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Detection of Herpes Viruses in Aggressive Periodontitis Patients in Mosul

Mahmoud Y. M. Taha[a*], Zeyad T. Al-Rassam[b], Rafea A. Al-Taleb[c]

[a]*Professor, Department of Dental Basic Science. College of Dentistry. Mosul University, Mosul, Iraq.*

[b]*Lecturer, Department of Biology. College of Science. Mosul University.*

[c]*Assist. Prof., Department of Oral Surgery. College of Dentistry. Mosul University.*

[a]*Email: tahadent04@yahoo.com*

[b]*Email: zeyadalrassam@yahoo.com*

Abstract

The aim of the study is to estimate the frequency of herpes viruses HCMV, EBV1 and HSV in periodontal pocket samples of aggressive periodontitis patients. This study was carried out on total number of 24 Aggressive Periodontitis patients, 14 (58%) males and 10 (42%) females, aging between 20-≥40 years old). Periodontal pocket samples were collected from three deepest pocket sites and nested PCR technique was used to detect HCMV, EBV and HSV–1. The nested PCR technique results showed detection of herpes viruses in 12(50%) patients pocket samples, these viruses were distributed as follow; 3(12.5%) of patients showed presence of EBV, 3 (12.5%) of patients showed presence of HSV-1. In this study EBV-CMV co-infection and EBV-HSV-1 co-infection were detected in 3 (12.5%) for each one, with absence of CMV or CMV-HSV co-infection. The comparison between the type of virus detected and age groups showed detection of viruses in 9 (75%) and 3 (25%) of patients at age groups ≥40 and 20-29 years respectively, while there was no detection of viruses at 30-39 years age group. EBV was detected at high percentage in ≥40 years age group with absence from 20-29 and 30-39 years age groups. This study showed significant association between the presence of EBV and the increase of age. In the present study, viruses were detected in males more than females but there is no significant association between gender and type of viral detected.

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* Corresponding author.

The comparison between periodontal pocket depth and type of viral detected showed detection of EBV and EBV–HSV in 7mm pocket depth and detection of CMV-EBV, HSV in 6mm and 5mm respectively with absence of viruses in 4mm pocket depth. Thus this result showed significant association between the increase pocket depth and presence of EBV.

Keywords: aggressive periodontitis; Herpes Viruses.

1. Introduction

Periodontitis is defined as "an inflammatory disease affected supporting tissues of teeth caused by specific microorganisms or groups of microorganisms leading to progressive destruction of the periodontal ligament and alveolar bone with pocket formation, recession, or both [1]. Aggressive periodontitis (AP; formerly known as juvenile periodontitis) is defined as an aggressive form of disease characterized by rapid progression of attachment loss, clear bone destruction and there is a predisposition to the disease within the family [2,3]. Generally AP affects systemically healthy individuals aged <30 years or may onset at around adolescence or early adulthood therefore AP is characterized by a prepubertal periodontitis [2,4,5] The prevalence of AP varies significantly between populations, and differences in race/ethnicities. Studies consistently show that AP is higher prevalent in Africa and in African-American populations than in whites population in Europe and North America with unclear difference between gender [6,7]. Herpes viruses specially herpes simplex virus (HSV), human cytomegalovirus (HCMV) and Epstein–Barr virus type-1 (EBV-1), are found more frequently in periodontal disease and might be involved in the pathogenesis of periodontal disease [8,9]. Recent study demonstrated an association of herpes viruses with periodontal disease, particularly detection of viral DNA in gingival tissue, gingival crevicular fluid (GCF) and subgingival plaque from periodontally diseased sites. Thus members of the herpes viruses family may play a putative role in the ethiophatogenesis of severe types of periodontitis via recovered at periodontal sites, therefore these viruses seem to be greater at diseased sites than healthy sites [10]. Recent study identified HCMV, EBV-1 and HSV-1 viruses in 72-78% percentage of AP patients [11]. These viruses may play a causal role in periodontitis pathogenesis [12]. Although herpes viruses may be associated with aggressive periodontitis but this hypothesis is still remains to be elucidated [13]. This study was conducted to evaluate the frequency of three members of herpes virus family in samples of periodontal pockets fluid of AP patients.

2. Materials and Methods

2.1. Subject Groups

This study was carried out on a total number of 24 AP. Patients were selected from subjects referred to the periodontic clinic in oral surgery, College of Dentistry/Mosul University, Iraq and some of private clinics in Mosul. Patients participated were 14 males and 10 females, aging between 20-≥40 years. All patients were systemically healthy and had not undergone periodontal therapy in the previous 6 months and anti-viral therapy in the previous 3 months. Smokers and alcoholisms were excluded from this study. Medical history and demographic data were determined by chart review and patient interview.

Diagnosis of AP was based on full mouth periodontal probing and analysis of the alveolar bone level on the perapical and inter proximal radiographs [14,15].

2.2. Collection of Samples

Before sampling, supragingival plaque was removed by a curette and the region was dried using a gentle blast of air and the sample sites were isolated with sterile cotton pellets. Samples were taken from one tooth with the deepest pocket ≥4 mm for a test site from each individual using sterile paper points and the paper points was gently inserted into the bottom of pocket and kept for 30 sec [16]. These samples were transferred into a sterile plastic microtubes (eppiendroff tubes) containing 100 µl of TE buffer pH = 7.5 and stored at -70 ^{o}C until nucleic acid extraction. DNA extraction was performed using InnuPREP Virus DNA and kept at -70°C until detection of viruses by PCR technique.

2.3. Nested PCR technique

A nested PCR technique was used to detect herpes viruses genomes. A nested PCR amplification was accomplished using eppiendroff thermal cycler (Master cycler).

Cytomegalovirus detection by *FAST PCR* of *IEA* region.

This system is a qualitative test that allows the DNA amplification by means of FAST PCR of *IEA* region of CMV genome.

Epstein-Barr Virus Detection using *FAST PCR* of *Bam HI-W* region

The EBV system is a qualitative test that allows the DNA amplification by means of *FAST PCR* of *Bam HI-W* region of EBV genome.

Herpes Simplex Virus (HSV 1-and 2) Detection using *FAST PCR* of the *gD* region.

The HSV 1 and 2 system is a qualitative test that allows the DNA amplification by means of *FAST PCR* of *gD* region of HSV 1 genome.

PCR products were subjected to electrophoresis using 1.5% agarose gel and TAE buffer. A 100bp DNA ladder was used as a marker of molecular size. Electrophoresis was run at 120 Volts for 15-30 minutes. The gels were stained using 0.5 µg mL^{-1} of ethidium bromide for DNA visualization. The final bands product were visualized under 300 nm UV light trans illuminator. Procedures of all kits were accomplished according to manufactory recommendation.

2.4. Statistical analysis

T-Test, Pearson Chi-Square Test, Pos Hoc Test, ANOVA Test, Duncan Test, Correlations, Mean, were used to analyse data.

3. Results

This study was carried out on total number of 24 AP patients, 14 (58%) males and females 10 (42%), aging between 20 - ≥40 years. (Figure 1).

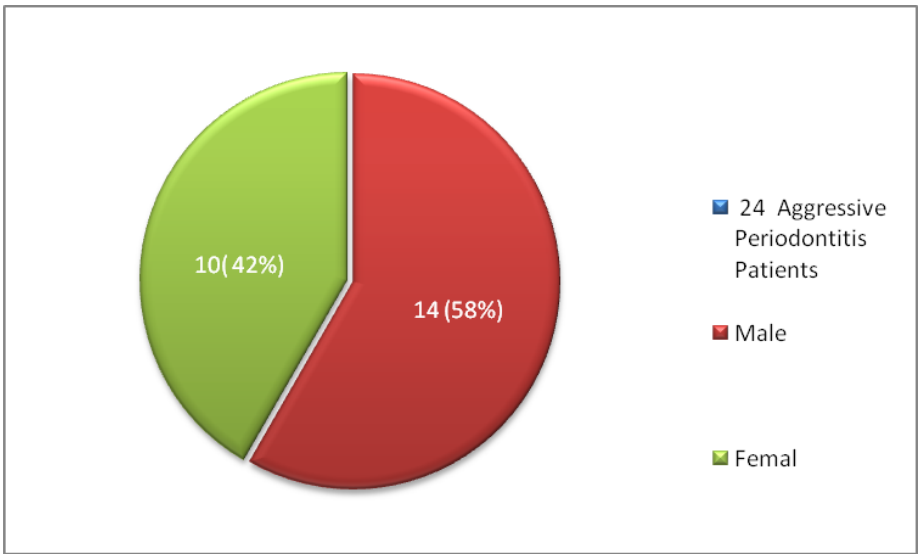

Figure 1: Percentages of aggressive periodontitis patients according to the gender

Virological detection of herpes viruses (EBV, CMV, HSV1) using Nested PCR showed presence of these viruses in 12 (50%) AP patients (viral detection AP group), whereas 12(50%) of AP patients showed negative result (Figure 2).

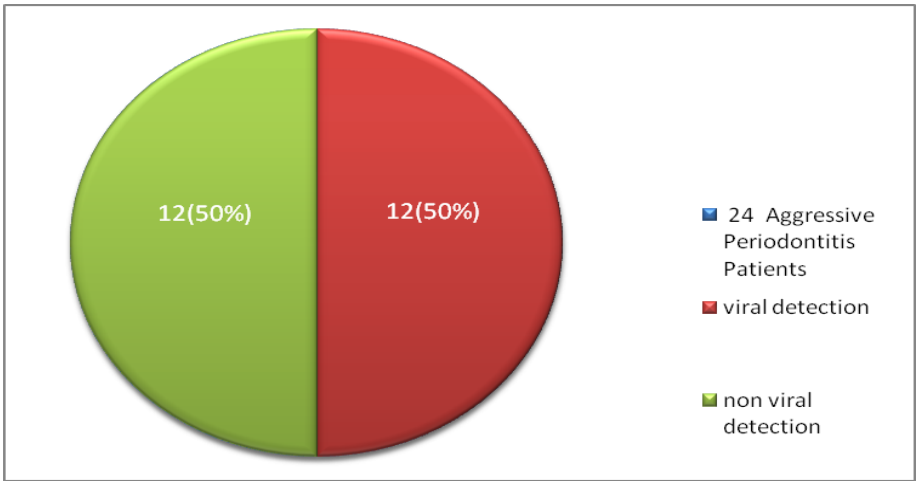

Figure 2: Percentages of percent under stud viruses in aggressive periodontitis patients

In this study viral detection AP group showed distribution of viruses in 8 (67%) males and 4 (33%) in females, but there was no significance association between gender and type of viral detected. (Figure 3).

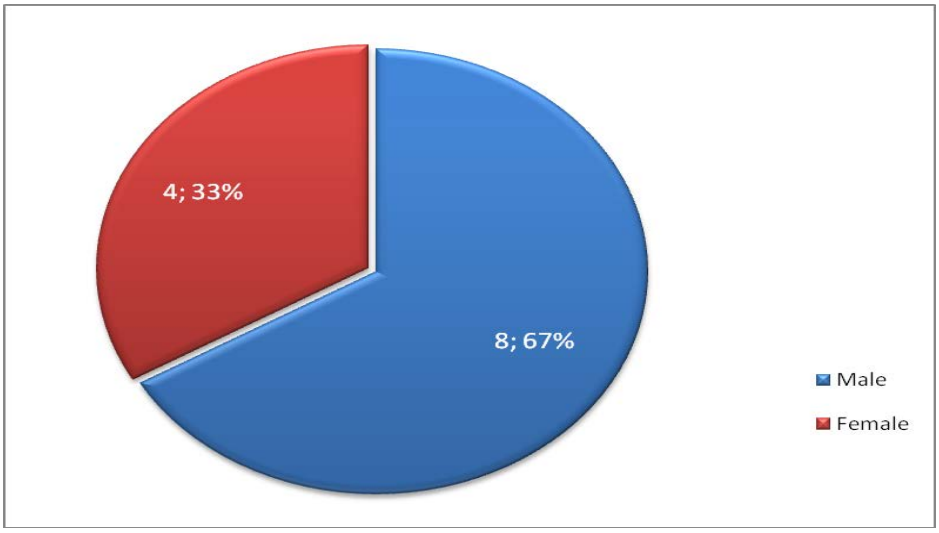

Figure 3: Percentages of gender in viral detection aggressive periodontitis group

In viral detection AP group, EBV was detected as a single infection in 3 (12.5%) of patients and also HSV was detected as a single infection in 3 (12.5%) of patients. Furthermore, presence of EBV& CMV as co-infection was detected in 3 (12.5%) of patients. EBV& HSV as co-infection was detected in 3 (12.5%) of patients, with absence of CMV as a single infection and HSV& CMV as co-infection. (Figure 4).

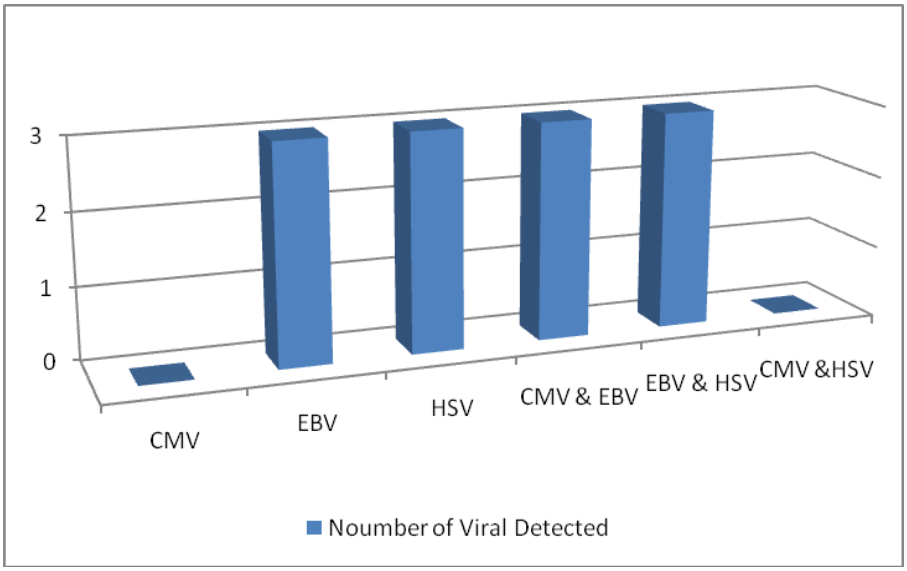

Figure 4: distribution of viruses in viral detection aggressive periodontitis group.

The comparison between the type of virus detected and age group showed detection of viruses in 9 (75%) and 3 (25%) of patients in ≥40 and 20-29 years age groups respectively, while there was no detection of viruses at 30-39 years age group (Figure 5).

In this study, EBV present at high percentage in ≥40 years age group, whereas absent in 20-29 and 30-39 years age groups. Thus, this study showed significant association between presence of EBV and the increase of age.

(Figure 6).

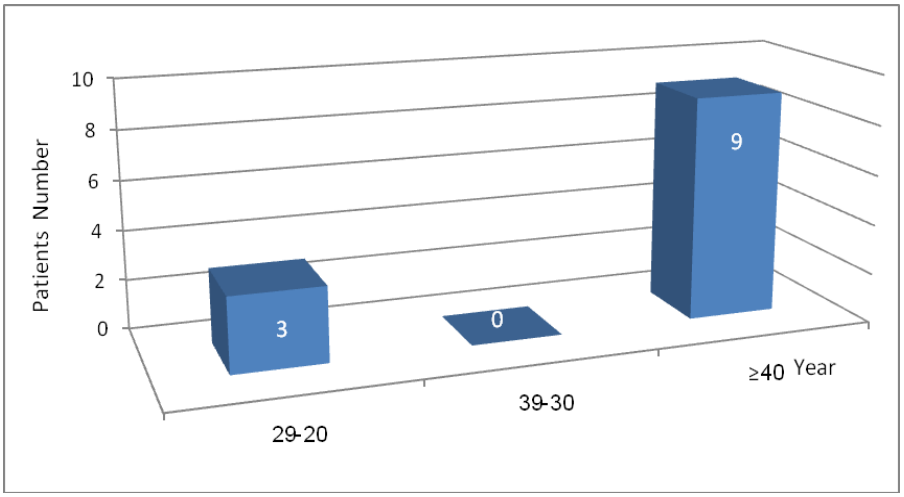

Figure 5: distribution of viral detection aggressive periodontitis patients according

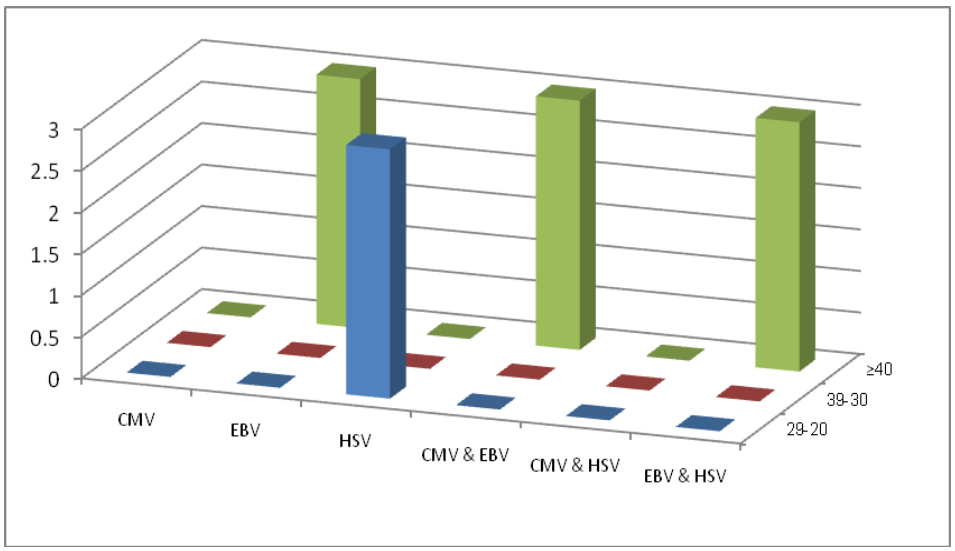

Figure 6: distribution of under stud viruses in aggressive periodontitis patients according to age groups

The comparison between periodontal pocket depth and type of viral detected showed presence of significant association between the increase pocket depth and presence of EBV. (Figure 7).

4. Discussion

In this study, AP is present in males 14 (58%) more than females 10 (42%) with no significant association between AP and gender. This result is compatible with several studies that showed equal distribution of the disease between genders [6,7,17,18]. However prevalence of AP varies significantly between populations, and differences in race and ethnicities, thus AP is most prevalent in Africa and in populations of African descent and is least prevalent in Caucasians in Europe and North America [6].

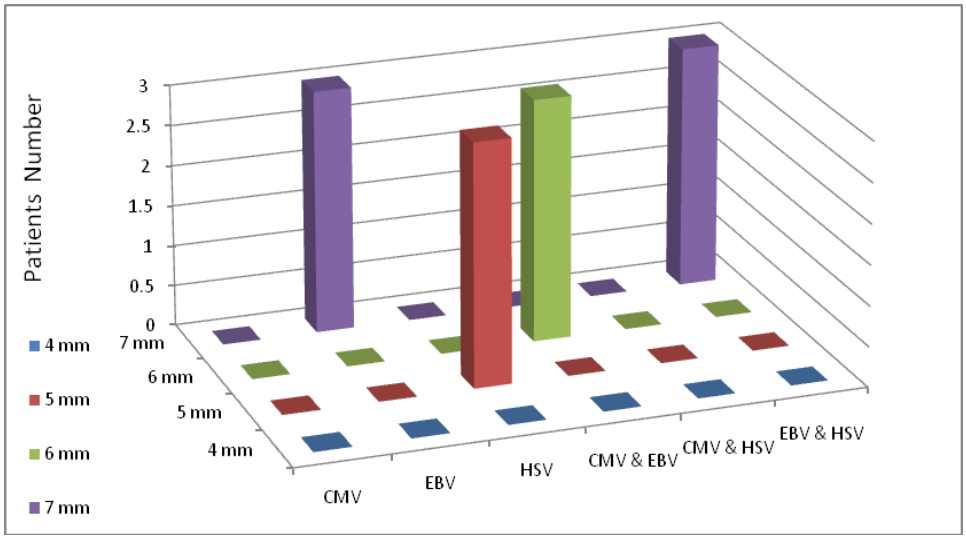

Figure 7: Distribution of under stud viruses in aggressive periodontitis patients according to pocket death

Virological detection of EBV, CMV and HSV using Nested PCR showed detection of viruses in 12 (50%) of AP patients. Viruses were detected in 8 (67%) males and 4 (33%) females, although the infected males were more than females but there was no statistically significant difference between male-female proportions. Most studies show comparable disease prevalence in male and female subjects [6,18]. Viral detection in AP group showed detection of EBV in 9 (75%) of patients; 6 (50%) of this patients showed presence of EBV as a co-infection with CMV and HSV. Moreover, HSV was detected as a single infection in 3 (25%) of patients, whereas absence of CMV as a single infection or as co-infection with HSV. This result showed dominance of EBV 9 (75%) which is compatible with several studies which showed that HCMV, EBV and HSV-1 detected in crevicular samples in high frequency (72-78%) of progressive AP [11]. Moreover, the prevalence of these viruses seems to be greater at diseased sites than healthy sites[10], whereas presence and amount of viral DNA is correlated with disease severity[19]. Bilichodmath et.al., found that herpes viruses were less frequently in AP than in chronic periodontitis patients [20]. Dawson et, al., concluded that EBV was commonly seen, with rarely present of CMV in patients with AP [19], whereas other study noted that CMV may be implicated in the pathogenesis of human periodontitis, and confirmed frequent presence of HCMV in AP lesions [21]. HCMV-EBV co-infection is closely associated with AP periodontal abscesses.[(22)] Moreover, this co-infection occurs more frequently in deep than in shallow periodontal sites [23,24].

Several studies mention that herpes viruses infection impaired periodontal defense and supported overgrowth and increased pathogenicity of periodontal microbiota [11,25]. Herpes viruses may cause direct cytopathic effects alter functions of microphages and macrophages cells causing reduce in its defense ability via induce abnormalities in its chemotaxis, adherence and phagocytic activities [26]. EBV is frequently detected in AP sites, EBV infection of lymphocytes can increase B lymphocytes counts towards predominance of B lymphocytes / plasma cells (B lymphocytes is prominent in AP lesions), with generation of neutropenia and antineutrophil antibodies, Infected B lymphocytes may shed viral antigens causing activation of T suppressor cell [27,28]. HCMV reactivation in periodontitis lesions seems to be linked to advancing disease via infecting periodontal monocytes/macrophages and T-lymphocytes [22]. HCMV can suppress antigen specific cytotoxic T

lymphocytes functions, resulting in decrease in circulatory CD4+ cells and increase in CD8 suppressor cells which lead to impairment of cell mediated immunity [28]. However, HSV-1 virus is involved only in advanced AP sites [27], while HSV-2 was not detected in any specimen of AP patients [20]. Although herpes viruses infections can reduce host immune defense mechanisms and alter the ability of tissues to withstand opportunistic periodontopathogenes [25], recent study did not suggest any contribution of HSV-1, EBV or HCMV to AP in a German population [12]. Furthermore other study noted that EBV and HCMV seem unlikely to play a major role in the pathogenesis of periodontitis. [(29)] In contrast Kaarthikeyan,et, al., study have revealed an association between herpesvirus (HCMV, HSV-1, and EBV) and AP [30]. Thus herpes viruses may be associated with AP but this hypothesis is still limited, because the exact role of herpes viruses in progressive of AP is still unclear and need further studies [13].

The comparison between the type of virus detected and age groups showed detection of viruses in 9 (75%) and 3 (25%) of patients in ≥40 and 20-29 years age group respectively with absence of viruses at 30-39 years age group. In this study dominance of EBV as single and co-infection was presence in ≥40 years age group, whereas presence of HSV only in 20-29 years age groups. Thus there was a significance association between presence of EBV and the increase of age. Herpes viruses were found less frequently in AP patients and their prevalence may vary according to the age and race of the patient [20].

In the present study the comparison between periodontal pocket depth and type of viral detected showed presence of significant association between the increase pocket depth and presence of EBV and CMV. HCMV and HSV. Viruses were detected with higher frequency in deep periodontal pockets. Furthermore viral co-infection occurred more frequently in deep than in shallow periodontal sites [24].

5. Conclusion

There is no clear association between aggressive periodontitis and the presence of under study viruses in periodontal pocket. We recommend further work on viruses in periodontitis to understand the association between viruses and aggressive periodontitis.

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