American Journal of
Biomedical Science & Research

www.biomedgrid.com
ISSN: 2642-1747

Research Article

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Influence of Oral Microbiota on the Development of Complications during Orthodontic Treatment

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To Cite This article: *Gholizadeh Akhranjani Mohammad, Ashrafi Rad Motahkhare, Saneeva Lada Vladimirovna, Influence of Oral Microbiota on the Development of Complications during Orthodontic Treatment. Am J Biomed Sci & Res. 2026 29(6) AJBSR.MS.ID.003870,*

DOI: 10.34297/AJBSR.2026.29.003870

Received: January 24, 2026; **Published:** February 02, 2026

Abstract

Introduction: Changes in the oral microbiota under the influence of orthodontic treatment can affect its clinical outcome and provoke the occurrence of complications such as the development of secondary caries and tooth root resorption.

Objective: The objective of this study was to investigate the relationship between changes in oral microbiota during orthodontic treatment and the risk of secondary caries and tooth root resorption, in relation to age and gender differentiation.

Relevance of the Study: Currently, orthodontic treatment is a lengthy process that brings psychological discomfort to patients. Moreover, with poor oral hygiene, complications often arise during it: secondary caries and resorption of tooth roots. Studying the composition of the oral microbiota during treatment, as a marker of ongoing processes, helps prevent complications and reduce the duration of orthodontic treatment, which can have a beneficial effect on the psychological state of patients.

Method: This longitudinal-cross-sectional study included patients undergoing fixed orthodontic treatment using brackets; changes in the microbiota were assessed in the study patients before treatment, three months later, and six months later. To determine the microbial composition of the oral microbiota, saliva and dental plaque samples were collected using 16S rRNA sequencing. Clinical indicators of secondary caries and root resorption were assessed using oral examination and radiographic examination. Statistical analysis, including the chi-square test, logistic regression, analysis of variance (ANOVA), and regression models with moderators and mediators, were performed using SPSS.

Results: It was found that the number of *Cariogenic bacteria*, including *Streptococcus mutans, Lactobacillus spp.* and *Actinomyces spp.*, as well as bacteria associated with tooth root resorption, such as *Porphyromonas gingivalis, Fusobacterium nucleatum* and *Aggregatibacter actinomycetemcomitans,* increased during orthodontic treatment in all age and gender groups ($p<0.05$); while the number of symbiotic bacteria *Veillonella spp.* significantly decreased. The highest rate of dental caries and root resorption and the weakest response to treatment were observed in the 12-14-year age group, especially in male adolescents. The mediating and moderating role of the response to treatment on the intensity and direction of the influence of oral microbiota, which determines the outcome of dental treatment, was revealed, which was especially pronounced in adolescents of both sexes and men; this pattern was statistically significant.

Conclusion: Orthodontic treatment causes an imbalance of oral microbiota and an increase in clinical morbidity; these consequences are more pronounced in the adolescent group, especially in male adolescents aged 12-14 years. Monitoring the microbiota response during treatment and paying increased attention to patients at high risk of complications, taking into account their age and gender, can prevent unwanted dental complications.

Keywords: Oral microbiota, Orthodontics, Dental caries, Root resorption

Introduction

To compare the results of this longitudinal-cross-sectional study with the results obtained by other scientists, we conducted an analysis of scientific sources covering similar topics. A number of authors [1-13] obtained similar results when conducting studies, *Alam MK, et al.* [14-17] also believe, as we do, that the use of modern innovative technologies allows us to avoid severe complications; *Marincak Vrankova Z, et al.* [15] argue that at the initial stage of treatment, significant changes in the composition of the oral microbiota do not occur, but do not exclude the possibility of such changes in a later period, which is confirmed by *Lucchese A, et al.* [18], who believe that significant changes in the oral microbiota are determined starting from the 15th day from the start of treatment, and *Reichardt E, et al.* [19] report that significant changes in the oral microbiota are noted during the first 6 months of treatment. *Xie Q, et* *al.* [16] report significant changes in the structure of oral microbiota during treatment and suggest using Metabol to minimize possible complications. Orthodontics, as a specialized branch of dentistry, plays a very important role in improving the coordination of the temporomandibular joint and correcting anomalies of the dental arches of the upper and lower jaws. In addition, it helps improve the aesthetic appearance of patients and improves the functional state of the oral cavity and the dental system [20-28]. Oral microbiota is the collection of microorganisms, including bacteria, viruses, fungi, and protozoa, that naturally inhabit the oral cavity and play an important role in maintaining the biological balance between symbiotic and pathogenic microorganisms [29,30].

The action of various general and local factors (dietary habits, oral hygiene level, medication intake, hormonal changes, systemic diseases, dental interventions, the presence of orthodontic structures in the oral cavity) can disrupt this balance and lead to significant changes in it [31,32]. These changes shift the composition of the microbiota towards species that increase the likelihood of developing secondary caries and gum disease [32-36]. Therefore, it is crucial to better understand the changes in the oral microbiota that influence patients' response to orthodontic treatment and the development of associated complications, such as secondary caries and gingivitis. This knowledge could pave the way for improving existing therapeutic and hygiene treatment methods and help develop more precise and targeted interventions that will not only facilitate orthodontic treatment but also prevent side effects [37]. For example, the use of new anti-plaque methods, topical disinfectants, or even probiotics can help maintain microbial balance and create conditions for better periodontal tissue restoration. Furthermore, studying the complex relationship between the oral microbiota and its response to orthodontic treatment can help better understand the biological factors influencing tooth mobility and root health [38]. This knowledge may ultimately lead to the development of personalized treatment protocols that provide specific care for patients at high risk of adverse events. This will not only improve the results of orthodontic treatment, but will also sustainably maintain the patients' oral health and improve their quality of life.

Objective

The objective of this study was to investigate the oral microbiota and its response to orthodontic treatment in connection with the likelihood of complications: secondary caries and resorption of tooth roots during their movement, which is of great clinical importance, since effective control of microbiota can reduce the number of complications, speed up the treatment process and increase patient satisfaction with its results.

Research Methodology

This longitudinal-cross-sectional stud was developed and conducted in 2025 in the dental clinics LLC "Doctor Confident" and "Iran Rad Clinic", which are clinical bases of: "Peoples' Friendship University of Russia named after Patrice Lumumba" (RUDN) and "Mashhad University of Medical Sciences (MUMS)". The statistical population included patients requiring fixed orthodontic treatment in the above-mentioned specialized clinics, of which at least 30 were purposefully selected from patient databases as meeting the inclusion criteria for the study: patients over 12 but under 22 years of age, in good health, requiring fixed orthodontic treatment. If patients were found to have systemic diseases, take antibiotics or drugs that affect the oral microbiota in the last three months, or were unwilling to continue cooperation, they were excluded from the study.

Each patient included in the study provided voluntary informed consent to participate. Microbiological samples were collected from saliva and dental plaque three times during the study: before treatment, three months later, and six months later. To assess caries and root resorption, periapical and panoramic radiographs were taken and analyzed in standard mode at regular intervals. Clinical examinations were also performed to assess the condition of the gums and periodontium. For microbiome analysis, microbial DNA was isolated from saliva and dental plaque samples, and the bacterial composition of the samples was determined using 16S rRNA sequencing. The obtained data were analyzed using bioinformatics software, examining quantitative and qualitative changes in the microbiota over time. In addition, statistical tests such as the chi-square test, logistic regression, and analysis of variance were used to evaluate the relationship between microbiota changes and the incidence of dental caries and root resorption. Data analysis was performed using SPSS statistical software.

All stages of the study were accompanied by obtaining informed consent from patients or their parents, while confidentiality of information was carefully maintained. The average age of the participants was 15.8 years with a standard deviation of 2.4 years. The study subjects were divided into three age groups: the highest number of participants were in the 15-17 years old age group (13; 43.3%), followed by the 12-14 years old age group (10; 33.3%) and finally the 18-21 years old age group (7; 23.4%). This distribution indicates that the majority of the sample was in adolescence, which is consistent with the high prevalence of need for orthodontic treatment in this age group. Regarding the gender of the adolescents, 16 participants were female (53.3%) and 14 were male (46.7%), which provided a relatively balanced distribution between the two genders and also allowed for gender comparisons. The analyzed data are reflected in Table 1 and Figure 1, 2.

Table 1: Demographic characteristics of study participants.

Variable	Age Grip (Years)	Quantity (n)	Percent (%)
Age	14-Dec	10	33,3%
	15-17	13	43,3%
	18-21	7	23,4%
Gender	Female	16	53,3%
	Male	14	46,7%

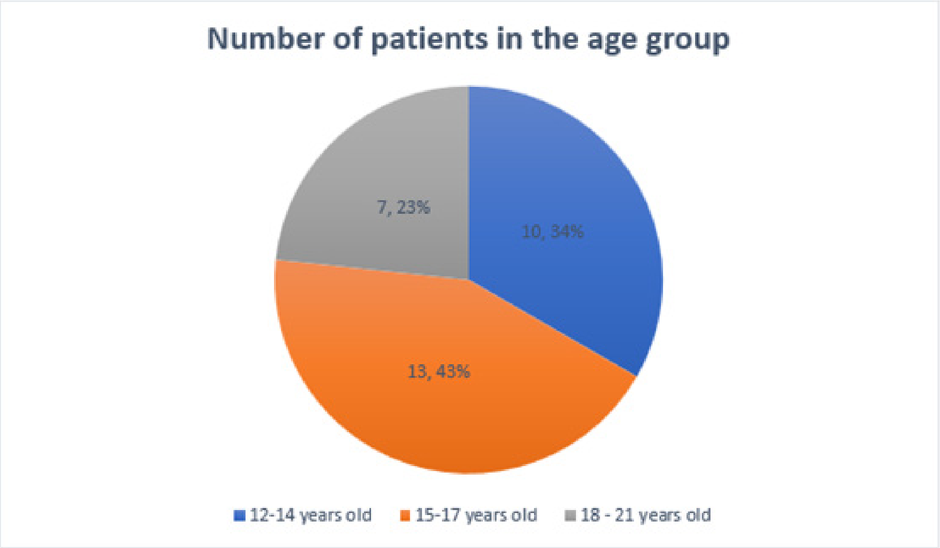


Figure 1: Age characteristics of study participants.

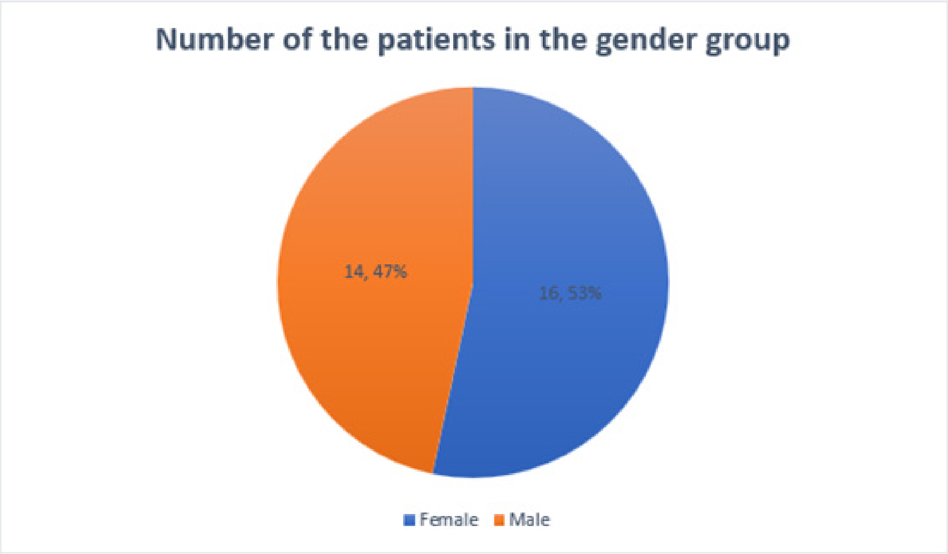


Figure 2: Gender characteristics of study participants.

In this study, the title of the following oral microbiota bacteria was studied: *Streptococcus mutans, Lactobacillus spp., Actinomyces spp., Porphyromonas gingivalis, Fusobacterium nucleatum, Aggregatibacter actinomycetemcomitans, Veillonella spp.*

Results

Descriptive analysis of variables

The results of the study are presented in Tables 2, 3, Figures 3-16 of this article.

Table 2: Relative mean changes in the abundance of individual oral microbiome bacteria during orthodontic treatment depending on age, gender, severity of dental caries and the effect of orthodontic treatment.

Groups	**Time of Sampling**	***Streptococcus Mutans* in (CFU)**	***Lactobacillus* spp. (CFU)**	***Actinomyces* spp. in (CFU)**	***Porphyromonas gingivalis* in (CFU)**	***Fusobacterium nucleatum* in (CFU)**	***Aggregatibacter Actinomycetemcomitans* in (CFU)**	***Veillonella* spp. (CFU)**	**Caries in (%)**	**Tooth root resorption (%)**	**Microbiome assessment with detailed description**
Response to orthodontic treatment 12-14-years	Before treatment	8.2	5.5	3.5	2	ss2.5	1.2	12.4	17.2	5.7	Poor condition (increase in cariogenic and resorptive bacteria, decrease in the titer of Veillonella spp.)
	In 3 months	12.6	9.1	4	2.8	3.2	1.6	9.7	25.7	7.6	Poor condition (increase in titer of carious flora, decrease in titer of symbiotic bacteria)
	In 6 months	15.3	11.8	4.5	3.4	3.8	2	8.5	31.6	9.2	Poor condition (progression of caries and root resorption, decrease in the titer of symbiotic bacteria)
15-17-years	Before treatment	7.6	4.9	3.2	1.8	2.3	1.1	13.2	15.7	5.2	Satisfactory condition (changes in the titer of microorganisms of the microbiome are less pronounced than in the previous group)
	In 3 months	11.2	8.3	3.7	2.5	2.9	1.5	10.1	23.2	6.9	Satisfactory condition (growth of carious flora titer, decrease of symbiotic bacteria titer is less pronounced than in the group of patients aged 12-14 years)
	In 6 months	13.9	10.7	4.2	3	3.4	1.8	8.9	28.8	8.2	Lower limit of a satisfactory condition: moderate changes (progression of caries and root resorption)
18-21-years	Before treatment	6.9	4.1	2.8	1.6	2	0.9	14	13.8	4.5	Satisfactory condition, moderate changes, expressed less than in the previous groups, Veillonella spp titer is higher than in the previous groups)
	In 3 months	10.4	7.5	3.3	2.3	2.6	1.3	11.5	21.2	6.2	Satisfactory condition, moderate changes
	In 6 months	12.7	9.3	3.8	2.8	3	1.6	9.8	25.8	7.4	Satisfactory condition, moderate changes
Female	Before treatment	7.5	5	3.3	1.7	2.2	1	13	15.8	4.9	Satisfactory condition, moderate changes

	In 3 months	11.3	8.6	3.8	2.4	2.8	1.4	10.4	23.7	6.6	Satisfactory condition, moderate changes
	In 6 months	13.8	10.9	4.3	2.9	3.3	1.7	9.1	29	7.9	Lower limit of a satisfactory condition is moderate changes
Male	Before treatment	7.9	5.2	3.4	1.8	2.4	1.1	12.9	16.5	5.3	Satisfactory condition, moderate changes
	In 3 months	11.8	8.9	3.9	2.5	3	1.5	9.8	24.6	7	Lower limit of a satisfactory condition is moderate changes
	In 6 months	14.2	11.2	4.4	3.1	3.5	1.8	8.7	29.8	8.4	Poor condition

Table 3: Results of changes and ANOVA test for microbiological and clinical orthodontic indicators.

Groups	**Variable (Microorganism)**	**Before Treatment (CFU)**	**In 3 Months (CFU)**	**In 6 Months (CFU)**	**F**	**p**	**Statistical Interpretation**
12-14-years	*Streptococcus mutans*	8.2	12.6	15.3	62.4	0.0003	Significant growth ↑
	Lactobacillus spp.	5.5	9.1	11.8	58.7	0.0005	Significant growth ↑
	Actinomyces spp.	3.5	4	4.5	9.1	0.01	Significant growth ↑
	Porphyromonas gingivalis	2	2.8	3.4	7.9	0.019	Significant growth ↑
	Fusobacterium nucleatum	2.5	3.2	3.8	8.3	0.017	Significant growth ↑
	Aggregatibacter actinomycetemcomitans	1.2	1.6	2	7	0.023	Significant growth ↑
	Veillonella spp.	12.4	9.7	8.5	23.1	0.0032	Significant reduction ↓
	Caries in (%)	17.2	25.7	31.6	52.1	0.0007	Significant growth ↑
	Root resorption in (%)	5.7	7.6	9.2	44.3	0.0012	Significant growth ↑
15-17-years	Streptococcus mutans	7.6	11.2	13.9	47.1	0.0011	Significant growth ↑
	Lactobacillus spp.	4.9	8.3	10.7	42.2	0.0018	Significant growth ↑
	Actinomyces spp.	3.2	3.7	4.2	8.3	0.017	Significant growth ↑
	Porphyromonas gingivalis	1.8	2.5	3	7.1	0.023	Significant growth ↑
	Fusobacterium nucleatum	2.3	2.9	3.4	7.8	0.019	Significant growth ↑
	Aggregatibacter actinomycetemcomitans	1.1	1.5	1.8	6.1	0.031	Significant growth ↑
	Veillonella spp.	13.2	10.1	8.9	14.9	0.015	Significant reduction ↓
	Caries in (%)	15.7	23.2	28.8	39.5	0.0023	Significant growth ↑
	Root resorption in (%)	5.2	6.9	8.2	26.7	0.0057	Significant growth ↑
18-21-years	*Streptococcus mutans*	6.9	10.4	12.7	31.5	0.0036	Significant growth ↑
	Lactobacillus spp.	4.1	7.5	9.3	29.2	0.0041	Significant growth ↑
	Actinomyces spp.	2.8	3.3	3.8	5.7	0.045	Significant growth ↑
	Porphyromonas gingivalis	1.6	2.3	2.8	5.9	0.042	Significant growth ↑
	Fusobacterium nucleatum	2	2.6	3	6.4	0.037	Significant growth ↑
	Aggregatibacter actinomycetemcomitans	0.9	1.3	1.6	5.1	0.052	Not a significant growth ↑
	Veillonella spp.	14	11.5	9.8	10.2	0.025	Significant reduction ↓
	Caries in (%)	13.8	21.2	25.8	22.3	0.008	Significant growth ↑
	Root resorption in (%)	4.5	6.2	7.4	18.8	0.011	Significant growth ↑
Female	*Streptococcus mutans*	7.5	11.3	13.8	37.8	0.0024	Significant growth ↑
	Lactobacillus spp.	5	8.6	10.9	35.2	0.0029	Significant growth ↑
	Actinomyces spp.	3.3	3.8	4.3	8.6	0.016	Significant growth ↑
	Porphyromonas gingivalis	1.7	2.4	2.9	7.2	0.022	Significant growth ↑
	Fusobacterium nucleatum	2.2	2.8	3.3	7.9	0.018	Significant growth ↑

	Aggregatibacter actinomycetemcomitans	1	1.4	1.7	6.8	0.025	Significant growth ↑
	Veillonella spp.	13	10.4	9.1	12	0.021	Significant reduction ↓
	Caries in (%)	15.8	23.7	29	34.1	0.0032	Significant growth ↑
	Root resorption in (%)	4.9	6.6	7.9	23.5	0.007	Significant growth ↑
Male	*Streptococcus mutans*	7.9	11.8	14.2	39.1	0.0023	Significant growth ↑
	Lactobacillus spp.	5.2	8.9	11.2	36.6	0.0027	Significant growth ↑
	Actinomyces spp.	3.4	3.9	4.4	9.5	0.012	Significant growth ↑
	Porphyromonas gingivalis	1.8	2.5	3.1	7.6	0.02	Significant growth ↑
	Fusobacterium nucleatum	2.4	3	3.5	8.1	0.018	Significant growth ↑
	Aggregatibacter actinomycetemcomitans	1.1	1.5	1.8	7	0.023	Significant growth ↑
	Veillonella spp.	12,9	9,8	8,7	11,7	0,022	Significant reduction ↓
	Caries in (%)	16,5	24,6	29,8	36,8	0,0025	Significant growth ↑
	Root resorption in (%)	5,3	7,0	8,4	24,1	0,008	Significant growth ↑

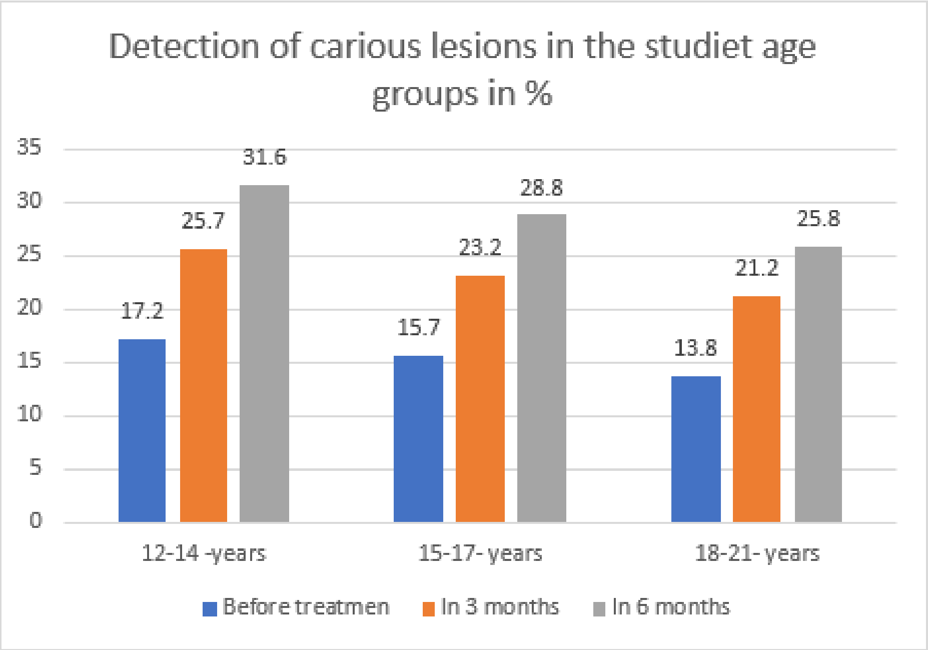


Figure 3: Prevalence of carious lesions in the age groups studied.

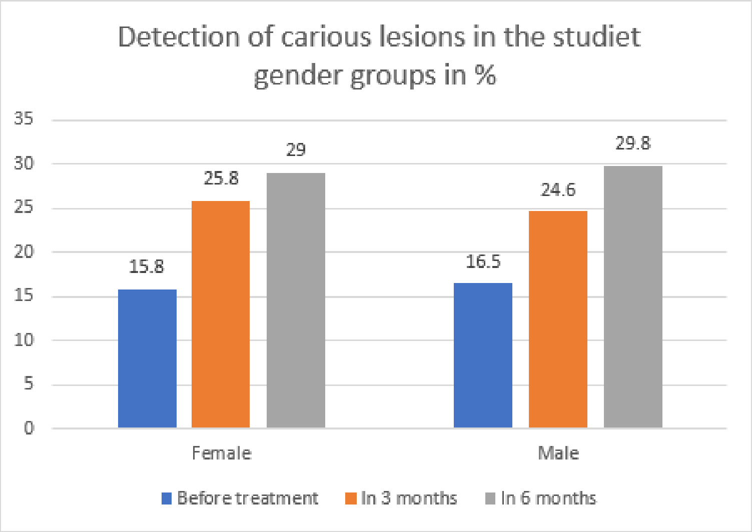


Figure 4: Prevalence of carious lesions in the studied gender groups группах.

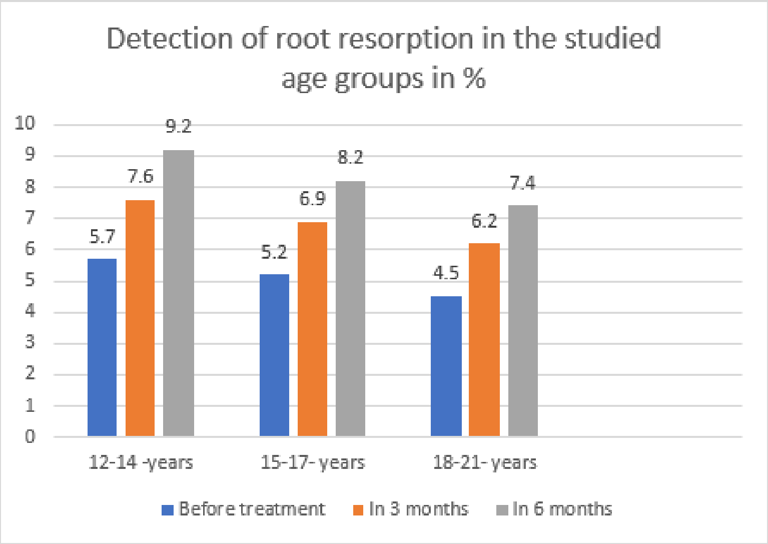


Figure 5: Prevalence of root resorption in the age groups studied.

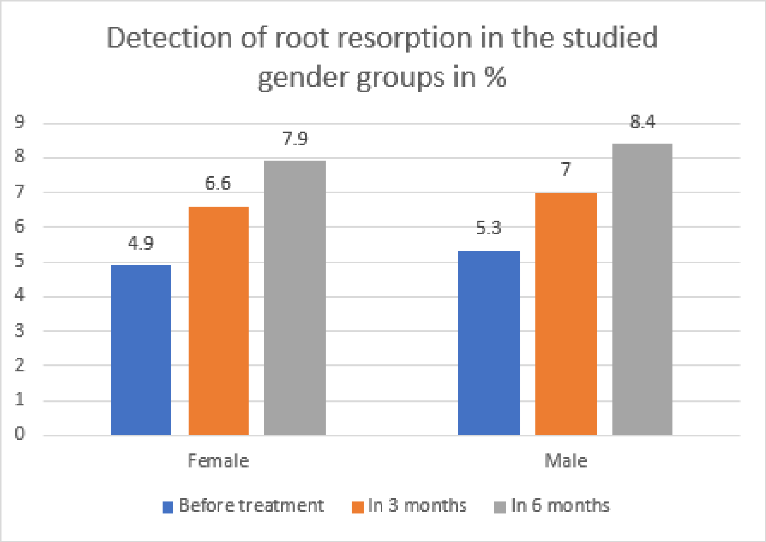
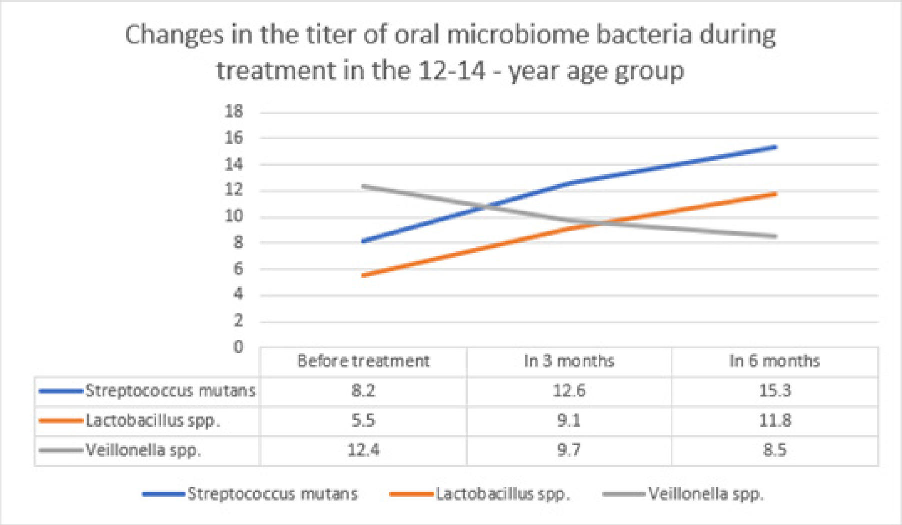
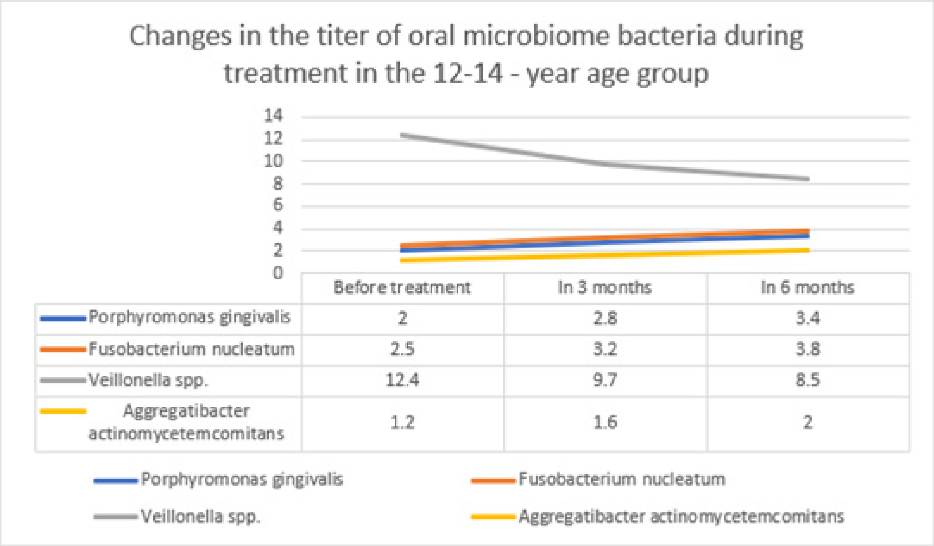


Figure 6: Prevalence of root resorption in the gender groups studied.



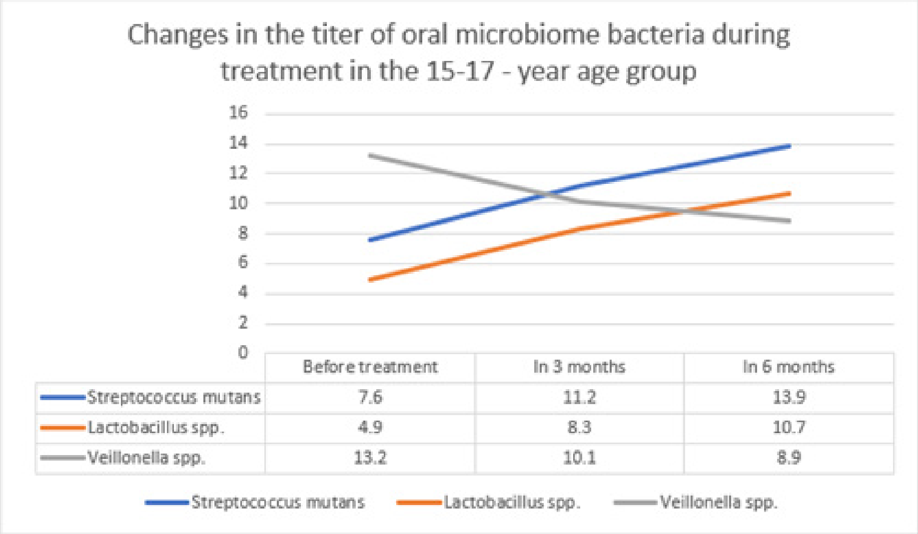
	Before treatment	In 3 months	In 6 months
Streptococcus mutans	8.2	12.6	15.3
Lactobacillus spp.	5.5	9.1	11.8
Veillonella spp.	12.4	9.7	8.5

Figure 7: Changes in the titter of oral microbiome bacteria during treatment in the 12-14 - year age group.



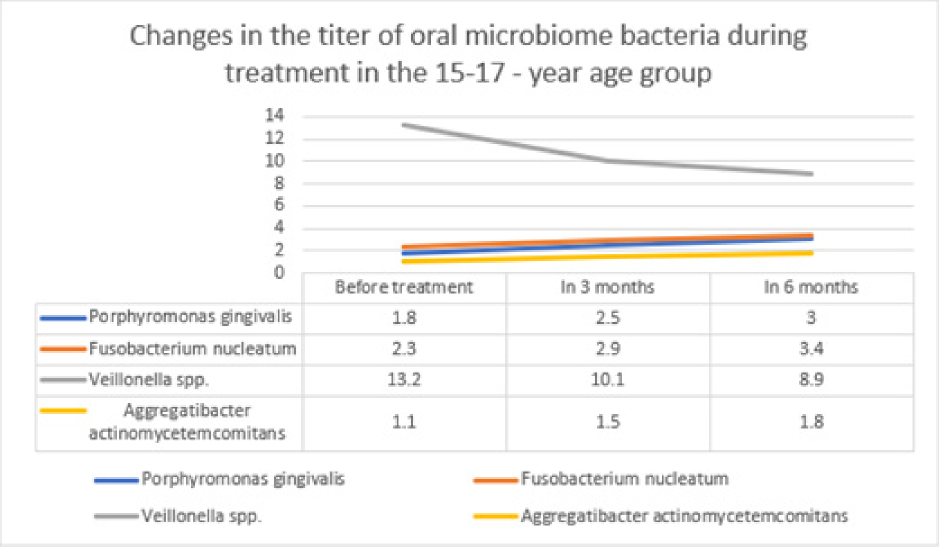
	Before treatment	In 3 months	In 6 months
Porphyromonas gingivalis	2	2.8	3.4
Fusobacterium nucleatum	2.5	3.2	3.8
Veillonella spp.	12.4	9.7	8.5
Aggregatibacter actinomycetemcomitans	1.2	1.6	2

Figure 8: Changes in the titter of oral microbiome bacteria during treatment in the 12-14 - year age group.



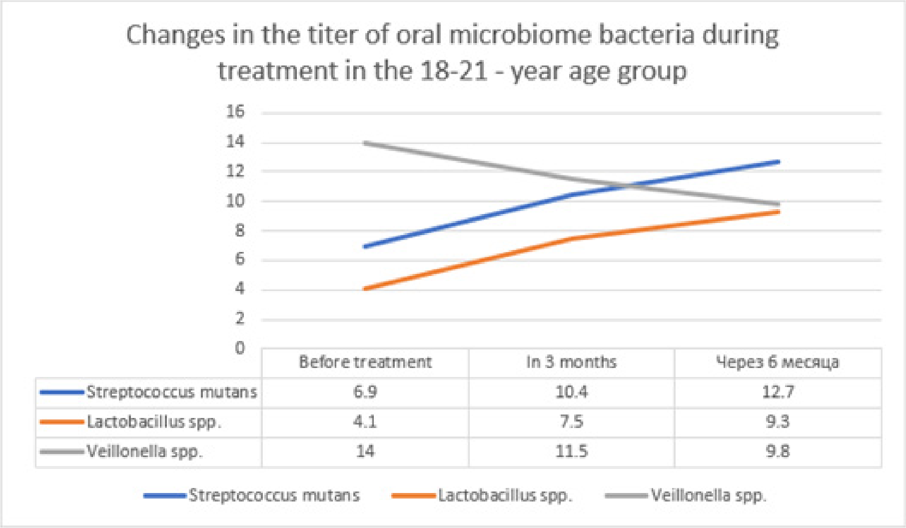
	Before treatment	In 3 months	In 6 months
Streptococcus mutans	7.6	11.2	13.9
Lactobacillus spp.	4.9	8.3	10.7
Veillonella spp.	13.2	10.1	8.9

Figure 9: Changes in the titter of oral microbiome bacteria during treatment in the 15-17 - year age group.



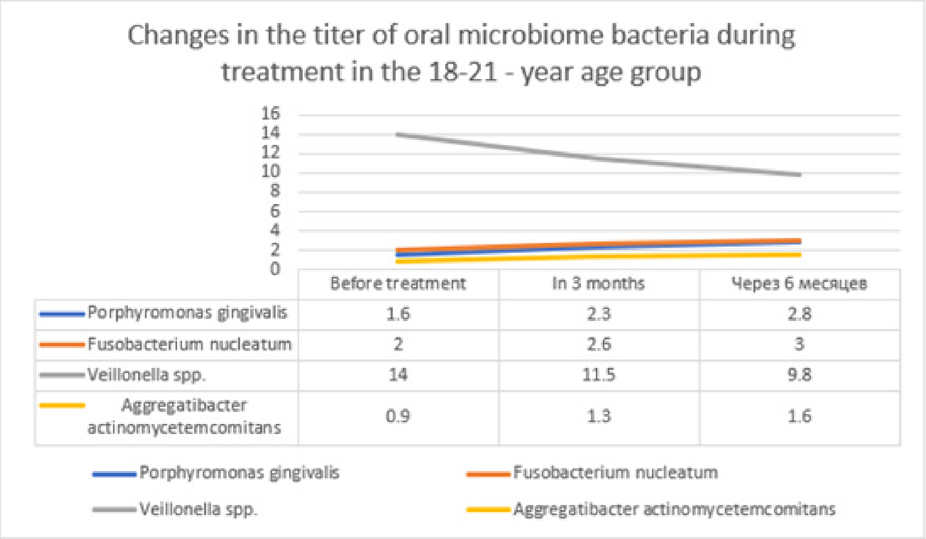
	Before treatment	In 3 months	In 6 months
Porphyromonas gingivalis	1.8	2.5	3
Fusobacterium nucleatum	2.3	2.9	3.4
Veillonella spp.	13.2	10.1	8.9
Aggregatibacter actinomycetemcomitans	1.1	1.5	1.8

Figure 10: Changes in the titter of oral microbiome bacteria during treatment in the 15-17 - year age group.



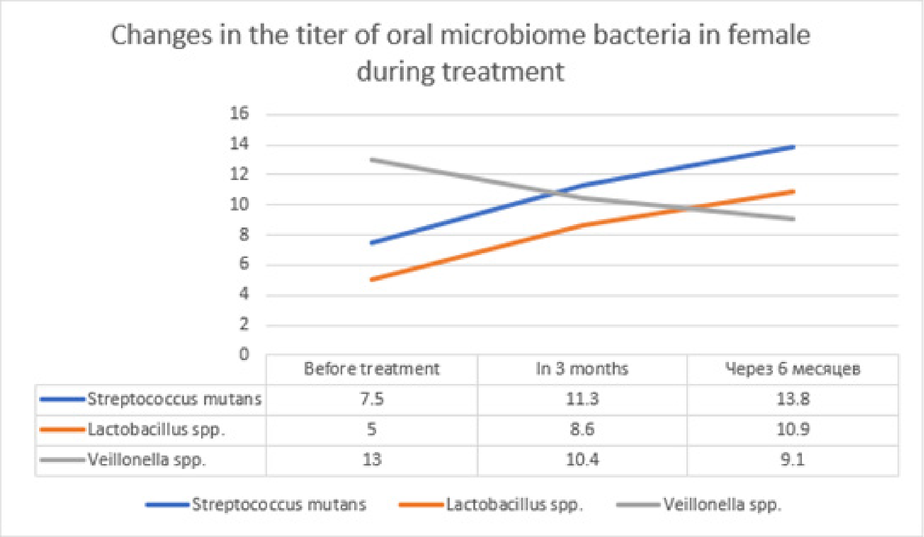
	Before treatment	In 3 months	Через 6 месяца
Streptococcus mutans	6.9	10.4	12.7
Lactobacillus spp.	4.1	7.5	9.3
Veillonella spp.	14	11.5	9.8

Figure 11: Changes in the titter of oral microbiome bacteria during treatment in the 18-21 - year age group.



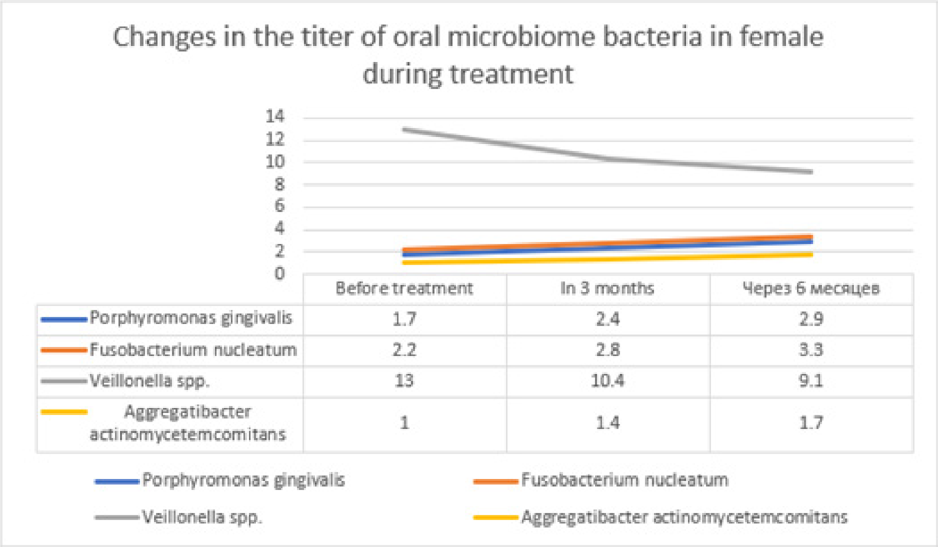
	Before treatment	In 3 months	Через 6 месяцев
Porphyromonas gingivalis	1.6	2.3	2.8
Fusobacterium nucleatum	2	2.6	3
Veillonella spp.	14	11.5	9.8
Aggregatibacter actinomycetemcomitans	0.9	1.3	1.6

Figure 12: Changes in the titter of oral microbiome bacteria during treatment in the 18-21 - year age group.



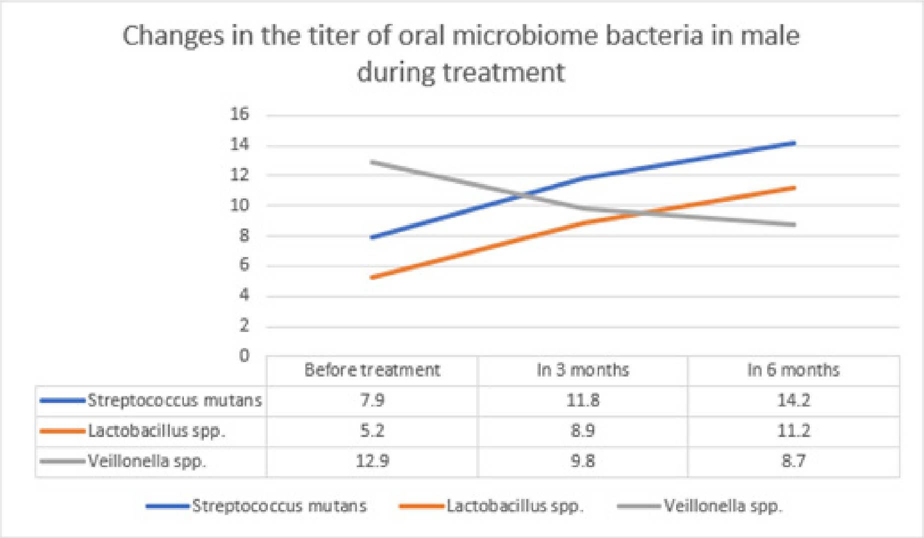
	Before treatment	In 3 months	Через 6 месяцев
Streptococcus mutans	7.5	11.3	13.8
Lactobacillus spp.	5	8.6	10.9
Veillonella spp.	13	10.4	9.1

Figure 13: Changes in the titter of oral microbiome bacteria in female during treatment.



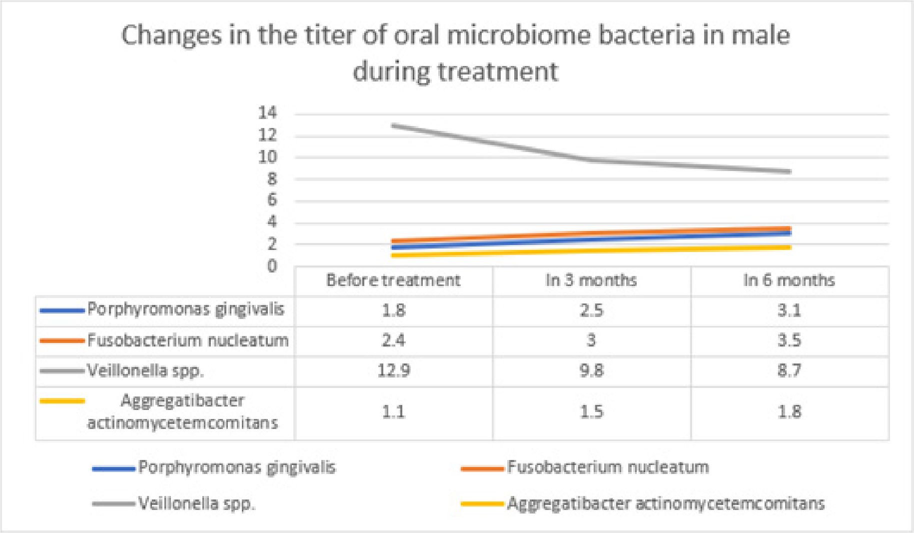
	Before treatment	In 3 months	Через 6 месяцев
Porphyromonas gingivalis	1.7	2.4	2.9
Fusobacterium nucleatum	2.2	2.8	3.3
Veillonella spp.	13	10.4	9.1
Aggregatibacter actinomycetemcomitans	1	1.4	1.7

Figure 14: Changes in the titter of oral microbiome bacteria in female during treatment.



	Before treatment	In 3 months	In 6 months
Streptococcus mutans	7.9	11.8	14.2
Lactobacillus spp.	5.2	8.9	11.2
Veillonella spp.	12.9	9.8	8.7

Figure 15: Changes in the titter of oral microbiome bacteria in male during treatment.



	Before treatment	In 3 months	In 6 months
Porphyromonas gingivalis	1.8	2.5	3.1
Fusobacterium nucleatum	2.4	3	3.5
Veillonella spp.	12.9	9.8	8.7
Aggregatibacter actinomycetemcomitans	1.1	1.5	1.8

Figure 16: Changes in the titter of oral microbiome bacteria in male during treatment.

The findings highlight that orthodontic treatment can cause significant changes in the oral microbiota, which, if not properly controlled, increase the risk of dental caries and root resorption. Therefore, special attention must be paid to oral hygiene, especially in sensitive groups, as well as to maintaining and improving the balance of bacteria, such as *Veillonella spp.,* which are essential for maintaining oral health during orthodontic treatment.

Inferential Analysis

Influence of Oral Microbiota on the Severity of Response to Orthodontic Treatment: Based on the data obtained from the statistical analysis, especially the ANOVA test, a significant and robust relationship was found between changes in oral microbiota and the quality of response to orthodontic treatment in adolescent patients (Table 4).

Table 4: Results of the analysis of the influence of oral microbiome on the severity of the response to orthodontic treatment.

Age Group	Gender	*Δ Streptococcus mutans*	*Δ Lactobacillus spp.*	*Δ* *Veillonella* spp.	Treatment response rate (% of group)	ANOVA & F F	-p value	η2 (size effect)
12-14 -years	Male	Significant growth ↑	Significant growth ↑	Significant reduction ↓	Poor condition (74%)	21.6	0.00003	0.51
12-14 -years	Female	Moderate growth ↑	Moderate growth ↑	Moderate reduction ↓	Poor condition (61%)	14.1	0.0007	0.33
15-17- years	Male	Moderate growth ↑	Small	Moderate reduction ↓	Satisfactory condition (41%)	11.4	0.002	0.26
15-17- years	Female	A slight increase ↑	Moderate growth ↑	A slight	Satisfactory condition (36%)	7.2	0.007	0.18
18-21- years	Male	Moderate growth ↑	No changes	Minor	Good condition (27%)	5.9	0.012	0.14
18-21- years	Female	No changes	No changes	No changes	Good condition (22%)	4.1	0.026	0.1

The findings indicate that in the 12-14-year age group, particularly in male adolescents, the severity of microbial dysbiosis was highest, with a significant increase in pathogenic bacteria such as *Streptococcus mutans* and *Lactobacillus spp.,* along with a significant decrease in *Veillonella spp.,* which coincided with the poorest levels of treatment response. In this group, more than 74% of patients demonstrated a weak response to treatment, and the high F values and low p value together with the large effect size (high η2) indicate a very strong statistical relationship. With increasing age, the severity of microbial changes decreased, and the population of protective bacteria partially returned to its original state.

As a result, in the age groups 15-17 and 18-21- years, the percentage of good responses to orthodontic treatment increases, and the statistical relationship between changes in microbiota and the quality of response weakens. In young adolescents, especially males, poor treatment response is not simply due to mechanical or genetic factors, but rather to a direct imbalance of oral microbiota. Conversely, the relative stability of the microbiota in this age group (18-21 years) and in females leads to better maintenance of clinical response. Therefore, monitoring and control of changes in oral microbiota during orthodontic treatment, especially in high-risk groups, should be given serious attention as part of clinical strategies and care.

Relationship Between Changes in Oral Microbiota and Dental Caries Incidence: Multivariate regression analysis in Table 5 (Table 5 in Appendix 1 of this article) showed that an increase in the number of *cariogenic bacteria,* especially S. mutans and *Lactobacillus spp.,* had a direct and significant relationship with an increase in the incidence of carious lesions ($p < 0.005$). Furthermore, a decrease in *Veillonella spp.* showed an inverse and significant relationship with an increase in caries development ($p < 0.01$).

Table 5: Results of the analysis of the relationship between microbial changes and the development of caries depending on age and gender.

Groups	Value of the Pearson Linear Dependence Coefficient (r) between the Titer of *Streptococcus mutans*, *Lactobacillus* spp. and *Veillonella* spp.	p-value	Δ *Lactobacillus* spp.	p-value	Δ *Veillonella* spp.	p-value	R2	Scientific Interpretation of the Correlation Coefficient of Pearson's Linear Dependence (r) between the titer of *Streptococcus Mutans*, *Lactobacillus* spp. and *Veillonella* spp. and Veillonella spp.
Female 12-14 - years	0,59	0,0007	0,45	0,0013	-0,36	0,0041	0,71	Strong positive correlation, strong negative correlation with *Veillonella* spp.
Female 15-17 - years	0,53	0,0012	0.4	0.0022	-0.32	0.0062	0.68	Moderate positive correlation, moderate negative correlation with *Veillonella* spp.
Female 18-21 - years	0.48	0.0019	0.34	0.0047	-0.27	0.0093	0.65	Positive correlation, but less pronounced than in previous age groups
Male 12-14 - years	0.62	0.0004	0.49	0.0009	-0.39	0.0029	0.75	Very strong positive correlation, strong negative correlation with *Veillonella* spp.
Male 15-17 - years	0,56	0,0008	0,44	0,0017	-0,33	0,0058	0,71	Positive and significant, negative correlation with *Veillonella* spp.
Male 18-21 - years	0,51	0,0016	0,38	0,0031	-0,29	0,0082	0,68	Positive correlation, weaker than in previous age groups

Relationship Between Microbiota and Tooth Root Resorption: The data presented in Table 6 indicate a significant relationship between changes in oral microbiota parameters and the rate of tooth root resorption in the studied patients undergoing orthodontic treatment. During the study, an increase in the number of *cariogenic bacteria* such as *Streptococcus mutans* and *Lactobacillus spp.* was observed in all age and gender groups, and a positive correlation and significant relationship was found between the increase in the number of these microorganisms and the intensity of tooth root resorption, which was especially pronounced in adolescents aged 12 to 14 years, where the greatest intensity of changes and the highest F values were observed; which indicates that this age group is more likely to experience damage to the tooth root structure, and microbiota plays an important role in this process.

Table 6: Results of ANOVA to determine the relationship between changes in microbiome parameters and root analysis (by age and gender).

Group	Indicator (Microorganism)	F-value	p-value	Presence of Statistical Significance	Scientific interpretation of the Correlation between Changes in the Oral Microbiome and Tooth Root Resorption
Female 12-14 - years	*Streptococcus mutans*	11,2	0,0015	Yes	Significant growth commensurate with tooth root resorption
	Lactobacillus spp.	7,8	0,0054	Yes	Positive correlation, but less pronounced than that of Streptococcus mutans
	Veillonella spp.	6,5	0,0103	Yes	A pronounced negative correlation is commensurate with tooth root resorption
Female 15-17 - years	*Streptococcus mutans*	9,4	0,0026	Yes	Scientifically proven strong positive correlation commensurate with tooth root resorption
	Lactobacillus spp.	6,2	0,0111	Yes	Moderate positive correlation commensurate with tooth root resorption
	Veillonella spp.	5,1	0,0204	Yes	A decrease in the Veillonella spp. titer is proportionate to the progression of tooth root resorption
Female 18-21 - years	*Streptococcus mutans*	7,1	0,0073	Yes	Positive correlation, expressed weaker than in the previous groups
	Lactobacillus spp.	4,8	0,0231	Yes	Less pronounced strong positive correlation
	Veillonella spp.	4,4	0,0294	Yes	Negative correlation with tooth root resorption, but less pronounced than in previous groups
Male 12-14 - years	*Streptococcus mutans*	13,7	0,0007	Yes	Strongest positive correlation was with tooth root resorption

	Lactobacillus spp.	9,9	0,0023	Yes	Strong positive correlation with tooth root resorption
	Veillonella spp.	7,6	0,0065	Yes	Strong negative correlation with tooth root resorption
Male 15-17 - years	*Streptococcus mutans*	10,5	0,0018	Yes	Significant positive correlation with tooth root resorption
	Lactobacillus spp.	7,3	0,0087	Yes	Moderate positive correlation with tooth root resorption
	Veillonella spp.	6,2	0,0119	Yes	Moderate negative correlation with tooth root resorption
Male 18-21 - years	*Streptococcus mutans*	8,2	0,0059	Yes	Positive correlation is significant, but weaker than in the previous groups
	Lactobacillus spp.	5,6	0,0174	Yes	Moderate positive correlation
	Veillonella spp.	5,0	0,0227	Yes	Negative correlation, less pronounced than in previous groups

At the same time, an increase in the number of symbiotic and protective bacteria *Veillonella spp.* has a pronounced inverse correlation and a significant relationship with tooth root resorption; which means that a decrease in the number of these microorganisms in the oral cavity is accompanied by an increase in the intensity of root destruction. This discovery highlights the importance of Veillonella in maintaining oral microbial balance and preventing the progression of tooth root resorption. Furthermore, the correlation between these parameters and root resorption was stronger in men than in women, which may be related to behavioural, hormonal, or biological factors. Overall, the obtained results highlight those changes in the microbiota during orthodontic treatment, especially in young adolescents and men, play a key role in the root resorption process and require more careful monitoring and care. This reinforces the importance of preventive measures and oral hygiene education for these patients. Based on statistical and conceptual data, it can be concluded that the response to orthodontic treatment plays a major mediating role in the relationship between oral microbiota and clinical outcomes (secondary caries, root resorption). However, some data also support the presence of a moderating role for the response to orthodontic treatment, for example, data have been obtained that the magnitude of the influence of microbiota on treatment outcome varies in people with different responses to it.

To Study the Influence of an Independent Variable (microbiota) on a Dependent Variable (Development of Dental Caries or Root Resorption) Through an Intervening Variable: Response to Treatment: The statistical analysis in SPSS software was performed using the macro tool developed by *Andrew F Hayes.* The results presented in Table 7 show that the quality and composition of the oral microbiota play a decisive role in the incidence or severity of dental caries and root resorption, and this effect affects not only directly, but also to a large extent indirectly, namely, it is associated with the response to treatment.

Table 7: Combined analysis of the effect of microbiome-mediated treatment response on dental caries and root resorption by age and gender.

Age/gender Group	Exodus	Total Microbiome effect (β)	Direct Microbiome Effect (β)	Indirect Effect through Treatment Response (β)	Ratio of Indirect to total Effect (%)	p Total Effect	p Direct Effect	p Indirect Effect	Meaning of the Method of Obtaining the Result
Male 12-14 - years	Caries	0.85	0.44	0.41	48	0.001	0.019	0.005	All significant
	Root resorption	0.73	0.38	0.35	48	0.004	0.027	0.011	All significant
Female 12-14 - years	Caries	0.66	0.38	0.28	42	0.018	0.049	0.024	All significant
	Root resorption	0.58	0.33	0.25	43	0.027	0.044	0.031	All significant
Male 15-17 - years	Caries	0.53	0.33	0.2	38	0.022	0.05	0.045	All significant
	Root resorption	0.48	0.31	0.17	35	0.029	0.054	0.047	All significant
Female 15-17 - years	Caries	0.46	0.28	0.18	39	0.036	0.062	0.049	Indirect and general methods are important
	Root resorption	0.42	0.26	0.16	38	0.04	0.065	0.052	Indirect and general methods are important

Across all age and gender groups, a positive and significant association was observed between microbiota changes and both clinical outcomes. However, the magnitude of this effect and the contribution of the indirect mediation pathway varied by age and gender. The data presented in the table indicate that male adolescents aged 12 to 14 are most vulnerable. In this group, both the overall effect of microbiota on the development of caries and tooth root resorption and the contribution of the indirect pathway (through treatment response) are particularly high (about 48% of the total effect). This finding is consistent with biological studies related to puberty, hormonal changes and immunology in adolescent males and calls for special attention to this high-risk group.

In contrast, in female adolescents aged 12 to 14 years and older adolescents (aged 15 to 17 years) of both sexes, there was a decrease in both the overall effect of microbiota and the contribution of the indirect pathway. Hormonal factors, healthy behaviours, and adaptive immune strategies in these groups appear to play a relative protective role and limit the severity of adverse microbiota effects. Analysis of the moderating effect of treatment response, including the influence of microbiota on the development of dental caries and root resorption, depending on age and gender In this study, the criterion for the significance of the interaction coefficient in the multiple linear regression model is the multiplicative interaction term, which was tested using the t-test. This can be implemented in SPSS using the linear regression method or the PROCESS 1 macromodel.

The results of this study, as shown in Table 7, indicate that treatment response not only plays a role as a mediating variable in the pathway by which microbiota influences dental outcomes (development of dental caries and root resorption), but also acts as a moderator; meaning that the strength and direction of the effect of microbiota on the outcomes obtained depend on the level of an individual's response to treatment. This issue was first examined and structured in the present study across age and gender groups. The largest and most significant moderating effect of treatment response was observed in the group of male adolescents aged 12-14-years. In this group, the interaction coefficient for caries was β=0.23 (p=0.011), and for root resorption, β=0.19 (p=0.022). These values were statistically significant and indicate that the negative impact of microbiota on clinical dental outcomes is significantly exacerbated when the treatment response is poor or less. From a practical point of view, this discovery demonstrates the double vulnerability of this group with the combination of an unfavourable microbial status and an inadequate response to treatment.

In contrast, in the subgroups of female adolescents aged 12-14 and 15-17-years, the interaction coefficient was low and insignificant (β=0.05 and 0.02 for caries development, 0.03 and 0.01 for tooth root resorption, respectively). Similarly, for male adolescents aged 15-17 years, the moderating effect was borderline significant. For dental caries: β=0.13, p=0.068. An important finding from this table is that tradition and gender significantly influence the magnitude and significance of the moderating role of treatment response. In younger male adolescents (12-14 years), the combination of an unfavourable microbiota and poor treatment response has the greatest negative impact on the development of dental caries and root resorption, while in younger female and older male adolescents this effect is reduced and largely insignificant.

Conclusion

The results of the study indicate that the composition and quality of the oral microbiota play an important role in the incidence and severity of dental caries and root resorption, and this effect is transmitted both directly and indirectly through the response to orthodontic treatment. This positive and significant relationship was observed across all age and gender groups, but the strength and contribution of the indirect mediation pathway varied by age and gender; thus, male adolescents aged 12 to 14 years are the most vulnerable, with approximately 48% of the total microbiota effect on clinical outcomes mediated by treatment response. In addition to its mediating role, treatment response also acts as a moderating factor, meaning that the magnitude and direction of the microbiota's impact on caries development and root resorption depends on the individual's level of treatment response.

This mitigating effect was particularly significant in male adolescents aged 12-14-years, with poor treatment response exacerbating these negative effects. In contrast, in female adolescents and older adolescents of both sexes, the moderate effect of treatment response was smaller and non-significant, suggesting a possible protective role of hormonal and behavioural factors in these groups. Statistical analysis including ANOVA test and multivariate regression, especially in younger age groups (12-14-years) and in males, showed the greatest severity of dysbiosis, the highest frequency of caries, tooth root resorption and the worst response to treatment. In contrast, in the older age group (18-21-years) and in women, the microbiota changes detected were milder and more controlled, and as a result, a more favourable clinical response to orthodontic treatment was reported. Biological mechanisms confirm that dysbacteriosis has a negative impact on the course of treatment and dental health, causing local inflammation, changing the pH of the oral cavity, weakening tissue regeneration and promoting the migration of pathogens into deep tissues.

Acknowledgement

None.

Conflict of Interest

The authors declare no conflict of interest.

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