressive periodontitis patients. Also, other studies were performed to evaluate the levels of HCMV virus, Kuber et al. demonstrated HCMV DNA in 78% of subgingival samples of aggressive periodontitis lesions and only 46% in chronic periodontitis patients.[9] Kuber et al. identified in another study using Real-Time PCR that 68.8% of HCMV was present in aggressive periodontitis.[14]

Ling et al.[12] hypothesized that active HCMV infection could be the relative cause for initiation and progression of localized juvenile periodontitis whereas on the contrary, Contreras & Slots results suggested that active HCMV replication may occur in any of the periodontal sites.[11] But this fact has remained unclear whether HCMV reactivation causes initiation or progression of destructive periodontal disease. In the present study, the conclusion for HCMV can be drawn as the destruction caused to the periodontium can be reliable but not decisive.

It has been estimated that, worldwide, 90% of the population infected with EBV is asymptomatic.4 Also, it has been observed that EBV is majorly present in sub-gingival plaque of patients with chronic periodontitis, periodontal abscesses and acute necrotizing ulcerative gingivitis. As mentioned earlier, HCMV infects mainly periodontal monocytes/ macrophages and T- Lymphocytes likewise, EBV infects B-Lymphocytes. With the increase in the freight of viruses, the virulence of the native bacteria increases its invasiveness into the epithelial cells. Thus smoothing the path of pathogenic bacteria to penetrate into connective tissue.[9]

The detection frequency of EBV in subgingival plaque in the present study is comparatively low as compared to HSV and HCMV.[15] In the present study, EBV detected was 23.3% in pre-treatment consistently decreasing it to 13.3% in post-treatment of chronic generalized periodontitis patients. On evaluating statistical analysis, the p value obtained for EBV virus was 0.001 which showed statistically significant value.

Studies were performed and reported their prevalence rates as 78.9% EBV by Bilichodmath et al.[4], 32% by Das et al., [32]. 0% by Mauricio et al.[8], 30.6% by Kazi and Bharadwaj.[8] The detection rate of Mauricio et al and Kazi & Bharadwaj showed some similarity.

Knowing the importance of association of viruses HSV, EBV and HCMV with chronic generalized periodontitis patients may prove to be substantial to understand the pathogenesis of the disease and it also helps one in proper diagnosis, monitoring and treatment of the disease.[16] An average statistical significance was found between overall detection of viruses and clinical parameters such as oral hygiene index simplified, plaque index, probing pocket depth, attachment loss and gingival index in the present study.

The findings of this present study explained clearly the association between the frequency of viruses and the increasing severity of periodontitis highlight for further affirmation for the role of viruses in pathogenesis of the disease.

On scrutinizing the present study results, a classic declaration can be procured as viruses are remarkably higher in pre-treatment than in post-treatment of the patients, bridging an association between the increase in the viruses and the severity of the pathology.

Altogether, viruses and their severity to cause periodontal pathology along with the clinical parameters evaluation at both the phases of treatment and also contemplating the previous studies, a speculation can be held at to state that an infection by HSV, HCMV and EBV transpiring concurrently may employ a pathogenic effect on chronic periodontitis.

## Conclusion:

### Intending towards the limitations of the study:

1. An individual to individual study results can infer with contrasting results.
2. The local population with the infection of periodontitis, lored with aggregation of viruses may diversify the obtained results.
3. Also, the deficient sample size may prove out to be insufficient to conclude.
4. Graduating the follow-up session may also bring changes to the results.
5. Even after considering the exclusion criteria, if the patient turns to be immunosuppressed, the results may bring disparity.

Within the limitations of the study, as per the previous studies conducted, our study also proved to be efficient to infer that HSV was majorly significant virus followed by HCMV and EBV in pre-treatment and a considerable reduction was obtained on follow up after non-surgical periodontal therapy. Hence, the non-surgical periodontal therapy proves out to be fruitful to reduce the virus in chronic periodontitis affected individuals.

The strides that have been made in the field of medical virology has also enabled us to unveil the pathogenesis and the treatment of viral diseases of mouth. Prevention and treatment based on this knowledge helps to avert the infection and disease progression caused by viruses. The fact that "the sky is the limit" for the number of researches needed to validate a given hypothesis. Our study was a genuine attempt to gain even more insight into this incessant quest for knowledge and we hope that the coherence of our results with the world literature further bolsters the grasp of this field.

## References:


1. Shah R, Mehta DS. Prevalence of herpesviruses in gingivitis and chronic periodontitis: relationship to clinical parameters and effect of treatment. J Indian Soc Periodontol 2016;20:279;5.
2. Saygun I, Sahin S, Ozdemir A, Kurtis B, Yapar M, Kubar A, et al. Detection of human viruses in patients with chronic periodontitis and the relationship between viruses and clinical parameters. J Periodontol 2002;73:1437-43.
3. Sharma M, Kotwal B, Mahajan N, Kharyal S, Slathia B, Tomar V. Role of Viruses in Periodontal Diseases Research Study. Int J Sci Stud 2017;5(7):59-64
4. Bilichodmath S, Mangalekar SB, Sharma DC, Prabhakar AK, Reddy SB, Kalburgi NB, et al. Herpesviruses in chronic and aggressive periodontitis patients in an Indian population. J Oral Sci 2009;51:79-86.
5. G Chandni, D Deepa. Role of periodontopathogenic virus in periodontal disease: a review
6. E Mohammad, W Firdous, W Iram, K Prabhjot, N Sheeba. Polymerase Chain Reaction (PCR): Back to Basics
7. Soben Peter. Essentials of Preventive and Community Dentistry (Public Health Dentistry), 4th Edition.
8. Kazi MM, Bharadwaj R. Role of herpesviruses in chronic periodontitis and their association with clinical parameters and in increasing severity of the disease. Eur J Dent 2017;11:299-304.
9. Imbronito AV, Grande SR, deFretas NM, Okuda O, Lotufo RFM, Nunes FD (2008) Detection of Epstein-Barr virus and human cytomegalovirus in blood and oral samples: comparison of three sampling methods. J Oral Sci 50, 25-31.
10. Parra B, Slots J. Detection of human viruses in periodontal pockets using polymerase chain reaction. Oral Microbiol Immunol 1996;11:289-93.
11. Contreras A, Slots J. Active cytomegalovirus infection in human periodontitis. Oral Microbiol Immunol 1998; 13:225-230.
12. Ling LJ, Ho CC, Wu CY, Chen YT, Hung SL. Association between human herpes viruses and the severity of periodontitis. J Periodontol 2004;75:1479-85.
13. Botero JE, Parra B, Jaramillo A, Contreras A. Subgingival human cytomegalovirus correlates with increased clinical periodontal parameters and bacterial co-infection in periodontitis. J Periodontol 2007;78:2303-10.
14. Botero JE, Vidal C, Contreras A, Parra B. Comparison of nested polymerase chain reaction (PCR), real-time PCR and viral culture for the detection of cytomegalovirus in subgingival samples. Oral Microbiol Immunol 2008;23:239-44.
15. Wu YM, Yan J, Ojcius DM, Chen LL, Gu ZY, Pan JP. Correlation between infections with different genotypes of human cytomegalovirus and Epstein-Barr virus in subgingival samples and periodontal status of patients. J Clin Microbiol 2007;45:3665-70.
16. Rotola A, Cassai E, Farina R, Caselli E, Gentili V, Lazzarotto T, Trombelli L. Human herpes virus 7, Epstein–Barr virus and human cytomegalovirus in periodontal tissues of periodontally diseased and healthy subjects. J Clin Periodontol 2008; 35: 831–837.
17. Chalabi M, Moghim S, Mogharehabed A, Najafi F, Rezaie F. EBV and CMV in chronic periodontitis: A prevalence study. Arch Virol 2008; 153:1917-9.
18. Grenier G, Gagnon G, Grenier D. Detection of herpetic

viruses in gingival crevicular fluid of patients suffering from periodontal diseases: prevalence and effect of treatment. Oral Microbiol Immunol 2009: 24: 506–509.

19. Elnifro EM, Ashshi AM, Cooper RJ, Klapper PE. Multiplex PCR: Optimization and Application in Diagnostic Virology. American Society for Microbiology, 2000;13[4]:559–570.
20. Das S, Krithiga GSP, Gopalakrishnan S. Detection of human herpes viruses in patients with chronic and aggressive periodontitis and relationship between viruses and clinical parameters. J Oral Maxillofac Pathol. 2012;16(2): 203–209