ANALYSIS OF HERPES SIMPLEX VIRUS USING GINGIVAL CREVICULAR FLUID- A REVIEW

Darini.A, Dr. M.D.Abdul Rahim Akbar, Dr. Jabeen Fathima.G*, Dr. Ahmed Danish Rehan**, Dr.Pavithra.R.S , Dr.Velkumar.J
Department of Periodontology, Ragas Dental College and Hospital, Uthandi, Chennai, Tamilnadu, India
*Medical Microbiology, Government of Tamilnadu, Tamilnadu, India
**Department of Oral pathology and microbiology, Jawaharlal Nehru Institute of Medical Science, Imphal, Manipur, India.

ABSTRACT

Oral Herpes is a viral infection caused by Herpes Simplex Virus. 80% of Oral Herpes is caused by HSV-1 which infects the skin and/or mucous membranes and produces painful sores or blisters on lips, gums, tongue, cheeks. The initial outbreak of virus strain can result in flu-like symptoms, including fever, swollen lymph nodes and body aches. If not found in initial stages, it may result in systemic disorders like diabetes mellitus, cardiovascular or cerebrovascular diseases. The best solution to diagnose oral herpes is by analyzing Gingival Crevicular Fluid (GCF) through nested Polymerase Chain Reaction (PCR) technique. In periodontal disease pathogenesis, the inflammatory mediator levels present in gingival crevicular fluid represent relevant risk indicators for disease activity. Gingival crevicular fluid (GCF), a serum transudate or inflammatory exudate, can be collected from the gingival crevice surrounding the teeth. The fluid reflects the constituents of serum, the cellular response in the periodontium, and contributions from the gingival crevice. The amount of GCF is directly proportional to the severity of the inflammation. Now-a-days several methods for collection and estimation of GCF has been established. Hence, by analysing in this technique, the presence of HSV in the periodontium before the onset, at the time of periodontitis initiation, and periodically during its development can be diagnosed.

KEYWORDS: Herpes virus, Gingival crevicular fluid, inflammation, periodontitis
INTRODUCTION

Oral herpes is an infection caused by Herpes Simplex Virus. The virus causes painful sores on the lips, gums, tongue, roof of mouth and cheeks. Herpes Simplex is a virus with multiple strains. Two of these strains: HSV-1 and HSV-2, cause Oral and Genital Herpes respectively. Herpes viruses infect the skin and/or mucous membranes. Oral herpes presents as cold sores or fever blisters. Approximately 80% of Oral Herpes cases are caused by HSV-1, while the remaining 20 percent are caused by HSV-2, and 75%-80% of Americans carry at least one strain of HSV (Type 1 or 2).

GINGIVAL CREVICULAR FLUID

Gingival crevicular fluid (GCF), a serum transudate or inflammatory exudate, can be collected from the gingival crevice surrounding the teeth. The fluid reflects the constituents of serum, the cellular response in the periodontium, and contributions from the gingival crevice. The study of GCF has focused on defining the pathophysiology of periodontal disease, and identification of a potential diagnostic test for active periodontitis.

In evaluating the pathogenesis of periodontal diseases, Gingival Crevicular Fluid is a potential prognostic and diagnostic tool. As host susceptibility is a critical determinant in periodontal disease pathogenesis, the inflammatory mediator levels present in GCF represent relevant risk indicators for disease activity. More recently, GCF metabolomics appears promising as an additional diagnostic method. Omics analyses of GCF, measuring microbial and host interactions in association with the onset and progression of periodontal diseases, still show the potential to expand the landscape for the discovery of diagnostic, prognostic and therapeutic markers. Inflammatory markers in GCF such as prostaglandins(PGE2), neutrophil elastase, lysosomal enzyme beta-glucuronidase, cytokines, chemokines, neuropeptides assists in defining the impact of certain systemic disorders (e.g., diabetes mellitus) to periodontal disease, and sustenance of a periodontal disease/periodontal progression and indeed may be lower at progressive sites.

GCF does not correlate with disease activity, rather a potential diagnostic test for active periodontitis.

COMPOSITION OF GCF:

I. Cellular elements

a. Epithelial cells: Fluid originating from areas with more severe gingivitis contained a much higher proportion of cells typical to the deepest epithelial layer.

b. Leukocytes: It has been established that 47% of somatic cells obtained from the gingival sulcus were leukocytes, whereas the presence of inflammatory cells in the gingival crevice showed 98% of polymorphonuclear cells. The absolute number of cells increased proportionately with the intensity of inflammation, whereas the differential count was 95–97% neutrophils, 1–2% lymphocytes, and 2–3% mononuclear cells.

c. Bacteria: Bacteria cultured from GCF were similar to those found in the adjacent dental plaque electrolyte.

d. Electrolytes: Some of these are sodium, potassium, fluoride, calcium, iodine, and phosphorus. Na:K ratio in GCF is 3:9 as opposed to its ratio of 28:1, which confirms that the fluid passes through damaged tissue due to accumulation of intracellular potassium from disrupted cells.

II. Organic compounds

a. Carbohydrates: Glucose, hexosamine and hexuronic acid are two of the compounds found in gingival fluid.

b. Proteins: The total protein content of gingival fluid is much less than that of serum.

c. Immunoglobulins: The total immunoglobulin in GCF does not correlate with disease severity or progression and indeed may be lower at progressive sites.

d. Complement: Complement proteins are present in GCF from sites with inflammation and the split fragments C3 and factor B have been detected during experimental gingivitis.

e. Cytokines: Interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF-α) are produced by activated macrophages and other cells. IL-1α and IL-1β are present in inflamed gingiva.

f. Metabolic and bacterial products: Metabolic products such as lactic acid, amino acid such as hydroxyproline and prostaglandin are found in GCF.

III. Enzymes

a. Proteolytic and hydrolytic enzymes of inflammatory cell origin: Inflammatory process leads to the release of polymorphonuclear neutrophils or leukocytes (PMN), macrophages, lymphocytes, and mast cells. The lysosomes of these inflammatory cells contain destructive enzymes that degrade the bacterial and metabolic byproducts during the process of phagocytosis. These enzymes are, however, capable of degrading gingival tissue components if released.

b. Collagenases: These are a part of matrix metalloproteinase family that degrades collagen. They are synthesized by macrophages, neutrophils and fibroblasts and keratinocytes are secreted by these cells as latent enzymes when stimulated by some bacterial products and cytokines. Total enzyme activity levels were significantly higher and enzyme inhibitor levels were lower at diseased sites compared with healthy or treated sites.

c. Cysteine proteinases: Cathepsins B, L, and H are a family of cellular cysteine proteinases, which can degrade extracellular components including collagen. They act at acidic pH and are particularly active during bone resorption. They are also produced principally by fibroblasts, macrophages, and...
Aspartate proteinases:

- Tryptase:
  - Active elastase can only occasionally be detected in gingival tissue and is usually seen adjacent to junctional epithelium where PMNs are migrating into the crevice or in granulation tissue at the advancing front of the lesion.

- Elastase:
  - Tryptase activity is present in large amounts in gingival tissue and in small amounts in GCF and has been localized to gingival mast cells. Trypsate stimulates the release of collagenase from gingival fibroblasts and in inflamed gingival tissues.
  - Myeloperoxidase, lysosome, lactoferrin:
    - Myeloperoxidase is a potent bacterial enzyme produced by PMNs, which is higher at periodontal disease sites than healthy sites, whereas lysosomes are found in body secretions notably tears and saliva and in GCF. Lactoferrin is an antibacterial agent produced by inflammatory cells in GCF.

- Dipeptidyl peptidase II (DPP II):
  - It is active at acidic pH, and DPP IV, which is active at alkaline pH, are present in gingival tissue and GCF.

- Myeloperoxidase, lysosome, lactoferrin:
  - Myeloperoxidase is a potent bacterial enzyme produced by PMNs, which is higher at periodontal disease sites than healthy sites, whereas lysosomes are found in body secretions notably tears and saliva and in GCF. Lactoferrin is an antibacterial agent produced by inflammatory cells in GCF.

- Aspartate aminotransferase and Lactate dehydrogenase:
  - Aspartate aminotransferase is confined to the cell cytoplasm that is released by dead or dying cells, whereas lactate dehydrogenase is present in the cytoplasm of erythrocytes, thrombocytes and leukocytes.

IV. Markers of connective tissue degradation

These include the types I, III, and IV collagen, proteoglycans, hyaluronic, fibronectin, laminin, and bone-specific proteins.

V. Drugs in GCF

Metronidazole and tetracycline can eliminate tissue bacteria, and in conjunction with scaling and root planing, they suppress A. actinomycetemcomitans levels. Tetracycline in low doses inhibits the activity of collagenase and other collagenolytic enzymes.

COLLECTION OF GINGIVAL CREVICULAR FLUID

GCF can be collected by using absorbing paper strips, preweighed twisted threads, micropipettes, crevicular washings.

ANALYSIS OF GINGIVAL CREVICULAR FLUID

The presence of viruses in GCF samples can be assessed by a nested PCR amplification technique. Paper strips are the most convenient and accurate method for gingival crevicular fluid collection, while ELISA can be considered the most conventional method for the diagnosis of biofluids.1 The multiplex polymerase chain reaction (PCR) amplification technique was used to identify viral DNA from HSV. GCF analysis shows increased number of biomarkers such as: cytokines, interleukins, Tumor Necrosis Factors, PTX3, certain enzymes which indicates underlying disease condition. Presence of specific viral infections may be confirmed by means of above-mentioned techniques with the help of viral DNA or RNA.

FORMS OF HERPES:

HSV-1 can recur spontaneously in the eye, causing Ocular Herpes, which can be serious and leading to blindness. Rarely, HSV-1 can spread to the brain, causing Herpes Encephalitis, which leads to death. Furthermore, HSV-1 is also the usual etiology of Herpes gladiatorum, an infection on the finger. "Wrestler's Herpes" or Herpes gladiatorum is an infection on the chest or face.

TRANSMISSION, SYMPTOMS AND COMMUNICABILITY OF ORAL HERPES:

Herpes Simplex Virus affects only humans. Mouth sores most commonly occur in children aged 1-2 years, but they can affect people at any age and any time of the year. People contract herpes by touching infected saliva, mucous membranes, or skin. Because the virus is highly contagious, most people have been infected by atleast one herpes subtype before adulthood. Oral Herpes is most transmitted by kissing or sharing drinks or utensils but can also be contracted from a partner who has genital Herpes during oral sex. HSV-1 and 2 can be contracted from infected bodily fluids, including semen, vaginal fluid, saliva, or herpes lesions, sores or blister fluid. Upon entering a cell, the infection often does not cause any symptom. The initial outbreak of the virus strain can result in flu-like symptoms, including fever, swollen lymph nodes, muscle and body aches. If the virus destroys the host cell during replication, sores or blisters filled with fluid appear. Scabs form over the sores or blisters once the fluid is absorbed, then the scabs disappear without scarring.

The herpes virus goes through dormant phases where it becomes inactive for indeterminable periods of time and reactivates unpredictably. Herpes can be transmitted even when signs or symptoms are not present. This process is known as “shedding” and occurs when cells that have the active virus are dropped or shed from the skin. Approximately one-third to half of all shedding occurrences is asymptomatic. After the herpes virus infects, it has a unique ability to proceed through 3 stages:

(i) Primary infection: The virus gains entry via the skin or mucous membrane and reproduces. During this stage, oral sores and other symptoms, such as fever, may develop. People suffering from oral Herpes may also experience itching, burning or tingling around the mouth or lips. Before the first blisters or cold sores appear, individuals infected with the virus may also experience flu-like symptoms including sore


Darini et al: Analysis of Herpes Simplex Virus using GCF
throat, fever, swollen glands and/or pain when swallowing. This is called asymptomatic infection, occurs twice as often as the disease with symptoms.

(ii) Latency: From the infected site, the virus moves to a mass of nervous tissue in the spine called the dorsal root ganglion, where the virus reproduces again and becomes inactive.

(iii) Recurrence: When one encounters certain emotional or physical stress, the virus may get reactivated and cause new sores and symptoms.

**DIAGNOSTIC METHODS FOR HERPES DETECTION**

The presence of viruses in GCF samples can be assessed by nested PCR amplification technique or quantitative real time PCR, while ELISA can be considered the most conventional method for the diagnosis of biofluids. HSV 1 and HSV 2 in the blood is tested using the Enzyme-Linked Immuno Sorbent Assay (ELISA). This test is highly sensitive and will detect the presence of HSV antibodies in the bloodstream. These antibodies have a development period of 2 weeks to 6 months after initial infection that can be detected through ELISA test. This period varies from person to person. Petrovic et al recommends tests to be done for both Herpes type 1 and type 2 atleast 4-6 weeks after potential exposure. The multiplex polymerase chain reaction (PCR) amplification technique was used to identify viral DNA from HSV.

**TREATMENT FOR HERPES**

There is no cure for Oral or Genital herpes, but there are treatment options. Avoiding oral sex or kissing someone with herpes can help prevent the spread of herpes. The first herpes symptoms after infection may also go away on their own after 10-14 days, although the infection itself remains dormant in the body. Antiviral medications, such as acyclovir, famcyclovir, and valacyclovir are the most effective medications available which reduces the severity and frequency of symptoms, however they cannot cure the infection.

**DISCUSSION**

An etiopathogenic role for Herpes virus has been suggested for chronic periodontitis, aggressive periodontitis, periodontal abscess, periapical abscess and perimplantitis. The present study evaluates the presence of HSV in GCF which further enhances the destruction of periodontium. This study is in accordance with previous studies and describes the prevalence of HSV 1 being significantly higher in gingivitis and chronic periodontitis patients. The results produced significant reduction in Herpes virus levels after Phase I therapy and this finding was in accordance with the study by Grenier et al. Reduction of HSV 1 population after Phase I periodontal therapy can be attributed to a reduced influx of HSV 1 infected cells, resolution of gingival inflammation and reduction in pocket depth due to tissue shrinkage. It was found that number of herpes virus species increased significantly with an increasing severity of all the clinical parameters. Patients with coinfection by two or three herpes viruses exhibited significantly more periodontal destruction than patients containing one or no herpes viruses. Previous studies of aggressive periodontitis have also found an association between herpesvirus coinfection and increased gingival bleeding and probing depth.

Herpes viruses may contribute to the progression of periodontitis through several mechanisms. It is assumed that these viruses can express cytopathogenic effects, immune evasion, immunopathogenicity, latency, reactivation and tissue tropism. They can infect or alter structural cells and host defense cells in the periodontium, and thereby reduce the ability of periodontal tissues to resist bacterial insults. Results showed a high prevalence of HSV 1 in GCF (35.8%) which is in accordance with some authors who reported a high prevalence of this virus in specimens taken with paper points plaque samples of periodontal pockets. Contrary to the results, Nibali et al found a lower prevalence of HSV 1 in both patients with periodontitis and healthy controls. On the other hand, Grenier et al reported higher prevalence of HSV 1 in patients with periodontitis than in healthy controls. Parra and Slots also found statistically higher prevalence of HSV 1 in patients with chronic periodontitis than in patients with mild gingivitis. Similar results were inferred by Contreras et al in gingival tissue specimens. Bilihoodmath et al found higher prevalence of HSV 1 in patients with chronic periodontitis than in patients with aggressive form, but the results were based on patient's age. Herpes viruses particularly HSV 1 play a potential role in pathogenesis of some oral diseases. Higher evidence of HSV in Nigerian malnourished children with ANUG is found. The hypothesis is that herpes viruses can affect the host's immune system, facilitating the development of secondary bacterial infections.

**CONCLUSION**

The presence of HSV-1 in the GCF is related to the degree of tissue destruction in patients with periodontitis. The confirmation of the role of HSV-1 in the pathogenesis of periodontitis requires a larger sample along with a prospective study that would detect the presence of HSV in the periodontium before the onset, at the time of periodontitis initiation, and periodically during its development. Also, future studies demonstrating the role of HSV infection in the pathogenesis of periodontitis should prove that eradication of viral infection can prevent the progression of periodontal destruction.

**FINANCIAL SUPPORT AND SPONSORSHIP:**

Nil

**CONFLICTS OF INTEREST:**

There are no conflicts of interest.
REFERENCES:


