Hilla University College Journal For Medical Science

Volume 2 | Issue 3 Article 7

2024

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ORIGINAL STUDY

Hilla Univ Coll J Med Sci

Impact of Salivary pH and Epstein-Barr Virus among Gingivitis Patients

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Abstract

Background: Gingivitis is an inflammation of the gums often caused by bacterial infections related to dental plaque, which forms a biofilm on teeth and influences oral health. The microbial composition in the mouth changes with environmental factors and pH fluctuations, impacting conditions like gingivitis, dental caries, and periodontitis. Saliva plays a crucial role in maintaining oral pH, buffering acids, and containing antimicrobial agents. Epstein-Barr virus (EBV), prevalent in the oral cavity, might affect periodontal health and systemic conditions.

Objectives: The study aimed to explore the connection between salivary pH levels and the presence of Epstein-Barr virus (EBV) in individuals with gingivitis compared to healthy controls.

Materials and Methods: A total of 90 participants (60 gingivitis cases and 30 healthy controls) were recruited from the Dental Specialized Center (Babylon) and the University of Babylon College of Dental. Saliva samples were collected and processed for pH measurement using pH test strips. EBV detection was performed via polymerase chain reaction (PCR) targeting the BNLF2a gene.

Results: The mean of pH values was 6.7 ± 0.78 for gingivitis patients and 7.1 ± 0.84 for controls, with no significant difference ($p = 0.074$). EBV was detected in all samples from both groups (patients and control), confirming a 100% prevalence. Gel electrophoresis revealed distinct 121-bp bands of BNLF2a for EBV-specific PCR products in all samples.

Conclusion: This suggests a more acidic salivary pH may be associated with poorer periodontal health; the relationship is complex and not solely dependent on pH levels. The study findings on the prevalence of Epstein-Barr virus (EBV) in both gingivitis patients and control samples emphasize the need to consider viral factors in periodontal disease.

Keywords: Gingivitis, pH, Epstein-Barr virus (EBV), Saliva

1. Introduction

As a burning condition of the gingival tissue, gingivitis is most commonly caused by bacteria. The gingival epithelium and connective tissue are the only areas where this condition can occur, unlike periodontitis, which does not involve attachment misfortune and hence does not result in junctional epithelium migration [1]. Plaque is the primary cause of periodontal disease and cavities, and it may be a classic example of a naturally occurring biofilm. In later cases, the species that prevail in health are no longer represented in the composition of the plaque flora. There are several ecosystems connected to the tooth's surface. Depending on the tooth in question and its level of environmental exposure, the bacterial community's makeup differs. Sub-gingival surfaces are more anaerobic than supra-gingival surfaces, and smooth surfaces are colonized by fewer species than pits and fissures [2].

Although it can vary greatly depending on several factors, the pH of the oral cavity typically ranges from 5 to 9. Although there has been much research on the connection between pH and dental caries, it is less evident how important pH is in terms of gingivitis and periodontitis [3]. *Porphyromonas gingivalis*

Received 29 July 2024; accepted 8 August 2024.
Available online 25 September 2024

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https://doi.org/10.62445/2958-4515.1031

and other oral bacteria linked to periodontal disease often prefer a pH range of 6.5 to 7.0 [4]. It is a well-known fact that Gram-negative anaerobic organisms are mostly linked to human periodontal illnesses. It is known that these bacteria colonize tooth surfaces at and apical to the gingival edge before deforming periodontal diseases appear. Persistent periodontal diseases require microbial colonization. Numerous investigations have noted that certain pH levels are conducive to the growth of periodontal bacteria upon colonization [5]. Saliva is made up of GCF, bacteria, leukocytes, desquamated epithelial cells, and secretions secreted by the salivary gland. The normal range of whole saliva production is 800–1500 mL/day or 1.0–3.0 mL/min. The pH of unstimulated whole saliva is between 6 and 7 [4].

Since EBV is the most prevalent virus that infects humans, about 90% of people worldwide get the infection in childhood. As a result, the illness remains asymptomatic for the duration of the person's life, with intermittent EBV shedding in the oral cavity. Approximately 80 percent of people have these viruses in their saliva [6]. Moreover, the degree of periodontal inflammation and oral hygiene were linked to Epstein-Barr virus (EBV) infection in the mouth [7]. Additionally, it has been discovered that diabetes and double infection with oral EBV and Porphyromonas gingivalis are significantly correlated [8]. Because of its epigenetic profile and the expression of several genes, EBV has significant molecular properties [9]. As a tail-anchored protein, BNLF2a enters the endoplasmic reticulum (ER) membrane post translationally. A hydrophobic C-terminal transmembrane segment and a hydrophilic N-terminal portion make up the protein. The C-terminal section faces the ER lumen, whereas the N-terminal region binds directly to the transporter linked to antigen processing complex [10].

1.1. The aim of the study

Investigate the relationship between salivary pH levels and the presence of Epstein-Barr virus (EBV) in gingivitis patients compared to healthy controls. The second goal was to analyze the participant demographic profiles and assess the prevalence of EBV using molecular techniques.

2. Material and methods

2.1. Subject

The present study is a case-control study that included a total of 90 study samples—60 gingivitis patients and 30 controls—from healthy people. Plaque samples and saliva were taken from both groups (gingivitis patients and controls). The sample collection from the Dental Specialized Center and dentistry faculty of the University of Babylon, both located in Babylon/Iraq, served as study populations. Samples were collected during the period from November 2023 to March 2024, and the age group was from 18 years to 55 years. The patient data collection consisted of structured questionnaires.

2.2. Collection of saliva

Saliva samples were collected properly in contamination-free cups from each patient. A portion of the saliva was then transferred into a plain tube and separated by centrifugation into supernatant and precipitate. The precipitate was transferred to another plain tube and frozen for molecular detection of EBV by PCR. pH test strips (Cybow/China) were used to measure the pH value of the supernatant.

2.3. Inclusion criteria

A. Age range (18–55).
B. Both genders.

2.4. Exclusion criteria

1. Patients with antibiotic therapy.
2. Smokers
3. Systemic diseases
4. Medications

2.5. Ethical approval

The Helsinki Declaration's ethical guidelines were adhered to throughout the investigation. Prior to samples being taken, patients gave their written and verbal consent. The ethical approval of the study protocol with document number 6798 at 12/12/2023.

2.6. Statistical analysis

The study used transferred to SPSS software. The analysis used descriptive and inferential statistical methods, with categorical data presented in frequencies and percentages and continuous numerical data expressed in means and standard deviations. To assess relationships between categorical variables, Fisher's exact test or the chi-square test were applied. On the other hand, differences between numerical variables were examined using the Mann-Whitney U test or the independent sample t-test.

Table 1. The demographic attributes of both control subjects and patients diagnosed with gingivitis.

Characteristic	Gingivitis No. = 60	Control No. = 30	*p-Value*
Gender			
Male, *n* (%)	24 (40%)	18 (60%)	0.07 NS
Female, *n* (%)	36 (60%)	12 (40%)	

No.: number of cases; SD: standard deviation; C: The chi-square test results indicate that there was no significant difference ($p \geq 0.05$), denoted as NS, in the variables being compared.

Table 2. Outlines the demographic profiles of patients diagnosed with gingivitis and the corresponding control subjects.

Characteristic	Gingivitis No. = 60	Control No. = 30	*p-Value*
Age (years)			
Mean ± SD	27.4 ± 9.5	30.77 ± 7.2	0.09 NS
Range	18–56	22–45	

No.: The number of cases examined, alongside their standard deviation (SD), was subjected to an independent samples t-test (I), revealing no significant difference (NS: $p \geq 0.05$).

3. Results

3.1. Demographic characteristics of the study samples

The study gathered a brigade of ninety patients, comprising 60 patients with gingivitis and 30 healthy subjects. According to the gender distribution presented in Table 1 and Fig. 2 the gingivitis group consisted of 40% males (24 patients) and 60% females (36 patients). In contrast, the control group comprised 60% males (18 subjects) and 40% females (12 subjects). In Table 2 the age mean ± SD was 27.4 ± 9.5 for the gingivitis group and 30.77 ± 7.2 for the control group. The observed contrast, as depicted in Table 1 and Table 2 did not yield statistical significance ($p \geq 0.05$).

3.2. pH levels among gingivitis patients and control groups

Table 3 compares the pH levels between control subjects and patients with gingivitis using the independent samples t-test for mean comparison, indicating no significant differences in pH levels ($p > 0.05$).

Table 4 categorizes both gingivitis patients and control subjects by specific pH levels, ranging from 5.5 to 8.5, and displays the data in counts and percentages. This table demonstrates a relative frequency distribution, which does not indicate significant differences.

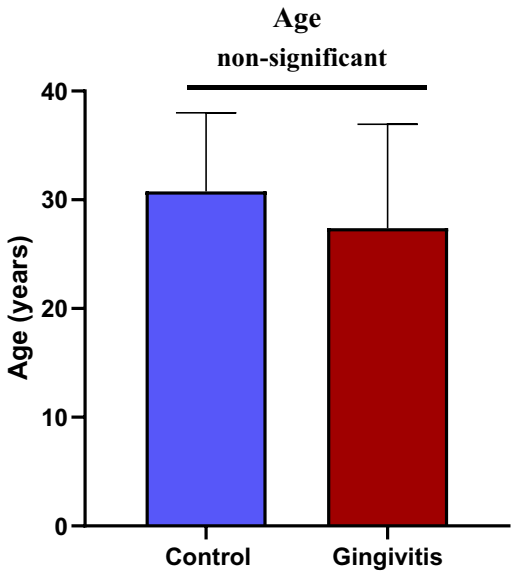

Fig. 1. Bar chart comparing the average age among gingivitis patients and control groups.

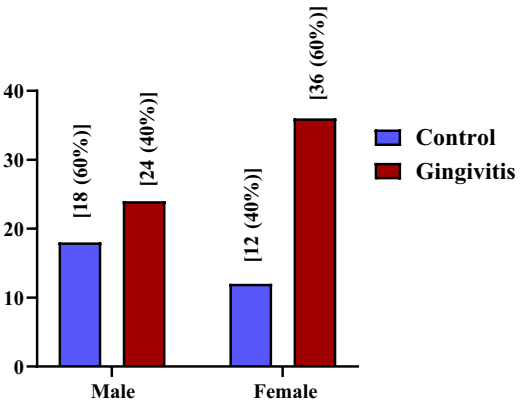

Fig. 2. Histogram showing the frequency distribution of gender for the control group and patients with gingivitis.

Table 3. Distribution of pH levels in control subjects and patients with gingivitis.

Characteristic	Gingivitis No. = 60	Control No. = 30	*p-Value*
pH			
Mean ± SD	6.7 ± 0.78	7.1 ± 0.84	0.074 NS
Range	5.5–8.5	5.5–8.5	

No.: The analysis included the number of cases observed, along with their corresponding standard deviations (SD). An independent samples t-test (I) was conducted, revealing no significant results (NS).

3.3. Detection of Epstein Barr Virus (EBV) among gingivitis patients by PCR

To find the Epstein-Barr virus (EBV), DNA was isolated from samples taken from 60 patients with

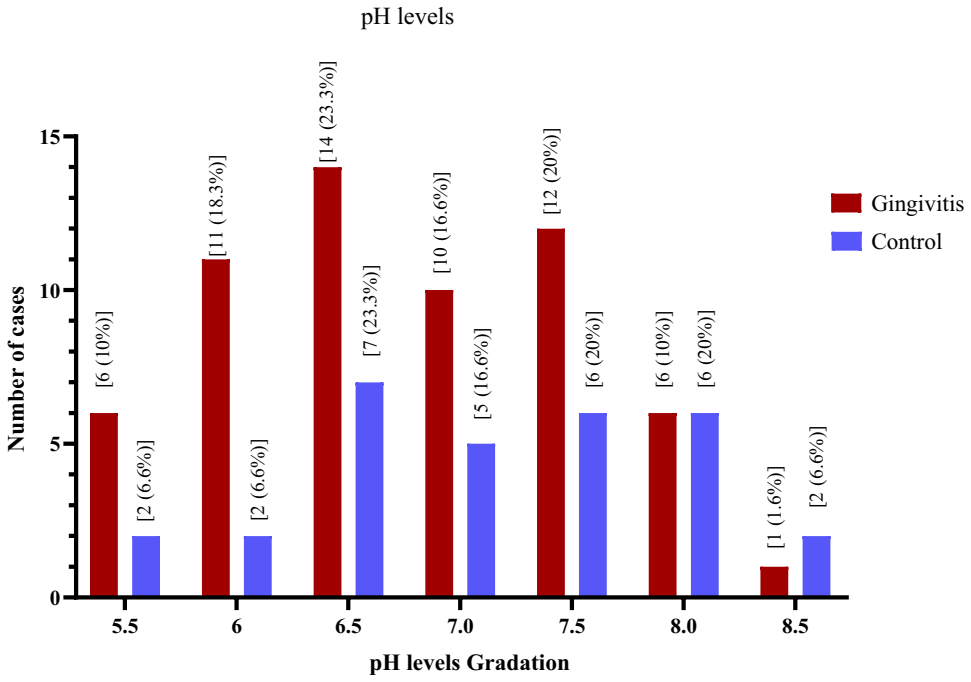

Fig. 3. A histogram depicting the frequency distribution of patients with gingivitis and the control groups based on their pH levels is presented.

Table 4. Provides an overview of the distribution of patients with gingivitis and the control groups concerning their pH levels.

pH level	Gingivitis patients	Control groups	*p-Value*
5.5	6 (10)%	2 (6.6)%	
6	11 (18.3)%	2 (6.6)%	
6.5	14 (23.3)%	7 (23.3)%	
7.0	10 (16.6)%	5 (16.6)%	0.52 NS
7.5	12 (20)%	6 (20)%	
8.0	6 (10)%	6 (20)%	
8.5	1 (1.6)%	2 (6.6)%	
Total	60 (100)%	30 (100)%	

gingivitis and 30 control persons. Conventional polymerase chain reaction (PCR) was used for detection, and particular primers were used to amplify a 121-bp section of the BNLF2a gene. After that, the PCR products were separated on a 2% agarose gel, with a 100-bp DNA ladder placed in lane L for reference. The extracted DNA's purity and concentration were evaluated. Gel electrophoresis was used to confirm the presence of the DNA bands. The study showed that detectable EBV, specifically the R and F genes, each presenting a unique band at 121-bp, were present in all samples from the gingivitis patients as well as the control group. As shown in Figs. (4A&B).

4. Discussion

Despite trends suggesting higher acidity in gingivitis patients, the study did not establish significant pH differences between groups, indicating a complex relationship between pH, microbial ecology, and gingival health. Further research is needed to elucidate these relationships and their clinical implications.

Recent study showed that the mean salivary pH was 6.58 in gingivitis patients, while in periodontitis, 6.24, and in healthy gingiva, 7.0. The findings indicate that a higher salivary pH that is more acidic could be linked to poorer periodontal health, even if the differences were not statistically significant [11]. The primary component of tooth enamel and dentin, hydroxyapatite crystals, are dissolved by the acids, which also cause the pH to drop below a crucial value and permeate the tooth through water [12]. According to a different study, salivary pH also shows no statistical difference between healthy, gingivitis, and periodontitis subjects [4]. A study conducted by [13] Showed that the periodontium is in better health the more alkaline the salivary pH is. However, there was no statistically significant difference (p value > 0.05) in salivary pH between the groups. Marsh (1994) has described oral illness as an illness that is initiated and promoted by alterations in the microbial ecology and environmental conditions. The microbial ecosystem's pathogenicity is increased by these modifications. We call it the ecological plaque theory [14]. In our examination of samples from individuals with gingivitis alongside control samples, we have detected the presence of the EBV virus in both sets at a prevalence rate of 100%. EBV is the most common virus infection in humans, accounting for around 95% of global

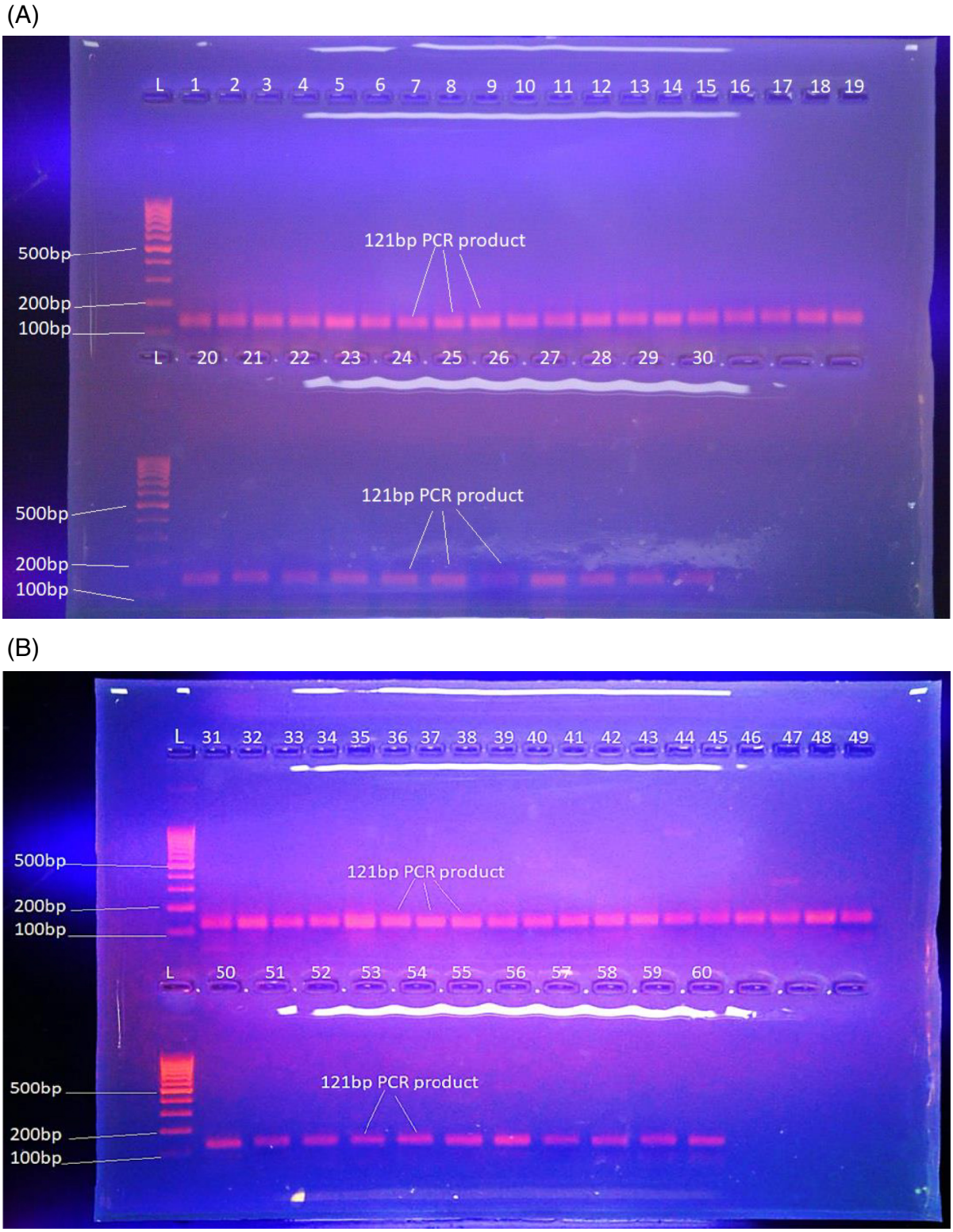

Fig. 4. (A) The detection of the Epstein-Barr virus was conducted using specific PCR techniques. Agarose gel electrophoresis was performed on a 2% gel at 100 volts for 45 minutes to separate the PCR-produced goods. These were first stained with ethidium bromide and then seen under a UV lamp. The analysis revealed a 121-bp PCR product of the BNLF2a gene in patients with gingivitis. Lane L contained a 100-bp molecular weight (ladder), while the other lanes displayed samples that tested positive. (B) Epstein-Barr virus detection was conducted using specific PCR and subsequent 2% agarose gel electrophoresis at 100 volts for 45 minutes. Following ethidium bromide staining, UV light was used to visualize the PCR products. The PCR product of the control group demonstrated a size of 121 base pairs corresponding to the BNLF2a gene. Additionally, the electrophoresis results featured a lane designated for the L100bp molecular weight ladder

infections, which are asymptomatic and persistent over a lifetime [15]. Based on epidemiological research, it is estimated that over 90% of the global population carries EBV [16]. Over 90% of adults worldwide are infected with the gamma-herpesvirus known as EBV [17]. Given that EBV can inhibit host immunity, it is plausible that EBV is the cause of periodontitis [18]. The Epstein-Barr virus genome contains the BNLF2a gene, express the immediate-early protein (EBV). It is essential to the EBV lytic cycle and contributes to immune evasion by preventing antigens from being presented by TAP, which reduces the susceptibility of cells to CD8+ T-cell identification [19]. Our objective was to explore the impact of naturally occurring BNLF2a expression during Epstein-Barr virus lytic replication by studying its effect on inhibiting the presentation of indicator antigens to CD8+ T lymphocytes, which leads to decreased surface MHC class I expression [20].

5. Conclusion

This suggests a more acidic salivary pH may be associated with poorer periodontal health; the relationship is complex and not solely dependent on pH levels. The study findings on the prevalence of Epstein-Barr virus (EBV) in both gingivitis patients and control samples emphasize the need to consider viral factors in periodontal disease.

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