® Postepy Hig Med Dosw (online), 2021; 75: 101-106
e-ISSN 1732-2693

www.phmd.pl
Original Article

Received: 12.05.2020
Accepted: 01.12.2020
Published: 25.02.2021

Dental bacterial biofilm and gingival status in cystic fibrosis adult patients

Biofilm bakteryjny płytki nazębnej a stan dziąseł dorosłych pacjentów chorujących na mukowiscydozę

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Summary

Introduction: The purpose of the study conducted on cystic fibrosis (CF) patients was the bacterial plaque accumulation and the gingival condition assessment, and microbial analysis of the subgingival biofilm.

Materials/Methods: The study included 22 CF adult patients and 22 healthy controls, sex and age compatible with the CF patients. The dental plaque was assessed using plaque index (PLI), and the gingival status using gingival index (GI). Analyses of the subgingival biofilm were performed by the real-time polymerase chain reaction (PCR) test.

Results: The mean value of GI in CF patients was 0.39 ±0.36 and was significantly lower comparing to the healthy controls (1.02 ±0.63), PLI was similar in both the groups (1.31 ±0.69) for the study group and 1.04 ±0.62 for controls). In CF patients there was no correlation between PLI and GI, which was observed in the control group. In both the groups there was a correlation between PLI and the total number of periopathogens. Furthermore, in the control group, there was a correlation between GI and the number of periopathogens. Such a correlation was not observed in the CF patients.

Conclusions: In patients with CF, the lack of correlation between the amount of tooth deposits and the gingival condition may indicate a stable, most likely pharmacologically conditioned oral biofilm ecology. Among the aetiological factors of gingivitis and periodontitis in patients with CF, the bacterial activity does not seem to be modified. However, the unsatisfactory oral hygiene found in the study participants does not exclude the possibility of disease development in the future.

Keywords: **cystic fibrosis, dental biofilm, gingival status, periopathogens**

GICID 01.3001.0014.7699
DOI: 10.5604/01.3001.0014.7699
Word count: 3 699
Tables: 1
Figures: 2
References: 22

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Abbreviations: **CF** – Cystic Fibrosis, **GI** – Gingival Index by Löe & Silness, **PCR** – Polymerase Chain Reaction, **PLI** – Plaque Index by Silness & Löe.

INTRODUCTION

Microorganisms form biofilms in nearly any moist environment, e.g. the respiratory tract, oral cavity [5]. Most oral microorganisms do not exist independently, but they form an organised bacterial structure, which is covered by organic and inorganic substances and surrounded by an extracellular polymeric substance produced by them, and can adhere to oral tissue surfaces [10]. The oral microbiome creates a specific ecological niche in the form of a biological membrane, called a bacterial plaque (biofilm). It is formed by a layered growth of microorganisms arranged in orderly structures [18]. Such a layered construction creates various environmental conditions that affect specific specialisation and production of various metabolic products, including toxins. Oral microorganisms are present on the tooth surface in the form of two types of biofilms, namely supra- and subgingival, which are considerably different in terms of bacterial flora. Supragingival plaque is dominated by gram-positive streptococci, while subgingival plaque is dominated by anaerobic gram-negative bacteria and causes plaque-induced gingivitis, leading to periodontitis [7, 18]. Subgingival bacteria form a specific, based on bacterial correlations, complex, in which all of the pathogens are divided into groups: the red complex, which includes Porphyromonas gingivalis, Tannerella forsythia, and Treponema denticola; the orange, including Fusobacterium nucleatum, Prevotella intermedia, Peptostreptococcus micros, Campylobacter rectus; the yellow comprises Streptococcus mitis, Streptococcus oralis, Streptococcus sanguis; and the green complex, which includes Capnocytophaga gingivalis, Campylobacter concisus, Eikenella corrodens, Aggregatibacter actinomycetem comitans serotype a. In addition, there are separate periopathogens such as A. actinomycetem comitans serotype b, Selenomonas-noxia and Actinomyces naeslundii genospecies which are not related to other groups [21].

Defence mechanisms, including repair processes, protect the oral cavity of the host from harmful factors. However, even slight dental plaque accumulation can cause tissue inflammation, which is a part of physiological immune surveillance [6]. Further, in case of microbial imbalance because of the prevalence of harmful factors (e.g., plaque deposition) or reduced effectiveness of the defence mechanisms, the symbiosis between the biofilm and immune and inflammatory reactions of the host cells is disturbed. Such dysbiosis contributes to the development of gingivitis. Therefore, plaque-induced gingivitis is an inflammatory reaction of the gingiva to prolonged bacterial plaque accumulation [12]. The initial symptoms of this inflammatory condition may be clinically unnoticeable, but redness and swelling develop over time and patients may report gingival bleeding during tooth brushing and/or halitosis [6, 12]. In many cases, gingivitis may go unnoticed for many years because of the symptom-free course, and because of the chronic and progressing nature of the disease, periodontitis may develop [19]. It is worth mentioning that the oral bacterial microbiota is a part of the upper respiratory tract microbiota, which is of particular significance in patients at risk of developing pulmonary autoinfection [9].

The purpose of the present study conducted on adult patients with cystic fibrosis (CF) was to clinically assess the bacterial plaque accumulation and the gingival condition, to conduct microbial analysis of the subgingival biofilm and to determine whether the clinical and laboratory results had a significant correlation.

MATERIALS AND METHODS

The research was conducted in accordance with ethical principles, including the World Medical Association Declaration of Helsinki. The study group included adult patients, treated in the Department of Pulmonology, Allergology and Respiratory Oncology of the Poznan University of Medical Sciences, Poland, in whom the diagnosis of CF was confirmed by a positive sweat test and a genetic test. The control group consisted of generally healthy people who came to the Department of Risk Group Dentistry for control visit. Theywere sex and age compatible with the patients in the study group. Both study groups were living in the same environment. All the study participants provided informed consent to participate in the study after the aim of the study and its procedure were explained to them. The study and control groups included 22 participants each (age: 29.14 ±6.63 years). An oral health status examination was conducted in accordance with the World Health Organization criteria for epidemiological surveys [22]. The participants were examined by two professionals who were calibrated prior to the clinical examination, and inter-examiner agreement was determined by Cohen's Kappa value of 0.85.

The Silness & Löe Plaque Index (PLI) was used for dental plaque assessment [20]. According to the recommendations of the index, four surfaces (buccal, lingual/palatal, mesial and distal) of six index teeth, namely upper first right molar (16), upper right central incisor (11), upper first left premolar (24), lower first left molar (36), lower left central incisor (31) and lower right premolar (44), were examined. The index involves a four-level scale that is scored as follows: 0: no visible plaque, 1: plaque detectable only with a probe, 2: a thin layer of plaque in the gingival area and 3: high accumulation of plaque. Then, the scores from the four surfaces were summed up and divided by four in order to obtain PLI for each tooth. PLI for each participant was obtained by summing the indices for all six teeth and dividing by six. A PLI score < 0.1 indicates no plaque, from 0.1 to 1.0 indicates a small amount of plaque, from 1.1 to 2.0 indicates a moderate amount of plaque and from 2.1 to 3.0 indicates a considerable amount of plaque.

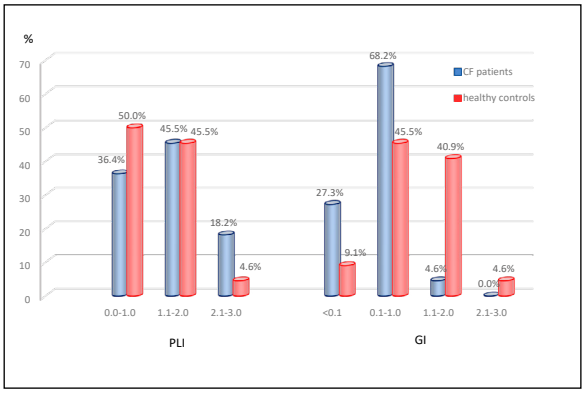

Fig. 1. Plaque score (PLI by Silness & Löe) and gingival score (GI by Löe & Silness) in cystic fibrosis patients and healthy controls; CF – cystic fibrosis; GI – Gingival Index by Löe & Silness; PLI – Plaque Index by Silness & Löe

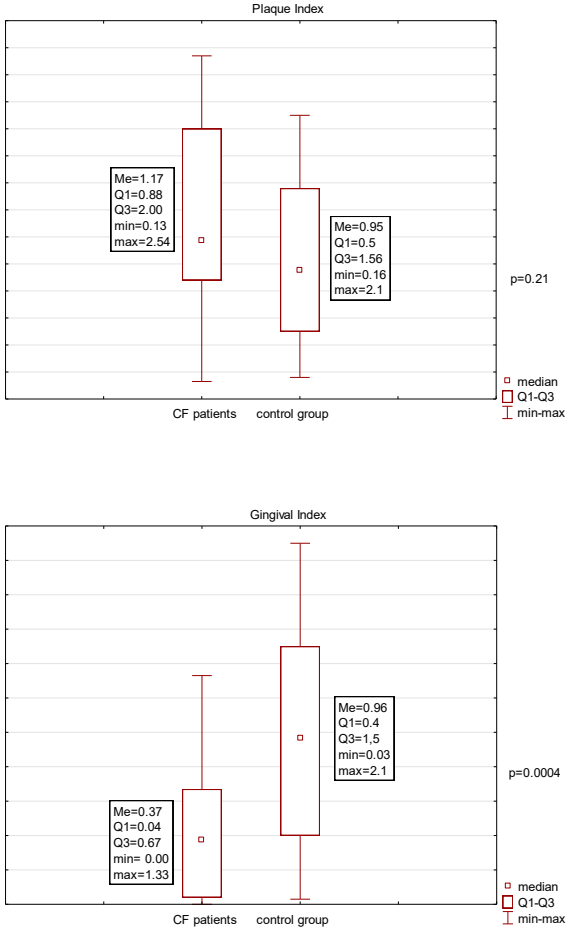

Fig. 2. Plaque index (PLI by Silness & Löe) and gingival index (GI by Löe & Silness) in cystic fibrosis patients and control group; CF – cystic fibrosis; GI – Gingival Index by Löe & Silness; PLI – Plaque Index by Silness & Löe

The gingival condition was assessed using the Löe & Silness Gingival Index (GI) [8]. GI was assessed for the same teeth as PLI [11, 15, 24, 31, 36, 44]. The assessment was performed with reference to the four-level scale of gingival condition proposed by the authors: 0 indicates healthy gums; 1 indicates slight colour changes, slight oedema and no presence of bleeding on probing; 2 indicates oedema with slight redness and bleeding on probing and 3 indicates severe oedema, redness, the presence of ulceration and a tendency for spontaneous bleeding. The respective values were summed up and divided by the number of teeth and surfaces covered during the examination. A GI score from 0.1 to 1.0 indicates mild inflammation, from 1.1 to 2.0 indicates moderate inflammation and from 2.1 to 3.0 indicates severe inflammation.

Qualitative and quantitative microbiological analyses of the subgingival biofilm were performed by the real-time polymerase chain reaction (PCR) test using the diagnostic PET Test® plus (MIP Pharma). The test allows the estimation of the total number of microorganisms and identification of nine periopathogens: Porphyromonasgingivalis, Tannerella forsythia and Treponema denticola belonging to the red complex; Fusobacterium nucleatum, Prevotella intermedia, Peptostreptococcus micros and Eubacterium nodatum belonging to the orange complex; Capnocytophaga gingivalis belonging to the green complex and Aggregati bacteractinomycetem comitans. The samples were collected from the gingival crevices of four teeth: two posterior and two anterior teeth (two upper and two lower). These were the same teeth for which the clinical indices PLI and GI were determined (16, 11, 36, 31). Following the manufacturer's recommendation, the supragingival plaque was mechanically removed before sample collection, following which the area was dried and saliva was removed using sterile swabs. Using sterile tweezers, a sterile paper point included in the kit was inserted in the dried area for 20 s. All the samples were then placed in the transportation kit and sent to the analytical laboratory (MPI Pharma GmbH, Hamburg, Germany).

Statistical analysis was performed using Statistica program, version 12 (StatSoft, Inc., Tulsa, United States). For all the test results, the arithmetic mean, standard deviation, median and quartiles, as the values of deviation from the median (25 quartile and 75 quartile), were calculated. The quantitative variables in both the groups were compared using the Mann–Whitney test. To determine the correlation between two variables, the Spearman's rank correlation coefficient was calculated, where $r = 0$ indicates no correlation, $r \leq 0.3$ indicates vague correlation, $0.3 < r \leq 0.5$ indicates medium correlation and $r > 0.5$ indicates strong correlation. The results were found to be statistically significant if the significance level was $p < 0.05$.

RESULTS

Figure 1 presents the amount of bacterial plaque accumulated (PLI) and gingival condition (GI) in the study and control groups. In the control group, a significantly strong correlation was observed between PLI and GI ($r = 0.77$; $p = 0.0001$); such correlation was not observed in the CF patients ($p = 0.1$). There was no significant difference in PLI between the study and control groups ($p = 0.21$). The mean value of PLI was respectively for the study and control group 1.3 ±0,69 and 1.04 ±062. However, GI was significantly lower in the study group than in the control group

Table 1. Number and percentage of cystic fibrosis patients and healthy controls colonised by particular periopathogens

Tested periopathogens		CF patients N (%)	Healthy controls *N* (%)	*p* value
Total tested periopathogens		11 (50.00)	18 (81.82)	0.07
Red complex	*Porphyromonas gingivalis*	1 (4.55)	1 (4.55)	1
	Treponema denticola	2 (9.09)	5 (22.73)	0.67
	Tannerella forsythia	1 (4.55)	7 (31.82)	0.43
Total red complex		2 (9.09)	8 (36.36)	0.46
Orange complex	*Fusobacterium nucleatum*	5 (22.73)	1 (4.55)	0.33
	Prevotella intermedia	1 (4.55)	4 (18.18)	0.51
	Peptostreptococcus micros	3 (13.64)	5 (22.73)	0.78
	Eubacterium nodatum	0 (0.00)	1 (4.55)	0.71
Total orange complex		6 (27.27)	8 (45.45)	0.49
Green complex	*Capnocytophaga gingivalis*	7 (31.82)	18 (81.82)	0.02

(respectively, the mean value for the study and the control group 0.39 ±0.36 and 1.02 ±0.63) (p = 0.0004) (Fig. 2).

The total number of microorganisms in subgingival biofilms was significantly lower in the study group (1.3×107 ±3.8×107) than in the control group (1.1×108 ±1.4×108) (p = 0.0001). On the other hand, no significant difference was noted in the percentage of participants in whom the presence of periopathogens was confirmed between the study and control groups as well as in the number of periopathogens between the samples collected from the two groups (p = 0.07) (Table 1). The percentage of participants in whom C. gingivalis was detected was significantly different between the two groups (p = 0.02). In both the groups, C. gingivalis was the most frequently existing bacterium; however, it was detected in a lower percentage of participants in the study group than in the control group (Table 1). A. actinomycetemcomitans was not found in none of the group.

In both the groups, statistical analysis showed a strong positive correlation between PLI and the total number of periopathogens (study group: r = 0.65; p = 0.001; control group: r = 0.54; p = 0.008) and a medium correlation between PLI and the total number of microorganisms (study group: r = 0.48; p = 0.02; control group: r = 0.32; p = 0.03). Moreover, in both the groups, a strong positive correlation was observed between the bacteria belonging to the red complex (P. gingivalis, T. forsythia and T. denticola) and those belonging to the orange complex (F. nucleatum, P. intermedia and P. micros). Furthermore, in the control group, a correlation was observed between GI and the number of periopathogens (r = 0.44; p = 0.03).

DISCUSSION

The literature referring to the presence of bacteria on tooth surfaces and the gingival condition [1, 3, 11, 13, 15, 16, 17] is based on different assessment methods. Martens et al. [11] and Aps et al. [1] visually assessed all surfaces of each tooth and noted the presence or absence of plaque. Dąbrowska et al. [2] used Debris Index (DI) by Green & Vermilion, which is based on numerical determinations representing the amount of dental plaque found on the one (buccal or lingual) tooth surfaces selected from four posterior and two anterior teeth (total six surfaces are examined). Only Peker et al. [17] used PLI, which was also used in our study. These prior studies have reported that there is no significant difference in plaque accumulation (propagation and thickness) between patients with CF and healthy controls [1, 2, 11, 15]. Another study on plaque propagation using the same group of patients with CF and healthy controls found a higher percentage of tooth surfaces covered with bacterial plaque in patients with CF [14]. In the examined adult patients with CF, there was significantly higher plaque propagation; however, there was no significant difference in the thickness of the deposited plaque between the patients with CF and healthy controls.

Martens et al. [11], Aps et al. [1], Narang et al. [17] and Ferrazzano et al. [3] assessed the gingival condition of patients with CF based on the existence or non-existence of bleeding as a result of bacterial plaque accumulation after careful examination. According to our knowledge, ours is the first study to analyse the advancement of the inflammatory condition in the gingiva. Martens et al. [11], Narang et al. [17] and Ferrazzano et al. [3] did not find a significant difference in gingival bleeding between patients with CF and healthy controls aged less than 18 years. However, in adults, Martens et al. [11] and Asp et al. [1] found significantly lesser gingival bleeding in patients with CF than in healthy controls, despite the lack of such differences in the propagation of visible bacterial plaque. The mean GI in patients with CF was 0.4 and that in healthy controls was 1.2, indicating mild and moderate inflammation,

respectively; this difference was significant, although the plaque thickness was similar on selected teeth in both the groups, but the accumulation was significant when analysing all teeth [14].

From among 700 oral microorganisms, Socransky et al. [21] singled out approximately 40 bacteria responsible for periodontal diseases, which they divided into five complexes, which are conventionally marked with colours depending on their metabolic preferences. The species belonging to the respective complexes are closely related, which allows a better use of nutrients and more effective protection from host defence mechanisms [4, 21]. The positive correlation found between the respective periopathogens belonging to the complexes in the study and control groups confirms the cooperation of bacteria within a given complex (community theory).

The physiological oral flora is individually diversified and usually contains a certain number of potentially pathogenic microorganisms. The correlation between the clinically confirmed thickness of deposits and the number of microorganisms in the study group and the correlation between the plaque thickness and the number of periopathogens in the study and control groups indicate only the microbiological composition. The aetiology of gingivitis is based on the assumption that the occurrence of the disease depends on the interaction between the plaque microorganisms and the host immune system cells [6, 12]. The initiation and progression of the disease are influenced by the amount of plaque, its composition and importantly, its microbial pathogenicity. The plaque thickness and number of periopathogens were similar in both the studied groups, and the observed gingival inflammation in the study group was significantly lower than that in the control group. The lack of correlation between the plaque thickness and gingival condition in the study group confirms the theory of a specific plaque, according to which only some microorganisms, and not all microorganisms grouped in the form of an unspecific biomass, of the plaque are responsible for the development of pathological lesions. It must be considered that in such patients, probably due to prolonged, frequent and long-lasting pharmacotherapy (also in the inhaled form), the pathogenic potential of the plaque may be changed (reduced), and such patients may demonstrate an individual and specific ecological biofilm balance [14]. Such presumption is confirmed by the lack of correlation between the total number of periopathogens and GI and between the number of bacteria belonging to the respective complexes and GI. Such correlations were observed in the control group. It must be emphasised that all the complexes assessed by PCR in our study may also occur in persons with a healthy periodontium, without any clinical manifestations. One cannot exclude that under adverse conditions, as a result of a disturbed bacterial ecosystem, potentially harmful microflora may become pathogenic to periodontal tissues.

The ability of oral cavity bacteria to form a biofilm is of significance when determining the pathogenesis of oral cavity diseases; it also helps determine the pathogenicity of microorganisms and their resistance to treatment. The gingival condition is the result of interaction between biofilm microorganisms and the reaction of host tissues, and the intensification and progression of the disease are due to many factors, such as the general health condition, nutrition level, hygiene and dietary habits, specifics of food chewing, malocclusions and importantly, the existence of pathogenic bacteria [6, 7, 10, 12].

CONCLUSIONS

In patients with CF, the lack of correlation between the amount of tooth deposits and the gingival condition may indicate a stable, most likely pharmacologically conditioned oral biofilm ecology.

Among the aetiological factors of gingivitis and periodontitis in patients with CF, the bacterial activity does not seem to be modified. However, the unsatisfactory oral hygiene found in the study participants does not exclude the possibility of disease development in the future.

ACKNOWLEDGMENTS

We are grateful to the individuals with cystic fibrosis and from control group for contributing to this study.

The authors would like to thank the staff of the Department of Pulmonology, Allergology and Respiratory Oncology, Poznan University of Medical Sciences who assisted in the project.

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The authors have no potential conflicts of interest to declare.