Original Article

# Detection of Virulence Factors of *Porphyromonas gingivalis* and Periodontal Diseases in Chronic Disease Patients

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## Abstract

**Background:** *Porphyromonas gingivalis* is a prominent pathogen associated with periodontal diseases, particularly in patients with chronic conditions such as diabetes and hypertension. **Objectives:** This study aims to investigate the virulence factors of *P. gingivalis* and their correlation with periodontal disease severity in these patient populations. **Materials and Methods:** A total of 100 patients diagnosed with chronic periodontal disease were selected, with equal representation of males and females suffering from either diabetes or hypertension. Clinical samples were collected from subgingival pockets using sterile instruments and cultured on selective anaerobic media, specifically Kanamycin-Vancomycin Blood Agar (KVBA), to isolate *P. gingivalis*. The protease activity, particularly from gingipains, was assessed through a series of biochemical assays, measuring absorbance at 405 nm to quantify enzyme concentrations. **Results:** Results indicated a significant increase in protease levels in samples from diabetic patients compared to those with hypertension, suggesting a stronger association between *P. gingivalis* virulence and periodontal disease severity in diabetes. Statistical analysis revealed a notable correlation between the concentration of proteases and the type of chronic disease, highlighting the role of *P. gingivalis* in exacerbating periodontal conditions. **Conclusion:** Results of this study indicated a significant increase in protease levels in samples from diabetic patients compared to those with hypertension, suggesting a stronger association between *P. gingivalis* virulence and periodontal disease severity in diabetes.

**Keywords:** Periodontal diseases, *Porphyromonas gingivalis*, virulence factors

## Introduction

Periodontal diseases, including periodontitis and gingivitis, are conditions of inflammation that influence the tissues surrounding and supporting the teeth[1] These diseases are primarily caused by the colonization and interaction of various bacteria in the oral cavity.[2,3] Among these bacteria, *Porphyromonas gingivalis* is recognized as a major pathogen associated with the development and progression of periodontal diseases. *P. gingivalis* possesses several virulence factors that contribute to its ability to establish infection and induce damage to the periodontal tissues.[4] Significant data over time have implicated a small number of bacteria that live in the subgingival position in the onset and development of periodontal infection. There is significant indication that *Porphyromonas gingivalis*, anaerobe G+ve, is the central species that increases the chronic periodontitis. The virulence factors enable the bacterium to evade the host immune response, invade the periodontal tissues, and promote inflammation and tissue destruction.[5]

Understanding the virulence factors of *P. gingivalis* is crucial for comprehending the mechanisms of disease pathogenesis and developing targeted strategies for prevention and treatment. Fimbriae[5,6]: *P. gingivalis* expresses fimbriae, which are hair-like appendages on the bacterial surface, and these fimbriae enable the bacterium to adhere to host tissues and colonize the periodontal

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Quick Response Code:

Website: https://journals.lww.com/mjby

DOI: 10.4103/MJBL.MJBL_854_24

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**Submission:** 04-Oct-2024 **Accepted:** 17-Nov-2025 **Published:** 29-Mar-2025



**How to cite this article:** Al-Janabi AO, Alzaidi JR, Al-janabi MA. Detection of virulence factors of *Porphyromonas gingivalis* and periodontal diseases in chronic disease patients. Med J Babylon 2025;22:212-5.

pocket, contributing to the establishment of infection. Lipopolysaccharide (LPS): *P. gingivalis* produces lipopolysaccharide, a component of the outer membrane in Gram-negative bacteria.[7]

The LPS of *P. gingivalis* has unique structural characteristics that can stimulate a strong immune response and induce the creation of pro-inflammatory cytokines, which contributes to tissue damage of tissue in periodontal diseases. Proteases: *P. gingivalis* secretes various proteases, including gingipains.[8]

Gingipains are a group of cysteine proteases that can degrade host proteins, such as extracellular matrix components, immunoglobulins, and cytokines.[9] The activity of gingipains facilitates the invasion of *P. gingivalis* into the periodontal tissues and contributes to tissue destruction. *P. gingivalis* produces hemagglutinin, which are proteins that enable the bacterium to bind and aggregate red blood cells.[10] This aggregation can promote bacterial colonization and evasion of the host immune response. *P. gingivalis* can form a capsule, a polysaccharide layer that surrounds the bacterial cell, which benefits the bacterium by escaping phagocytosis by immune cells and providing protection against antimicrobial agents.[11,12]

## Materials and Methods

### Clinical sample collection

One hundred patients with chronic periodontal disease were selected, equally distributed between males and females. The main chronic diseases suffered by the patients included hypertension and diabetes. Sterile instruments, such as cotton swabs or sterile curettes, were used to collect samples from the subgingival pockets. The period of sample collection extended from April to September 2024. The instrument was gently inserted deep into the periodontal pocket and then withdrawn with a slight rotation to collect the suspended biomaterial. For each patient, at least two samples were collected from different sites of the gums to ensure accurate representation of the bacteria. The collected samples were placed in tubes containing a suitable carrier medium for anaerobic bacteria, such as VMGA (Viability Medium Glycogen Anaerobic). The samples were stored in a refrigerated container (4°C) until transported to the laboratory within a period not exceeding 24 h to maintain the quality of the samples.

### Identification of *Porphyromonas gingivalis*

These bacteria are cultured on Kanamycin-Vancomycin Blood Agar (KVBA). Trypticase Soy Agar with 5% sheep blood. Selective media were incubated under completely anaerobic conditions using anaerobic bags containing oxygen-absorbing chemicals. Incubation is at 37°C for 5 to 7 days.[13]

### Estimating protease activity in *Porphyromonas gingivalis*

The estimation of the protease activity of *P. gingivalis* was performed using various methods. Here's a detailed protocol for estimating protease activity.[14,15]

### Sample preparation

*P. gingivalis* was isolated from clinical samples and grown under anaerobic conditions on selective media such as KVBA. The bacterial cells were harvested by centrifugation (e.g., 10,000 × g for 10 min) and the pellet was resuspended in a suitable buffer, such as Tris-HCl (pH 7.4), and sonicated to lyse the cells. The pellet was centrifuged again to remove cell debris, and the supernatant containing the soluble proteins, including proteases, was collected.

### Reaction conditions

The enzyme extract was incubated with the substrate at an optimal pH (usually pH 7.5–8.0 for gingipains). The reaction mixture is typically incubated at 37°C for 30–60 min. To stop the reaction, add a suitable inhibitor or stop solution, such as trichloroacetic acid (TCA).

### Measurement

For azocasein, centrifuge the mixture after adding TCA to remove the precipitate, and measure the absorbance of the supernatant at 405 nm. For BAPNA and BLPN, measure the release of p-nitroaniline at 405 nm, which indicates the protease activity.

### Ethical approval

The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. The study was carried out after obtaining verbal consent prior to sample collection. The study was approved by the Committee on Publication Ethics at the AnaberHealth Directorate under the reference number 0299 on Dec 12, 2023.

## Results

Table 1 shows the concentration of the protease enzyme in patients with diabetes and blood pressure patients in both genders, where a clear increase in the concentration of the enzyme was observed from bacteria isolated from diabetic

**Table 1: Concentration of protease of *Porphyromonas gingivalis* in diabetic and blood pressure patients**

| Gender | Type of disease | Mean | Std. deviation |
|---|---|---|---|
| Male | Diabetic | 18.60 | 1.140 |
| | Blood pressure | 5.80 | 2.280 |
| | Total | 12.20 | 6.957 |
| Female | Diabetic | 16.40 | 1.140 |
| | Blood pressure | 4.80 | 2.168 |
| | Total | 10.60 | 6.328 |

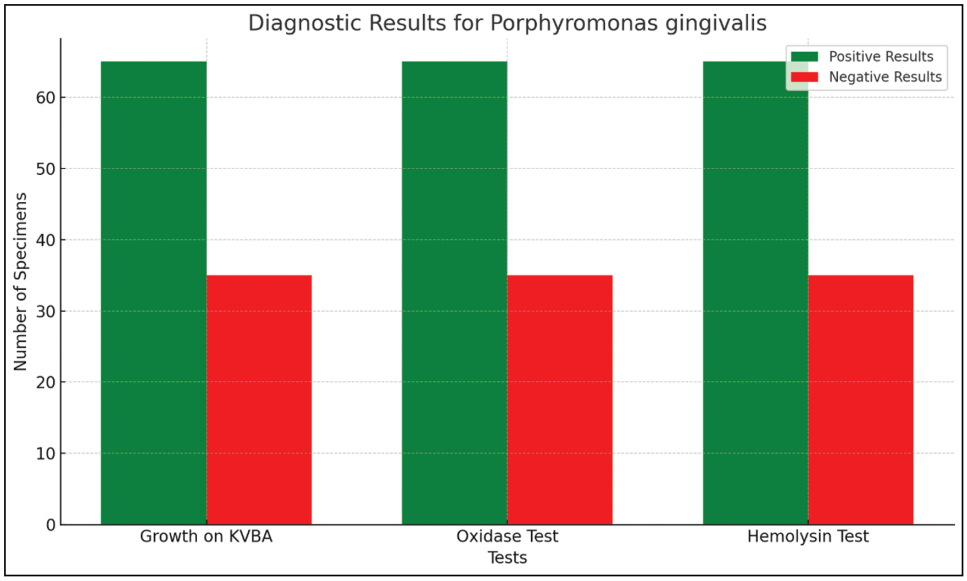


**Figure 1:** The diagnosis of *Porphyromonas gingivalis* in 100 samples, divided into positive and negative results for three different tests: growth on selective medium (Kanamycin-Vancomycin Blood Agar), oxidase test, and hemolysin test

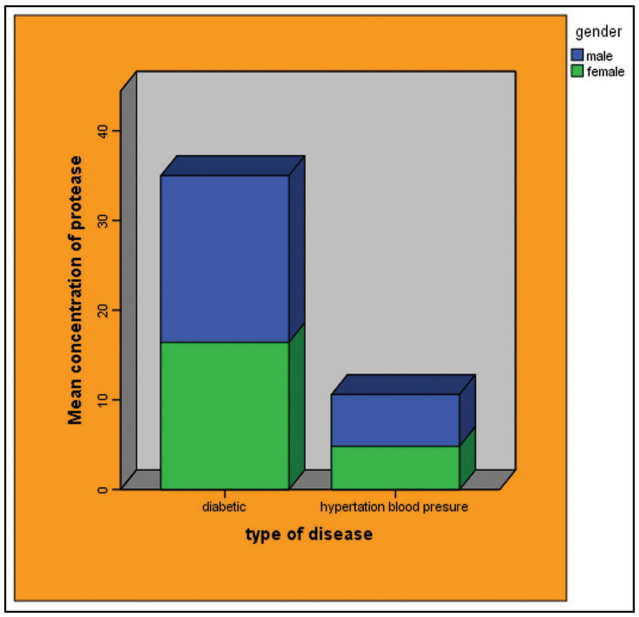


**Figure 2:** Protease of *P. gingivalis* diseases in diabetic and blood pressure patients

patients in males (18.60) and in females (16.40) compared to patients with blood pressure (5.80) in males and (4.80) in females [Figures 1 and 2].

Result in Table 2 demonstrated the variance to determine the concentration of the protease enzyme in patients with diabetes and blood pressure.

## Discussion

Results indicated a significant increase in protease levels in samples from diabetic patients compared to those with hypertension, suggesting a stronger association between *P. gingivalis* virulence and periodontal disease severity in diabetes. Statistical analysis revealed a notable correlation between the concentration of proteases and the type of chronic disease, highlighting the role of *P. gingivalis* in exacerbating periodontal conditions. Other studies have reported associations between P. gingivalis infection, periodontal diseases, and systemic conditions like diabetes and hypertension. These studies suggest that the presence of *P. gingivalis,* including its proteases, may contribute to the progression or severity of these conditions.[16,17]

This study showed that the relationship between gender, periodontal diseases, and *P. gingivalis* proteases, including any potential differences in diabetic or hypertensive patients, requires further investigation. The results of this study indicated a clear increase in the concentration of the protease enzyme that was observed from bacteria isolated from diabetic patients, with males (18.60) and females (16.40) compared to patients with high blood pressure (5.80) in males and (4.80) in females.[17-19]

The chronic inflammation and tissue destruction in the periodontium may exacerbate systemic inflammation and impact the overall health of individuals, including those with diabetes or hypertension. However, more research is needed to establish a direct link between *P. gingivalis* proteases and the progression or severity of these systemic conditions. Immune modulation: *P. gingivalis* proteases can modulate the host immune response, which may have implications for individuals with diabetes or hypertension. They can affect immune cell function, alter cytokine production, and inhibit the complement system,

**Table 2: ANOVA of protease concentration of *P. gingivalis* in diabetic and high blood pressure patients**

| Source | Type III sum of square | dt | Mean of square | *t* | Sig. |
|---|---|---|---|---|---|
| Corrected model | 758.800[a] | 3 | 252.933 | 80.939 | 0.000 |
| Intercept | 2599.200 | 1 | 2599.200 | 831.744 | 0.000 |
| Gender | 12.800 | 1 | 12.800 | 4.096 | 0.060 |
| Disease | 744.200 | 1 | 744.200 | 238.144 | 0.000 |
| gender * disease | 1.800 | 1 | 1.800 | 0.576 | 0.459 |
| Error | 50.000 | 16 | 3.125 | | |
| Total | 3408.000 | 20 | | | |
| Corrected total | 808.800 | 19 | | | |

[a]: *R* squared = 0.938 (Adjusted *R* squared = 0.927)

potentially leading to dysregulation of the immune response. This dysregulation could potentially impact the inflammatory status in diabetic and hypertensive patients, although further investigations are necessary to understand the specific mechanisms involved.[19-21]

## Conclusion

Results of this study indicated a significant increase in protease levels in samples from diabetic patients compared to those with hypertension, suggesting a stronger association between *P. gingivalis* virulence and periodontal disease severity in diabetes.

### Financial support and sponsorship

Nil.

### Conflicts of interests

There are no conflicts of interest.

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