

PATHOGENIC MICROORGANISMS ASSOCIATED WITH PERIODONTAL DISEASE IN INDIVIDUALS WITH TYPE 2 DIABETES

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ABSTRACT

This study examined the presence of periodontal pathogens in 29 patients with type 2 diabetes, categorized into three age groups: Group I (18–30 years), Group II (31–50 years), and Group III (51+ years). *Porphyromonas gingivalis* was detected in various degrees across all groups, with no significant correlation between pathogen presence and patient age. The study also highlighted the prevalence of *Aggregatibacter actinomycetemcomitans*, *Bacteroides forsythus*/Tannerella forsythia, and *Fuzobacterium nucleatum*, which were present in 29 patients, indicating a strong association with periodontal disease. Additionally, *Eikenella corrodens* and *Capnocytophaga* were found in patients with hyperglycemia, with positive results more frequent in Group II. Less common pathogens, including *Treponema denticola*, *Prevotella intermedia*, and *Campylobacter rectus*, were detected in smaller numbers, but their presence suggests a significant role in periodontal pathology, particularly in older patients. These findings reinforce the link between oral microbial agents and periodontal disease in patients with type 2 diabetes. Effective oral hygiene and periodontal treatment are essential in reducing pathogen load and improving glycemic control, potentially lowering the risk of systemic complications such as cardiovascular disease.

Keywords: periodontal pathology, age, bacteria, type 2 diabetes

INTRODUCTION

Recent in vitro and in vivo studies have demonstrated that gingival inflammation can arise in the presence of pathogenic agents without a single specific cause. In type 2 diabetes patients with poor oral hygiene, bacterial plaque or dental tartar accumulates on the teeth. This often leads to gingival inflammation within 10 to 21 days, which is frequently accompanied by mild gum overgrowth or hyperplasia.

The predominant microbial flora in the oral cavity was determined and studied from the gingival groove by numerous authors [2] who classified the groups of microbes like this:

Gram-positive:

- facultative aerobes: *Streptococcus* (*S. gordonii*, *S. mitis*, *S. auralis*, *S. salivarius*), *Staphylococcus* (*S. aureus*, *S. epidermidis*), *Enterococcus* (*E. faecalis*), *Lactobacillus* (*L. case*, *L. fermentum*), *Corynebacterium* (*C. matruchotii*), *Actinomyces* (*A. naeslundii*, *A. israelii*, *A. viscosus*), *Arachnia* (*A. propionica*), *Rothia* (*R. dentocariosa*).

- anaerobes: *Bacillus* (*B. cereus*), *Propionibacterium* (*P. acnes*), *Peptostreptococcus* (*P. micros*, *P. anaerobius*).

Gram-negative:

- facultative aerobes: *Campylobacter* (*C. rectus*, *C. concisus*, *C. gracilis*), *Actinobacillus* (*A. actinomycetemcomitans*).

- anaerobes: *Fusobacterium* (*F. nucleatum*), *Porphyromonas* (*P. gingivalis*), *Prevotella* (*P. melaninogenica*, *P. oralis*, *P. intermedia*).

Culturing pathogenic species from marginal periodontal diseases is complicated due to the multitude of pathogens that act successively and lead to the appearance of lesions specific to periodontal disease. They form according to their pathological effects bacterial complexes: the yellow complex with a role in early bacterial colonization, the green complex and the purple complex present in periodontal diseases, the orange complex allows the colonization of bacteria with an accentuated virulence, and the red complex correlated with the loss of the periodontal junction [1].

Bacterial groups were first classified by Socransky in 1998 [3]. The red complex includes the species: *Porphyromonas gingivalis*, *Treponema denticola*, *Tannerella forsythensis*. The orange complex: *Fuzobacterium nucleatum*, *Prevotella intermedia*, *P. nigrescens*, *Peptostreptococcus micros*, *Eubacterium nodatum*, *Campilobacter rectus*, *C. Showae*, *C. gracilis*, *Streptococcus constellatus*. The yellow complex: *Streptococcus sanguis*, *S. oralis*, *S. mitis*, *S. gordonii*, *S. intermedius*. The green complex includes the species *Capnocytophaga*, *Eikenella corrodens*, *Actinobacillus actinomycetemcomitans* serotype a *Camplobacter concisus*. The purple complex: *Actinobacillus actinomycetemcomitans* serotype b, *Veillonella parvula*, *Actinomyces odontolyticus*, *Actinomyces naeslundii*, *Selenomous noxia*.

The first group of bacteria linked to periodontal disease is known as the orange complex, consisting of gram-negative anaerobic species such as *Prevotella intermedia*, *P. nigrescens*, *P. micros*, and *Fusobacterium nucleatum*. As periodontal disease progresses, this complex tends to shift towards the more advanced red complex, which includes *Tannerella forsythia*, *Treponema denticola*, and *Porphyromonas gingivalis*. Both the red and orange complexes are commonly found in advanced stages of periodontal disease [4].

The pathogenic action of the microbial flora present in periodontal diseases has been investigated in numerous studies.

1. *Porphyromonas gingivalis*, a key bacterium in the red complex, is known for its high virulence and presence in subgingival biofilm. It has a polysaccharide capsule that protects it from phagocytosis, and its outer membrane contains lipopolysaccharides that contribute to its endotoxin activity, leading to bone resorption and tissue destruction [1,4].

It is present in young patients, in patients with type 2 diabetes, without causing periodontal disease. It is present when there are risk factors with weakened local or general defense factors. Due to the presence of this bacterium, there is a deficit in the defense function of neutrophils, and it can cause the appearance of local periodontal lesions. Chronic inflammation from periodontal disease is a risk factor in diabetic patients, and *Porphyromonas gingivalis* is the bacterium frequently detected in patients' atherosclerotic plaques [5].

Porphyromonas gingivalis can enter the bloodstream, leading to the formation of a prothrombotic state, while evading detection by the immune system and influencing periodontal immune response. Additionally, it can activate both humoral and cellular hemostatic factors [6,7].

2. *Aggregatibacter actinomycetemcomitans*, part of the green complex, is found in subgingival biofilm and exhibits hemolytic activity. It targets and destroys monocytes and neutrophils, producing a cytotoxin with immunosuppressive effects while stimulating cytokine production in the bloodstream. This bacterium thrives when risk factors are present or when local or systemic defense mechanisms are weakened, such as in metabolic conditions like diabetes. Impaired neutrophil function can lead to localized periodontal lesions [8,9].

3. *Bacteroides forsythus/Tannerella forsythia*, part of the red complex, is a major pathogen in gingivitis and periodontitis with aggressive forms and easily penetrates the tissues [10,11].

4. *Fuzobacterium nucleatum* which is part of the orange complex is a Gram-negative bacterium, important in the formation of the bacterial plaque biofilm. It causes the secretion of pro-inflammatory cytokines and the

destruction and disappearance of fibroblasts [12]. The presence of this bacteria in the oral cavity can cause periodontal disease, halitosis, dental pulp infection, and oral cancer [13,14].

5.*Eikenella corrodens*, belonging to the green complex, is a Gram-negative bacillus that develops colonies and erodes the gelatin. It is present in patients with aggressive and advanced forms of periodontal disease but also in healthy oral cavities [8,15].

6.*Capnocytophaga*, which is part of the green complex, is present in periodontal diseases but also in healthy oral cavities. Hyperglycemia stimulates the action of these germs [16]. It causes important inflammatory diseases in immunodeficient people [17,18].

7.*Treponema denticola*, a part of the red complex, is a Gram-negative bacterium with cytotoxic action on epithelial cells, collagen, fibroblast cells, and erythrocytes. It inhibits the adhesion and locomotion of cells involved in phagocytosis and is present in the gingival sulcus and severe forms of periodontal disease [8,19].

8.*Prevotella intermedia*, a part of the orange complex, produces hydrolases with proteolytic and hemolytic action. It is present in advanced forms of periodontal disease. It has an antiphagocytic role, secretes lytic enzymes, and has toxic effects on epithelial cells [8,20].

9.*Eubacterium nodatum*, which is part of the orange complex, is a Gram-positive bacterium. It is an anaerobic species found in severe forms of periodontal disease [8,21].

10.*Campylobacter rectus*, a part of the orange complex, are Gram-negative bacteria, highly mobile, present in periodontal diseases with advanced dento-periodontal destruction. They are dominant bacteria in AIDS patients [8].

11.*Peptostreptococcus micros*/*Micromonas micros* are part of the orange complex, and they are Gram-positive bacteria grown in severe lesions of periodontal disease or aggressive periodontopathies with early onset [22].

Pathogenic bacteria groups capable of initiating periodontal disease have been detected within periodontal pockets: *Actinobacillus actinomycetemcomitans*, *Campylobacter rectus*, *Tanarella forsythia*, *P. gingivalis*, *Treponema denticola*, *P. Intermedia* [23].

The specific objectives of the scientific research in this chapter pursued:

- the qualitative identification of pathogens associated with periodontal disease in patients with type 2 diabetes, previously presented pathogens.

- identification of a bidirectional relationship between type 2 diabetes and the degree of damage to the oral cavity by periodontal disease according to the intensity and presence of groups of pathogenic bacteria.

MATERIALS AND METHODS

Twenty-nine patients with type 2 diabetes mellitus were recruited and included in this study. We followed the Ethical principles of the 2008 World Medical Association (WMA) Declaration of Helsinki on Ethical Principles for Medical Research involving human subjects and the Approval of the Ethics Commission of the County Emergency Clinical Hospital "St. Apostle Andrei" with number 35278/25.06.2024. The subjects knew the study methodology and the treatment stages. They signed the acceptance and informed consent for their inclusion in the study on identifying pathogens associated with periodontal disease in patients with type 2 diabetes.

Patients with type 2 diabetes were categorized by age into three groups as follows: Group I consisted of 10 patients aged between 18 and 30 years.

Group II consisted of 17 patients aged between 31 and 50 years.

Group III consisted of 2 patients aged over 51 years.

The inclusion criteria for patients with type 2 diabetes in the study were as follows:

- Subjects with fasting blood glucose levels below 125 mg/dl;

- Subjects with glycated hemoglobin levels under 9%;

- Both male and female individuals aged 18-70 years;

- Those who had received oral cavity hygiene treatment and completed dento-periodontal treatment;

- Exclusion of patients undergoing orthodontic treatment;

- Participants with a body mass index (BMI) below 30;

- Blood pressure not exceeding 140/90 mmHg;

- Non-smokers with no history of alcohol consumption;

Bacteria	Weakly positive	Strongly positive	Positive	Negative	Bacteria present $\Sigma a,b,c$
	a	b	c	d	
I	1	x	1	x	2
II	1	x	1	x	2
III	1	x	1	x	2
IV	x	x	2	x	2
V	1	x	x	1	1
VI	1	x	1	x	2
VII	x	x	1	1	1
VIII	1	1	x	x	2
IX	1	1	x	x	2
X	1	1	x	x	2
XI	2	x	x	x	2
Total	a=10	b=3	c=7	d=2	a+b+c=20

- after the completion of the General Condition Assessment Questionnaire and the Informed Consent Form from the patient.

Oral cavity sample collection method

We performed the micro-IDent tests based on the chain polymerization technique and showed high specificity for the presence of the periodontopathogenic bacteria and their relative quantity. The tests are more sensitive than bacterial culture because they identify germs by DNA regardless of viability [24].

Samples were collected using sterilized forceps and a paper stick from the collection kit, which was then inserted into the periodontal pocket in the most affected oral area where the gingival pocket depth was 3-5mm. The stick remained in the periodontal pocket for 10-11 seconds and was then placed into a transfer tube in the blue collection kit, along with a slip containing the serial number (from 1 to 29) and the patient's initials. The transportation to the laboratory does not pose any issues as it involves DNA determination. The sample remains stable for one week at 2-8 degrees Celsius. The method used is a multiplex PCR chain reaction with colorimetric detection. Pathogenic germs were identified and reported as weakly positive (a), strongly positive (b), positive (c), and negative (d) for each germ.

We qualitatively determined the pathogens identified in the tables with the number corresponding to the Roman numerals.

Each pathogen, noted from I to XI in the table, corresponds to the qualification a=weakly positive, b=strongly positive, c=positive, and d=negative.

RESULTS

We collected the results from each pathogen for every patient with type 2 diabetes and presented them in Tables 1-3.

I. *Porphyromonas gingivalis*. **II.** *Aggregatibacter actinomycetemcomitans*. **III.** *Bacteroides forsythus/Tannerella forsythia*. **IV.** *Fuzobacterium nucleatum*. **V.** *Eikenella corrodens*. **VI.** *Capnocytophaga*. **VII.** *Treponema denticola*. **VIII.** *Prevotella intermedia*. **IX.** *Eubacterium nodatum*. **X.** *Campilobacter rectus*. **XI.** *Peptostreptococcus micros/Micromonas micros*.

Table 1. Results in patients aged between 18 and 30 years

Bacteria	Weakly positive	Strongly positive	Positive	Negative	Bacteria present $\Sigma a,b,c$
	a	b	c	d	
I	2	4	1	3	7
II	2	4	1	3	7
III	1	1	4	4	6
IV	0	0	2	8	2
V	0	1	1	8	2
VI	3	0	3	4	6
VII	1	0	0	9	1
VIII	0	0	2	8	2
IX	0	0	2	8	2
X	0	0	0	10	0
XI	0	0	2	8	2
Total	a=9	b=10	c=18	d=73	a+b+c=37

Table 2. Results in patients aged between 31 and 50 years

Bacteria	Weakly positive	Strongly positive	Positive	Negative	Bacteria present $\Sigma a,b,c$
	a	b	c	d	
I	6	0	6	5	12
II	4	0	4	9	8
III	3	1	7	6	11
IV	2	1	10	4	13
V	4	0	3	10	7
VI	1	2	10	4	13
VII	0	0	4	13	4
VIII	4	0	5	8	9
IX	2	0	1	14	3
X	3	6	0	8	9
XI	3	0	3	11	6
Total	a=32	b=4	c=59	d=92	a+b+c=95

Table 3. Results in patients over 51 years

The results showed a clear increase in bacterial presence in the middle age group (31–50 years), while the youngest and oldest groups

had significantly lower bacterial counts. The standard deviation also reflected the variation in bacterial counts, with the middle age group having the highest variability. Since the calculated F-statistic (22.59) was much larger than the critical value (3.32), the result was statistically significant at the 0.05 level. This means we reject the null hypothesis and conclude that there are significant differences in bacterial counts across the three age groups.

DISCUSSIONS

When analyzing the pathogen *Porphyromonas gingivalis* in the first age group, it was found to be weakly positive in 2 cases, strongly positive in 4 patients, positive in one, and negative in 3. We observed that the second group tested weakly positive for both sexes in 6 patients, positive in 6 patients, and negative in only 5 patients. The age of diabetic patients does not seem to be related to the presence or absence of this pathogen. Other authors [25] have confirmed the presence of the pathogen *Porphyromonas gingivalis* in patients with type 2 diabetes, finding a percentage of 27.03%. Other studies described the pathogen as implicated in the initiation and progression of diabetes [26]. At the age of 30-50 years, it is a risk factor for cardiovascular disorders and esophageal cancer. *Porphyromonas gingivalis* is commonly found in adult patients, including those with type 2 diabetes, without causing periodontal disease [27]. The pathogen is typically present in the bacterial plaque, and its removal has been associated with positive outcomes for patients with type 2 diabetes. Periodontitis may be linked to gut microbiota, which plays a crucial role in the development of diabetes [28]. Treatment of periodontal disease lowers fasting blood glucose and the number of pathogens after effective periodontal hygiene [28,29].

Analyzing the pathogen *Aggregatibacter actinomycetemcomitans* showed a similar fingerprint to *Porphyromonas gingivalis* but was more pronounced. Other authors [30] emphasize the role and importance of oral hygiene, noting the presence of this pathogen in the bacterial plaque. *Actinobacillus actinomycetemcomitans* worsens periodontal disease in patients with type 2 diabetes and

impairs the role and functions of neutrophils [27].

The pathogen *Bacteroides forsythus/Tannerella forsythia* was active in twenty-nine cases; it is considered a major pathogen in gingivitis [10,11].

The pathogenic agent *Fuzobacterium nucleatum* was also present in twenty-nine patients. Patients with type 2 diabetes may have *Fuzobacterium nucleatum*, a pathogenic bacterium, present in the oral microbial flora and biofilm [31]. Consequently, the presence of these microbial agents can, under specific conditions, contribute to the development of periodontal pathology.

Eikenella corrodens is also found in healthy patients, with two values highlighted, one intensely positive and one positive.

Capnocytophaga is present in patients with hyperglycemia or healthy patients. In the first collection, there were three patients with a weakly positive value and three with a positive value.

The pathogenic agents *Treponema denticola*, *Prevotella intermedia*, *Eubacterium nodatum*, *Micromonas micros*, *Campilobacter rectus*, and *Peptostreptococcus micros/Micromonas micros* were detected in only one patient. These findings are consistent with similar results reported in the specialized literature [32,33].

Upon analyzing the Table 1 we can see that the combined positive results for all pathogens amounted to 37, while the negative results added up to 73. Notably, the pathogens *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, and *Bacteroides forsythus/Tannerella forsythia* exhibited intensely positive values.

Consequently, the presence of these microbial agents can, under specific conditions, contribute to the development of periodontal pathology.

Eikenella corrodens was found in 7 patients with type 2 diabetes in the group from 31 to 50 years. This pathogen resides in the oropharynx and can be particularly virulent in individuals with compromised immunity, especially those with diabetes who are at risk of lung infections [34].

The pathogen *Capnocytophaga* is commonly found in healthy individuals, but it can also lead to various dental and periodontal complications in patients with type 2 diabetes.

The test results have shown positivity in 13 patients in the second group. The pathology of this pathogen is closely associated with blood sugar levels and oral hygiene [34].

The pathogen *Treponema denticola* is found in severe cases of periodontal disease and is typically located within periodontal pockets [29,30]. In patients over 40 years old, *Prevotella intermedia* has been detected in 9 cases [35]. Low immunity and hyperglycemia can perpetuate periodontal disease, and they also represent a risk factor for cardiovascular diseases.

Eubacterium nodatum exhibited a positive result in two patients, aged 48 and 50, respectively. This pathogen is linked to severe periodontal disease and plays a significant role in periodontal pathology during pregnancy.

Campylobacter rectus proved 9 positive results in the group of 31-50 years (3 weakly positive and 6 strongly positive). *Peptostreptococcus* proved positive 3 weakly positive and 3 strongly positive results.

Both patients of the oldest group showed weak positive and positive results with a combined nominal value of 20. Negative results were obtained for the pathogens *Eikenella corrodens* and *Treponema denticola*.

The results were strongly positive for the pathogens *Prevotella intermedia*, *Eubacterium nodatum*, and *Campylobacter rectus*.

CONCLUSIONS

Conflict of interest: the authors declare no conflict of interest associated with this paper.

Institutional Review Board Statement: the study was conducted according to the guidelines of the Declaration of Helsinki on Ethical Principles for Medical Research involving human subjects and the Approval of the Ethics Commission of the County Emergency Clinical Hospital "St. Apostle Andrei" with number 35278/25.06.2024.

Informed Consent Statement: informed consent was obtained from all subjects involved in the study.

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1. The presence of periodontal pathogens in patients with type 2 diabetes was significant across all three groups studied. Pathogenic agents developed in the microbial biofilm were found in patients with dental tartar, gingivitis, and compromised immune systems.

2. Evidence suggested a bidirectional relationship between the age of patients with type 2 diabetes and periodontal disease. In Group I, the presence of pathogens such as *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, and *Bacteroides forsythus/Tanerella forsythia* indicated low immunity in young people with type 2 diabetes who also have gingivitis and bacterial plaque. In Group II, the presence of pathogens like *Bacteroides forsythus/Tanerella forsythia*, *Fuzobacterium nucleatum*, and *Capnocytophaga* were characteristic of periodontal disease. In Group III, pathogens such as *Prevotella intermedia*, *Eubacterium nodatum*, and *Campylobacter rectus* were associated with advanced and aggressive periodontal diseases.

3. Regular dental check-ups are essential for diagnosing and treating odonto-periodontal issues. Professional oral hygiene and personalized maintenance are crucial in reducing or partially eliminating the specific microbial flora associated with periodontal diseases.

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