Al-Qadisiyah Journal of Pure Science

Volume 26 | Number 4 Article 30

8-15-2021

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Recommended Citation
Al-Bdery, Alaa Shahid Jassim and Al-Yasseen, Ahlam Kadhum (2021) "Prevalence Of Epstain–Bar Virus Among Patients With Periodontitis In Al–Najaf Al–Ashraf / Iraq," *Al-Qadisiyah Journal of Pure Science*: Vol. 26: No. 4, Article 30.
DOI: 10.29350/qjps.2021.26.4.1424
Available at: https://qjps.researchcommons.org/home/vol26/iss4/30

Al-Qadisiyah Journal of Pure Science
ISSN(Printed): 1997-2490 ISSN(Online): 2411-3514
DOI: /10.29350/jops.
http://qu.edu.iq/journalsc/index.php/JOPS

Prevalence of Epstain–Bar Virus among Patients with Periodontitis in Al–Najaf Al–Ashraf / Iraq

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Article History
Received on: 1/7/2021
Revised on: 30/7/2021
Accepted on: 5/8/2021
Keywords:
Periodontitis, EBV,
dental plaque

DOI: https://doi.org/10.29350/jops.2021.26. 4.1424

ABSTRACT

Periodontitis, a complex chronic inflammatory disease caused by subgingival infection, is among the most prevalent microbial diseases in humans which characterized by periodontal damage, alveolar bone resorption, pain, and eventual tooth loss. Epstein–Barr Virus (EBV) has widely infected >90% adults in the world and is associated with many human diseases, so that this study aimed to investigate the association between EBV and periodontitis.

Patients and samples: Subgingival paper point samples were collected from 100 patients with periodontitis and 30 healthy people. All samples undergo direct DNA extraction to amplified EBVs DNA using PCR technique.

The results indicated a high percentage of EBV infections (18%) in patient suffering from periodontitis while there was no EBV infection were detected in healthy persons. A high percentage of EBV was detected in female (56%) in comparison with male (44%).

The results improved the association between EBV and periodontitis suggesting that EBV may serve as a pathogenic factor leading to periodontitis among patients.

1-Introduction

Almost 800 different types of microorganisms have been found in the oral cavity, some of these microorganism known as microbiota, microflora or microbome, forming a biofilm of dental plaque on the soft tissues of the oral mucosa and hard tissues [9]. The majority of oral microbes are bacteria that formed 94% of total microbiota such as Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, Spirochaetes, Fuscobacteria. The imbalance of microorganism intervening in the oral cavity which called dysbiosis, lead to the onset of inflammation and thus the disease it self[18,4]. Periodontitis is an infectious disease in which plaque bacteria have been considered as the causative agents of periodontitis for many than years, but the role of such bacteria in such disease was not well explained [24,7].

Herpes virus which belong to herpes family has been suggested as another cause of periodontitis due to its present in dental plaque, gingival tissue and periodontal acute fluids from periodontitis[6]. Epstein-Barr virus (EBV) is a double-stranded DNA which affects human only and responsible for more than 90% of adult infection worldwide [23]. EBV leads to viral progeny and contributes to the pathogenesis of many human diseases, including infectious mononucleosis, Autoimmune disorders, a number of malignancies [14]. Although there is a causal relationship between periodontitis and EBV , and there was a positive association reported between periodontitis and EBV infection [10]. Activation of latent EBV results in viral progeny and contributes to the pathogenesis of several human

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disease including infections mononucleosis, autoimmune disorders, and a number of malignancies [25]. Therefore, the virus may deteriorate immune stability in the gum disease by contributing to the overgrowth and aggressiveness of inflammatory bacteria Surrounding pathogens, thus favoring the initiation and development of tissues collapse [5]. Indeed, the interaction of EBV and bacterial aetiology is bidirectional [22], as structural components of specific anaerobic bacteria have the potential to induce EBV and virus may affect the overgrowth of pathogenic bacteria by affecting the potential Adhesion to infected host cells and alteration of inflammatory cells involved in immunity response [22]. Regarding the pathogenesis, it seems that the presence of EBV could be related to an increase in the inflammatory response with the consequent increase in markers such as the macrophage inflammatory protein-1β (MIP-1β) and tumor necrosis factor-α (TNF- α) [16]

Preliminary studies have shown that (EBV) may be related to the occurrence of localized juvenile periodontitis, acute necrotic ulcerative gingivitis and chronic periodontitis [13]. So that this study aimed to improve the correlation between EBV and periodontitis in patient suffering from periodontal disease.

2- Methods

Patient: one hundred paper point samples (50 samples from males and 50 from females) have been collected from patient suffering from periodontitis who admitted to the health centers and the Specialized Dental Center in Najaf, Iraq, and 30 paper point samples have been collected from healthy persons. All samples were preserved at -80°C for DNA extraction process.

DNA extraction: A high purity nucleic acid kit (Intron, Korea) has been used for direct extraction of viral DNA from the paper point sample as recommended by the manufacture.

PCR Technique: Amplification of EBV DNA was carried out using a set of primer as described previously[11] which include F, 5'-AAAGTTGACGTCATGCCAAG-3' and R, 5'-AGCAGTGGCCAGCTCATG-3'. PCR mixture consist of 25µl of master mix (Go Taq® G2 Green Master Mix), 4 µl of both forward and revers primer (final concentration of each primer were 10 µM/µl), 4 µl of DNA template and final volume of mixture was completed to 50 µl by adding 13 µl of nuclease free water. The PCR mixture was centrifuged for 1min at 1000 rpm then the PCR tubes were transferred to the thermocycler (QLS, UK). The amplification condition involved a primary denaturation at 94 °C for 5 min followed by 35 cycles of denaturation at 95°C for 30s, annealing at 56 °C for 30 s, extension at 72 °C for 60 s, and a final extension at 72 °C for 5 min. The amplicons resulted from amplification were electrophoresed on 2% agarose gel at 90 volts/cm for 40 min. Then photographed using Gel Documentation system (Cleaver, UK).

Ethical approval: The consent of the patiant and healthy was obtained.

3-Results

The agarose gel electrophoresis of 130 DNA samples extracted from paper point of patient (100 samples) and healthy (30 samples) showed that only 18 samples (18%) of patients gave positive results for amplification of EBV by appearance of amplicons with molecular weight 331 bp [Figure 1], while a negative results were obtained for DNA extracted from healthy person which showed no amplicons for EBV.

Out of 18 positive samples for EBV, the results showed that the percentage of male infected with EBV were 44% (8/18) in comparison with female that gave 56% (10/18) of infection with no significance difference between both group at P-Value <0.05 ([Figure 2].

A high rate of infection with EBV was observed among age group 10-19 years old of both male and female which were 37.5% and 40% respectively with a significance difference between both group at P-Value <0.05 (Table 1)

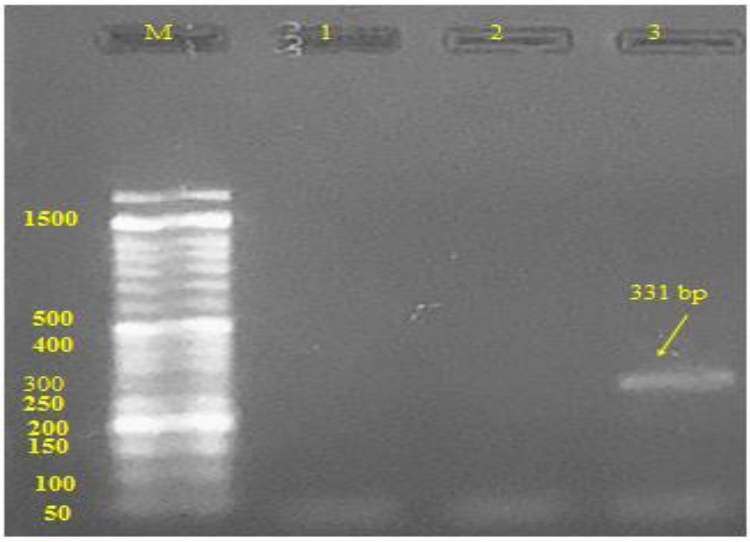

Fig. 1- Agarose gel electrophoresis of PCR product of EBV(331bp) line M: DNA Marker 100bp (Intron, Korea) lines: 1 and 2 negative results for amplification. Line3: Positive results for amplification.

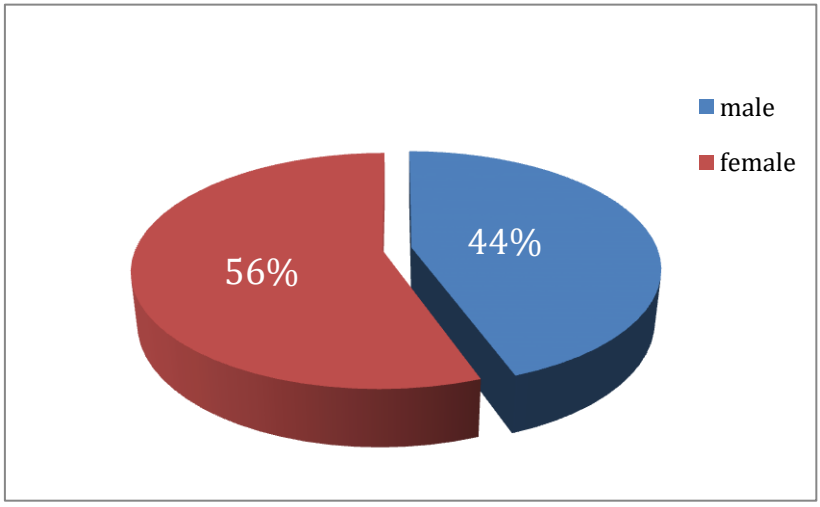

Fig. 2– Distribution of EBV in patients with periodontitis among both sex.

Table 1- Distribution of EBV in patients with periodontitis according to six and age group.

Six	Age Groups (Years)					Total Number	Calculated Chi-Square value	Table Chi-Square value
	10-19	20-29	30-39	40-49	50-59			
Male	3(37.5%)	1(12.5%)	2(25%)	1(12.5%)	1(12.5%)	8(44%)	**0.893**	**0.484**
Female	4(40%)	0	2(20%)	2(20%)	2(20%)	10(56%)	**0.714**	

4-Discussion

Periodontitis involves an oppugnancies' between host and periodontal pathogens leading to disruption of host homeostasis and periodontal tissue destruction. Herpes viruses do not appear to be only passive by standers to gingival inflammation in periodontitis lesions, may cause periodontal pathosis as a direct result of virus infection and replication[1]. Although a number of putative bacteria are considered to be associated with chronic periodontitis, it has become increasingly clear that herpes viruses are involved in the etiology of several types of periodontitis. Bacterial activity alone is not sufficient to explain the following clinical characteristics of periodontitis: rapid periodontium destruction with minimal plaque; site specificity in periodontal disease; and presence of disease activity and quiescence phases [10,2]. [2,3] focused a light on the assumptions of anaerobic bacteria such as *Porphyromonas gingivalis* on periodontitis, despite of that herpes virus is suggested to be involved in causing periodontitis, the bacterial etiology alone does not adequately explain the different clinical aspects between viral and bacterial infection.

Several previous studies showed statistically significant levels of EBV DNA in patients with chronic periodontitis compared with that in healthy controls where a high level of EBVs DNA were detected in gingival creviscular fluid and saliva of patients with periodontitis and chronic periodontitis [10,3,20]. Also a correlation has been founded between presence of subgingival bacteria and EBV in patients with periodontitis where EBV was detected in 30% of plaque samples of patient, while it was not detected in periodontal health/mild gums Inflammation patients [21]. It is believed that EBV infection is associated with chronic periodontal disease and a correlation between EBV prevalence in periodontal patients and periodontal pocket depth was demonstrated [17]. Although emerging evidence implicates an association between EBV and periodontal diseases, the mechanisms of EBV reactivation in the oral cavity and activated EBV progressing to periodontal disease have not yet been determined. The EBV passes through the oropharyngeal epithelium to ß lymphocytes, where it establishes lifelong, latent infection [8]. EBV gingival epithelial extent Infection is associated with the severity of periodontitis [19]. Furthermore, Previous reports, as well as the current study, indicated that EBV also contributes to the progression of periapical periodontitis [26,15]. Periodontitis can be attributed to pathological immune changes in periodontal tissues such as gingival micro-ulcerations, increased vascular cell infiltration and cytokine-mediated epithelial attachment destruction, induced by the periodontal-viral synergistic mechanism[12].

5-conclusion

The Epstein-Barr Virus has a certain relationship with periodontitis infection among both male and female with age group (10-19). Additional molecular studies were required to investigate the correlation of certain strains of EBV with periodontitis disease and its effect on immune system of patients.

Acknowledgements

We extend our thanks to Biological Department /Faculty of Education for Girls/Kufa University and Dental Caries Center , the health clinic and a Specialized Center of Dentistry in Al- Najaf Al-Ashraf city to facilitate the process of research.

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