Collection of Gingival Crevicular Fluid from Children Using Periotron 8010

Most investigations concerning periodontal disease and its relationship to gingival crevicular fluid had been focused on adults.
Very little interest had been given to children and young adolescents.

Gingival crevicular fluid (GCF) is a biological exudate and quantification of its constituents is a current method to identify specific biomarkers with reasonable sensitivity. At sites in the absence of inflammation and subgingival plaque, the production of GCF is mediated by passive diffusion of the extracellular fluid by an osmotic gradient. In this situation, the GCF is considered as a transudate. When an inflammatory response is provoked by compounds of microbial origin, the permeability of the epithelial barrier and the underlying vasculature increases and the GCF protein concentration is now modulated by extent of plasma protein exudation. Subsequently, the GCF is considered an inflammatory exudate.

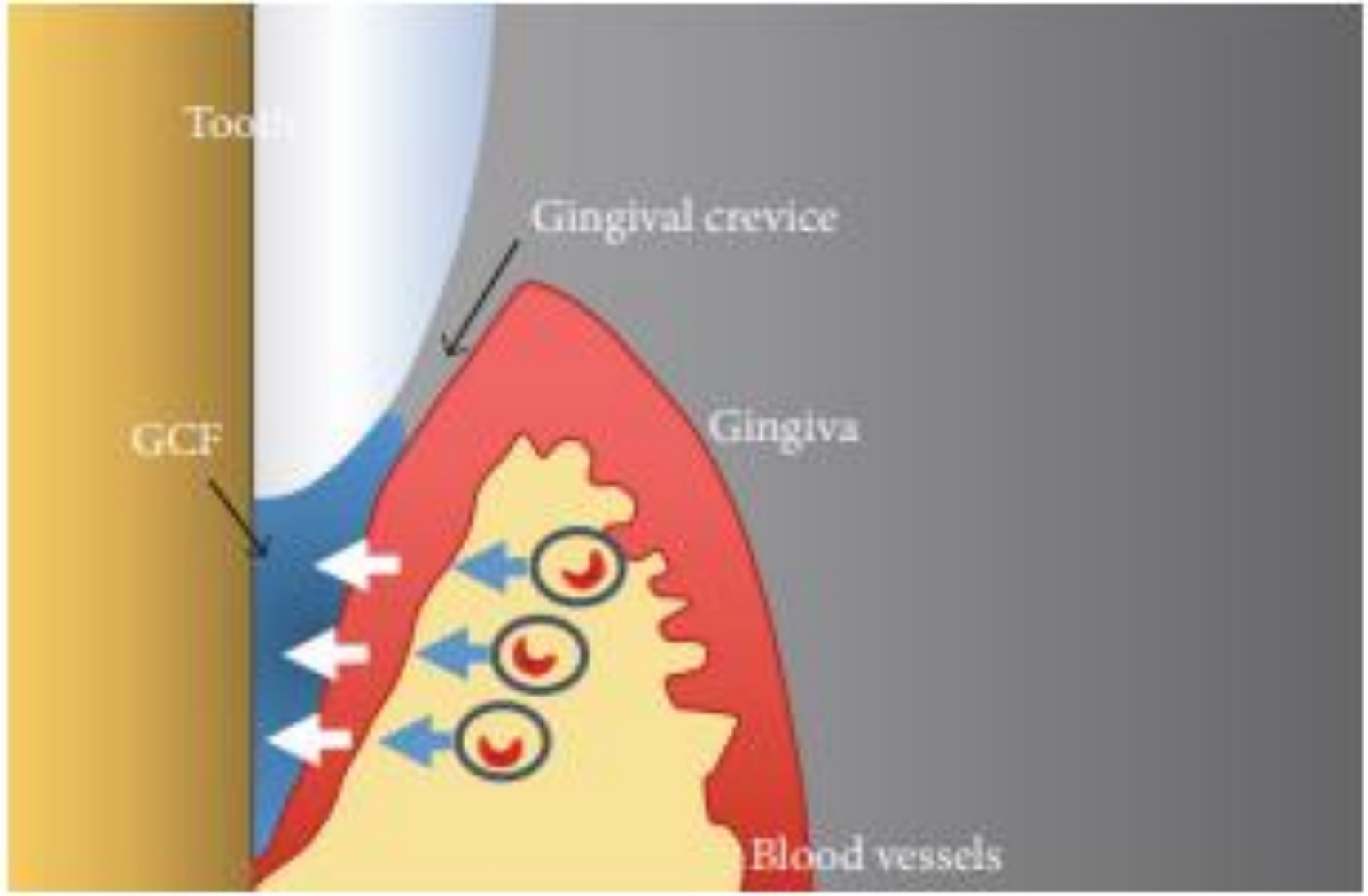

FIGURE 1: The gingival crevicular fluid (GCF) formation. The GCF flow is an interstitial fluid which appears in the crevice as a result of an osmotic gradient.

Gingival crevicular fluid (GCF) is physiologically produced in small amounts and its composition resembles blood serum. In a healthy state, GCF is responsible for leaching pathogens and toxic metabolites from the periodontium. However, during inflammation, the microcapillary framework enhances the secretion of fluid containing various inflammatory biomarkers [1]. The inflammatory exudate components include bacteria, damaged tissue fragments, proteins, complement system components, immune cells, immunoglobulins, and inflammatory mediators [2–4].

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The analysis of GCF is a very useful diagnostic instrument.
The correlations between the levels of many host GCF biomarkers and periodontal diseases have been extensively studied.
In orthodontics, biomarkers related to bone deposition (bone alkaline phosphatase and osteoprotegerin) represent new possibilities for the understanding of bone growth and remodeling .The possibility of identifying the bone turnover in children and juvenile subjects can help orthodontists to decide when to intercept a malocclusion. The biomarkers found in GCF also permit the monitoring of the orthodontic movement and consequences of the forces applied through its level’s variations.

Table 1: Main GCF host biomarkers according to biological significance.

Bone deposition and mineralization
Bone alkaline phosphatase
Osteoprotegerin
Bone resorption
Osteonectin
Bone phosphoprotein
Osteocalcin
Cross-linked carboxyterminal telopeptide of type I collagen
Receptor activator of nuclear factor kappa-B and its ligand
Inflammation
Cytokines (interleukins, tumor necrosis factors, interferons, growth factors, and colony-stimulating factors)
Arachidonic acid derivates (prostaglandins, leukotrienes)
Neutrophil alkaline phosphatase
Hydroxyproline
Collagen cross-linking peptides
Others
Cell death or tissue damage
Aspartate aminotransferase
Lactate dehydrogenase
Hydroxyproline
Collagen cross-linking peptides
Glycosaminoglycans
Metalloproteases (proteolytic enzyme)
Cathepsin B (proteolytic enzyme)
Antibodies

Identification of Growth Phase

The decision to intercept orthopedically on a growing patient depends primarily on the identification of his skeletal maturation phase. The most desirable time for treatment is different in various malocclusions.

Different established methods are used to identify the growth phase. The analysis of cervical vertebra maturation (CVM) is a method based on assessing the shape of the cervical bodies, as seen in lateral cephalograms. The CVM method shows great reliability. Another radiographic method is the hand-wrist analysis that calculates the mean age for the appearance of each of the various centers of ossification or the epiphyseal closure and variations in these ages.

Alternative methods to identify the growth phase are analysis of dentition, chronological age, and dental maturation. These methods are mainly morphological and recent studies affirm that those are not reliable assessments of growth phases. New possibilities might be offered by the biochemical markers. Collection of gingival crevicular fluid avoids radiographic exposure and the biomarkers represent agents that are directly involved in bone growth and remodeling.

The alkaline phosphatase (ALP) has been investigated as reliable biologic indicator of skeletal maturation in different studies, where the ALP levels are compared with other methods to identify the skeletal maturation in growing patients . The bone alkaline phosphatase is synthesized by the osteoblasts and is presumed to be involved in the calcification of bone matrix. It is considered to be a highly specific marker of the bone-forming activity of osteoblasts.

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Cytokine profile in gingival crevicular fluid of children with inflammatory bowel disease

The term inflammatory bowel disease (IBD) is the generic name for two major categories of chronic inflammatory intestinal disorders: Crohn’s disease (CD) and ulcerative colitis (UC)

Extra-intestinal manifestations including oral cavity are more commonly seen at the disease onset in pediatric IBD patients than adults

there are some studies demonstrating the increased risk of periodontitis in patients with IBD

A quarter of all IBD patients are diagnosed during childhood or adolescence and up to 25% of pediatric IBD patients experience extra-intestinal manifestations including chronic, severe inflammation of the gingiva/periodontium.

Aphthous stomatitis has proven to be the one of the two most common extra-intestinal manifestations in pediatric IBD patients with a range from 3.2% to 7.3%.3 Despite the high prevalence of IBD and IBD related oral manifestations, like aphthous stomatitis, in children, the data about local periodontal immunoinflammatory response (gingival crevicular fluid (GCF) cytokine profile) and the relationship of local and systemic inflammatory response (serum cytokine profile) in this population are limited.

A relation between the increased GCF volume, which was a marker of gingival inflammation, and increased inflammatory activity of IBD had been found. Moreover, moderate and severe gingival inflammations were observed in greater rates in active disease and treatment-resistant groups, respectively than IBD patients with remission and control group. (Children with IBD had higher gingival inflammation than systemically healthy children.)

IL-1β is a proinflammatory cytokine that stimulates most of the events in the pathogenesis of periodontal disease and there are several evidences which demonstrate that IL1β is elevated at the regions affected by periodontal disease

IL-4 is an anti-inflammatory cytokine that downregulates the progression of periodontal disease and it has been stated that GCF IL-4 level displays a significant increase in periodontal health

IBD had an impact on the cytokine profile of GCF. The clustering patterns of cytokines in both GCF and serum differed based on IBD activity. Considering the increased complexity of cytokine interactions and the increased severity of gingival inflammation in patients with active disease, it can be concluded that IBD activity might have an impact on gingival inflammation in pediatric patients with IBD.

Bruxism Influence on Volume and Interleukin-1β Concentration of Gingival Crevicular Fluid

Interleukin-1 (IL-1) is one of the proinflammatory nuclear factor-κB dependent cytokines present in gingival fluid, and is responsible for acute phase response induction .The beta form (IL-1β), prevalent in periodontal tissues, is produced by macrophages. IL-1 is the most important biomarker associated with chronic periodontitis. Interleukin-1β is sometimes referred to as a mediator of periodontal bone loss, as it stimulates the formation of osteoclasts and affects bone resorption.

Moreover, young sleep bruxers potentially have higher cardiovascular risk due to the increased concentrations of inflammatory and stress markers .Interestingly, phasic sleep bruxism seems to be positively correlated with snore intensity despite body position

Patients diagnosed with probable bruxism, as a result of occlusal overload, are more prone to microinflammation in periodontal tissues compared to people without visible signs. The measurement of gingival fluid volume and the concentration of interleukin-1β may be reliable predictors for diagnosing periodontal microinflammation due to clenching and grinding of the teeth in patients with probable bruxism. Non-invasive detection of potential markers in GCF shows that subclinical periodontitis could allow faster diagnosis and the prevention of further inflammatory processes.

sleep deprivation affects general health, and oral health is also essential. We analyzed oxidative stress biomarkers in gingival crevicular fluid in sufficient sleep versus insufficient sleep groups in a pediatric and adolescent population, and MDA, H_2O_2, and GSH were good oxidative stress markers. Sleep deprivation increases oxidative stress markers in gingival crevicular fluid and impacts oral redox balance

Malondialdehyde (MDA) is a commonly used indicator to measure oxidative lipid damage caused by free radicals

Glutathione is considered a major free radical scavenger, reflecting the extent to which tissues are challenged by oxidation stress

H_2O_2 is one of the organisms' most common reactive oxygen species molecules

Evaluation of chemokines in gingival crevicular fluid in children with dental caries and stainless steel crowns

Aims and Objectives: The study was conducted to detect the presence of macrophage inflammatory protein-1α (MIP-1α) and MIP-1β and estimate their levels in gingival crevicular fluid (GCF) in children with dental caries and stainless steel crowns. **Materials and Methods:** A total of 80 children with primary dentition were selected and categorized into four groups with twenty in each group; Group 1 - healthy subjects, Group 2 - dental caries, Group 3 - dental caries involving the pulp, and Group 4 - stainless steel crowns. GCF samples were collected by an extra-crevicular method with microcapillary pipettes. The GCF samples were quantified by ELISA and the levels of MIP-1α and MIP-1β were determined. **Results:** MIP-1α and MIP-1β were detected in all the samples. Highest mean concentration in GCF was obtained for Group 3 followed by Groups 2 and 4 while the lowest concentration was seen in Group 1. This suggests that MIP-1α and MIP-1β levels in GCF increased proportionately with the inflammation. **Conclusions:** GCF serves as a noninvasive diagnostic fluid to measure biomarkers released during dental caries initiation and progression. MIP-1α and MIP-1β chemokines can be considered as novel biomarkers, in biological mechanism underlying the pathogenesis and inflammation in children with dental caries and stainless steel crowns.

Macrophage inflammatory protein-1α (MIP-1α) expression in gingival epithelial cells is induced by lipopolysaccharide (LPS), and it is important in initiating inflammation

Both MIP-1α and MIP-1β have shown to be potent chemoattractants for macrophages, lymphocytes, eosinophils, natural killer cells, and dendritic cells