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Identification of periodontal pathogens in type 2 diabetic patients with fixed orthodontic retainers

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ABSTRACT

This study assessed the impact of orthodontic fixed retainers on periodontal health in 40 patients with type 2 diabetes, divided into two age groups: 18-30 years (n = 18) and over 30 years (n = 22). Bacterial loads were measured before and after a 10-14 day oral hygiene regimen using the micro-IDent test. In the younger group, the positive bacterial load decreased from 66.6% to 33.3% after treatment, while older patients showed less improvement. Pathogens like *Treponema denticola* and *Prevotella intermedia* were more resistant to treatment, especially in older patients. The study concluded that oral hygiene improves periodontal health and may benefit glycemic control in diabetic patients, but some pathogens require ongoing management for optimal outcomes.

Keywords: type 2 diabetes, periodontal health, glycemic control

INTRODUCTION

In the scientific literature, there is ongoing debate regarding the impact of orthodontic retainers on periodontal health. Some studies have reported no significant differences in periodontal outcomes between stainless steel fixed retainers bonded to anterior teeth and canines, as observed at 12-month and 3-year follow-ups. These findings suggest that orthodontic fixed retainers may be compatible with periodontal health or, at a minimum, not associated with severe negative effects on the periodontium. Conversely, other researchers have documented increased plaque accumulation, gingival recession, deepened periodontal pockets, and heightened calculus buildup in individuals wearing fixed retainers. The microorganisms that adhere to the biofilm exhibit different properties than free-floating planktonic bacteria, demonstrating resistance to antibiotics, disinfectants, and the body's immune responses. Among patients with type 2 diabetes and poor oral hygiene, soft deposits linked to bacterial plaque or dental tartar form on their teeth. Without proper hygiene for 10-21 days, these individuals may experience gingival inflammation and slight hyperplasia [1,2].

The dominant microbial flora in the oral cavity has been researched by various authors who have classified the microbial groups based on studies from the gingival sulcus [3].

In 1998, Socransky pioneered the classification of bacterial groups into microbiological complexes,

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which marked the inception of modern periodontal microbiology [4]:

- the red complex: *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: *Capnocytophaga, Eikenella corrodens, Actinobacillus actinomycetemcomitans serotype a, Camplobacter concisus.*
- the violet complex: *Actinobacillus actinomycetemcomitans serotype b, Veillonella parvula, Actinomyces odontolyticus, Actinomyces naesludii, Selenomous noxia.*

The orange complex, comprising gram-negative anaerobic species such as *Prevotella intermedia, P. nigrescens, P. micros,* and *Fusobacterium nucleatum,* is the first bacterial group implicated in periodontal disease. As the disease progresses, these species transition towards the red complex, which includes *Tannerella forsythia, Treponema denticola,* and *Porphyromonas gingivalis.* Both the orange and red complexes are present during the advanced stages of periodontal disease, contributing to its pathogenesis [5].

Porphyromonas gingivalis (from the red complex) is a highly virulent bacterium in the subgingival biofilm. It is present in young patients and patients with type 2 diabetes without causing periodontal disease. Porphyromonas gingivalis is present when there are risk factors and weak local or general defense factors. It can cause local periodontal lesions and a deficit in the defense function of neutrophils. Chronic inflammation from periodontal disease is a risk factor in diabetic patients, and Porphyromonas gingivalis is the bacterium frequently detected in patients' atherosclerotic plaques [6].

Porphyromonas gingivalis may enter the systemic circulation, leading to a prothrombotic state, evading detection by the immune system and the periodontal immune response and triggering the activation of humoral and cellular hemostatic factors [7].

Aggregatibacter actinomycetemcomitans, also known as *Actinobacillus actinomycetemcomitans,* a part of the green complex that is present in the subgingival biofilm, has hemolytic activity, acting on monocytes and neutrophils and destroying them. It weake local or general defense, and metabolic conditions such as diabetes, which involve a deficiency in the defensive capabilities of neutrophils. It can cause the appearance of local periodontal lesions [8].

Tannerella forsythia, also known as Bacteroides forsythus from the red complex, is a significant pathogen found in gingivitis and aggressive forms of periodontitis, with a notable ability to penetrate the surrounding tissues [9].

Fusobacterium nucleatum, a member of the orange complex, is a Gram-negative bacterium that plays a crucial role in the genesis of bacterial plaque biofilm. It triggers the secretion of pro-inflammatory cytokines and leads to the destruction and depletion of fibroblasts [10].

Eikenella corrodens (from 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 [11].

Capnocytophaga, a bacterium from the green complex, can be found in both periodontal diseases and healthy oral cavities. Hyperglycemia promotes the activity of pathogenic bacteria [12] and can lead to significant inflammatory conditions in individuals with immunodeficiency [11,12].

Treponema denticola, a member of the red complex, is a Gram-negative bacterium that exerts cytotoxic effects on epithelial cells, collagen, fibroblast cells, and erythrocytes. It disrupts the adhesion and movement of cells involved in phagocytosis and is commonly found in the gingival sulcus and severe cases of periodontal disease [13].

Prevotella intermedia, part of the orange complex, produces hydrolases with proteolytic and hemolytic properties. It is found in advanced stages of periodontal disease and plays a role in evading phagocytosis, secreting lytic enzymes, and exerting toxic effects on epithelial cells [14].

Eubacterium nodatum from the orange complex. It is a Gram-positive, anaerobic species and is present in severe forms of periodontal disease [3,15].

Campylobacter rectus is part of the orange complex. It's a type of Gram-negative bacteria that is highly mobile, commonly found in periodontal diseases characterized by advanced dental-periodontal destruction. It is the dominant bacteria in AIDS patients [3].

Peptostreptococcus micros (or *Micromonas micros*) is part of the orange complex. These gram-positive bacteria proliferate in severe lesions associated with periodontal disease or aggressive periodontopathies with early onset [3].

Pathogenic bacteria capable of initiating periodontal disease have been identified within periodontal pockets: *Actinobacillus actinomycetemcomitans, Campilobacter rectus, Tanerella forsythia, P. gingivalis, Treponema denticola, P. Intermedia* [15].

The specific objectives of the scientific research were to:

- Qualitatively identify pathogens associated with periodontal disease in patients with type 2 diabetes wearing fixed orthodontic retainers

before and after oral cavity hygiene and complete dental-periodontal treatment.
– Identify a bidirectional relationship between type 2 diabetes and the extent of damage to the oral cavity caused by periodontal disease.

MATERIALS AND METHODS

A total of 40 patients diagnosed with type 2 diabetes mellitus wearing uni maxillary or bimaxillary orthodontic fixed retentions were carefully selected to participate in this study. The research adhered to the ethical guidelines outlined in the 2008 World Medical Association (WMA) Declaration of Helsinki, focusing on the standards for medical research involving human subjects. The study received approval from the Ethics Commission of the County Emergency Clinical Hospital "St. Apostle Andrei" under reference number 35278/25.06.2024. Before their inclusion, we informed all participants about the study's methodology, the clinical and paraclinical examination processes, and the subsequent treatment stages. They provided their informed consent to participate in the research that explored the correlation between periodontal disease pathogens and patients with type 2 diabetes.

Patients with type 2 diabetes were divided by age into two groups of both sexes, gender not influencing periodontal pathology, as follows:

Group I, aged between 18-30 years, 18 patients.

Group II, aged over 30 years, 22 patients.

The criteria for including patients with type 2 diabetes in the study were:

– subjects with blood glucose levels lower than 125 mg/dl;
– subjects with glycated hemoglobin less than 9%;
– age 18-70 years, both sexes;
– before and after an oral cavity hygiene treatment and completed dental-periodontal treatment.
– orthodontic fixed retainers wearers
– BMI lower than 30 (in degree 1 obesity).
– blood pressure 140/90 mmHg.
– non-smokers and no history of alcohol consumption.
– after completing the General Condition Assessment Questionnaire and the Informed Patient's Agreement.

The statistical analysis of the data included qualitative measurements, presented in tables, highlighting the prevalence of pathogens in two groups of patients with type 2 diabetes. Bacterial load was assessed using categories such as weakly positive, strongly positive, positive, and negative. Additionally, the total bacterial load was calculated. Sample sizes were determined based on the prevalence of periodontal pathogens associated with type 2 diabetes.

The samples from the oral cavity were collected using micro-IDent tests, which utilize the chain polymerization technique and demonstrate high specificity in identifying periodontal-pathogenic bacteria and determining their relative abundance [16]. These tests are more sensitive than bacterial culture as they identify bacteria based on their DNA, irrespective of viability.

Samples were collected using sterile forceps with a paper stick from the collection kit inserted into the periodontal pocket in the most affected oral area, where the gingival pocket is about 2-4mm. The stick remains in the periodontal bag for 10-11 seconds, then inserted into a transfer tube in the blue collection kit with a slip with the serial number (from 1- to 40), the patient's initials, and their age. The transport to the laboratory does not raise any problems since it is about DNA determination. The stability of the sample is one week at 2-8 degrees Celsius, the method being a multiplex PCR chain reaction with colorimetric detection.

The presence of pathogenic microorganisms was in patients with type 2 diabetes, with each one identified based on its bacterial load: weakly positive (denoted as a), strongly positive (denoted as b), positive (denoted as c), and negative (denoted as d). Using the micro-IDent test, we qualitatively determined the prevalence of specific pathogens represented by Roman numerals in the tables as follows:

The sampling was carried out in two stages:

a. Pathogen identification in the oral cavity without prior oral hygiene and periodontal treatment.

b. Following a 10-14 day period, the oral cavity underwent oral hygiene through gingival-dental brushing performed by the patient and completed by the doctor to eliminate bacterial plaque. It included gingival debridement, scaling, polishing of dental surfaces, and local antiseptic substances (mouthwashes). After 6-10 days, we identified pathogens using micro-IDent tests, maintaining the initial conditions of the laboratory samples. Based on the doctor's recommendations, patients were instructed to sanitize their oral cavity at least twice daily.

RESULTS

Table 1 shows the results regarding the bacterial load in group 1 of diabetic patients aged between 18 and 30 years.

Table 2 shows the results regarding the bacterial load in group 1 of diabetic patients over 30 years old.

DISCUSSIONS

The presence of the pathogen *Porphyromonas gingivalis* in patients with type 2 diabetes is con-

TABLE 1. Bacterial load in the first age group of 18-30 years old

	Before oral hygiene					After oral hygiene				
Pathogenic agents	Weakly positive	Strongly positive	Positive	Negative	Positive	Weakly positive	Strongly positive	Positive	Negative	Positive
	a	b	c	d	Σa,b,c	a	b	c	d	Σa,b,c
I. *P. gingivalis*	5	5	2	6	12	5	0	1	12	6
II. *Agg. actinom.*	4	5	1	8	10	5	0	1	12	6
III. *Bact. forsyt.*	2	2	6	8	10	4	0	0	14	4
IV. *F. nucleat.*	2	0	2	14	4	1	0	0	17	1
V. *E. corrod.*	2	1	3	12	6	4	0	1	13	5
VI. *Capn.*	3	0	5	10	8	4	0	0	14	4
VII. *T. dentic.*	3	0	0	15	3	1	0	0	17	1
VIII. *Pr. interm.*	1	0	3	14	4	2	0	0	16	2
IX. *Eub. nod.*	2	0	2	14	4	1	0	0	17	1
X. *C. rectus*	0	0	1	17	1	1	0	0	17	1
XI. *P. micros*	1	0	3	14	4	3	0	0	15	3
Total	**25**	**13**	**28**	**132**	**66**	**31**	**0**	**3**	**164**	**34**

TABLE 2. Bacterial load in the second age group of patients older than 30 years

	Before oral hygiene					After oral hygiene				
Pathogenic agents	Weakly positive	Strongly positive	Positive	Negative	Positive	Weakly positive	Strongly positive	Positive	Negative	Positive
	a	b	c	d	Σa,b,c	a	b	c	d	Σa,b,c
I. *P. gingivalis*	7	0	8	7	15	6	0	3	13	9
II. *Agg. actinom.*	6	0	7	9	13	5	0	4	13	9
III. *Bact. forsyt.*	4	1	9	8	14	10	0	4	8	14
IV. *F. nucleat.*	2	1	13	6	16	7	1	5	9	13
V. *E. corrod.*	7	0	3	12	10	8	0	0	14	8
VI. *Capn.*	2	3	11	6	16	9	0	2	11	11
VII. *T. dentic.*	0	0	6	16	6	4	0	4	14	8
VIII. *Pr. interm.*	6	1	5	10	12	6	0	1	15	7
IX. *Eub. nod.*	4	1	1	16	6	5	0	0	17	5
X. *C. rectus*	5	1	6	10	12	5	0	0	17	5
XI. *P. micros*	5	0	3	14	8	3	0	1	18	4
Total	**48**	**8**	**72**	**114**	**128**	**68**	**1**	**24**	**149**	**93**

LEGEND:
I. *Porphyromonas gingivalis.* II. *Aggregatibacter actinomycetemcomitans.* III. *Bacteroides forsythus/Tanerella 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*. Σa,b,c = numerical sum a+b+c;
a = weak positive; b = intensely positive; c = positive; d =negative

firmed by several researchers, some describe its presence as 27.03% [17].

Before oral hygiene, the first group had a total positive bacterial load with a nominal value of 12, representing 66.6% of young patients aged between 18 and 30 years. After oral hygiene, this decreased to a nominal value of 6 (33.3%). The highly positive bacterial load went from a nominal value of 5 to 0.

Effective dental-periodontal hygiene and appropriate treatment of periodontal disease lowers fasting blood sugar and the number of pathogens [18,19]. Also, through proper hygiene and treatment of gingivitis in patients with type 2 diabetes, blood glucose intake is reduced [20]. Porphyromonas gingivalis is present in adult patients, in patients with type 2 diabetes, without causing periodontal disease, and is a risk factor in cardiovascular disorders and esophageal cancer [21]. The pathogen is typically present in the bacterial plaque, and its removal leads to favorable outcomes for patients with type 2 diabetes. Notably, there exists a correlation between periodontal disease in patients with type 2 diabetes, with the pathogen playing a role in the onset and progression of diabetes [22].

The initial bacterial load before oral hygiene showed positive results for 15 patients in the second age group. Subsequently, the positive results decreased to 9 after oral hygiene, while negative re-

sults increased from 7 to 13 patients. It is noteworthy that no strongly positive bacterial load was observed.

Before oral cavity hygiene, the pathogen *Aggregatibacter actinomycetemcomitans* was found with a similar mark as Porphyromonas gingivalis in the first group, but with a higher bacterial load. It demonstrated strongly positive results in 5 cases, positive results in 4 cases, and weakly positive results in 4 cases. After oral hygiene, only 6 positive cases persisted, while the negative results increased from 8 to 12.

For the pathogen *Aggregatibacter actinomycetemcomitans*, in the group of patients older than 30, a weakly positive result was maintained even after oral hygiene, slightly increasing the number of patients with a negative result from 9 to 13. *Actinobacillus actinomycetemcomitans* causes the exacerbation of periodontal disease in patients with type 2 diabetes and reduces the role and functions of neutrophils [23]. The role and importance of oral hygiene were emphasized by authors who noted the presence of the pathogen in the bacterial plaque [24].

The pathogen *Bacteroides forsythus/Tannerella forsythia* was active in 10 cases. After oral hygiene, there were 4 weakly positive values, and the negative bacterial load increased from 8 to 14. This pathogen is considered a contributor to gingivitis pathogenesis [25].

Tanerella forsythia is more prevalent in patients with type 2 diabetes, with pathogen reproduction influenced by blood sugar levels. *T. forsythia* is a significant pathogen in periodontal disease, along with *P. gingivalis* and *Treponema denticola*, forming the red complex. The interplay between *T. forsythia* and *F. nucleatum*, combined with hyperglycemia, contributes to the development of periodontal pathology. It is a pathogen for which oral hygiene does not positively influence the bacterial load [26]. The combined challenge of *T. forsythia* and *F. nucleatum* leads to a stronger inflammatory reaction that contributes to the combined effects on alveolar bone loss [27]. In our study, we observed that out of 10 initially positive cases in the 18-30 age group, only four remained positive after oral hygiene procedures. In the group over 30 years old, all 14 initially positive cases remained positive after oral hygiene procedures. However, we did note a slight decrease in the bacterial load after the therapeutic procedures.

The bacterial load of *Fusobacterium nucleatum* decreased after oral hygiene. The bacterial load of *F. nucleatum* diminished, and there were no strongly positive bacterial loads, only weakly positive. This fact belongs to the presence of microbial flora in the microbial biofilm. Thus, the presence of microbial agents can, under certain conditions, determine a periodontal pathology [28]. *Fuzobacterium nucleatum*, *P. gingivalis*, and *T. forsythia* are oral bacteria as potential biomarkers for periodontal disease and type 2 diabetes. *F. nucleatum* has a higher bacterial load in patients with uncontrolled type 2 diabetes [29]. This is why oral hygiene has minimal impact on the microbial load. Among the two initially positive cases in the 18-30 age group, only one tested positive after oral hygiene procedures. In the group over 30 years old, all 13 initially positive cases remained positive after oral hygiene procedures. Nonetheless, we had a minor reduction in the bacterial load following the therapeutic procedures.

Eikenella corrodens signals the onset of periodontal disease in young people [30]. Oral hygiene had minimal impact on reducing the bacterial load of *E. corrodens* in the first age group, only decreasing it from the nominal value of 6 to 5. Eikenella corrodens was detected in 10 patients with type 2 diabetes in the second age group. Following oral hygiene interventions, the pathogen was found in 8 patients, indicating a small reduction in pathological values. Oral hygiene barely diminished the bacterial load. *Eikenella corrodens* is commonly found in the oropharynx and can exhibit virulence in individuals with compromised immunity, particularly in patients with diabetes predisposed to lung infections [30].

Capnocytophaga is present in hyperglycemic or healthy patients [31]. In the first collection of the first group, there were 8 patients with weakly positive and positive values. Oral hygiene reduced the positive bacterial load from the nominal value of 5 to 0, and the negative bacterial load increased to 14. The pathogen Capnocytophaga is present in healthy patients but also in patients with type 2 diabetes, where it can cause multiple dental-periodontal complications. The results do not decrease significantly after oral hygiene. The pathology of this agent is consistent with glycemia and oral hygiene [31,32]. Poor oral hygiene in adults and the elderly is linked to the presence of the pathogen Capnocytophaga [31]. In the group of individuals over 30, there were 11 positive cases at the start, which remained the same after oral hygiene procedures. However, we observed a decrease in bacteria following the therapeutic procedures.

To eradicate the pathogen *Treponema denticola*, maintaining oral hygiene is crucial. Before oral hygiene, the overall positive bacterial load in the 18-30-year-old patients was rated at a nominal value of 3 for weakly positive, which subsequently decreased to 1 after the hygiene regimen. We did not determine strongly positive and weakly positive bacterial loads. Treponema denticola indicates the severity of clinical periodontal parameters in patients with aggressive periodontal disease [33]. It is also found in severe cases of periodontal pathology and located within periodontal pockets; improved

oral hygiene does not lead to a significant change [33]. In the case of the first age group in our study, from three initially weakly positive values, only one weakly positive value remained after oral hygiene. In the second group of patients over 30 years old, we found that after completing the oral hygiene procedures, the number of cases with positive values of *Treponema denticola* increased from 6 to 8.

Prevotella intermedia is poorly represented in the 18-30 age group, with the total bacterial load being 4. Oral hygiene does not significantly change the presence of pathogens, but it eliminates the positive load to the nominal value of 0, with the total bacterial load falling to 2. *Prevotella intermedia* is more present in patients over 30 years old. The total bacterial load before oral hygiene is nominally 12 and diminished at 7 after finishing procedures. Low immunity and hyperglycemia can maintain periodontal disease, being also a risk factor in cardiovascular diseases [34].

Eubacterium nodatum has a weak positive value in the first age group. Dental-periodontal hygiene brings a significant result by removing bacterial plaque. Thus, the positive charge with the nominal value 4 is reduced to 1. *Eubacterium nodatum* plays a significant role in the pathology of periodontal diseases in pregnant women [35]. *Eubacterium nodatum* in controlled type 2 diabetic patients reduces pathogenic microbial flora of periodontal disease compared to healthy subjects [35]. *Eubacterium nodatum* exhibited significant resistance to oral hygiene procedures in the group of patients aged over 30 years. The initial bacterial load in this group was substantial, with a nominal value of 6. However, it was significantly impacted by the implemented procedures, ultimately decreasing to a nominal value of 5.

The Campylobacter rectus bacterial load is low in patients aged between 18 and 30; there is only one case with a weakly positive load with a positive effect after sanitization. Oral hygiene has a positive influence and reduces the bacterial load. The bacterial load in the group aged more than 30 had a nominal value of 12, which was reduced after oral hygiene procedures to 5. *Campylobacter rectus* in patients with controlled type 2 diabetes reduces the pathogenic microbial flora in periodontal disease compared to healthy subjects [36].

The bacterial load values of *Peptostreptococcus micros* before and after oral hygiene showed no significant difference. In the first age group, the number of positive cases decreased from 4 to 3 after procedures, while in the second age group, the number decreased from 8 to 4. These findings are consistent with those reported in the specialized literature [36,37].

CONCLUSIONS

The study highlights the complex relationship between orthodontic fixed retainers and periodontal health, particularly in patients with type 2 diabetes. Research indicates increased risks of plaque accumulation, gingival recession, and periodontal pocket depth in retainer wearers. Effective oral hygiene practices significantly reduced the bacterial load and improved periodontal health in diabetic patients, particularly for younger individuals. However, pathogens such as Treponema denticola and Porphyromonas gingivalis were more resistant to hygiene interventions, particularly in older patients.

The results also underscore the importance of maintaining rigorous oral hygiene to mitigate the bacterial load in patients with diabetes, which could, in turn, improve glycemic control and reduce the risk of cardiovascular complications. Pathogens like *Aggregatibacter actinomycetemcomitans* and *Fusobacterium nucleatum*, which contribute to periodontal disease, were reduced through periodontal treatment, though some, such as *Tannerella forsythia* and *Prevotella intermedia*, showed more resistance.

While periodontal pathogens are prevalent in individuals with type 2 diabetes, targeted oral hygiene and periodontal treatments can significantly reduce their presence, improve periodontal health, and potentially influence systemic health outcomes. Nevertheless, long-term management and further research are necessary to fully understand the impact of orthodontic retainers on the periodontal health of diabetic patients.

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