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The Distribution of *Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponema denticola* and *Aggregatibacter actinomycetemcomitans* in Patients with Alcoholic Disease: A Pilot Study*

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article

Abstract

Background. Both drinking and periodontal disease are serious health and social problems. Findings on the effect of alcohol consumption on periodontal disease are inconclusive.

Objectives. The aim of this study was to evaluate, in patients with alcoholic disease, the composition of the main periopathogens: *Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponema denticola* and *Aggregatibacter actinomycetemcomitans*.

Material and Methods. The study was conducted on 25 alcoholics from the Department of Alcohol Addiction Closed Treatment and 25 non-alcoholic patients from the Department of Periodontology, Wrocław Medical University. Subgingival biofilm samples were obtained from the 4 deepest sites (≥ 4 mm). The presence of 4 bacterial taxa was analysed using the PCR technique.

Results. The prevalence of bacterial species was significantly different between groups. Alcoholics showed significantly higher mean DNA counts for *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis* and *Treponema denticola*. In the qualitative analysis, no difference was observed between the groups. The study showed no statistically significant association between the amount of alcohol consumed and the composition of subgingival flora in patients suffering from alcoholism.

Conclusions. Alcoholics demonstrated the presence of pathogenic bacteria in similar amounts to people diagnosed with chronic periodontal disease, but showed significantly higher mean DNA counts for *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis* and *Treponema denticola* but there is no correlation between the amount of alcohol consumption and the level of periopathogens (Adv Clin Exp Med 2016, 25, 2, 243–248).

Key words: risk factors, periodontal disease, alcoholism.

Both drinking and periodontal disease are serious health and social problems. According to the World Health Organization, alcohol is the third highest risk factor for the health of a population, and over 60 types of diseases and injuries are associated with its consumption [1]. There are direct and indirect toxic damages related to alcohol consumption, such as alcohol liver disease (ALD), coronary heart diseases, gastrointestinal diseases, diabetes and neuropsychiatric diseases [2, 3]. The available data shows that about 23 million Europeans are depen-

dent on alcohol [1]. In Poland, about 4.5 million people abuse alcohol, and among these 600–900 thousand are dependent and require treatment.

Periodontal disease is a multifactorial disease modulated by environmental, behavioral and genetic factors. Periodontitis is an oral polymicrobial disease caused by the coordinated action of a complex microbial community, which results in inflammation and destruction of the periodontal tissues in susceptible hosts [4]. *Porphyromonas gingivalis*, *Treponema denticola*, *Tannerella forsythia* and

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Agreggatibacter actinomycetemcomitans are recognized as the most important pathogens in periodontal disease. These bacteria are usually found together in periodontal pockets and it is suggested that they may cause destruction of the periodontal tissue in a cooperative manner [5–7]. On the other hand, different immunological aspects are suspected to play roles in the development and progression of periodontal diseases [8].

Alcoholism is related to harmful effects on host response. Alcohol consumption may have a significant impact on the homeostasis between periodontal bacteria and host response [9, 10]. Numerous studies have investigated the association between alcohol consumption and periodontal disease but this relationship is still unclear [11, 12]. However, not many studies have examined the distribution of periopathogens and its relationship with periodontal disease development in alcoholic patients. The objective of the present study was to evaluate the microbiological status of periodontal tissues in patients suffering from alcoholism and to examine the relationship between the level of alcohol consumption and periodontal pathogens.

Material and Methods

The study included 25 patients (5 female, 20 male) with alcohol dependence (ChA-alcoholics). Subjects were hospitalized in the Alcohol Addiction Closed Treatment Department of the Specialized Mental Health Care Hospital in Wrocław for a 10-day detoxification period. The control group (non-alcoholic – CP) included 25 patients (10 female, 15 male) from the Department of Periodontology at Wrocław Medical University, who did not have any alcohol problems in their medical history and had not been submitted to antibiotic medication or periodontal therapy in the previous 3 months. In both groups, subjects were diagnosed with generalized chronic periodontitis (> 30% of sites with a clinical attachment level ≥ 3 mm and a probing depth ≥ 4 mm).

The age of participants ranged from 21 to 73 years among alcoholics and from 38 to 67 years in the control group. All subjects were informed about the nature of the study and gave written consent to participate. The study protocol was approved by the Bioethics Commission at Wrocław Medical University, Poland.

A questionnaire was used to collect information on individual-alcohol consumption in milliliters per day with differentiation in terms of the type of beverage. The study assessed alcohol consumption on the basis of the amount of grams of ethanol per day.

The periodontal examination of all the subjects was performed by a single researcher (A.S-J). The following periodontal measurements were recorded at 4 sites (at the disto-, mid-, and mesio-buccal as well as the mid-lingual positions) on all teeth except the third molars: the number of teeth, probing depth (PD), clinical attachment loss (CAL), modified sulcus bleeding index (mSBI), and the presence or absence of dental plaque in the interdental spaces (API – approximal plaque index).

The subgingival biofilm samples were obtained from 4 deepest sites selected during the clinical examination. In both groups, sample pockets were ≥ 4 mm deep in conjunction with an attachment level ≥ 3 mm. The samples were taken using sterile paper points which were placed in the periodontal pocket for 30 sec and then were placed in individual tubes. The presence and level of periopathogenic bacteria were determined by polymerase chain reaction (PCR) in the Department of Molecular Techniques, Wrocław Medical University. Bacterial DNA was isolated using a QIAamp® DNA Mini Kit (Qiagen) [13, 14]. *Agreggatibacter actinomycetemcomitans* and *Treponema denticola* were determined by a single-stage PCR and *Porphyromonas gingivalis* and *Tannerella forsythia* were determined using Nested PCR.

The primer pairs for *Agreggatibacter actinomycetemcomitans* were:

F 5'AAACCCATCTCTGAGTTCTTCTTC3' and R 5'ATGCCAACTTGACGTAAAT3'; for *Treponema denticola*: F 5'TAATACCGAATGTGCTCATTACAC3' and

R 5'TCAAAGAAGCATTCCCTCTTCTTCTTA3'. The primer pairs in the first PCR for *Porphyromonas gingivalis* and *Tannerella forsythia* were: 5'AGAGTTTGATCMTGGCTCAG 3' and 5'TACGGYTACCTTGTTACGACTT3'. The primer pairs in the second PCR for *Porphyromonas gingivalis* were: F 5'TGTAGATGACTGATGGTGAAC3' and R 5'ACGTCATCCCCACCTTCCTC3' and for *Tannerella forsythia*: F 5'TACAGGGGAATAAAATGAGATACT3' and R 5'ACGTCATCCCCACCTTCCTC3'.

The reaction was assured by Qiagen PCR Master Mix® (Qiagen) and carried out using the GeneAmp PCR System 9700 (Applied Biosystems). The PCR products were detected by agarose gel electrophoresis stained with ethidium bromide. Quantitative analysis was carried out using the ABI PRISM 7900HT Fast Real-Time PCR System (Applied Biosystems) with Brilliant III Ultra-Fast SYBR QPCR MasterMix (Agilent Technologies). Special pairs of primers were designed. For quantification, a standard curve method was applied together with the quantification analysis proposed by Satio et al. [13].

Statistical analysis was performed using the statistical software package EPIINFO v. 3.5.2 (dated 17-12-2010). Verification of the hypothesis of equal sized groups of parameters was performed by the non-parametrical Kruskal-Wallis signed rank test (because the number of cases was too small to use parametrical tests). Statistical significance between frequencies was calculated by χ^2 test with Yate's correction or if expected value was less than 5, by Fisher exact test. Person's correlation coefficient was calculated in order to examine possible correlations between the investigated variables (r). Results were considered statistically significant at $p \leq 0.05$.

Results

Qualitative Analysis

A positive result for the presence of *A. actinomycetemcomitans* was achieved in 44% of patients with alcohol disease and in 48% of subjects in the control group. In the case of *T. forsythia* and *T. denticola* the study showed similar results, 100% and 76% in ChA group and 92% and 88% in CP group, respectively. *P. gingivalis* was present in 76% of samples from patients with alcohol dependence and 56% of patients with chronic periodontitis. For all periopathogens, there were no statistically signif-

icant differences between the groups. The results of the qualitative analysis of the selected periopathogens of patients with alcohol dependence and chronic periodontitis are shown in Table 1.

Quantitative Analysis

Alcoholics showed significantly higher mean DNA counts for *A. actinomycetemcomitans*, *Porphyromonas gingivalis* and *Treponema denticola*. The results of the quantitative analysis are presented in Table 2.

The level of alcohol consumption in alcoholics is shown in Table 3. There were no correlations between the level of alcohol consumption and the level of periopathogens in periodontal pockets in alcoholics (Table 4).

Discussion

Long-term alcohol consumption may impair systemic health and it is also a potential risk factor for oral infections. The direct effects of alcohol on the oral cavity are related to caries, periodontal diseases and oral and oropharynx cancers [15–18]. Numerous studies have shown that alcoholic disease negatively affects periodontal tissues. Associations have been suggested between alcohol consumption and dental health, particularly poor oral

Table 1. Qualitative analysis of periopathogens of alcoholic (ChA) and non-alcoholic (CP) subjects

Periopathogens	Non-alcoholics (N = 25)	Alcoholics (N = 25)	p-value
<i>A. actinomycetemcomitans</i> ±	13/12	14/11	0.544
<i>T. forsythia</i> ±	2/23	0/25	0.490F
<i>T. denticola</i> ±	6/19	3/22	0.463F
<i>P. gingivalis</i> ±	6/19	11/14	0.232

F – Fisher test.

Table 2. Quantitative analysis of periopathogens in alcoholic (ChA) and non-alcoholic subjects (CP)

DNA concentration	<i>A. actinomycetemcomitans</i>		<i>T. forsythia</i>		<i>T. denticola</i>		<i>P. gingivalis</i>	
	CP	ChA	CP	ChA	CP	ChA	CP	ChA
N – number	16	11	24	25	21	23	20	17
X – mean counts	1.51 E-07	5.35 E-06	8.29 E-03	4.69 E-03	4.65 E-06	1.55 E-05	4.81 E-05	3.50 E-04
SD – standard deviation	2.99 E-07	9.39 E-06	1.93 E-02	7.32 E-03	8.86 E-06	3.05 E-05	9.23 E-05	4.89 E-04
P-values	0.0002*		0.435		0.0194		0.0189*	

* The Kruskal-Wallis one-way analysis of variance by ranks.

Table 3. The level of alcohol consumption among alcoholics

Parameters	N	X	SD
Duration of alcohol disease (years)	25	12.8	11.8
Beer (mL/day)	19	2710.5	1575.1
Ethanol (g/day)	19	108.4	63.0
Vodka (mL/day)	20	712.5	550.1
Ethanol (g/day)	20	237.5	183.4
Wine (mL/day)	1		
Ethanol (g/day)	1		
Total amount of ethanol (g/day)	25	276.4	205.0

X – mean; N – number; SD – standard deviation.

Table 4. Correlation between total level of alcohol (g/day) consumption and periopathogens in alcoholics (ChA)

The concentration of DNA	The total amount of ethanol (g/day)		
	no. of patients	r – Pearson's correlation coefficient	p-value
<i>A. actinomycetemcomitans</i>	11	0.31	0.350
<i>T. forsythia</i>	25	–0.32	0.115
<i>T. denticola</i>	23	–0.26	0.224
<i>P. gingivalis</i>	17	0.10	0.711

hygiene [19, 20]. Some studies have evaluated the effects of drinking on the clinical periodontal condition [15–17]. Authors have reported relationships between alcohol consumption and probing depth (PD) and clinical attachment loss (CAL) [18, 21–23]. There are also studies which have reported that the education of alcohol-dependent individuals may contribute to their attention to dental health and desire to change their habits [19, 24]. Alcoholics tend to also smoke tobacco that increases their risk for periodontal disease.

Numerous studies showed associations between different periopathogens, the immune host response and chronic periodontitis [25]. Present of periopathogens: *A. actinomycetemcomitans*, *P. gingivalis*, *T. forsythia* and *T. denticola* are considered to be the main periodontal pathogens and strongly associated to the clinical status of periodontal tissues and progression of the periodontal disease [26, 27]. Meanwhile, only a few studies have investigated the association between alcohol consumption and subgingival bacteria. Tezal et al. used the immunofluorescence technique to show that heavy drink-

ers had a higher concentration of *T. forsythia* and *P. gingivalis* than patients in the control group [18]. In our study, alcoholics showed significantly higher mean DNA counts for *A. actinomycetemcomitans* ($p = 0.0002$), *P. gingivalis* ($p = 0.0189$) and *T. denticola* ($p = 0.0194$). There was no statistically significant difference between the groups with regard to *T. forsythia*. Using the PCR technique, Amaral et al. showed no statistically significant difference in the subgingival microflora of alcoholics and non-alcoholics, although they obtained a slightly higher average number of *A. actinomycetemcomitans*, *P. gingivalis* and *F. nucleatum* in the material of the pockets of people with alcohol dependence. In the case of shallow pockets (< 4 mm) in patients with alcoholism, they achieved a higher average number of *P. gingivalis*, *T. forsythia* and *F. nucleatum*. Similar correlations were shown in the deep pockets (≥ 4 mm). The surprising finding was that people who abuse alcohol had a higher concentration of *P. gingivalis*, *F. nucleatum* and *T. forsythia* in shallow pockets vs. the deep periodontal pockets of non-drinkers [28]. Also findings from the Lages et al. study showed that alcohol-dependent users have worst periodontal status and higher levels of *P. intermedia*, *E. corrodens* and *F. nucleatum* and demonstrated the negative influence of alcohol consumption on periodontal and microbiological parameters [29].

The limitations of this study should be considered. Periodontitis is a multifactorial disease. Meanwhile, most alcoholics are smokers, which increases the risk of developing periodontal disease [30]. Moreover, alcoholics may have poor oral care, abnormalities in immune system, which might be also a potential risk factors for periodontal disease.

Therefore, there is a need for further studies including larger populations to investigate both alcohol consumption measured at different points in time and long-term alcohol consumption and periodontitis progression over time. There is also a need for studies including a group of healthy volunteers to compare the periodontal flora with the group of alcoholics. Because of the size and complexity of the problem, periodontal prevention programs focusing on oral health behavior should be mandatory.

The authors concluded that alcoholics demonstrated the presence of pathogenic bacteria in similar amounts to people diagnosed with chronic periodontal disease and showed significantly higher mean DNA counts for *A. actinomycetemcomitans*, *P. gingivalis* and *T. denticola*. Because of the potential negative influence of alcohol consumption on periodontal and microbiological parameters, there is still need for further studies to corroborate the results.

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