



RELATIONSHIP OF SEVERAL RISK FACTORS IN PERIODONTITIS: SMOKING, GENDER, AGE AND MICROBIOLOGICAL FINDING

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ABSTRACT

Introduction: Literature data discuss the importance of some risk factors in conjunction with the periodontitis' severity and extent – age, gender, periodontal pathogens, smoking habits, diet, emotional stress, systemic diseases, osteoporosis, medication, and socio-economic status. The role of microorganisms in the initiation and progression of periodontal diseases is well documented at present. The presence of certain bacteria may be related with the elevated risk of periodontitis, as well as with the severity of the destructive process.

Aim: Evaluation of the risk factors: smoking, gender and age in relation to the bacterial finding in periodontitis.

Material and methods: In this study, 20 subjects with periodontitis were included. Important periodontal parameters were evaluated to confirm the diagnosis. Subgingival plaque samples were taken from periodontal sites with probing depth ≥ 6 mm. The presence of periodontal pathogens was assessed according to gender, age and smoking.

Results: In the current study, significant differences were found between the microbial parameters and the gender and age of the patients. Two pathogenic bacteria - *Treponema denticola* and *Prevotella intermedia*, showed significantly higher levels in the male gender – $p=0.05$ and $p=0.07$, respectively. Periodontal pathogens *Tannerella forsythia* and *Peptostreptococcus micros* were found in elevated levels in individuals older than 47 years.

Conclusion: No significant differences were found in the bacterial presence between smokers and non-smokers. Only *Treponema denticola* was found in elevated levels in smokers ($p=0.9$). The male gender may be associated with representatives of periodontal microbiota.

Keywords: gender, pathogens, periodontitis, smoking,

INTRODUCTION:

Periodontitis is initiated by the pathogenic subgingival bacteria that provoke host reactivity. In health, a balance between the primary etiologic agents – microorganisms) and the macroorganism's immune response. In periodontal disease, this homeostasis is destroyed because of many factors, including environmental risk factors: virulent capacities of subgingival microbiota, gender, age, genetic conditions, smoking, socio-economic status, diet, diabetes etc. [1-4]. In a result of the interaction of these different components of host homeostasis, destructive elements may dominate protective and thus determines the development and progression of periodontitis [3-6].

The impact of smoking on the composition of the subgingival microbiota in periodontitis conditions is unclear. Some studies showed higher levels of certain species in smokers, while other studies failed to detect differences in the microbiota between subjects with different smoking histories [7, 8].

Another risk factor that may influence periodontal disease is gender. The male gender has been associated with the severity of periodontitis and the presence of higher levels of periodontal pathogenic bacteria [1-3].

Literature data demonstrated that age is a risk factor in periodontitis which is related to the presence of attachment loss in the persisting periodontal disease and bacterial stimuli [1, 2, 6].

The **purpose** of the present investigation was to examine the prevalence, proportions and levels of the subgingival species in adult subjects with periodontitis in regard to smoking, age and gender conditions.

Subjects' selection: 20 patients with a diagnosis of moderate or severe generalized periodontitis – based on appropriate clinical and radiological periodontal examination. The information about the smoking history was obtained using a short questionnaire. Informed consent was signed by all participants in this study.

Inclusion criteria were: the presence of a minimum of 20 teeth, the presence of periodontal pockets with PPD ≥ 6 mm, patients without periodontal therapy for a period of 6 months, patients without systemic antimicrobial treatment in the last 6 months.

Exclusion criteria were: pregnant women, patients with systemic diseases and the presence of systemic medication.

The following **clinical parameters** were evaluated: Hygiene Index (HI); Bleeding on probing (BOP); Periodontal pocket depth (PPD); Clinical attachment loss (CAL), and bone loss (BL) as a part of the standardized periodontal examination. Clinical measures were taken at 6 sites per tooth at all teeth with graduated periodontal probe UNC-15 (HuFriedy®).

MICROBIOLOGICAL AND MOLECULAR METHODS:

Subgingival plaque samples were obtained from 20 patients with periodontitis. Subgingival plaque samples were taken from periodontal sites with probing pocket depth equal or more than 6 mm. After that, they were assayed individually for counts of 9 subgingival species (using PETtest – MIP Pharma, Real-time polymerase chain reaction (PCR) analysis). The samples were analyzed by qPCR using TaqMan probes and specific primers to determine the presence and count of *Aggregatibacter actinomycetemcomitans* (Aa), *Fusobacterium nucleatum* (Fn), *Peptostreptococcus / Micromonas/ micros* (Pm), *Porphyromonas gingivalis* (Pg), *Prevotella intermedia* (Pi), *Tannerella forsythia* (Tf), *Treponema denticola* (Td), *Eubacterium nodatum* (En), and *Capnocytophaga gingivalis* (Cg)

Statistical Analysis:

Data analysis was implemented by the statistical package IBM SPSS Statistics 19.0.

RESULTS:

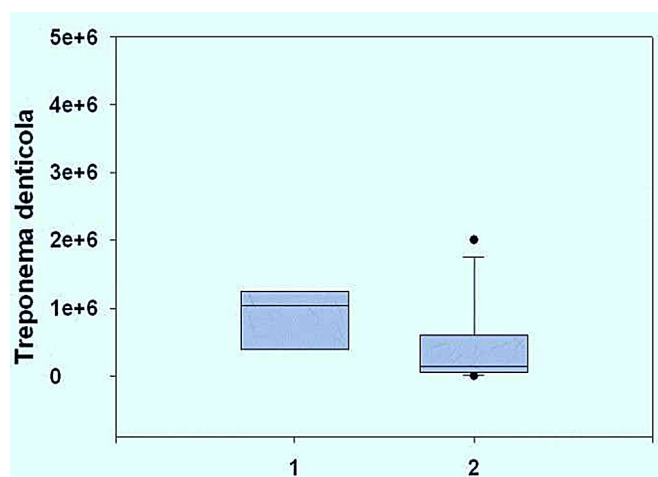
Twenty patients were included in the current study - 8 men and 12 women; 12 smokers and 8 non-smokers. The average age of the participants in the study was 47.55 ± 6.42 years.

Results related to received statistical differences

1. Significant differences depending on the gender of the patients

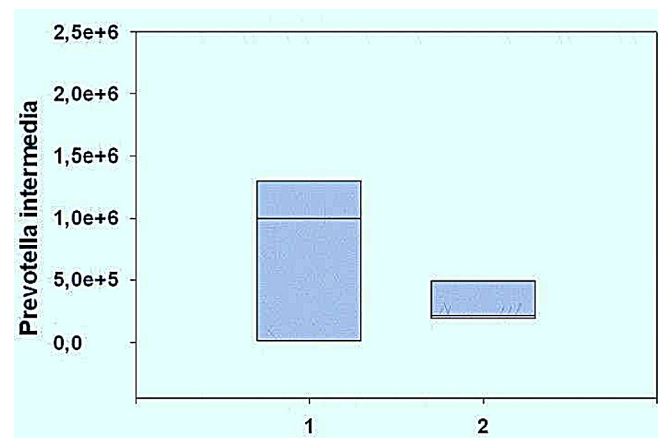
✓ The levels of *Treponema denticola* ($p = 0.05$) show significant differences depending on gender. In men, the levels of this bacteria are significantly higher than in women.

Fig. 1. *Treponemadenticola* and gender conditions (1 - men; 2 - women).



✓ The levels of *Prevotellaintermedia* ($p=0.07$) show significant differences depending on gender. In men, the levels of this bacteria are significantly higher than in women.

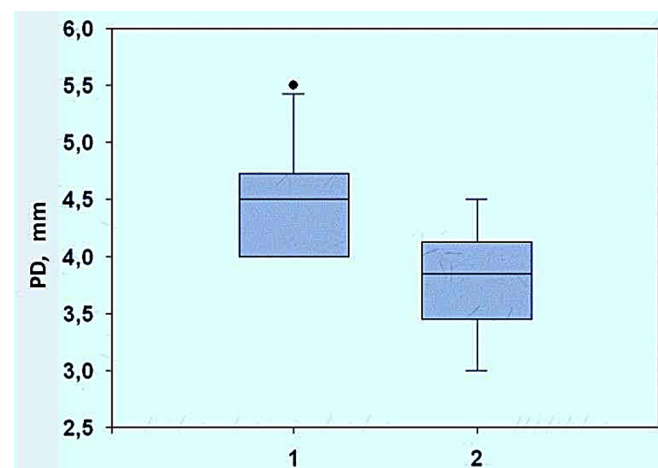
Fig. 2. *Prevotellaintermedia* and gender conditions (1 - men; 2 - women).



2. Significant differences depending on the smoking factor

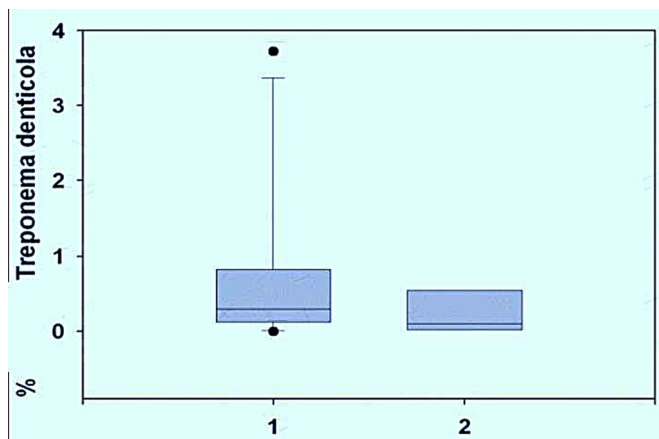
✓ Significant differences were found for the PD, and smokers showed significantly higher pocket depth values compared to non-smokers.

Fig. 3. PD and smoking (Axis-PD; 1 - smokers; 2 - non-smokers).



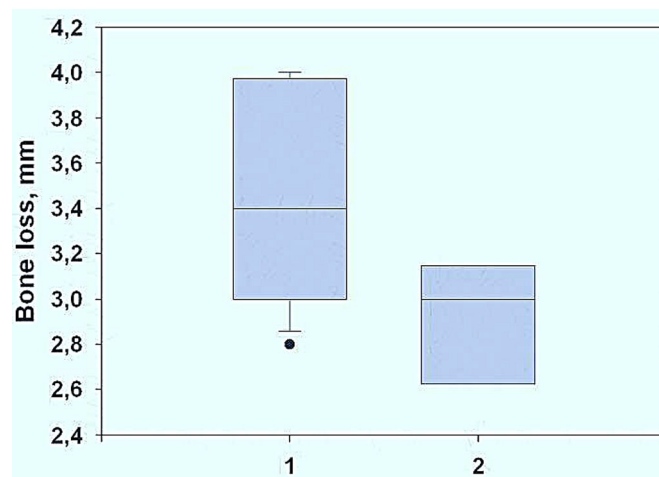
✓ Received outcomes showed only a tendency for differences between the smokers and non-smokers in relation to the prevalence of *Treponemadenticola* ($p=0,9$) in periodontal sites with $PDE \geq 6$ mm. However, there were higher levels of this pathogen in smokers.

Fig. 4. *Treponemadenticola* and smoking 1 - smokers; 2 - non-smokers).



✓ Received outcomes showed only a tendency for differences between the smokers and non-smokers in relation to bone loss ($p=0,9$) in periodontal sites with $PD \geq 6$ mm. Higher bone loss was observed in smokers compared to non-smokers.

Fig. 5. Bone loss and smoking (1 - smokers; 2 - non-smokers).



3. Significant differences depending on the age factor

In the current investigation, the patients were divided based on age criterion and using the median line in two groups: 1-st – underage of 47 years, 2-nd – over 47 years. We observed differences in the prevalence of both bacteria *Tannerella forsythia* è *Peptostreptococcus micros*. These pathogens showed higher levels in the second group of investigated subjects.

Fig. 6. *Tannerella forsythia* and age.

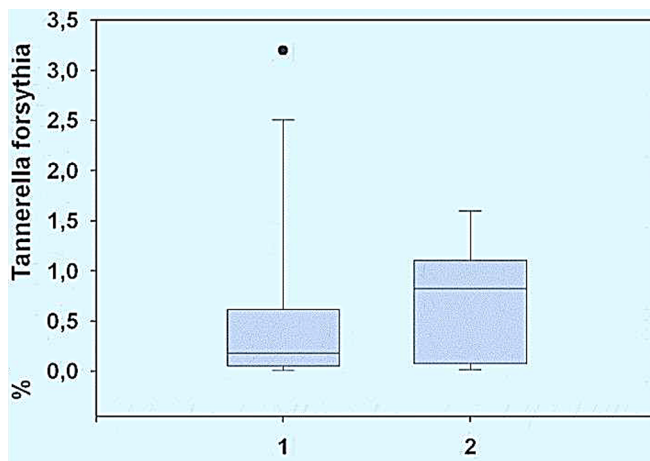
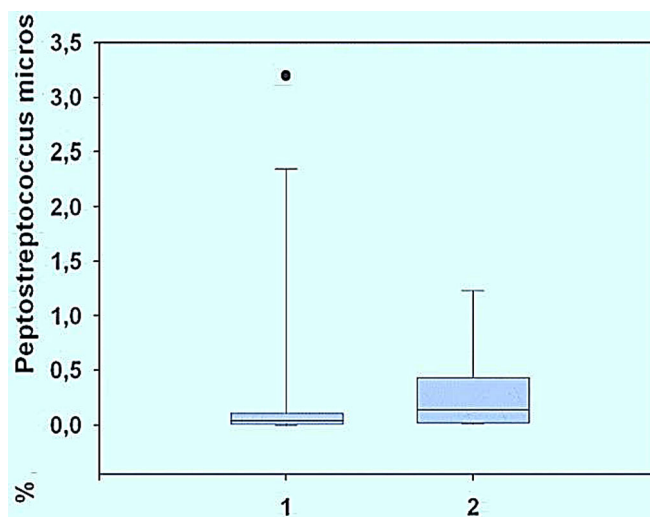


Fig. 7. *Peptostreptococcus micros* and age.



DISCUSSION:

In the present study, significant differences were found between the studied parameters depending on the gender of the patients. *Treponema denticola* and *Prevotella intermedia* showed significantly higher levels in the male gender. This can be explained by poor oral hygiene in men. Moreover, significant differences were found depending on the smoking factor. Smokers have a higher percentage of *Treponemadenticola* and a higher bone loss. These facts for smokers appeared to be due to greater microbial levels at pocket depths ≥ 6 mm, oral hygiene habits, and tissue changes affected by smoking as an important risk factor impacted in the pathogenesis of periodontitis [9-12].

Authors demonstrated that *E. nodatum*, *F. Nucleatum*, *P. intermedia*, *P. micros*, *P. nigrescens*, *T. forsythia*, *P. gingivalis* and *T. denticola* were significantly more prevalent in current smokers compared to non-smokers. Their study included 272 individuals, and they found that the presence of microbial species depend on smoking habit, age and gender ($p < 0.000001$) [10].

Although evaluating the influence of smoking was not a major objective of the present study, our findings in this regard are consistent with those of Tomita et al. (2013), and we did not detect significant differences in the profiles of the nine microbial species [13]. Our data showed similar characteristics between smokers and non-smokers, but these results should be interpreted with caution since, in larger series, other authors have found significantly higher prevalences of Td and Tf and significantly higher levels of Aa, Pg, and Tf in smokers with chronic periodontitis [14,15].

Authors evaluated that heavy smokers (over 20/day) had higher plaque levels during the maintenance phase of a periodontal treatment plan and the poorest response to treatment, but did not significantly differ from light smokers [16].

Grossi et al. (1997) investigated the effect of on patients' clinical and microbiological responses to mechanical therapy. They found that smokers had less healing and

reduction in subgingival *T. forsythia* and *P. gingivalis* after treatment compared with non-smokers. These authors concluded that smoking cessation might contribute restoration of the normal healing tissue response [17].

CONCLUSION:

No significant differences were found in the bacterial amount between smokers and non-smokers. With regard to pathogens, only *Treponemadenticola* was found in important value in smokers, but this may be interpreted as a tendency because of nonrepresentative status. In the current investigation, significant differences were found in conjunction with the subjects' sex. Both periodontal pathogens *Treponemadenticola* and *Prevotellaintermedia* were elevated significantly in men. Our research has some limitations. This was probably due to the small sample size (only 20 periodontal patients). However, further studies with a larger sample size are needed to achieve more validate data.

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