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The Relationship Between the Presence of *Prevotella Intermedia* and *Porphyromonas Gingivalis* and Increased Severity of Gingivitis

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ABSTRACT: Gingivitis and periodontitis are prevalent oral diseases caused by the disruption of the oral microbiota, with Porphyromonas gingivalis and Prevotella intermedia recognized as key pathogenic bacteria. This study aimed to investigate the prevalence and copy number of P. gingivalis and P. intermedia in patients with gingivitis and periodontitis, and to examine their association with age and gender. Ninety dental samples were collected from patients aged 2–68 years in Diwaniyah Governorate and analyzed using quantitative real-time polymerase chain reaction (qPCR). The results showed that 34.4% of samples tested positive for P. gingivalis and 82.2% for P. intermedia. Mean age differences between infected groups were not statistically significant, nor were differences in prevalence by gender. In pediatric patients aged 2–12 years, P. intermedia was associated with halitosis, regardless of the presence of caries. Higher bacterial copy numbers were observed in older age groups, indicating an age-related increase in periodontal pathogen burden. These findings highlight the significant role of P. gingivalis and P. intermedia in the progression of gingivitis and periodontitis and underscore the importance of early detection and targeted preventive strategies to maintain oral health.

Keywords: *P. gingivalis*, *P. intermedia*, Real-Time Quantitative Polymerase Chain Reaction

1. INTRODUCTION

Gingivitis, commonly known as gum disease, is a condition that affects the tissues surrounding the teeth as a result of pathogenic bacterial accumulation in the oral cavity. It is among the most prevalent oral diseases and, if left untreated, may progress into periodontal disease. Over time, this progression can lead to tooth loss due to damage to the protective layer covering the teeth. The disease is strongly associated with the buildup of dental plaque, which consists of pathogenic bacteria and antigens that trigger chronic inflammation. Understanding the role of these bacteria in causing inflammation is a crucial step toward improving treatment strategies and preventive measures [1].

The oral microbiota represents a complex ecosystem composed of bacteria, fungi, archaea, and viruses, and it plays a critical role in maintaining both oral and overall health. The balance of this ecosystem is influenced by various factors, including diet, smoking, alcohol consumption, lifestyle, and underlying medical conditions. Disruption of this balance can result in several oral health problems, such as tooth decay, gingivitis, periodontitis, oral candidiasis, and halitosis [2].

According to the World Health Organization (WHO), gum disease—including gingivitis—affects approximately 50% of the global population to varying degrees. Acute periodontitis affects about 10–15% of adults and is considered the leading cause of tooth loss worldwide. The condition often begins in adolescence or early adulthood due to plaque accumulation and poor oral hygiene. Its prevalence increases markedly with age, with 70–80% of individuals over 65 years suffering from some form of periodontal disease [3].

In low- and middle-income countries, the prevalence exceeds 60% due to limited access to healthcare services, while in developed countries, around 42% of adults are affected by chronic periodontitis. Periodontal disease also imposes a substantial financial burden on global healthcare systems, with annual costs estimated in the billions of dollars. Treatment of chronic periodontitis is particularly costly in advanced stages, often requiring surgical interventions [4].

Oral bacteria are a diverse group of microorganisms that naturally inhabit the oral cavity, helping to maintain the balance of the oral microbiome under healthy conditions. However, when this balance is disrupted by factors such as plaque accumulation, weakened immunity, or poor oral hygiene, pathogenic species begin to proliferate, including Porphyromonas gingivalis, Tannerella forsythia, and Treponema denticola. These bacteria stimulate the immune system and release virulence factors that damage the tissues surrounding the teeth, ultimately leading to gingivitis and the destruction of supporting bone structures [5].

Socransky et al. (1998) classified periodontal pathogens into two main groups based on their association with disease severity. The first is the red complex, which includes P. gingivalis, T. forsythia, and T. denticola, species strongly associated with advanced periodontitis. The second is the orange complex, which is less strongly associated with disease progression and includes Prevotella intermedia, Fusobacterium nucleatum, Campylobacter rectus, Campylobacter gracilis, and Peptostreptococcus micros. Among these, P. intermedia and P. gingivalis play a key role in chronic gingivitis due to their production of enzymes and toxins that contribute to tissue destruction.

These bacteria are well adapted to low-oxygen environments within the oral cavity, which enhances their pathogenic potential in advanced stages of the disease. Elevated levels of these microorganisms are closely linked to worsening clinical symptoms, including gingival bleeding, swelling, and progressive loss of supporting periodontal tissues [6].

Quantitative screening of oral bacterial species is an effective method for assessing the severity of gingivitis, as it enables precise measurement of bacterial counts within oral tissues. This approach facilitates accurate disease staging and supports the development of targeted treatment strategies. Moreover, quantitative analysis provides valuable data for monitoring disease progression and evaluating treatment efficacy [7].

Prevotella intermedia is a well-recognized pathogen associated with oral diseases such as gingivitis, periodontitis, and tissue inflammation. A strain of P. intermedia was isolated from an infected root canal in a Chinese patient with periodontitis, and genomic analysis revealed genetic features linked to environmental adaptability and virulence. These characteristics enhance the ability of P. intermedia to cause diverse infections and adapt to multiple niches, including the oral cavity and the respiratory tract [8].

The primary factor in the initiation and progression of periodontitis is the biofilm present in dental plaque, which consists of a cluster of microorganisms residing within the intercellular matrix. The results showed that, with the development of advanced diagnostic tools, it was realized that the occurrence of periodontitis does not only require an increase in plaque size, but also depends on complex interactions between bacteria and the host. They also indicated that some bacteria residing in the biofilm are not pathogenic, as beneficial bacteria play a role in maintaining the symbiotic relationship between the microbes in plaque and the host's immune-inflammatory response, preventing the development of pathogenic organisms and the onset of pathological changes [9].

The primary factor in the initiation and progression of periodontitis is the biofilm formed within dental plaque, which is composed of clusters of microorganisms embedded in an intercellular matrix. Advances in diagnostic tools have revealed that the development of periodontitis is not solely dependent on the quantity of plaque but also on the complex interactions between microbial communities and the host. Research has further demonstrated that not all bacteria within the biofilm are pathogenic. Beneficial species contribute to maintaining a symbiotic relationship between the microbial community and the host’s immune-inflammatory response, thereby preventing the overgrowth of pathogenic organisms and the onset of pathological changes [9].

This study holds significant importance for enhancing diagnostic and therapeutic approaches to periodontitis through quantitative investigations of Prevotella intermedia and Porphyromonas gingivalis, while seeking to better understand their pathological dynamics. The findings may contribute to the development of novel therapeutic strategies that directly target these bacteria, thereby reducing the complications of periodontitis and improving overall oral health.

The primary aim of this study was to examine the relationship between the presence of P. intermedia and P. gingivalis and the severity of gingivitis. A further objective was to quantify these bacterial species in patients in order to clarify their role in the initiation and progression of gingivitis.

2. MATERIALS AND METHODS

2.1. Sample Collection

A total of ninety dental paper points were collected and divided into two groups, consisting of 46 females and 44 males. All patients were clinically diagnosed by a dentist as having gingivitis, presenting with symptoms such as gingival redness, bleeding, pain, pocket discharge, and halitosis. The patients, aged between 18 and 60 years, attended private dental clinics in Diwaniyah from March 2024 to August 2024. For each patient, demographic and clinical information—including name, age, gender, medical condition, and date of sample collection—was recorded. The samples were then transported to the laboratory under appropriate conditions for preservation.

2.2. Bacterial DNA Extraction

DNA was extracted from the bacterial isolates using the classical method with the Presto Mini gDNA Bacteria Kit (Geneaid, USA). The procedure began by adding saline and tubes containing dental needle tips to a centrifuge tube, followed by centrifugation to remove the supernatant. The resulting pellet was resuspended in GT solution, after which Proteinase K was added, and the mixture was incubated at 60 °C with periodic agitation. Subsequently, GB buffer was added, and the sample was incubated at 70 °C. Ethanol was then introduced, and the mixture was transferred to a GD column for centrifugation. The column was washed sequentially with W1 and Wash Buffer, followed by complete drying. Finally, the column was placed into a clean tube, heated elution buffer was added, and the sample was centrifuged to obtain purified DNA. The extracted DNA was stored at −20 °C until further use.

2.3. Estimating DNA Concentration

The concentration of the extracted genomic DNA was assessed using a NanoDrop spectrophotometer, which measures DNA content in nanograms per microliter (ng/µL) and evaluates purity based on absorbance at 260/280 nm. Prior to measurement, the instrument was calibrated by cleaning the measurement pedestal with 2 µL of sterile ionic distilled water applied twice using a sterile pipette. For analysis, 1 µL of each DNA sample was placed directly onto the pedestal, and the measurement was initiated. The pedestal was cleaned between each sample to prevent cross-contamination and ensure accuracy. DNA purity was considered acceptable when the 260/280 absorbance ratio was approximately 1.8.

2.4. Real-time quantitative PCR for P. gingivalis and P. intermedia

To determine the copy number of the target bacteria in samples isolated from patients with dental infections, commercial kits were employed (PorGin detec-qPCR F100 and Preint detec-qPCR F100 kits, GPS, Genetic PCR Solutions, Spain). These kits contain specific target-species primers and probes for each bacterium. Briefly, the extracted bacterial DNA was used as the template for the qPCR reaction. The qPCR mixture composition and cycling conditions are summarized in Table (1), with a final reaction volume of 20 µL. Real-time amplification was performed using the Stratagene Mx3005P system (Agilent, USA). Data analysis was conducted with MxPro qPCR software (v4.10, Agilent, USA) to determine bacterial copy number. A decimal dilution series of the positive control was used to generate a standard calibration curve, which served as the basis for copy number quantification.

Linear regression of the logarithm of the bacterial copy number against the cycle threshold (Ct) generates the constants represented by the y-intercept and the slope of the standard curve (Equation 1). Using this regression equation, the copy number in each sample can be calculated (Equation 2).

$$Ct = Y \text{ intercept} + \text{Slope} \times \log(\text{copy number}) \quad (1)$$

$$\text{Copy number} = 10 (ct - Y \text{ intercept}))/\text{Slope} \quad (2)$$

Table 1. Quantitative PCR conditions for *Porphyromonas gingivalis* and *Prevotella intermedia*

ct		Time (sec.)	Temperature °C
40	Activation	2	95
	Denaturation	5	95
	Hybridization/Extension and data collection	20	60

2.5. Statistical analysis

Statistical analysis was performed using IBM SPSS software (version 23). Data were summarized in terms of frequency (number of cases) and relative frequency (percentage). Pearson's correlation coefficient was calculated to assess relationships between variables, with significance levels set at $p < 0.01$ and $p < 0.05$ [4].

3. RESULTS AND DISCUSSION

The present study was designed as a case study and included 90 samples from patients suspected of having gingivitis or periodontitis. These samples were collected between July 2024 and January 2025. The study population comprised both males and females, aged 18 to 68 years, with a mean age of 36.77 ± 8.85 years, from Diwaniyah Governorate (Table 2). Analysis of the 90 samples revealed that 31 (34.4%) were positive for P. gingivalis, while 74 (82.2%) were positive for P. intermedia. The results of bacterial detection and growth in the samples are illustrated in Figure 1.

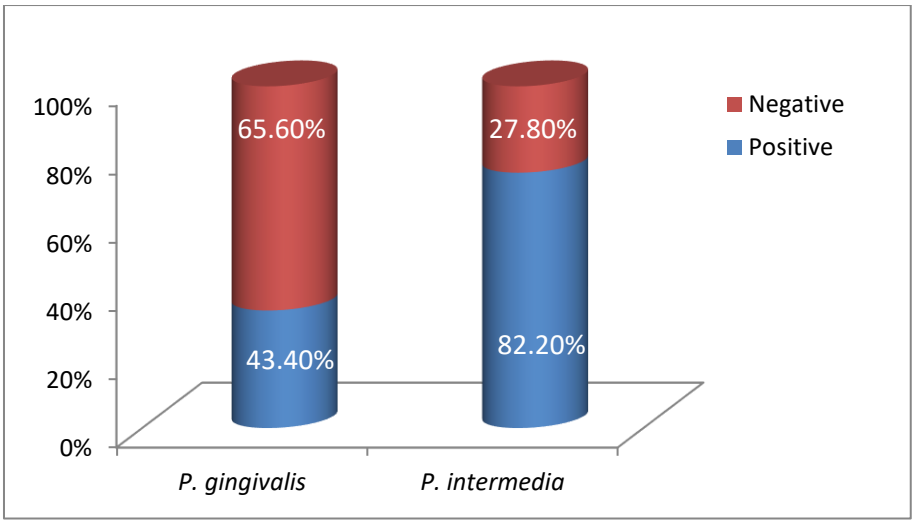

FIGURE 1. Results of bacterial growth in the samples

Table 2. Relative distribution of *P. gingivalis* and *P. intermedia* using real-time polymerase chain reaction

Result	number	Percentage	number	Percentage
Positive	31	34.4%	**74**	82.2%
Negative	59	65.6%	**16**	17. 8%
Total Number	90	100%	**90**	100%
p value	**0.003***		**0.001***	

*Represents a significant difference at $p \leq 0.05$. Data are expressed as mean ± standard deviation.

The results of the current study are consistent with those reported by Papone et al. (2015), who investigated 51 patients with chronic periodontitis. Using conventional microbiological techniques, they detected A. actinomycetemcomitans in 33% of samples and black-pigmented anaerobic bacteria in 100% of samples. Multiplex PCR analysis revealed that the most prevalent species were Fusobacterium nucleatum (100%), Tannerella forsythia (92%), and P. gingivalis (88%), whereas Prevotella intermedia (39%) and A. actinomycetemcomitans (33%) were among the least prevalent. These findings also align with those of Van der Weijden et al. (1994), who reported the presence of P. intermedia in 49 patients, A. actinomycetemcomitans in 35 patients, and P. gingivalis in 30 patients.

The findings of the current study are also consistent with those reported in [9], which showed that P. gingivalis was less frequently isolated (33.3%) from patients with periodontitis compared to other gingivitis-associated bacteria. That study included 30 patients with peri-implant disease (14 males and 16 females), aged 35–60 years (mean age 45.8 years). Among the peri-implant and gingival samples, 26 isolates were obtained, including 19 P. intermedia and 7 P. gingivalis strains. Of the 73 peri-implant samples, only four samples (5.48%) contained all four P. intermedia strains,

and a P. gingivalis strain was isolated. Furthermore, among the 73 gingival samples, nine samples (12.33%) contained 15 P. intermedia strains, while one sample (1.37%) contained seven P. gingivalis strains.

In studies of Brazilian patients with periodontitis, P. intermedia and Fusobacterium nucleatum were identified as the most prevalent periodontal pathogens, with a detection rate of 67%. Less prevalent species included P. gingivalis (26.7%), Tannerella forsythia (20%), and A. actinomycetemcomitans (13%) [10]..

In a study investigating the isolation of gingivitis-associated bacteria in patients with oropharyngeal cancer, P. intermedia was detected in 83.3% (20/24) of patients, P. gingivalis in 66.7% (16/24), and T. forsythia in 41.7% (10/24) of patients with lesions. These results indicate that these three periodontal pathogens were more prevalent in patients compared to healthy controls (Table 3). Over 700 bacterial species have been identified in the oral cavity, reflecting a high level of microbial diversity [11]. Oral diseases are frequently associated with microbial imbalance, with dental caries and periodontal disease being among the most common outcomes in adults [12].

However, the study differed in its isolation of gingivitis pathogens from saliva samples, where Fusobacterium nucleatum was the most prevalent species and showed significant differences compared to all other bacteria, followed by P. anaerobius, P. gingivalis, Eikenella corrodens, Campylobacter rectus, and T. denticola. The DNA copy numbers of P. intermedia, T. forsythia, and A. actinomycetemcomitans were notably lower than those of the previously mentioned species. Discrepancies in bacterial proportions between studies may result from differences in geographical, ethnic, etiological, genetic, environmental factors, and oral hygiene practices, as well as variations in sampling techniques and DNA extraction methods [12]..

The frequency distribution of age groups and mean age was compared between patients infected with P. gingivalis and those infected with P. intermedia. The results are presented in Table 4. The mean age of patients with P. gingivalis infection was 39.16 ± 8.31 years, while that of patients with P. intermedia infection was 36.47 ± 8.67 years. The difference in mean age between the two groups was not statistically significant (P = 0.292). Similarly, there was no significant difference in the frequency distribution of patients across age categories (P = 0.748).

Regarding gender distribution, the P. gingivalis group included 12 males (38.7%) and 19 females (61.3%), whereas the P. intermedia group included 38 males (51.4%) and 36 females (48.6%). No significant difference was observed between the two groups in terms of gender distribution (P = 0.237) (Table 5).

TABLE 3. Demographic characteristics of patients with positive bacterial infections

characteristics	***P. gingivalis***	***P. intermedia***	***P***
Age			
Mean ±SD	39.16 ± 8.31	36.47 ± 8.67	**0.292**
Range	22-68 years	20-68 years	
< 25, *n* (%)	4 (12.9 %)	14 (18.9 %)	**0.748**
25-45, *n* (%)	18 (58.1 %)	41 (55.4 %)	
≥ 45, *n* (%)	9 (29.0 %)	19 (25.7 %)	
sex			
Male, *n* (%)	12 (38.7 %)	38 (51.4 %)	**0.237**
Female, *n* (%)	19 (61.3 %)	36 (48.6 %)	

TABLE 4. Relative distribution of *P. gingivalis* and *P. intermedia* in patients with gingivitis and periodontitis by real-time polymerase chain reaction

P. gingivalis		
Result	***number***	***Percentage***
Positive	31	34.44%
Negative	59	65.56%
Total Number	90	100%

p value	0<0.003*	
P. intermedia		
Result	***number***	***Percentage***
Positive	74	82.22%
Negative	16	17.78%
Total Number	90	100%
p value	0<0.0001*	

*Represents a significant difference at $p \leq 0.05$. Data are expressed as mean ± standard deviation.

TABLE 5. Comparison between the cycle threshold (Ct) and the number of copies of *P. intermedia* and *P. gingivalis* in the infected group

P. gingivalis	
results	value
Cq	26.85±3.71
Copy number	745.95±2531.8
P. intermedia	
results	value
Cq	33.69±4.45
Copy number	533.612± 1547.4

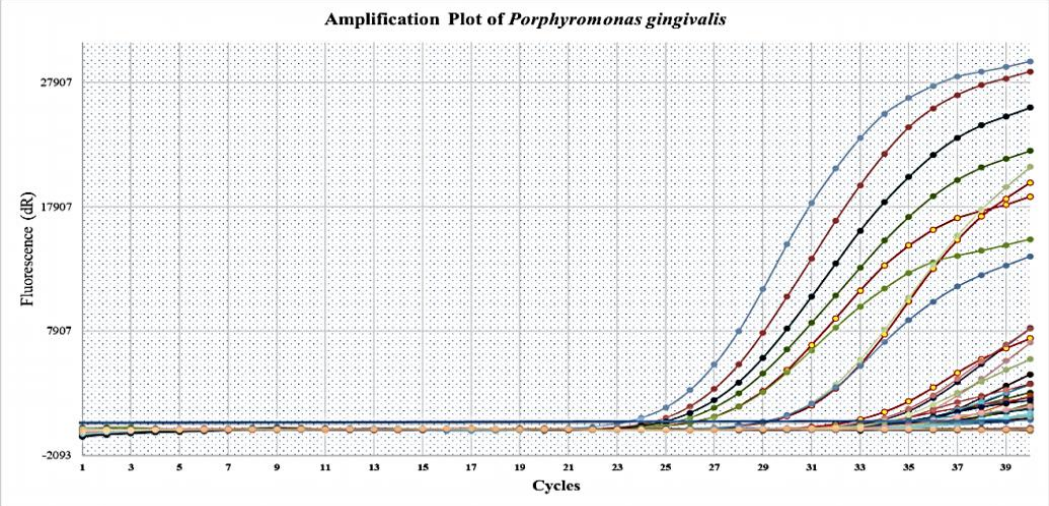

FIGURE 2. Determination and measurement of copy number in *P. gingivalis* bacteria by real-time polymerase chain reaction. This is the first cycle in which only 90 samples were performed, and it represents the amplification of a standard curve. The other lines represent the amplification of patient samples. dR means the baseline corrected fluorescence without reference.

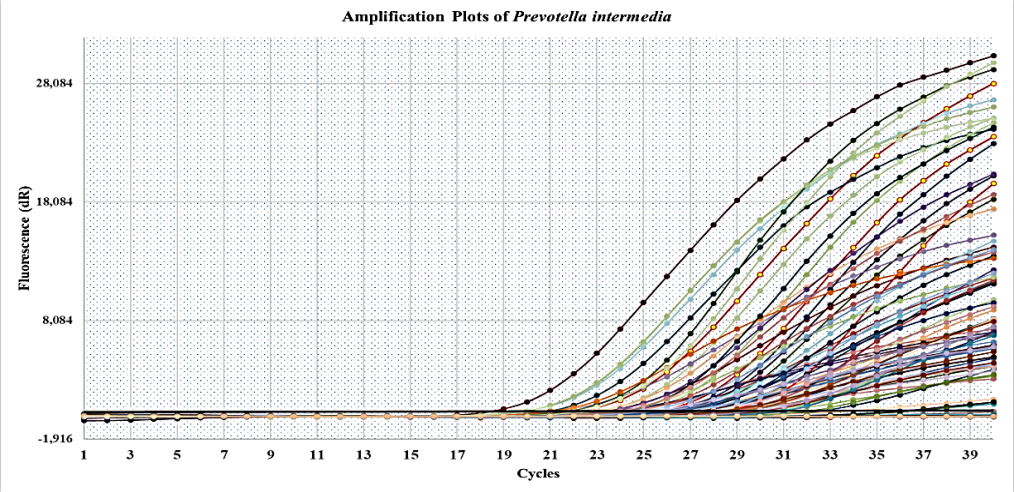

FIGURE 3. Identification and measurement of *P. intermedia* copy number using real-time polymerase chain reaction. This is the first run in which only 90 samples were tested, and represents amplification of a standard curve. The other lines represent amplification of patient samples. dR stands for baseline corrected fluorescence without reference.

Our results are consistent with several studies that have investigated the detection of P. gingivalis in patients with periodontitis and gingivitis using both conventional methods, such as culture, and molecular techniques, including conventional PCR and real-time PCR (Figures 2 and 3). These findings align with those of [13], who compared real-time PCR and culture for detecting P. gingivalis in subgingival plaque samples. In their study, P. gingivalis was detected in 60% of patients in the second group and 93.33% of patients in the third group, whereas only one patient in the first group tested positive. Similar results were reported in studies [14, 15, 16], which analyzed subgingival plaque samples from 259 adult patients with severe periodontitis. In these studies, P. gingivalis was detected in 111 samples (43%) by culture and in 138 samples (53%) by real-time PCR.

The variation in bacterial prevalence observed in the periodontitis group may be attributed to differences in geographical location, inclusion criteria, detection methods, and sample size. Several studies have reported that P. gingivalis is not only present in patients with chronic periodontitis but can also colonize healthy individuals, albeit at lower levels. This suggests that the presence of P. gingivalis may serve as a potential risk factor for disease development in otherwise uninfected individuals. Furthermore, these findings imply that non-virulent strains of P. gingivalis may acquire pathogenic characteristics over time, transforming into virulent strains that contribute to disease progression [17].

The results of the present study are consistent with those of Mahdi et al. (2019), who examined 30 samples from patients undergoing endodontic treatment. They detected six isolates (20%) of P. intermedia using molecular methods, while culture and biochemical tests identified ten isolates (33.3%). These findings are broadly aligned with other studies indicating that polymerase chain reaction (PCR) often shows higher detection rates for P. intermedia (33%) compared to culture-based methods (13%) [18].

In a separate study, [19] used PCR to identify P. intermedia in subgingival plaque samples from 97 adults with periodontitis, finding 38 positive samples (39%). Several studies have also demonstrated that the detection rates and abundance of gingival pathogens in saliva differ significantly between healthy individuals and those with periodontitis. Specifically, mean levels of P. gingivalis, P. intermedia, and T. forsythia were significantly higher in patients with periodontitis, reaching up to 20 times the levels observed in healthy individuals [20, 21, 22].

3.1. Distribution of *P. gingivalis* and *P. intermedia* by sex

The distribution of P. gingivalis by sex is presented in Table 6. The mean copy number of P. gingivalis was 805.45 ± 2214.3 in males and 361.93 ± 956.3 in females. For P. intermedia, the mean copy number was 748.7 ± 3059 in males and 742.9 ± 1863 in females. No statistically significant differences were observed between males and females for either bacterial species.

TABLE 6. Comparison of the cycle threshold (Ct) and copy number of *P. gingivalis* and *P. intermedia* by sex

P. gingivalis			
results	Patients		P-value
	Male (N=12)	Female (N=19)	
Ct	32.59±4.64	34.39±4.3	0.293
Copy No.	805.45±2214.3	361.93±956.3	
P. intermedia			
results	Patients		P-value
	Male (N=38)	Female (N=38)	
Ct	27.2±3.5	26.4±3.8	0.385
Copy No.	748.7±3059	742.9±1863	

Significant differences were considered at $p \leq 0.05$, and data are expressed as mean ± standard deviation. The results of the present study are consistent with previous research. For example, [23] investigated the transmission of P. gingivalis from caregivers (mothers and fathers) to children and found no significant differences between males and females in bacterial presence. Similarly, [24] studied 40 individuals, with 60% female, and reported no significant differences in sex distribution among participants across study groups.

In a study by [25], the prevalence of P. gingivalis was compared between males and females in both a chronic periodontitis group and a control group. Among 59 males with chronic periodontitis, 48 samples (81.35%) tested positive, whereas 20 of 75 healthy males (26.66%) were positive. Among females, the prevalence was 77.04% in the chronic periodontitis group and 33.33% in the control group. When comparing prevalence between sexes across both groups, more females (58.49%) tested positive for P. gingivalis than males (50.74%), although the difference was not statistically significant.

In another study involving 200 apparently healthy individuals and 200 patients with chronic periodontitis, aged 18–60 years and including both sexes, analysis of all samples revealed that 72% tested positive for P. gingivalis by bacterial culture, while 75% were positive by real-time PCR [26]. The findings of the current study are consistent with several previous studies that detected periodontal pathogens, including P. intermedia, in saliva and found no significant difference in bacterial prevalence between males and females. However, total bacterial counts were significantly higher in men than in women, which may reflect differences in the composition of the gingival microbiota. In men, multi-species bacterial communities are more prevalent, potentially influenced by hormonal factors, and these communities differ from those observed in women. Additionally, males have been reported to exhibit poorer oral hygiene behaviors than females [26].

Fusobacterium nucleatum has been reported to be present at significantly higher levels in men than in women (Choi et al., 2018). In studies analyzing chronic pulp infections using bacterial culture, P. gingivalis, Porphyromonas endodontalis, P. intermedia, and P. nigrescens were detected. Among 33 samples analyzed, 75.6% contained P. intermedia by PCR, while in another set of 60 samples, 31.7% tested positive for P. intermedia, with no significant differences observed between male and female patients. Overall, men exhibited higher bacterial isolation rates than women.

Co-detection rates of F. nucleatum and T. forsythia were 100% in men, compared to 83% in women. Similarly, the co-detection of three pathogens (F. nucleatum, T. forsythia, and P. gingivalis) occurred in 93% of men

and 70% of women. As more pathogens were included in co-detection (F. nucleatum, T. forsythia, P. gingivalis, and P. intermedia), the overall detection rates decreased; however, rates remained significantly higher in men (39%) than in women (26%) [27].

In contrast, [28] reported, using real-time PCR, that P. intermedia levels were higher than P. gingivalis in subgingival biofilm samples from women in the second and third trimesters of pregnancy. This increase is likely due to hormonal changes during pregnancy, which can exacerbate pre-existing gingivitis, resulting in increased gingival swelling and bleeding [29].

3.2. Distribution of *P. gingivalis* by age

The results of the age distribution of *P. gingivalis* bacteria were used, as shown in Table (7), which showed the highest number of copies recorded in patients over 45 years of age, reaching 2532±954.9, followed by patients under 25 years of age, reaching 869.08±436.4, and finally, patients aged 25-45 years, reaching 969.9±344.6. The results of the distribution of *P. intermedia* by age were based on the results shown in Table (7), which showed the highest number of copies recorded in infected individuals aged >65 years, with a percentage of 1002.13±2074, followed by infected individuals aged 45-65 years, with a percentage of 436.4±869.08, and finally, infected individuals aged <45 years, with a percentage of 257.4±560.9.

TABLE 7. Comparison between the cycle threshold (Ct) and copy number in *P. gingivalis* and *P. intermedia*, by age

P. gingivalis			
Ages	number	Gene expression	
		Ct	**Copy number**
<25	4		436.4±869.08
25-45	18		344.6±969.9
>45	9		954.9±2532
P value	0.855		
P. intermedia			
Ages	number	Gene expression	
		Ct	**Copy number**
<45	14	26.17±2.4	257.4±560.9
45-65	41	27.35±3.8	794.03±3089
>65	19	26.26±4.19	1002.13±2074
P value	0.437		

*Represents a significant difference at $p \leq 0.05$. Data are expressed as mean ± standard deviation.

The results of the present study are consistent with those reported by Kulkarni et al. (2019), who conducted a study on 40 individuals divided into three groups. The first group consisted of uninfected individuals with a mean age of 29.4 ± 3.80 years. The second group included 15 individuals with periodontitis, with a mean age of 40.60 ± 5.27 years, and the third group comprised individuals with chronic periodontitis, with a mean age of 45.66 ± 5.08 years. P. gingivalis was detected in 10% of uninfected individuals, 60% of those with periodontitis, and 93.33% of those with chronic periodontitis.

These findings also align with [29], which reported a significantly higher prevalence of P. gingivalis in the 41–50 and 51–62-year age groups, while differences in the 21–30 and 31–40-year age groups were not statistically significant.

The findings of the present study are consistent with those of [30], who reported that the 50–59-year age group was more prevalent among patients with periodontitis. In their study, P. gingivalis was detected in ten patients (33.3%) compared to two healthy controls (6.4%). Similarly, Kareem et al. (2022) investigated the presence of P. gingivalis in COVID-19 patients, reporting that the mean age of infected individuals was 51.04 ± 13.25 years, while the mean age of the uninfected group was 47.08 ± 11.45 years. The most frequent age group was 40 years and older, whereas patients under 40 years comprised 20% of the study population. No statistically significant differences were observed in age or

sex between the two groups (P > 0.05). Additionally, the prevalence of P. gingivalis and periodontal status indicated that the mean age of patients with periodontitis was 51.4 years, compared to 49.2 years in uninfected individuals [31, 32, 33].

There were differences with some studies. Tanaka et al. (2008) reported that the bacterial pathogens of gingivitis in children with and without caries were not significantly different from each other, as they were associated with halitosis and positive for *P. intermedia*. Sampling children with and without caries and children with gingivitis and periodontitis aged 2 to 12 years. The prevalence of pathogens detected by PCR in individuals without gingivitis and periodontitis was 0.0%, 6.0%, and 25.0% for *P. intermedia*. When selecting individuals with a mean age of 22 years (range 17–27) based on their gingivitis diagnosis, 73% of samples tested positive for the bacterial species presumed to *P. intermedia* [34,35].

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The findings of the present study are consistent with Alazemi et al. (2020), who reported that the 50–59-year age group was the most prevalent among individuals with periodontitis. In their study, Prevotella spp. was detected in 27 patients (90%) and in 30 healthy controls (96.7%). Furthermore, in adults aged 60 years or older, the presence of IgG antibodies against P. intermedia was associated with lower cognitive performance.

However, the results differ from those of [34], who collected samples from 60 individuals aged 30 years and found P. intermedia to be the most prevalent bacterial species in individuals with gingivitis (97%), followed by F. nucleatum as the most prevalent gingival bacterium in patients. The differences between study groups were statistically significant. Additionally, mothers, fathers, and children from 18 families were tested for the presence of P. intermedia.

In six of the 18 families tested, all three family members were found to carry P. intermedia. The ages of the mothers and fathers ranged from 36 to 49 years (mean 42.6 years), while the children ranged from 4 to 15 years (mean 11.0 years). A total of 293 strains of P. intermedia were isolated from the 18 individuals and identified using polymerase chain reaction (PCR), of which 115 were confirmed as P. intermedia isolates. The number of isolates per person ranged from 5 to 20, and 7 of the 18 individuals (39%) were positive for P. intermedia, with no significant differences observed [36, 37].

The study also noted that the number of P. intermedia increased with age, which is attributed to the longstanding association of aging with periodontal disease. Most researchers suggest that the severity of periodontitis rises with age due to cumulative tissue destruction over a lifetime, rather than age-related host immunodeficiency [38, 39, 40].

4. CONCLUSIONS

The study concluded that among 90 gingivitis and periodontitis samples, 31 (34.4%) tested positive for P. gingivalis, while 74 (82.2%) tested positive for P. intermedia. The mean age of patients with P. gingivalis infection was slightly higher than that of patients with P. intermedia infection, although the difference was not statistically significant. Similarly, no significant differences were observed in the frequency distribution of age categories between the two groups.

Regarding gender, patients with P. gingivalis infection included 12 males and 19 females, while patients with P. intermedia infection included 38 males and 36 females. No significant differences were observed in the frequency distribution by gender.

The study also found that bacterial pathogens associated with gingivitis in children, both with and without caries, were not significantly different, although P. intermedia was consistently associated with halitosis. Sampling included children aged 2–12 years with gingivitis and periodontitis, with or without caries.

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