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OCCURRENCE OF PERIODONTAL PATHOGENS IN PATIENTS TREATED WITH FIXED ORTHODONTIC APPLIANCES

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A b s t r a c t

Patients with fixed orthodontic appliances tend to exhibit signs of gingivitis and gingival enlargement with false pockets. The aim of this study was to evaluate the occurrence of periodontal pathogens in patients with clinical manifestation of plaque-associated gingivitis treated with fixed orthodontic appliances. Thirty-two consecutive orthodontic patients – 11 females (age range from 10 to 32; mean age 17.43 years) and 21 males (age range from 12 to 28; average age 16.25 years) were included in this study. All individuals were diagnosed with clinical signs of gingivitis. CPI and PBI were evaluated. Subgingival plaque samples were collected after periodontal examination. Genomic DNA was extracted from these samples and bacterial detection was performed by polymerase chain reaction (Test VariOr-Dento). We recorded the following occurrence of periodontal pathogens: *Actinobacillus actinomycetemcomitans* – 25 %, *Porphyromonas gingivalis* – 31.25 %, *Treponema denticola* – 43.75 %, *Tannerella forsythensis* – 59 %, *Prevotella intermedia* – 65.6 %, *Peptostreptococcus micros* – 75 %, and *Fusobacterium nucleatum* – 100 %. Two and more pathogens were detected simultaneously in 93.75 % of the patients. Simultaneous occurrence of *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* was recorded in 9.37 % of the patients. Likewise, simultaneous occurrence of *Porphyromonas gingivalis, Treponema denticola*, and *Tannerella forsythensis* was registered in 15.6 % of the patients. The number of *Porphyromonas gingivalis* bacteria in the sample correlated significantly (p=0.01) with the values of the CPI index (evaluated by the Kruskal-Wallis ANOVA test). In this study 80 % of the patients treated with the fixed orthodontic appliance and with clinical manifestations of plaque-induced gingivitis are bearers of periodontal pathogens in a quantity significant for the damage of periodontium. To treat these patients successfully, collaboration with a periodontologist is highly recommended.

K e y w o r d s

Gingivitis, Fixed orthodontic appliance, Periodontal pathogen, Polymerase chain reaction

INTRODUCTION

The oral cavity is colonised by natural microflora, which is relatively stable in individuals and the composition of which is the result of a long-term relationship between the microorganisms and the host. This balance can easily be disrupted

by the action of numerous external and internal factors. Microorganisms play an important role in the development of many pathological states in the oral cavity; they participate in the origin of caries and periodontal disease. There is a wide range of pathogens in the oral cavity; nevertheless, it seems that certain differences in their distribution between a healthy periodontium and inflammatory states, gingivitis or periodontitis (with particularly increased incidence of G- anaerobes), do exist *(Tab. 1)* (*1*). According to a currently accepted hypothesis, periodontitis is an infectious disease of bacterial origin induced by microorganisms present in the dental plaque. Its development, however, is affected by many endo- and exogenous factors and their mutual interactions, which decide about the response of the periodontal tissues to this primary infectious stimulation.

Diseases of periodontium often begin as gingivitis. It typically develops during adolescence, i.e. from 15 to 18 years of age. At the same time many patients of this age undergo orthodontic treatment with a fixed appliance; this represents a new element in the oral cavity with higher requirements for hygiene. The orthodontic appliance also creates additional retention surfaces and spaces for adhering of microorganisms and growth of biofilm, i.e. an organised structure of microorganisms in mutually supporting colonies.

Patients with fixed orthodontic appliances tend to exhibit signs of gingivitis and gingival enlargement with false pockets *(Figs 1, 2)*. However, very little information is available on the microbiological changes that these periodontal tissues experience during orthodontic treatment. The aim of this study was to evaluate the occurrence of periodontal pathogens in patients with clinical manifestation of plaque-associated gingivitis treated with fixed orthodontic appliances.

MATERIALS AND METHODS

Thirty-two consecutive orthodontic patients – 11 females (age range from 10 to 32; mean age 17.43 years) and 21 males (age range from 12 to 28; average age 16.25 years) were included in this study. The criteria for inclusion in the study were the presence of clinically manifested gingivitis or gingival enlargement with false pockets and an orthodontic appliance fixed at least to one jaw for a period of at least three months in patients who had not been cured with antibiotics in the preceding six months. Written approval for inclusion in the study was obtained from all patients (or legal representatives in children).

The patients were clinically examined, history was taken, and CPI (Community Periodontal Index) and PBI (Papilla Bleeding Index) indexes were determined. A panoramic x-ray image (OPG) was taken and assessed to exclude more serious forms of periodontitis.

To detect periodontal pathogens, the VariOr®Dento test was used (Gen-Trend, s.r.o., České Budějovice, Czech Republic, www.gentrend.cz); this test enables detection of the presence of seven periodontal pathogens (shown in *Table 1* in red colour). All supragingival plaque must be removed prior to sampling. Sulcus liquid from the subgingival space (sulcus or periodontal pocket) was collected with endodontic paper points (endodontic points ISO 40) in several teeth (so-called multi-site sampling). After their removing from the examined site, all the points were air-dried, placed into a transporting plastic box, and sent to the Gen-Trend laboratory where they were evaluated as a sample of one patient. The VariOr®Dento test works with the DNA of the pathogens, therefore necrosis of microorganisms exposed to the air during the transport does not play any role. The target genomic

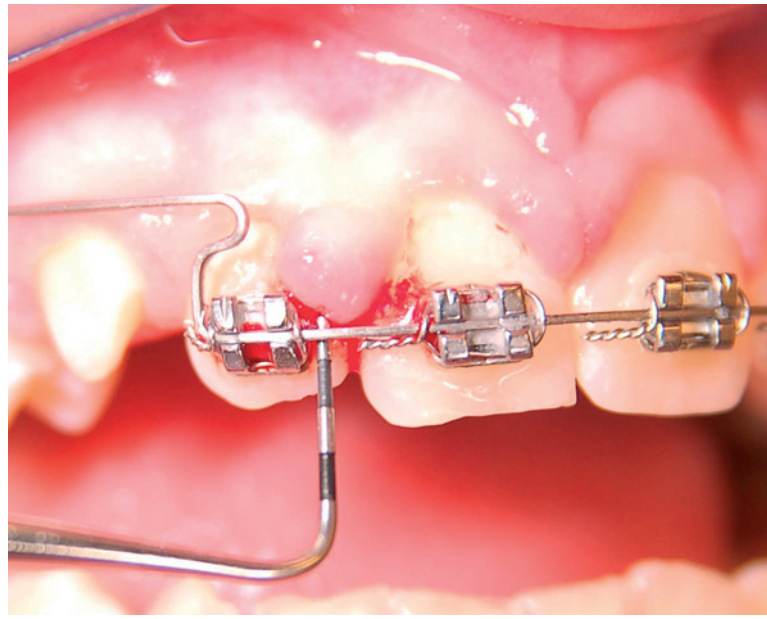


Fig. 1
Detail of upper dental arch with the installed fixed orthodontic appliance
with gingival enlargement and false pockets

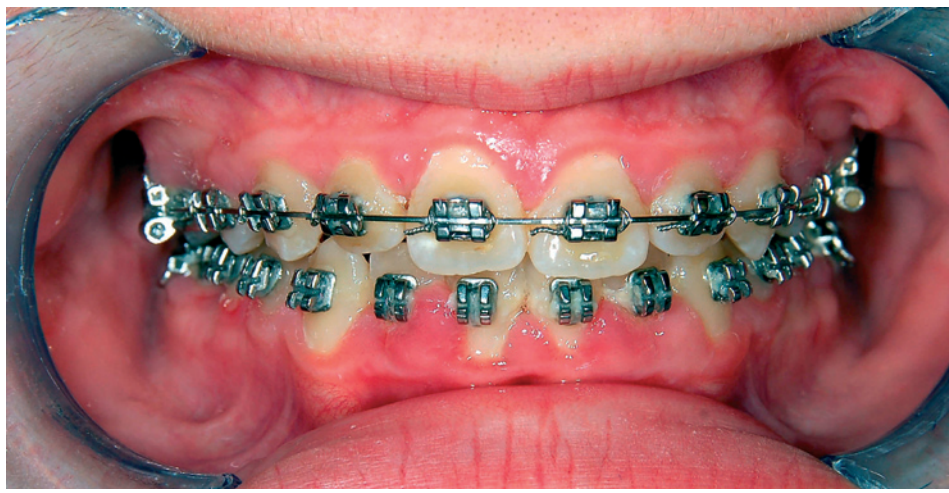


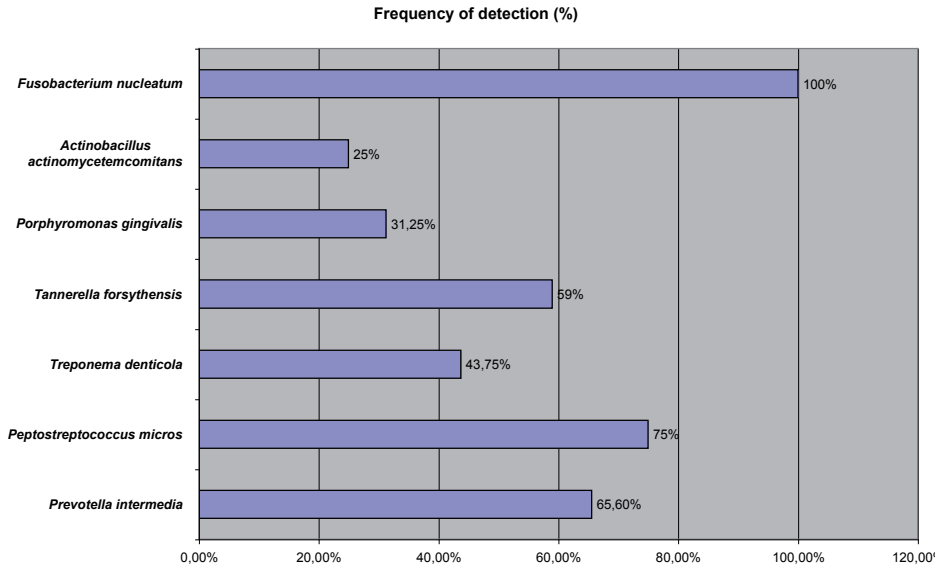
Fig. 2
Intraoral view in the patient with the fixed appliance in both dental arches with gingivitis

region, containing multiple highly variable parts, is amplified and labelled by PCR using a mixture of universal primers. The denatured product is then hybridised to a DNA-macroarray platform with immobilised specific probes spotted in duplicates. After high-stringency washing of non-hybridised probes the macroarrays are digitally evaluated. The test provides very reliable results because it uses three independent variable genomic regions for the detection of each pathogen. The diagnostic aim of this test is to semiquantitatively analyse the levels of the selected pathogens. A physician receives the result in a report containing the examined patient's data (name and surname, year of birth, date of sample collection, sample acceptance and processing, and sample number) and the identification data of the physician who performed the examination. The laboratory report contains a table with the results where the individual pathogens are marked as follows: (-) undetected, which corresponds to the number of bacteria less than 10^3, (+) slightly positive corresponding to the number of bacteria 10^3 to 10^4, (++) positive; this corresponds to the number of bacteria 10^4 to 10^5, (+++) and strongly positive, with the number of bacteria higher than 10^5. Subsequently, depending on the quantity of the pathogens determined and their mutual combinations, the test estimates the so-called total risk of resorption of the periodontal tissues as follows: without risk, low risk, medium, and high risk.

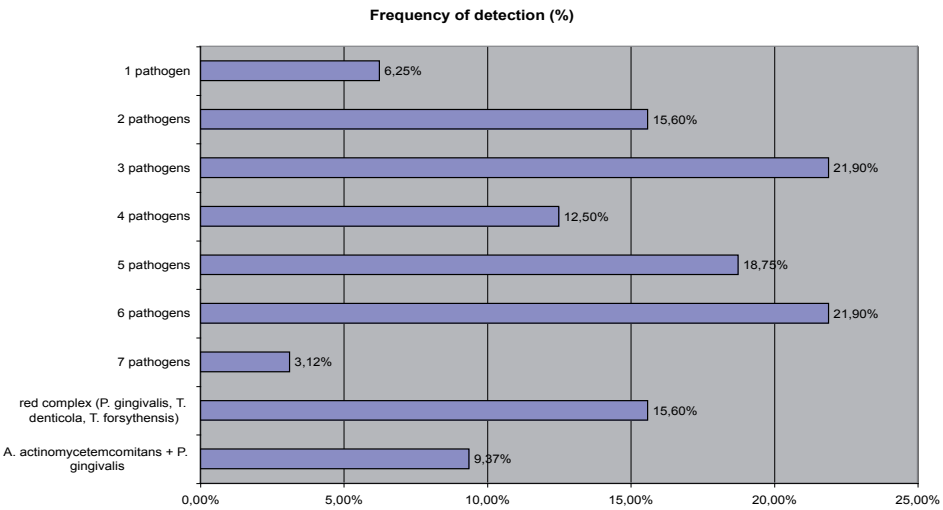
To find out whether the number of the pathogens detected correlates with some of the determined indexes (CPI or PBI), and considering the data distribution, we used nonparametric analysis using the Kruskal-Wallis ANOVA test.

RESULTS

The frequency of occurrence of the individual periodontal pathogens determined by the VariOr®Dento test is given in *Graph 1*. *Graph 2* shows the occurrence of combinations of the individual pathogens. Two and more pathogens were detected simultaneously in 93.75 % of the patients. The presence of all three pathogens of the so-called red complex (*Porphyromonas gingivalis, Treponema denticola, Tannerella forsythensis*) was recorded in 15.6 % of the patients. Simultaneous occurrence of *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* was detected in 9.37 % of the patients. *Table 2* shows the frequency of occurrence of *Actinobacillus actinomycetemcomitans*. This pathogen was not detected, i.e. the number of the bacteria present was lower than 10^3, in 75 % of the patients (53.1 % males and 21.9 % females). The finding in 21.9 % of the patients (9.4 % males and 12.5 % females) was slightly positive (number 10^3 to 10^4) and, in one male (3.1 %), positive (number 10^4 to 10^5). *Table 3* summarises the frequency of occurrence of *Porphyromonas gingivalis.* This pathogen was not detected in 68.75 % of the patients (56.25 % males and 12.5 % females); the finding in 18.75 % (6.25 % males and 12.5 % females) was slightly positive, in one male, aged 28.6 years, it was positive and in three females, aged 21–33 years, strongly positive. The number of *Porphyromonas gingivalis* bacteria in the sample correlated significantly ($p=0.01$) with the values of the CPI index (evaluated by the Kruskal-Wallis ANOVA test). The total risk of resorption of the periodontal tissues estimated by the VariOr®Dento test is shown in *Graph 3*.



Graph 1
Frequency of detection of individual periodontal pathogens determined with the VariOr-Dento test



Graph 2
Frequency of detection of combinations of periodontal pathogens determined with the VariOr-Dento test

Table 1
Typical microbiological finding in the clinical states (adopted after Listgarten, 1994)

Health	*Streptococcus sanguis* *Streptococcus mitis* *Veillonella parvula* *Actinomyces viscosus* *Rothia dentocariosa*
Gingivitis	*Actinomyces species* *Streptococcus species* *Veillonella species* *Treponema denticola* *Prevotella intermedia* *Fusobacterium nucleatum*
Adult periodontitis	*Treponema denticola* *Prevotella intermedia* *Porphyromonas gingivalis* *Tannerella forsythensis* *Fusobacterium nucleatum* *Peptostreptococcus micros* *Actinobacillus actinomycetemcomitans* *Campylobacter rectus*
Juvenile periodontitis	*Actinobacillus actinomycetemcomitans*
Rapidly progressive periodontitis	*Actinobacillus actinomycetemcomitans* *Porphyromonas gingivalis* *Tannerella forsythensis*
Refractory periodontitis	Actinobacillus actinomycetemcomitans Porphyromonas gingivalis Tannerella forsythensis Prevotella intermedia Peptostreptococcus micros Candida species

Table 2
Occurrence of *Actinobacillus actinomycetemcomitans*

	Males	Females
Undetected	17 (53.1 %)	7 (21.9 %)
Slightly positive	3 (9.4 %)	4 (12.5 %)
Positive	1 (3.1 %)	0
Strongly positive	0	0

Table 3
Occurrence of *Porphyromonas gingivalis*

	Males	Females
Undetected	18 (56.25 %)	4 (12.5 %)
Slightly positive	2 (6.25 %) (age 13–16 years)	4 (12.5 %) (age 13–19 years)
Positive	1 (3.1 %) (28.6 years)	0
Strongly positive	0	3 (9.4 %) (age 21–33 years)

DISCUSSION

The role of microorganisms in the aetiopathogenesis of individual periodontal diseases has been discussed in the literature for many years. First, *Actinobacillus actinomycetemcomitans* was found in localised aggressive juvenile periodontitis (*2, 3, 4*). Lately, it was also proved in the so-called adult forms of destructive periodontitis and was found to have a number of serotypes, some of them incurring in the healthy periodontium (*5*). *Zadeh et al*. (*6*) suggested that destruction of the periodontium induced by *Actinobacillus actinomycetemcomitans (A.a.)* is caused by the interaction between this pathogen and immune response of the host. Its presence may be considered a risk factor for the development of periodontopathies; therefore it is part of many tests including the VariOr®Dento test used for detection of periodontal pathogens in our study. A positive test implies a higher risk of the development of the aggressive form of the disease.

Porphyromonas gingivalis and *Tannerella forsythensis* (formerly *Bacteroides forsythus*) are further pathogens with proved significant association with destructive forms of periodontitis (*7*). *Mayanagi et al*. (*8*) detected the occurrence of 25 bacterial species in supragingival and subgingival plaque in patients with periodontitis and a healthy periodontium. The authors demonstrated that the occurrence of bacteria in supragingival and subgingival plaque is similar and supragingival plaque can thus serve as a reservoir for bacteria that can subsequently invade the subgingival spaces. The assessment of occurrence of the individual pathogens confirmed the presence of *Porphyromonas gingivalis* only in the periodontitis patients. *Prevotella intermedia* and *Treponema denticola* were detected more frequently in patients with periodontitis but they occurred in the healthy periodontium as well. *Fusobacterium nucleatum* was found in all of the subjects, both healthy and periodontitis patients. *Tannerella forsythensis* also occurred in healthy subjects. Therefore, the authors suggested that these two microorganisms represent an initiative (triggering), or supporting, factor

for the periodontitis development. In the set of our patients, we also demonstrated 100% occurrence of *Fusobacterium nucleatum*, which supports the hypothesis mentioned above.

Okada et al. (*9*) analysed the occurrence of *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* in a set of 104 children 2 to 12 years old. They proved that both the pathogens rarely occur in children with the healthy periodontium (*A. actinomycetemcomitans* in 4.8 %, *P. gingivalis* in 4.8 %). The frequency of the occurrence, however, increases in children with gingivitis (*A. actinomycetemcomitans* in 6.8 %, *P. gingivalis* in 9.6 %) and periodontitis (*A. actinomycetemcomitans* in 20 %, *P. gingivalis* in 20 %). Coincidence of both pathogens was observed in 2.9 % of children with inflammation of periodontal tissues (gingivitis or periodontitis). In the set of our patients, we observed the occurrence of *A. actinomycetemcomitans* in 25 %, *P. gingivalis* in 31.25 %, and coincidence of both pathogens in 9.37 %. We concluded that a higher frequency of pathogens studied in the examined set was related to the presence of the fixed orthodontic appliance and might also be affected by the age distribution of our set.

Only a few studies have dealt with the effect of a fixed orthodontic appliance on the oral bacterial flora. *Lee et al*. (*10*) reported a significantly higher occurrence of *Treponema denticola* and *Tannerella forsythensis* in adult patients with gingivitis and an attached fixed orthodontic appliance. There were no differences in the frequency of occurrence of *Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis*, and *Prevotella intermedia* compared to the control group of adult patients with gingivitis and without the orthodontic appliance. Conversely, *Paolantonio et al.* (*11*) proved that *Actinobacillus actinomycetemcomitans* colonised subgingival plaque only on teeth with an attached fixed orthodontic appliance. *Anhoury et al.* (*12*) studied whether the bacteria colonising the oral cavity preferred any particular kind of surface. They found out that *Treponema denticola, Actinobacillus actinomycetemcomitans*, and *Fusobacterium nucleatum* occurred on metal compared to ceramic orthodontic brackets in a significantly higher quantity. *Sallum et al.* (*13*) compared the frequency of the occurrence of periodontal pathogens in the final phase of curing with the fixed orthodontic appliance and after 30 days after the removal of the appliance and subsequent professional prophylactic and hygienic treatment. They determined a 50% reduction in the level of *Actinobacillus actinomycetemcomitans* and a significant reduction in the level of *Tannerella forsythensis.*

Periodontal pathogens are able to invade cells and survive in them. *Fusobacterium nucleatum* and *Tannerella forsythensis* are considered to be potential mucosal invaders. *Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis*, and *Prevotella intermedia* have the capacity to penetrate into the buccal epithelial cells in the oral cavity. The released buccal epithelial cells can participate in the transmission of periodontal pathogens among individual localities in the same individual or among several individuals (e.g. transmission between parent and child) (*14*). *Leung et al*. (*15*) reported that the capacity of bacteria (especially

Actinobacillus actinomycetemcomitans) to invade buccal epithelial cells increased after fixing the orthodontic appliance; a probable reason being physical damage of cells by individual components of the orthodontic appliance.

Recently, methods for the detection of periodontal pathogens have undergone great development. The former cultivation methods have been replaced with DNA techniques. Periodontal pathogens are prevailingly anaerobic microorganisms; therefore, the cultivation methods were demanding for collecting samples, their transport, and further processing in a specialised laboratory. These methods can only identify live microorganisms, and furthermore, some pathogens (e.g. *Treponema)* are non-culturable. The DNA techniques working with DNA of both live and "dead" pathogens have brought a complete change in the detection of microorganisms (*16, 17*). These methods are non-demanding in terms of sample collection and transport but their routine application in practice is limited by rather higher financial costs. In this study, we chose the VariOr®Dento test of the laboratory Gen-Trend České Budějovice for the detection of periodontal pathogens. Besides research purposes, the DNA tests have begun to be used, although not quite routinely, in dental surgeries for curing periodontal diseases and also before providing financially demanding prosthetic or implantological treatment.

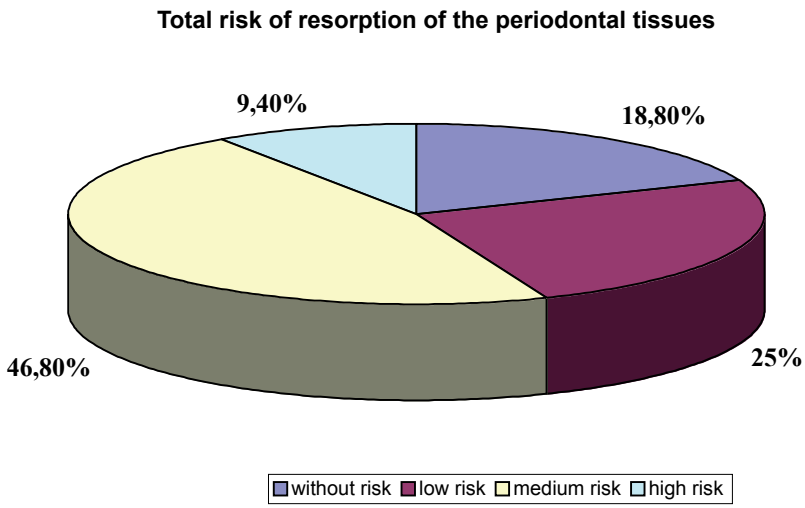
It is evident that periodontal pathogens play an important role in the aetiopathogenesis of periodontitis. Today, however, their role in the origin and development of general diseases, such as cardiovascular diseases, cerebral vascular diseases, and low birth weight in infants, has been considered more and more frequently (*18, 19, 20*). When performing various interventions in the oral cavity (e.g. in professional hygienic treatment), it is necessary to keep in mind that periodontal pathogens may penetrate into the patient's vessel system and their "systemic" operation. For this reason it is advisable to perform "risky" interventions in patients with positive tests, confirming the presence of pathogens, under antibiotic cover.

CONCLUSIONS

Periodontal pathogens represent a significant risk not only for the periodontal disease but also for the patient's general health. Orthodontic anomalies and their treatment with a fixed orthodontic appliance create favourable conditions for colonisation of subgingival plaque with periodontal pathogens and their following invasion into the cells. In this study 80 % of the patients treated with a fixed orthodontic appliance and with clinical manifestations of plaque-induced gingivitis are bearers of periodontal pathogens in a quantity significant for the damage of periodontium. To treat these patients successfully, collaboration with a periodontologist is highly recommended.

A c k n o w l e d g e m e n t s

The study follows the project of the Research Centre of Stomatology SVC No. 1M0528.



Graph 3
Total risk of resorption of the periodontal tissues determined with the VariOr-Dento test

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