SMOKING ELEVATES THE RISK OF DYSBIOSIS ASSOCIATED WITH DENTAL DECAY.

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ABSTRACT

BACKGROUND: Dental caries, commonly known as cavities or tooth decay, is a major oral health problem characterized by the breakdown of tooth structure due to acids produced by bacteria [1]. This condition arises when bacteria metabolize sugars from food, leading to acid production, which can contribute to the demineralization of hard tissues in the teeth, primarily the enamel and dentine [1]. The global prevalence of dental caries remains alarmingly high, making it a significant public health concern. According to recent data, dental caries affects approximately 2.3 billion people worldwide, particularly impacting permanent teeth, and 560 million children with deciduous teeth [2]. The global burden of disease study indicates that the prevalence of dental caries in primary teeth is around 46.2%, while for permanent teeth, it stands at 53.8% [2,3]. Dental caries is not only prevalent but also poses serious health implications, including impacts on quality of life and long-term health consequences such as infections that may result in systemic diseases or tooth loss, further exacerbating health disparities [3,4].

The oral microbiota plays a crucial role in maintaining oral health and influencing overall systemic health through complex interactions with the host. The oral microbiome consists of over 700 bacterial species that exist in a balanced ecosystem. This balance, known as eubiosis, is essential for protecting against the overgrowth of harmful microorganisms that can lead to diseases such as dental caries and periodontal disease [5,6]. Conversely, dysbiosis, or an imbalance in the oral microbiota, can lead to various oral diseases, including dental caries [6,7].

Several environmental and lifestyle factors contribute to the risk of dental caries through their effects on the oral microbiota[8–11]. Socioeconomic status, oral hygiene practices, dietary habits, fluoride exposure, knowledge and attitudes, and ethnicity and cultural factors significantly influence the prevalence of dental caries in different regions [3,12–15].

Cigarette smoking disrupts the delicate balance of the oral microbiota, leading to dysbiosis, which is characterized by an overgrowth of pathogenic bacteria and a reduction in beneficial species. Studies have shown that smokers harbor a higher proportion of cariogenic bacteria, such as Streptococcus mutans and Lactobacillus, compared to non-smokers. This shift in microbial composition is linked to an increased risk of dental caries [16–18]. However, there is no evidence specifically addressing the impact of smoking on the oral microbiota in Turkish populations.

AIM: This study investigates the role of smoking in influencing shifts in oral microbiota associated with dental caries in a Turkish population.

METHODS

Study design and sampling

An observational case-control study was conducted between January and June 2024 among young adults with and without dental caries referred to a dental clinic. Participants were interviewed face-to-face using a structured questionnaire, and oral examinations were performed based on the methods and criteria suggested by the World Health Organization[19]. Sociodemographic and behavioral characteristics of the participants were assessed. A group matching approach for age, gender, education level, sugar consumption (sachets/day), and tooth brushing habits was employed, followed by purposive sampling to assign participants with caries (patients)and without caries (control group). Eventually, a total of 270 participants were included in the study, comprising 180 patients with active dental caries (case group) and 90 participants with no detectable caries (control group), to evaluate the effect of smoking on oral microbiota imbalance.

Inclusion criteria were adults aged 18-44, and exclusion criteria of recent antibiotic use and underlying systemic diseases. Participants in each group who met the inclusion and exclusion criteria were purposively selected. Samples were collected via sterilized oral swabs directly from dental plaques and the tongue. The isolated samples were preserved using PBS and glycerol. The preservative medium was prepared by mixing 70% Phosphate Buffer Saline (PBS) and 30% glycerol in a reagent bottle. The medium was autoclaved for 15 minutes at 121ºC to avoid any contamination. One ml of the preservative medium was transferred into each Eppendorf tube, and the isolated samples were inoculated into each tube. The preserved samples were stored at -20ºC until further processing.

The collected samples were inoculated on a general-purpose medium for bacterial growth, specifically Nutrient Agar. The samples were then incubated for 24-48 hours at 37°C. To identify different bacterial species, pure cultures of bacterial colonies were prepared using various selective media, including MacConkey Agar, Mannitol Salt Agar, Blood Agar, and De Man, Rogosa, and Sharpe (MRS) Agar. Biochemical tests such as Catalase, Coagulase, Sugar Fermentation, Simmons Citrate, and Methyl Red were performed.

Bacterial species determination

The cultured samples were evaluated via gram staining, biochemical tests, and morphologically to determine the bacterial species, as summarized in Table 1.

Statistical analysis

Statistical analyses were conducted using R Project for Statistical Computing, Version 4.3.2 (R Foundation, Vienna, Austria). Categorical variables were expressed as the number of patients/controls (n) and relative frequency (%) using descriptive statistics. Descriptive statistics were calculated for demographic variables. Chi-square tests were used to compare the prevalence of smoking between cases and controls. Logistic regression was performed to assess the association between smoking and dental caries, with significance set at $p < 0.05$.

RESULTS

The present study involved a cohort comprising 180 samples from dental caries patients and 90 caries-free individuals, including other demographic parameters. Table 2 summarizes the distribution of demographic variables between patients and healthy participants. The patients had a mean age of 30.6 ± 7.9 years, with a male-to-female ratio of 86:94, while the healthy controls had a mean age of 31.3 ± 8.6 years, with a male-to-female ratio of 52:38. Among the patients, 36.1%, 41.1%, and 22.7% had primary, secondary, and higher education, respectively, whereas the corresponding relative frequencies among healthy participants were 37.7%, 31.1%, and 31.1%.

Regarding sugar consumption, 36.1% of patients consumed one sachet/day, 41.1% consumed 2 sachets/day, and 22.7% consumed it ≥3 sachets/day. In comparison, the respective sugar consumption rates among healthy individuals were 37.7%, 31.1%, and 31.1%. The majority of participants in both groups brushed their teeth at least twice a day. Except for smoking, which was the primary factor under study, no significant disparities were observed between patients and the no caries group regarding other key factors. Evidence showed an association between smoking and dental decay ($p = 0.03$). Furthermore, smokers were 1.28 times more likely to develop dental caries compared to non-smokers ($p = 0.04$) (Table 3).

A total of 15 bacterial species were identified in both groups: Staphylococcus aureus, Neisseria sp., Staphylococcus epidermidis, Corynebacteria, Streptococcus viridans, Lactobacillus, Lactococci, Enterobacter, Micrococcus luteus, Escherichia coli, Pseudomonas aeruginosa, Actinobacteria, Streptococcus mutans, Streptococcus sobrinus, and Lactobacillus acidophilus. Table 4 illustrates the observed frequency of oral microbiota in the patient and healthy control groups. Significant disparities were noted in the observed frequency of Neisseria sp., Streptococcus viridans, Micrococcus luteus, Escherichia coli, Pseudomonas aeruginosa, Streptococcus mutans, Streptococcus sobrinus, Lactobacillus acidophilus, and Actinobacteria between the patient and healthy control groups.

The observed frequency of Escherichia coli, Pseudomonas aeruginosa, Actinobacteria, Streptococcus mutans, Streptococcus sobrinus, Lactobacillus acidophilus, and Streptococcus viridans was significantly higher in patients ($p \leq 0.01$), whereas Neisseria sp. and Micrococcus luteus species were less frequently observed in patients compared to healthy individuals ($p \leq 0.02$).

The observational frequency of Staphylococcus aureus, Staphylococcus epidermidis, and Lactobacillus was relatively higher in those with caries, whereas Corynebacteria, Lactococci, and Enterobacter were more frequent in individuals without caries, although these differences were not statistically significant ($p \geq 0.05$).

DISCUSSION

An imbalance in the oral microbiota, known as dysbiosis, can have significant negative effects on oral health and lead to tooth decay [6,20]. The current study contributes to the body of oral health knowledge and provides persuasive evidence regarding the association between cigarette smoking and dysbiosis. The research results revealed how smoking enhances the dominance of cariogenic bacteria in the oral cavity.

The results support the association between smoking and a higher likelihood of developing dental caries. The relationship between smoking and dental caries has been extensively studied, with evidence indicating that smoking significantly increases the risk of dental decay [21]. It is thought that a combination of biological, behavioral, and environmental factors underlie this association. Smoking is known to alter salivary composition, reducing the levels of protective factors such as secretory IgA, which plays a crucial role in the immune response against dental caries. Lower salivary flow and changes in salivary pH can also contribute to an increased risk of caries [22]. Smokers tend to have higher plaque accumulation, which is a significant risk factor for dental caries. This accumulation can be attributed to the effects of smoking on oral hygiene practices and salivary function [23]. It has been observed that smokers often exhibit poorer oral hygiene practices and may consume sugary foods and beverages more frequently, further exacerbating the risk of developing dental caries [24,25]. Smoking promotes the growth of cariogenic bacteria like Streptococcus mutans, Lactobacilli, and Candida albicans. Nicotine in cigarettes also enhances the adherence and acid production of these bacteria, leading to more caries. In contrast, commensal bacteria like Streptococcus sanguinis are outcompeted or are displaced in the presence of cigarette smoke [18,21].

In the current study, certain bacterial species were more prevalent in patients, while others were less common in healthy controls. This information is important for understanding how smoking may influence dysbiosis, which in turn could be associated with dental caries. Specifically, Escherichia coli, Pseudomonas aeruginosa, Actinobacteria, Streptococcus mutans, Streptococcus sobrinus, Lactobacillus acidophilus, and Streptococcus viridans were significantly more prevalent in patients with caries. In contrast, Neisseria sp. and Micrococcus luteus species were less commonly observed in patients with caries compared to healthy individuals. E. coli has been found in the oral cavity of individuals, particularly those with poor oral hygiene and chronic periodontitis. The association of E. coli with poor oral health and dental caries is supported by evidence of its prevalence in the oral cavity, its observed role in periodontal disease, and its contribution to dysbiosis. These factors collectively suggest that E. coli may play a significant role in the pathogenesis of dental caries and other oral health issues [26,27]. P. aeruginosa is known for its ability to form biofilms, which are complex communities of bacteria that adhere to surfaces. This characteristic enhances its survival in the oral environment and contributes to its pathogenicity. The presence of P. aeruginosa in biofilms can disrupt the balance of the oral microbiome and promote conditions favorable for dental caries[8]. Evidence suggests that Actinobacteria, particularly Actinomyces species, are associated with oral health and the development of dental caries. Their ability to produce acids, form biofilms, and thrive in dysbiotic conditions underscores their role in the pathogenesis of dental caries[8]. S. mutans and S. sobrinus are strongly associated with dental caries due to their ability to produce acids and form biofilms. The Streptococcus viridans group includes both commensal and potentially cariogenic species, underscoring the complex interplay between different members of the oral microbiome in health and disease [29–31]. Lactobacillus acidophilus is associated with dental caries primarily due to its acidogenic properties and ability to form biofilms, which can contribute to the cariogenic environment. While it has potential probiotic benefits, its role in caries development underscores the complexity of the oral

microbiome and the need for a balanced microbial community [32,33].

The evidence indicates that Neisseria species are more prevalent in caries-free individuals and may play a protective role against dental caries. While specific data on Micrococcus luteus is less direct, its association with healthy oral conditions suggests that its abundance may also be reduced in patients with caries [34]. While Staphylococcus aureus, Staphylococcus epidermidis, and Lactobacillus are found at relatively higher frequencies in patients compared to healthy individuals, Corynebacteria, Lactococci, and Enterobacter seem less frequent. However, while there are observable differences in these bacteria, the statistical significance has not been specified. Staphylococcus aureus is associated with dental caries and oral infections, while Staphylococcus epidermidis may be less prevalent in caries-affected individuals. Lactobacillus species are strongly linked to caries development due to their acidogenic properties. The interplay between these bacteria highlights the complexity of the oral microbiome and its impact on oral health[35–37]. Corynebacteria, particularly Corynebacterium matruchotii and Corynebacterium durum, are associated with oral health and are more prevalent in caries-free individuals, suggesting a protective role against dental caries. Lactococci may contribute to the balance of the oral microbiome, while the role of Enterobacter in dental caries remains less clear [38,39].

Research limitations

This study provides valuable insights into the association between smoking and oral microbiota dysbiosis; however, several limitations may affect the generalizability and broader applicability of the findings. Firstly, the relatively small sample size of 270 participants restricts the generalization of the results to a wider population. To validate these findings and extend them to diverse demographic and geographic regions, larger and more varied samples are required. Moreover, the observational case-control design employed in this study allows for the identification of associations but does not establish causality. Longitudinal studies would be necessary to determine whether smoking directly induces changes in the oral microbiota that lead to dental caries. Another limitation is the reliance on self-reported data for smoking habits and oral hygiene practices, which introduces the potential for recall bias or social desirability bias, thereby potentially compromising the accuracy of the data. Although the study attempted to control for confounding factors such as age, gender, education level, and sugar consumption, other relevant variables—such as dietary habits, frequency of dental visits, and genetic predispositions—were not fully accounted for, which could have influenced the results. Finally, the study concentrated on a specific subset of bacterial species associated with dental caries. However, the oral microbiome is highly complex, and other bacterial species or microbial interactions that were not examined may also play significant roles in dysbiosis and caries development. Additionally, this study did not assess the impact of vaping (e-cigarette use) on the oral microbiota. Although vaping is reputed to produce aerosol with fewer toxins than conventional cigarette smoking, its long-term oral health effects remain unclear due to its relatively recent emergence. Future studies should investigate whether vaping induces similar dysbiotic shifts in the oral microbiome as observed with traditional smoking. Addressing these limitations in future research would enhance the robustness of the findings and provide a more comprehensive understanding of the relationship between smoking, oral microbiota dysbiosis, and dental caries.

CONCLUSION

There is decisive evidence that smoking has an impact on oral health and the development of dental caries, primarily through its effects on the oral microbiota and respective factors. Results indicate smoking promotes dysbiosis, which enhances the dominance of cariogenic bacteria. Meanwhile, smoking can reduce the abundance of beneficial bacteria, and in turn affect poor oral health of clients. Therefore, smoking, alongside subsequent factors such as poor oral hygiene practices and higher plaque accumulation have notable contribution to caries prevalence.

DECLARATIONS

Ethics Approval and Consent to Participate:
This study received ethical approval from the Institutional Review Board (IRB) of Bandırma Onyedi Eylül University (Approval No: :E-67961857-050.04-167793). Participants were thoroughly informed about the study, and written consent was obtained. The study was conducted in accordance with ethical guidelines, including the Declaration of Helsinki and its subsequent revisions.

Consent for publication: Not applicable.

Availability of data and material: The authors confirm that all data supporting the finding of the study are available within the article, and the raw data supporting the findings were generated and available at the corresponding author on request.

Competing interests: The authors have no conflict of interest to declare.

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Authors' contributions: RH, MH: Conceptualization, Methodology, Formal analysis. RH: Data management. AHH: Data analysis. MH, RH, MR, SÖ: Writing, Reviewing and Editing. All authors have read and approved the manuscript.

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APPENDIX

Table 1. bacterial species characteristics.

Bacterial Specie	Morphology	Gram staining	Hemolysis test	Methyl red test	Suger fermentation test	Simon citrate test	Coagulase test	Catalase test
Staphylococcus aureus	cocci	Gram-positive	β hemolysis	positive	positive	positive	positive	positive
Neisseria sp.	diplococci	Gram-negative	γ- hemolysis	Negative	positive	positive	Negative	positive
Staphylococcus epidermidis	cocci	Gram-positive	γ- hemolysis	Negative	positive	Negative	Negative	positive
Corynebacteria	rods	Gram-positive	γ hemolysis	positive	positive	Negative	Negative	positive
Streptococcus viridans	cocci	Gram-positive	α hemolysis	Negative	positive	positive	Negative	Negative
Lactobacillus	rods	Gram-positive	γ hemolysis	Negative	positive	Negative	Negative	Negative
Lactococci	cocci	Gram-positive	γ hemolysis	positive	positive	Negative	Negative	positive
Enterobacter	rods	Gram-negative	γ hemolysis	Negative	positive	positive	Negative	positive
Micrococcus luteus	cocci	Gram-positive	γ hemolysis	Negative	Negative	positive	Negative	positive
Escherichia coli	rods	Gram-negative	γ hemolysis	positive	positive	Negative	Negative	positive
Pseudomonas aeruginosa	rods	Gram-negative	β hemolysis	Negative	positive	positive	Negative	positive
Actinobacteria	Cocci	Gram-positive	γ hemolysis	positive	positive	Negative	Negative	Negative
Streptococcus mutans	Cocci	Gram-positive	α hemolysis	Negative	positive	Negative	Negative	Negative
Streptococcus sobrinus	Cocci	Gram-positive	α hemolysis	Negative	positive	Negative	Negative	Negative
Lactobacillus acidophilus	rods	Gram-positive	-	Negative	positive	Negative	Negative	Negative

APPENDIX

Table 2. Demographic variables distribution disparity of participants

Variable	Parameter	Patient	Healthy	P value
Age	mean	30.6±7.9	31.3±8.6	0.5
Gender	Male	86 (47.7%)	52 (57.7%)	0.1
	Female	94 (52.3%)	38 (42.2%)	0.1
Education level	Primary	65 (36.1%)	34 (37.7%)	0.8
	Secondary	74 (41.1%)	28 (31.1%)	0.1
	Higher	41 (22.7%)	28 (31.1%)	0.1
Suger consumption	≤1 sachet/day	65 (36.1%)	34 (37.7%)	0.8
	2 sachets/day	74 (41.1%)	28 (31.1%)	0.1
	≥3 sachets/day	41 (22.7%)	28 (31.1%)	0.1
Tooth brush	≤ 1 time a day	84 (46.6%)	41 (45.5%)	0.8
	≥2 times a day	96 (53.3%)	49 (54.4%)	0.8
Smoking	smokers	150 (83.3%)	65 (50.0%)	0.03
	nonsmokers	30 (16.6%)	25 (27.7%)	0.03

Table 3. Frequency distribution of smoking habits among participants and the relevant relative risk

	Dental caries	Healthy oral	Relative risk	Confidence interval	P value
Smokers	150	65	1.28	0.70 - 2.34	0.048
Non-smokers	30	25	0.78		
P value	0.03				

Table 4. Observational disparity of oral microbiota in dental decay patients versus healthy individuals

Bacterial Specie	Observed frequency		P value
	Patients	Healthy Control	
Staphylococcus aureus	30 (16.6%)	22 (24.4%)	0.12
Neisseria sp.	5 (2.7%)	9 (10.0%)	0.01
Staphylococcus epidermidis	22 (12.2%)	14 (15.5%)	0.4
Corynebacteria	23 (12.7%)	5 (5.5%)	0.14
Streptococcus viridans	48 (26.6%)	12 (13.3%)	0.01
Lactobacillus	35 (19.4%)	25 (27.7%)	0.12
Lactococci	35 (19.4%)	10 (11.1%)	0.08
Enterobacter	23 (12.7%)	9 (10.0%)	0.5
Micrococcus luteus	7 (3.8%)	10 (11.1%)	0.02
Escherichia coli	20 (11.1%)	3 (3.3%)	0.03
Pseudomonas aeruginosa	19 (10.5%)	3 (3.3%)	0.04
Actinobacteria	29 (16.1%)	5 (5.5%)	0.01
Streptococcus mutans	100 (55.5%)	10 (11.1%)	< 0.00001
Streptococcus sobrinus	92 (51.1%)	9 (10%)	< 0.00001
Lactobacillus acidophilus	95 (52.7%)	5 (5.5%)	< 0.00001