

Botero JE, Parra B, Jaramillo A, et al. Subgingival Human Cytomegalovirus (HCMV) correlates with increased clinical periodontal parameters and bacterial co-infection in periodontitis. J Periodontol 2007;78(12):2303-10.

Purpose: To compare the subgingival frequency of HCMV in South American subjects affected by periodontitis to that in periodontally healthy subjects. This investigation also assessed the correlation of HCMV to periodontal clinical parameters and periodontopathic bacteria.

Materials and Methods: A group of 30 subjects with periodontitis (periodontitis group [PG]; mean age, 47.0 years), comprising 20 subjects with chronic periodontitis (CP; mean age, 44.0 years) and 10 with aggressive periodontitis (AgP; mean age, 24.3 years), were studied. Exclusion criteria - Diabetes, heart disease, human immunodeficiency virus infection, pregnancy, heavy cigarette smoking (>15 cigarettes/day), previous periodontal treatment (4 months), and antibiotic intake (4 months). Twenty-two subjects (mean age, 31.2 years) were included as controls (control group [CG]). A full-mouth clinical examination was performed on each subject. PD and clinical attachment level (CAL) were recorded using a computerized periodontal probe with a standardized pressure (15 gm). Subgingival plaque samples were obtained in duplicate at the same session from the six deepest sites in subjects with periodontitis and from six healthy sites in the CG group by inserting paper points, which were kept in place for 20 seconds. Six additional paper points were transferred to a viability medium, anaerobically prepared III (VMGA III) screw cap vial and processed immediately for bacterial culture. DNA extraction and nested polymerase chain reaction (PCR) for the detection of HCMV was carried out. Differences in clinical parameters between groups were established with the two-tailed Student t test. The frequency of detection of HCMV and periodontopathic bacteria were analyzed between the groups with the X² test. The Mann-Whitney test was used to assess the cultivable subgingival microbiota composition between groups.

Findings: HCMV was more prevalent in PG subjects (53.3%) compared to CG subjects (18.1%) and this difference was statistically significant ($P < 0.05$). After stratification, the prevalence was 60% and 40% in the CP and AgP groups, respectively. No differences in PI or BOP were observed between HCMV-positive and -negative subjects in the PG, CP, and AgP groups, but they were increased in those groups compared to healthy subjects (Fig. 1). In contrast, PD and CAL in sampled sites were increased in HCMV-positive subjects compared to HCMV-negative subjects in all groups. The difference was more pronounced in AgP subjects than in CP subjects. Even in the CG, there was a tendency for increased PD and CAL when HCMV was positive, but it did not reach statistical significance. The frequency of detection of important periodontopathic bacteria, such as *P. gingivalis*, *P. intermedia* /*P. nigrescens*, and *E. corrodens* was increased in HCMV-positive periodontally diseased subjects compared to healthy subjects. After CP and AgP stratification, *P. intermedia*/*P. nigrescens* and *E. corrodens* were increased in HCMV-positive/CP subjects ($P < 0.05$), whereas *P. gingivalis* and *P. intermedia*/*P. nigrescens* were most prevalent in HCMV-positive/AgP subjects ($P < 0.05$). In contrast, *A. actinomycetemcomitans* was less frequent in HCMV-positive subjects in the CP and CG groups and was not detected in the AgP group. The odds ratio (OR) for coinfection with

periodontopathic bacteria and HCMV was 7.6 ($P < 0.01$; 95% confidence interval [CI] which means that simultaneous infection with high levels of periodontopathic microorganisms (>9% cultivable microbiota) and HCMV could increase the risk for periodontitis.

Conclusions: HCMV-positive subjects presented increased PD and CAL at sampled sites compared to HCMV-negative/periodontitis and healthy subjects. Important periodontopathic bacteria were associated positively with HCMV. HCMV may be involved in the pathogenesis of periodontal disease.

Contreras A, Slots J. Mammalian viruses in human periodontitis. Oral Microbiol Immunol. 1996; 11: 381-6

Purpose: This study aimed to determine the frequency of HCMV, EBV-I, EBV-2, herpes simplex virus (HSV) and human immuno-deficiency virus (HIV) in pts with periodontitis and gingivitis.

Materials and Methods: Subgingival samples of 27 adults (13M and 14F mean age of 45 years) were obtained from a periodontitis and a gingivitis site. Pts had atleast 1 pocket greater than 7mm and they were systemically healthy. Samples from deep pockets were either pooled (2-3 sites) when available or taken from a single site. A nested-PCR method was used to detect viral DNA of HCMV, EBV-I, EBV-2, HSV and HIV using previously described primers.

Findings: Twenty-four subjects (89%) yielded at least one of the five test viruses from deep periodontal pockets, whereas only 15 (56%) showed viruses from shallow periodontal sites ($P = 0.015$; chi-square test). Moreover, 12 deep pocket samples (44%) yielded 2-3 viruses, whereas only 3 shallow pocket samples (11%) showed viral co-infection ($P=0.015$; chi-square test) (Table 1). Only 3 patients (11%) failed to yield viruses in either deep or shallow pockets. HCMV was detected with higher frequency in deep than in shallow periodontal sites ($P=0.023$).

Discussion: HCMV and EBV can infect and alter functions of polymorphonuclear leukocytes, lymphocytes and macrophages. It may be hypothesized that a virus-induced dysfunction of polymorphonuclear leukocytes in periodontal sites can set the stage for overgrowth of subgingival periodontopathic bacteria and subsequent progression of destructive periodontal disease. Secondly, gingival viral infections may promote the attachment and colonization of periodontopathic bacteria in the subgingival ecosystem, similar to enhanced adherence to virus-infected cells displayed by some bacteria in medical infections. Thirdly, human viruses may produce cytopathic effects on fibroblasts, keratinocytes, endothelial cells, on migrating cells such as polymorphonuclear leukocytes, lymphocytes, macrophages and possibly on bone cells. Since these cells are key constituents of the periodontium, virus induced cytopathic effects may hamper the turnover and repair of periodontal tissues. Fourthly, viral infections can give rise to altered inflammatory mediator and cytokine responses. Fifthly, viruses can produce tissue injury as a result of immunopathological responses to viral-infected cells. Thus the role and importance of HCMV and other mammalian viruses in the initiation and progression of destructive periodontal disease merits further investigation.

Conclusions: In summary, this article presents a novel concept of the pathogenesis of human periodontal disease. The hypothesis presented by the authors is that gingival infection with certain herpesviruses lowers the resistance of the periodontal tissues, thereby permitting subgingival overgrowth of anaerobic and other periodontal pathogenic bacteria. The transient immunosuppressive effect of viral reactivation in periodontal tissues might explain in part the episodic progressive nature of human periodontitis. The absence of viral infection or viral reactivation could allow for some individuals carrying

periodontopathic bacteria in their subgingival microbiota while maintaining periodontal health. If some types of destructive periodontal disease represent the result of a virus-mediated opportunistic bacterial infection, a new approach to preventing and treating periodontitis may focus on controlling the virus(es) that enable overgrowth of periodontal pathogenic bacteria. The role of human viruses in the etiology and pathogenesis of destructive periodontal diseases merits further investigation.

Kamma JJ, Slots J. Herpesviral-bacterial interactions in aggressive periodontitis. J Clin Periodontol 2003;3;420-6.

Purpose: To review evidence supporting the role for herpesviruses in the development of aggressive periodontitis.

Materials and Methods: Review article.

Findings:

- Herpes virus is a DNA virus and 8 different human herpes viridae have been identified and named. HCMV and EBV-1 are the 2 most common types that have been implicated in the etio-pathogenesis of periodontal disease.
- Kamma et al. (2001) examined 16 patients with aggressive periodontitis during the maintenance phase and found that HCMV, EBV-1 and HSV occurred with higher frequency in periodontitis active sites. These sites also demonstrated increased numbers of *P.gingivalis*, *D. Pneumosintes*, *A. actinomycetemcomitans*, and *B. forsythus*.
- The authors speculate that this herpesviral-bacterial interaction is probably bidirectional. Herpes virus have the potential to reduce the host resistance and thereby increase the risk of bacterial infection. Bacterial-induced gingivitis has the potential to facilitate homing of herpesvirus-infected cells in the gingival tissue.
- Michalowicz et al. (2000) found that the most parsimonious multivariate model for localized juvenile periodontitis included HCMV, and *P.gingivalis* (OR=6.6), and the odds of having LJP increased exponentially when both HCMV and *P. gingivalis* were present (OR = 51.4).
- Ting et al. (2000) found that HCMV activation was associated with disease active sites in LJP patients.
- Slots and Contreras (2000) proposed that periodontal herpesvirus activation results in suppression of periodontal immune defenses, overgrowth of periodontal bacterial pathogens, release of proinflammatory cytokines, and subsequently periodontal tissue breakdown. Bacteria induced gingival breakdown allows viruses to enter the periodontium. HCMV resides in macrophage-granulocyte progenitors and peripheral blood mononuclear cells and EBV in lymphocytes. Subsequent reactivation may aggravate the inflammatory response and accelerate the existing disease.

Conclusions: Definitive role of herpes virus in the etiology of periodontal disease is difficult to ascertain.

Manor A, Lebediger M, Shiffer A et al. Bacterial invasion of periodontal tissue in advanced periodontitis in humans. J Periodontol 1984; 55: 567-73.

Purpose: To survey the presence of bacteria in the soft tissue wall of the pocket in cases of advanced periodontitis in humans.

Materials and Methods: Seven patients (age 32-47) with a diagnosis of chronic generalized advanced periodontitis were included in the study. The patients reported otherwise healthy. Teeth with a poor prognosis were extracted along with the gingival wall of the pocket. The selected teeth must present marked mobility, advanced bone loss radiographically, CAL at least 8mm and PD greater than 5mm. No systemic antibiotic was used in the past 3 months and no pocket probing was done for at least 1 wks prior to extraction. A straight horizontal incision was made on the buccal aspect of the tooth about 2 mm apical to the base of pocket; two vertical incisions extending from the horizontal incision to the center of the interdental papilla and connected with a lingual internal bevel incision. The tooth was then being extracted atraumatically. The teeth were trimmed with a bur with a water spray coolant until there's only a thin layer of dental tissue remained to the soft tissue. The specimens were then fixed, sectioned and examined under an electron microscope.

Findings: Bacterial invasion of the soft tissue of the pocket wall was observed in all seven specimens. In four cases, bacteria were present in both epithelium and connective tissue of the apical zone of the pocket and JE whereas in three cases bacterial invasion was limited to the pocket and JE without invasion within the connective tissue. In the junctional epithelium, free bacteria were observed in widened intercellular spaces only in the most coronal area. In the more apical zone, bacteria were found only as phagocytosed inclusions in PMNLs. The most apical zone of the junctional epithelium and coronal zone of the dento-gingival fibers were free of bacteria. In the connective tissue, bacterial invasion was accompanied by severe destruction of the collagen fibers, almost complete lysis of the collagen fibers and degeneration of fibroblast. The depth of bacterial penetration into the connective tissue was roughly estimated about 400 to 600µm on the connective tissue side of the basal lamina.

Conclusions: this study demonstrated bacterial penetration into the soft tissues of the apical zone of the pocket in all seven cases. However, in the study of Frank and Saglie et al., bacterial invasion was found in most but not all cases studied. It is possible that periodontitis is a collective term for a group of disease which may be caused by different microbial agents and which follow different pathogenic mechanisms.

Modeer T, Ljunggren O, Lerner UH. Bradykinin-2 receptor-mediated release of H-arachidonic acid and formation of prostaglandin E-2 in human gingival fibroblasts. J Periodont Res 1990;25: 358-363.

Purpose: To study whether bradykinin can stimulate the biosynthesis of prostaglandins in human gingival fibroblasts.

Materials and Methods: Cultures of fibroblast-like cells were established from gingival biopsies obtained following surgical treatment of impacted teeth in 3 pts. Cells were incubated differently for the analysis of arachidonic acid and determining prostaglandin production. The amount of PGE-2 determined by radioimmunoassays . Analysis of arachidonic acid release was done by liquid scintillation counter. Statistical analysis was done with ANOVA.

Findings: Bradykinin, at a conc. of 1 μ mol/l caused a rapid (30s) increase of biosynthesis of PGE-2 in human gingiva fibroblasts. The response was saturated after 3-5 min and the amount of PGE-2 remained unchanged for 15 min. This could be blocked by indomethacin, meclofenamic acid and flurbiprofen via cyclooxygenase pathway. The PGE-2 response to bradykinin was dose-dependent and could be seen at and above 10 nmol/l. Bradykinin also causes a rapid (30 s) stimulation of the release of arachidonic acid from gingival fibroblasts. The stimulation progressively increased at least for 15 min. This could not be blocked by indomethacin.

Conclusion: It was demonstrated in the study that bradykinin can stimulate prostaglandin production in human gingival fibroblasts. Consequently, gingival fibroblasts may contribute to the enhanced amounts of prostanoids found in gingival tissues and crevicular fluids in pts with periodontal disease.

Mullally BH, Coulter WA, Hutchinson JD, Clarke HA. Current oral contraceptive status and periodontitis in young adults. J Periodontol 2007; 78: 1031-36

Purpose: To investigate the relationship between oral contraceptive use and the presentation of aggressive periodontitis in young females in Northern Ireland.

Materials and Methods: Private practice patients between the ages of 18 and 35 who have aggressive periodontitis each received an examination which consisted of a six-site full-mouth periodontal charting plus a plaque and gingival index scoring. Each patient also completed a questionnaire which consisted of a full medical history and which detailed their contraceptive pill experience, including type of pill and duration of medication.

Findings: Of the 50 patients included in the study, 42 % were taking the contraceptive pill. The probing depths of pill users were deeper when compared to non-pill users. The mean probing depth of pill users was 3.3mm while in non-pill users it was 2.7mm. Pill users also had more bleeding sites than non-users. 44% of sites bled for users while 31.1% bled for non-users. Smoking was also found to have a significant negative effect on attachment loss

Conclusions: Users of the contraceptive pill had deeper probing depths, more frequent bleeding upon probing and more extensive and severe periodontal attachment loss when compared to those not taking the pill. The contraceptive pill has a negative effect on periodontal health.

Nibali L. Association between interleukin-6 promoter haplotypes and aggressive periodontitis. J Clin Perio 2008;35:193-198.

Purpose: The aim of this study was to investigate the relationship between five polymorphisms in the IL-6 gene promoter and their haplotypes and the presence of Aggressive Periodontitis, by comparing genotype frequencies in Aggressive Periodontitis patients and healthy controls.

Material and Methods: A case-control association study on 224 Aggressive Periodontitis patients and 231 healthy controls was performed in order to detect differences in genotype distributions of five single nucleotide polymorphisms (SNPs) located in the promoter region of the IL-6 gene. DNA was extracted from 10ml blood sample and analyzed with Real-time PCR allele discrimination.

Findings: Increases in allele distribution in the patient group (GAgP and LAgP) were detected for $_174$ G, $_1363$ G and $_1480$ C alleles (no deletion). These allelic differences between AgP and healthy controls were marked (although not statistically significant) in the Caucasian population ($p=0.014$ for $_174$, 0.013 for $_1363$ and 0.019 for $_1480$, respectively, not presented in the table), but not in the Black population. Only among Caucasians, logistic regression analysis adjusted for confounders revealed limited evidence of association with a diagnosis of AgP for $_174$ [$p=0.036$], $_1363$ ($p=0.013$) and $_1480$ ($p=0.037$). When AgP patients of all ethnicities were compared with all controls adjusting for gender, smoking and ethnicity, logistic regression revealed a statistically significant association with a diagnosis of AgP for the $_1363$ polymorphism ($p=0.006$). When looking at the different disease entities (LAgP and GAgP), significant associations were detected between LAgP and healthy controls. In the Caucasian group (144 healthy controls and 24 LAgP patients, see Table 3), logistic regression analysis revealed statistically significant associations for $_1480$ ($p=0.007$), and $_6106$ ($p=0.010$) polymorphisms having adjusted for gender and smoking. When the analysis was extended to subjects of all ethnicities, logistic regression analysis revealed statistically significant associations with LAgP for $_1480$ ($p=0.003$), having adjusted for gender, smoking and ethnicity. When analyzing haplotypes remarkable associations were detected in the LAgP Caucasian subgroup $P=0.01$ for the haplotype made up of all five SNPs and $p=0.001$ for a constrained model including only $_1363$ and $_1480$. In LAgP patients, the lowest p-value for association with the disease phenotype was also found for the $_1363$ and $_1480$ haplotypes (adjusted $p=0.002$).

Conclusions: In conclusion, this study supports the theory that IL-6 polymorphisms might play a role in the genetic profile predisposing to LAgP. It also highlights the importance of two IL-6 polymorphisms ($_1363$ and $_1480$) in modulating disease phenotype and susceptibility.

Novaes AB, Ruben MP, Kon S, Goldman HM, Novaes Jr. AB. The development of the periodontal cleft. A clinical and histopathological study. J Periodontol 75;46:701-9.

Purpose: 1. To discuss the factors that favor the formation of the periodontal cleft.
2. To discuss the development of the periodontal cleft clinically and histopathologically.

Materials and Methods: The formation of gingival and periodontal clefts may be related to a no. of etiological and local environmental factors. The inflammation is constant. The spread of inflammatory exudate occur not only apically-directed, but there is also lateral progression toward the outer aspect of gingiva and alv. mucosa. Connective tissue destroyed, covered partly by proliferating and migratory pocket epi. which eventually leads to anastomosis of the gingival and pocket epi. as the C.T. is progressively lost. Its slow process. Other factors, anatomic in nature, which favor the formation of gingival or periodontal dehiscence include 1) Bucco-lingual dimension of soft tissue wall 2) Nature of bony septa at the site which may lead to anatomic deviation like a) narrow zone of attached gingiva b) thin marginal gingiva, therefore, greater potential for tissue retractability from the tooth which not only increases the possibility of increased sulcular depth but also submarginal accrual of microbial plaque which causes inflammation c) wide zone of alv. mucosa – a loose C.T. rich in matrical and vascular components, qualities which structurally favor spread of inflam. In cases where TFO proceed the development and spread of gingival inflammation, TFO may first induce septal resorption with concomitant replacement by periosteal-cemental attachment which is structurally inf. to osseous septa and PDL, thus favoring extension of exudates from gingiva to mucosal-periodontal sites. The thin labial-buccal septum may also be affected by pressure of ortho forces or periodontal/oral surgery. Histologically, there is progressive thinning of gingival-mucosal corium of C.T. under the influence of resorptive enzymes secreted by osteoclasts, macrophages induced by activated lymphocytes. As the lesion progresses laterally, an epi. lined fistula forms with eventual perforation of the soft tissue wall. When the perforation occurs in a linear fashion occlusoapically, the soft tissue dehiscence is created.

Findings and Conclusions: The inflammation is constant for the formation of periodontal cleft. Other factors like thin B-L soft tissue wall, narrow zone of attached gingiva, thin marginal gingiva and wide zone of alveolar mucosa (which occur mostly in the labial/buccal prominence of teeth) in the presence of inflammation may lead to periodontal cleft formation. The thin labial-buccal septum may also be affected by pressure of ortho forces or periodontal/oral surgery.

Novak MJ. Necrotizing ulcerative periodontitis. Ann Periodontol 1999;4:74-77.

Review: In patients with no known systemic disease or immune dysfunction, necrotizing periodontitis (NUP) appears to share many of the clinical and etiologic characteristics of necrotizing ulcerative gingivitis (NUG) except that patients with NUP demonstrate loss of clinical attachment and alveolar bone at affected sites. In these patients, NUP may be a sequela of a single or multiple episodes of NUG or may be the result of the occurrence of necrotizing disease at a previously periodontitis-affected site. The existence of immune dysfunction may predispose patients to NUG and NUP, especially when associated with an infection of microorganisms frequently associated with periodontal disease such as *Treponema* and *Selenomonas* species, *Fusobacterium nucleatum*, *Prevotella intermedia*, and *Porphyromonas gingivalis*. The role of immune dysfunction is exemplified by the occasionally aggressive nature of necrotic forms of periodontal disease seen in patients with HIV infection or malnutrition, both of which may impact host defenses. Clinical studies of HIV-infected patients have shown that patients with NUP are 20.8 times more likely to have CD4+ cell counts below 200 cells/mm³. However, these same studies have demonstrated that most patients with CD4+ cell counts below 200 cells/mm³ do not have NUP, suggesting that other factors, in addition to immunocompromisation, are involved. Further studies are needed to define the complex interactions between the microbial, or viral, etiology of necrotic lesions and the immunocompromised host. It is, therefore, recommended that NUG and NUP be classified together under the grouping of necrotizing periodontal diseases based on their clinical characteristics.

Pini Prato G. Mucogingival deformities. Ann Periodontol 1999;4:98-101.

Purpose: To review the classification of mucogingival deformities.

Materials and Methods: Literature review and authors opinion.

Findings and Conclusions: the term mucogingival is currently defined as a “generic term used to describe the mucogingival junction and its relationship to the gingiva, alveolar mucosa, frenula, muscle attachments, vestibular fornices and the floor of the mouth. The glossary of Periodontal Terms defines mucogingival surgery as “periodontal surgical procedures designed to correct defects in the morphology, position and/or amount of gingiva”. The Consensus Report on Mucogingival Therapy from the 1996 world workshop in periodontics defines mucogingival therapy as “non-surgical and correction of defects in morphology, position, and/or amount of soft tissue and underlying bone” and periodontal plastic surgery as “surgical procedures performed to prevent or correct anatomical, developmental, traumatic, or plaque disease-induced defects of the gingiva, alveolar mucosa, or bone”. Rationale for classification for mucogingival deformities may be based on clinical, chronological, etiological, and/or morphological criteria. The chronological criterion (congenital versus acquired) is not appropriate, since it is difficult to determine if some deformities have been present since birth. An etiologic criterion is usually preferred for clinical classifications, but may not be adequate as the primary criterion for mucogingival deformities.

Clinical criterion will divide mucogingival deformities into 3 main categories:

1. Soft tissue deformities associated with teeth
2. Soft tissue deformities associated with implants
3. Soft tissue deformities associated with edentulous ridges.

A classification of mucogingival deformities should provide a method for identifying the different conditions in order to improve diagnosis, etiologic identification, research, treatment, and insurance evaluation.

Rowland RW. Necrotizing ulcerative gingivitis. Ann Periodontol 1999; 4: 65-73

Purpose: To review the data that exists in relation to Necrotizing Ulcerative Gingivitis (NUG) as a clinical diagnosis. Deliver information for a comprehensive understanding of NUG as a clinical diagnosis and delivery of appropriate therapy.

Materials and Methods: Literature review

Findings: NUG is different from other periodontal diseases, because it presents with interdental gingival necrosis described as “punched out” ulcerated papillae, gingival bleeding, and pain. If all three of these characteristics are not present then a diagnosis of NUG cannot be made. Loss of attachment and bone are not common findings but can occur after multiple occurrences. Bleeding occurs with little or no provocation. Pain is the characteristic most associated with NUG. It is intense and the reason why patients seek treatment. Other signs and symptoms which are associated with NUG, but are not pathognomonic are lymphadenopathy, fetid odor, fever and malaise. Episodes of NUG will typically resolve within a few days after receiving appropriate treatment. Treatment involves removing the bacterial challenge using mechanical debridement and/or antibiotic therapy. NUG is an infectious disease caused by microbial plaque, more specifically, fusiform spirochetes are most commonly found in patients with NUG. The constant cultivable flora found in NUG is *Provetella intermedia*, *Fusobacterium* species, *Treponema* species and *Selenomonas* species. Although NUG has a bacterial etiology it is not a communicable disease. Development of NUG is closely associated with the specific predisposing factors of psychological stress, immune suppression, smoking, malnutrition, pre-existing gingivitis and tissue trauma. Acute psychological stress is particularly associated with NUG. NUG patients have been found to have increased levels of corticosteroids, which is associated with increased stress and can cause immunosuppression. Increased cortisol has been associated with depression of PMN function and may provide essential nutrients for bacterial growth. In the past, NUG was thought to be associated with HIV/AIDS, but the current thinking is that those which HIV/AIDS should be diagnosed with NUP not NUG. This is due to the fact that HIV/AIDS patients have severe loss of attachment and bone, which is not usually associated with NUG. NUG has also been associated with malnutrition. It is a disease of young children in the developing world, and this appears to be a result of inadequate protein intake and secondary to viral infections such as measles.

Conclusions: The primary etiologic agents in NUG are opportunistic bacteria. Acute psychological stress, tobacco smoke and pre-existing gingivitis are non-specific predisposing factors involved in its development. Yet, the predominant factor in the development of NUG is immunosuppression.

Saglie R, Newman MG, Carranza FA Jr. A scanning electron microscopic study of leukocytes and their interaction with bacteria in human periodontitis. J Periodontol 1982;53:752-61

Purpose: What are the morphological and surface changes of leukocytes in human periodontitis utilizing scanning electron microscopy (SEM) while in 1) gingival blood vessels and 2) connective tissue, 3) on surface pocket epithelium, 4) in the nonattached plaque zone?

Materials and Methods: Eight extracted teeth from 6 patients with advanced periodontal disease were utilized in this study. The patients had not received antibiotics or been probed in the past 2 months. Prior to extraction, 2 vertical incisions and 1 horizontal incision were made outlining a rectangle of tissue including the apical extension of the pocket wall. The teeth were carefully extracted, the soft tissue separated, and split in half longitudinally using one half for side view of the pocket epithelium and adjacent connective tissue and the other for frontal view of the inner pocket wall. The tissue was processed and prepared for examination under SEM. Shape, size, surface morphology, topographical relationships, phagocytosis, and locomotion were evaluated. Exudative leukocytes were studied in different locations, starting with the blood vessels and their surrounding connective tissue, traversing the basement lamina, entering the pocket epithelium, junctional epithelium and then the pocket.

Findings: 1) Inside the vessels, leukocytes had an oval shape; during migration across vessel endothelium, they acquired an irregular shape which seemed to vary according to their locomotive needs. Similar shapes were seen in leukocytes traversing the basement lamina and in the junctional epithelium. 2) Leukocytes in the gingival connective tissue had an irregular shape and a smooth surface and varied from 7 to 10 μm . 3) Leukocytes on the surface of the pocket epithelium were bigger than in other locations reaching a size of 8 to 15 μm . The interaction with and phagocytosis of bacteria were clearly seen in the surface of pocket epithelium and also deep in the gingival tissue. 4) Immediately coronal to the remnants of the junctional epithelium, leukocytes were seen attached to the cemental surface. Some showed a shape compatible with phagocytosis with filipodia extending from the cell body.

Conclusions: The results from this study demonstrated the presence of leukocytes in the gingival blood vessels, gingival connective tissue, basement lamina, sectioned pocket epithelium, surface of pocket epithelium, junctional epithelium, and cementum surface in biopsies from patients with advanced adult periodontitis. The process of degranulation and the interaction of leukocytes and bacteria in the phases of recognition, attachment, and engulfment of bacteria was observed.

Saygun I, Kubar A, Sahin S. Quantitative Analysis of Association between herpesviruses and bacterial pathogens in periodontitis. J Perio Res. 2007: 1-8.

Purpose: The purpose of this study is to identify associations among human cytomegalovirus, Epstein-Barr virus and six putative periodontopathic bacteria in periodontitis lesions.

Materials and Methods: A total of thirty patients, fifteen with periodontal disease (nine with aggressive periodontitis and six with chronic) and fifteen with normal and healthy periodontium and all patients were systemically healthy. Microbiological samples were collected from the deepest periodontal probing depth, using a curette, a real time Taqman® polymerase chain reaction assay was used to determine the subgingival counts of human cytomegalovirus, Epstein-Barr virus, *P. gingivalis*, *T. forsythia*, *P. Intermedia*, *A. actinomycetemcomitans*, *F. nucleatum* and *C-bacter rectus*.

Findings and Conclusions: All clinical differences between the periodontitis patients and the periodontally normal subjects were statistically significant, showing an average in probing depths of 4.3mm in periodontitis patients and 1.9mm in normal patients 4.8mm / 1.9mm Cal, 78.3/11.7% BOP. Human cytomegalovirus was detected in 8 (PL) periodontitis lesions and 1 in (NPS) normal periodontal site, bacteria was detected 6-15 PL and 1-11 NPS, *P. gingivalis* and *T forsythia* were the most frequently detected bacteria in PL. The study confirmed that periodontal human cytomegalovirus and Epstein-Barr virus are associated with major periodontopathic bacteria and with the severity of periodontal disease.

Slots J, Kamma JJ, Sugar C. The herpesvirus-Porphyromonas gingivalis-periodontitis axis. J Periodont Res 2003;38:318-323.

Purpose: To examine whether periodontal human cytomegalovirus (HCMV), Epstein-Barr virus-1 (EBV-1), or herpes simplex virus (HSV) are linked to the presence of subgingival *Porphyromonas gingivalis* (*P. gingivalis*) and destructive periodontal disease.

Materials and Methods: Sixteen adult Greek patients presenting with aggressive periodontitis were evaluated for the study. Each of the patients contributed subgingival paper-point samples from 2 periodontitis lesions of 5.9 ± 0.8 mm mean probing depths that had undergone a loss of attachment of roughly 2 mm over the preceding 3-6 months. The patients also contributed paper-point samples from 2 periodontitis lesions of 5.2 ± 1.0 mm mean probing depths that had remained stable during the same 3-6 month period. To reduce bias, identifications of the infectious agents were performed without the knowledge of the clinical status of the sample sites. HSV, EBV-1, and HCMV were identified via polymerase chain reaction (PCR). The major periodontopathic bacteria including *P. gingivalis*, *Dialister pneumosintes*, *Bacteroides forsythus* (*Tannerella forsythia*), and *Actinobacillus* (*Aggregatibacter*) *actinomycetemcomitans* were also identified using the PCR amplification of signature sequences of bacterial 16S rRNA genes. Statistical analyses were performed in order to determine the following: 1) whether any of the 4 test bacteria and any of the 3 test viruses were associated with each other; 2) whether individual viruses, combinations of viruses, or other covariates were, on their own, associated with periodontal presence of *P. gingivalis*; and 3) to identify the best multivariate model for predicting the presence of subgingival *P. gingivalis* and periodontitis active disease with various clinical variables.

Findings: The presence of HCMV was associated with the presence of HSV and the presence of EBV-1 was associated with the presence of HSV; however, no significant relationship was found between EBV-1 and HCMV. No significant relationships were determined among the 4 test bacteria. All 3 test viruses were positively associated with probing pocket depth. HCMV, EBV-1, and HSV were found to be associated with periodontitis disease activity as determined by changes in probing attachment level. Through logistical regression analysis, HSV was mildly significant and HCMV was borderline significant in being associated with the presence of subgingival *P. gingivalis*. EBV-1 was found to be a poor predictor in all statistical models, indicating that the virus is not predictive of the presence of subgingival *P. gingivalis*. The strongest relationships with *P. gingivalis* were found with probing attachment level, periodontitis disease activity, patient age, gingival bleeding upon probing and probing pocket depth. The periodontitis disease risk associated with herpesvirus-*P. gingivalis* combinations depended on both patient-specific factors and site-specific factors.

Conclusions: The present study showed significant associations among HCMV, *P. gingivalis* and progressive periodontitis. All statistical analyses demonstrated that significant associations among HCMV-*P. gingivalis*-active periodontitis supports the notion that HCMV and *P. gingivalis* serve as cofactors in periodontal breakdown. There

was no significant relationship between EBV-1 and *P.gingivalis*. However, while EBV-1 was not predictive of the presence of subgingival *P. gingivalis*, the virus was correlated with periodontal disease activity, suggesting a promotional role in disease activity (i.e. through interactions with periodontopathic species other than *P. gingivalis*). HSV may demonstrate a probable role in the etio-pathogenesis of some types of aggressive periodontitis. The results of the study also show that both HCMV and HSV cooperate with *P. gingivalis* in the destruction of periodontal tissues. Further studies are required to investigate the spectrum of periodontopathogenicity of herpesviruses and effective management of these viruses in sites with active periodontal disease.

Taichi Ito, Akiyo Komiya-Ito, Tomohiko Arataki, et.al. Relationship Between Antimicrobial Protein Levels in Whole Saliva and Periodontitis. J Periodontol 2008;79:316-322 .

Purpose: To compare the amounts of two types of antibacterial protein, cystatin (cystatin SA and cystatin C) and lysozyme, in saliva between healthy persons and subjects with Periodontitis.

Materials and Methods: 40 subjects (24 females and 16 males) with Periodontitis aged 26 to 86 yrs and 27 healthy persons(13 females and 14 males) were evaluated. The saliva was collected by requiring all subjects to expectorate into a sterile 2ml tube. The samples were centrifuged to remove particulate matter and bacteria. The salivary levels of cystatin SA and cystatin C, and lysozyme were determined by enzyme-linked immunsorbent assay or immunoblot assay.

Findings: The cystatin SA level in saliva from the periodontally diseased group showed a mean value of 0.063mg/ml which was statistically lower than that of the healthy group. The cystatin C level in the periodontally diseased group was 2.27ng/ml which was lower than that in the healthy group 3.79ng/ml. The lysozyme levels in subjects with Periodontitis and healthy subjects were 16.75and 30.03g/ml respectively. The lysozyme level in the Periodontitis group was significantly lower than in the healthy group.

Conclusion: Evaluating the levels of antibacterial proteins in saliva is a useful tool for determining the onset and progression of Periodontitis. The severity and degree of periodontal disease can be assessed by using a specific monoclonal antibody to detect salivary cystatin levels.

Tonetti MS and Mombelli A. Early-onset periodontitis. Ann Periodontol 1999;4:39-52.

Purpose: To discuss the criteria to classify early-onset periodontitis (EOP) and review the evidence justifying a subclassification into particular subgroups of EOP.

Materials and Methods: Literature review.

Findings: EOP syndromes have been primarily defined using the following criteria.

- (1) Relationship to systemic conditions: Periodontal diseases in children and young adults may represent an oral manifestation of a systemic disease condition. On the other hand, EOP may also be present in children and young adults in the absence of obvious systemic conditions. In this two different clinical situations, EOP syndromes would be classified according to their relationship to a systemic condition, or lack of the things mentioned.
- (2) Age of onset and puberty: Age would be a valuable discriminator if there were evidence to support for an age-dependent window of susceptibility. However, in the absence of proof for an age-dependent modulation of susceptibility, patients diagnosed on the basis of an age-dependent classification will change diagnosis as they grow older, although they still suffer from the same disease. In addition, using age as a classification criterion is limited by the fact that age of diagnosis does not necessarily mean age of onset of the disease.
- (3) Involvement of primary/permanent dentition: Substantial evidences showed that early-onset periodontal destruction may affect both the primary and permanent dentition.
- (4) Distribution of lesions: Progression from a localized to a more generalized form of disease may happen in distinct patient subsets characterized by either infection with specific pathogens and/or by an increased susceptibility.
- (5) Severity of destruction: The problem with severity as a criterion for classification is related to the lack of evidence indicating that periodontal disease progress continuously.
- (6) Rate of progression: Correct application of this criterion requires availability of clinical or radiographic data from more than one time point.
- (7) Response the therapy: The major problem of this criterion is that the lack of treatment response should not be due to improper or inadequate therapy. Lack of response to proper treatment represents the most important rationale to subdivide a specific syndrome.

In addition, in spite of the great progress in the understanding of EOP etiology and susceptibility, there is insufficient knowledge to classify these syndromes.

Conclusions: Old classification of early-onset periodontitis should be revised.

*Background: Old classification of EOP

- Prepubertal periodontitis (PPP): Attachment and alveolar bone loss evident only in the primary dentition. Onset – started from 4 years of age. Moderate accumulation of plaque and calculus. Moderate signs of inflammation. Absence of systemic conditions, recurrent infections, and local factors.

- Localized juvenile periodontitis (LJP): Attachment loss of 4 mm or more on at least 2 permanent first molars and incisors. Age of onset – between puberty and 25-30 years of age. Familial aggregation. Absence of local factors.
- Generalized juvenile periodontitis (GJP): Attachment loss of 4 mm or more affecting at least 8 teeth. Age of onset – before age 35.