Prevalence of TT Virus in patients with chronic periodontitis, patients with aggressive periodontitis and healthy controls - a pilot study

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Abstract
Torque teno virus (TTV), a novel DNA virus resides in peripheral blood mononuclear cells and replicates when these cells get activated. The TTV replication shifts the immunobalance. **Aim:** To determine the presence of TTV in the gingiva of patients with aggressive periodontitis, patients with chronic periodontitis, and healthy controls, and to correlate the presence of TTV with probing pocket depth and clinical attachment level. **Methods:** Forty-two subjects (22 males and 20 females) aged 21 to 55 years were recruited for this study. Subjects were stratified into aggressive periodontitis (Group I), chronic periodontitis (Group II) and healthy controls (Group III). Gingival tissue biopsy was taken from all the subjects and the presence of TTV was analyzed using PCR and 2% agarose gel electrophoresis. **Results:** TTV was identified in half of the subjects and more number of subjects with periodontitis have TT virus compared to controls. There was significant association between presence of TT virus and pocket depth, clinical attachment level. **Conclusions:** The findings from the present study shows that there was no significant association between TT virus and periodontitis, even though it was isolated from more number of subjects with aggressive periodontitis, and TTV was associated with pocket depth and clinical attachment level. These findings need to be investigated in further studies.

Keywords: clinical attachment level, periodontitis, pocket depth, polymerase chain reaction, torque teno virus.

Introduction
Since 1997, groups of novel nonenveloped DNA viruses with a circular, single-stranded (negative sense) DNA genome of 3.6-3.9 kb, 3.2 kb, or 2.8-2.9 kb in size have been discovered and designated torque teno virus (TTV), torque teno midi virus (TTMDV), and torque teno mini virus (TTMV), respectively, in the floating genus Anellovirus⁵.

TTV belongs to the circoviridae family and it was first isolated from the serum of Japanese patients with post transfusion hepatitis⁶. Initially the mode of transmission of this virus was considered to be through blood and blood products and hence it was known as transfusion transmitted virus⁷. But other modes of transmission like nasal secretions, sexual transmission and oral fecal route also exist⁸. The anelloviruses frequently and ubiquitously infect humans, and the infections are characterized by lifelong viremia and great genetic variability. However, the
level of virus replication may vary among different individuals and this may represent an important marker of pathogenic role for TTV. TTV viral loads have been shown to increase in human immunodeficiency virus (HIV)-infected patients who are progressing toward AIDS, and a high TTV viral load was associated with a low CD4 cell count, indicating a potential role of the immune system in controlling TTV replication. The frequent identification of TTV in peripheral mononuclear blood cells (PMBC) may suggest that the virus replicates in lymphoid cells.

TTV has been associated with many chronic diseases like asthma, bronchiectasis, and hepatic failure. The previous study has shown association between periodontitis and TTV virus, but there was no clear cut distinction between chronic and aggressive periodontitis, and their association with TT virus.

Hence, the purposes of the present study were to determine the presence of TTV in the gingiva of patients with aggressive periodontitis, patients with chronic periodontitis, and healthy controls, and to correlate the presence of TTV with probing pocket depth and clinical attachment level.

Material and methods

Study Population

The study population consisted of 42 subjects who reported to the department of Periodontics, Saveetha Dental College and hospitals, Chennai. Subjects included 22 males and 20 females belonging to the southern Indian population. Fourteen subjects were periodontally healthy and 28 subjects were diagnosed with periodontitis. Patients with periodontitis were stratified into an aggressive periodontitis group (Group I) and a chronic periodontitis group (Group II) based on the criteria of AAP 1999. Group III included the healthy controls.

Subjects taking any medications, those who underwent any periodontal treatment within past six months, smokers, systemically ill subjects like diabetes mellitus, hypertensive subjects, patients with cardiac problem, immunocompromised subjects, patients under medications for any systemic diseases like asthma, bronchiectasis, and hepatic failure. The previous study has shown association between periodontitis and TT virus, but there was no clear cut distinction between chronic and aggressive periodontitis, and their association with TTV virus.

For the control group, tissue samples were collected during orthodontic purposes. The gingival tissue was then washed with sterile saline solution, put into a sterile centrifuge tube containing Earles balanced salt solution (EBSS) viral transport medium and kept in a freezer at -20°C. TT viral DNA extraction was carried out on weighed tissue samples using QIAgen DNA extraction kit according to manufacturer’s protocol. Extracted viral DNA was eluted in 50 µL TE buffer and stored at -80°C until PCR amplification.

PCR Reaction Mix (Takahashi et al., 1998)

25 µL reaction volume containing 12.5 µL of 2 X PCR master mix, 0.5 µL of Taq 1.0 µL of forward and reverse primers 5 µL of Nuclease free water and 5 µL of DNA was taken into the reaction mix. The forward primer sequence 5'-GCTACGTCACTAACCAGTG-3' (T 801, sense primer) and the reverse primer 5'-CTCCGGTGTGAAACTCACC-3' (T935, antisense primer) were used for PCR amplification.

Reaction Cycle

The reaction mix was kept at 95 °C for 10 min for reverse transcriptase inactivation and was subjected to the following thermo cycling profile in a thermocycler (Perkin Elmer cetus, USA). Denaturation at 94°C for 20 s, annealing at 60°C for 20 s and template extension at 72°C for 30 s. The cycle was repeated 55 times.

The PCR amplified products were analyzed on a 2% agarose gel with intercalating ethidium bromide dye. 10 µL of the amplified product was mixed with 1 µL of 10X loading dye and loaded into the wells along with 1 µL of molecular weight marker (50 bp ladder). The electrophoresis was run at 100 volts in 0.5X TBE buffer. The gel was visualized under UV transillumination and the products were compared with molecular weight marker as shown in figure 1.

Statistical Analysis

The descriptive statistic analysis was expressed as a mean ± standard deviation. ANOVA and Post Hoc Tukey test were performed to test for significance of means between groups. Unpaired t-test was performed to test for the difference between two means. Statistically significant values were set at p<0.05.

Results

Of the 42 subjects, 20 had positive results for the presence of TTV in gingival tissue. Descriptive statistical analyses are reported in Table 1 and the analysis of presence of TTV with mean pocket depth and mean clinical attachment.
TTV viral loads than controls with mild rhinitis in neonates and children hospitalized with acute respiratory disease or with bronchiectasis showed higher viral loads in nasal specimens were shown to have worse spirometric values, and TTV was suggested to contribute to the pathogenesis of asthma.

### Discussion

Although the gram negative bacteria is essential in initiating and perpetuating the periodontal destruction, the viruses have been shown to have a role in the etiology of periodontitis, especially the viruses belonging to the Herpetoviridae family like Herpes simplex, Epstein barr virus and cytomegalovirus play an important role in periodontal destruction. The main pathogenic mechanism of the above mentioned viruses tends to be the cytopathic effect on macrophages and monocytes. TTV has been associated with many diseases. For instance, infection with TTV coincided with mild rhinitis in neonates and children hospitalized with acute respiratory disease or with bronchiectasis showed higher TTV viral loads than controls. In addition, children with high TTV loads in nasal specimens were shown to have worse spirometric values, and TTV was suggested to contribute to the pathogenesis of asthma. The possible pathogenic mechanism of TTV in the above mentioned disease includes the following, TTV infects hematopoietic cells but only replicate when these cells are activated. The TTV replication could twist the immunobalance towards the T helper 2 cell (Th2) response. This shift in immunobalance is known to have a role in the pathogenesis of asthma. The other possible pathogenic mechanisms would be the open reading frame protein ORF 2 of TTV interferes with the activity of NF-κB, a well-characterized intracellular signal transcription factor known to play a myriad of roles in inflammation and immunomodulation.

Hence, the aim of the present study was to determine whether there was any association between the presence of TTV and periodontitis.

The present study did not find statistically significant association between gender and presence of TTV, which agrees with the study of Masia et al. Overall the results of the present study shows that half of the subjects examined including test and control group harbored TTV in gingiva. Also, the TTV was isolated from a larger number of subjects with periodontitis compared to healthy controls. The number of subjects with TTV was larger in the aggressive periodontitis group compared to chronic periodontitis and control groups. This is an important finding and further research is required to analyze the role of TTV in the etiopathogenesis of aggressive periodontitis.

It was also observed that the presence of TT virus correlated with pocket depth and clinical attachment level (p = 0.005 and p = 0.009). Within the groups, the TTV was also isolated in more subjects from deeper pocket regions (p = 0.015). A possible explanation for this can be ascribed to the ability of TTV to replicate in locally stimulated resident lymphoid cells. It is also possible that the periodontal inflammatory process attracts in situ peripheral lymphocytes that contain TTV. Thus, the presence of TTV correlated with increasing pocket depth and clinical attachment level and also TTV was isolated from more subjects with aggressive periodontitis. The major limitation of this study was the smaller sample size and hence further research using larger sample sizes are required.

In conclusion, the findings of the present study show that there was significant association between TT virus and pocket depth and clinical attachment level in TTV-positive subjects, even though there was no significant association between patients with periodontitis and healthy subjects. Further studies using larger populations and genotyping of TTV in periodontitis are needed.

### References

1. de Villiers, EM, zur Hausen H, editors. TT viruses; the still elusive human pathogens. Berlin: Springer; 2009.


