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Prevalence of periodontal pathogenic bacteria at different oral sites of patients with tongue piercing — results of a cross sectional study☆,☆☆

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ABSTRACT

Aim of this cross-sectional study was to investigate the prevalence of selected potentially periodontal pathogenic bacteria in different sites of patients with tongue piercing (TP) in comparison to a control group (C).
Fifty participants in each group were recruited. Samples from the biofilm originating from the piercing surface (TP group), periodontal pocket, tongue as well as cheek surface were examined regarding presence of 11 selected potentially periodontal pathogenic bacteria based on polymerase-chain reaction (PCR).
In the periodontal pocket of the participants, the majority of examined bacteria were more frequently detected in TP compared to C group ($p_i < 0.05$). At tongue and cheek surface, the prevalence of *Treponema denticola* ($P < 0.01$) and *Prevotella intermedia* ($P < 0.01$) was significantly higher in TP. For the majority of bacteria, a significant correlation between TP surface and periodontal pocket was detected ($P < 0.05$).
In conclusion TP must be considered as potentially important ecological niche and reservoir for periodontal pathogens.

1. Introduction

The oral cavity is an important habitat for a huge variety of bacteria including about 700 different predominant species (Dewhirst et al. 2010; Krishnan et al. 2017). These bacteria, summarized within the oral microbiome, are highly associated to oral health and disease (Krishnan et al. 2017, Costalonga et al. 2014, Zhang et al. 2018). Thereby, the colonization of the oral cavity is characterized by significantly different ecological niches like tooth surface, periodontal pocket or tongue which harbor distinct microbial communities (Zhang et al. 2018). Besides these physiological niches, several unnatural opportunities for bacterial colonization can exist. One potential artificial niche could be an intraoral piercing, of which tongue piercings represent the most prevalent form (Hennequin-Hoenderdos et al. 2012). This oral body art can be associated to different oral complications, including tooth decay, gingival recession as well as periodontal disease progression (Tomaževič et al. 2017, Plastargias et al. 2014, Hennequin-Hoenderdos et al. 2016). While the occurrence of hard and soft tissue trauma might be explained by mechanical reasons, the association to periodontal disease severity could be more complex.

Periodontitis is an inflammatory disease of multifactorial origin, in which a dysbiosis of oral microbiota play an important role (Kinane et al. 2017). This bacterial imbalance in favor of potentially periodontal pathogenic bacteria can be supported by different factors and environmental influences (Kinane et al. 2017). In this context, the presence of a tongue piercing as a possible habitat for potentially pathogenic bacteria could be of relevance. Accordingly, a few studies are available, which investigated microbiological parameters at tongue piercings (Borges et al. 2016; Kapferer et al. 2011; Lupi and Zaffe 2010; Ziebolz et al. 2009). One main issue of these examinations was the influence of piercing material on microbiological colonization (Borges et al. 2016; Kapferer et al. 2011). Only 1 previous study investigated the occurrence of common potentially periodontal pathogenic bacteria at tongue piercing in a small group of participants (Ziebolz et al. 2009). However, there is up until now no knowledge whether the colonization of the tongue piercing with periodontal pathogens would affect the prevalence of these bacteria in other different oral niches, especially within the periodontal pocket.

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Accordingly, the current cross-sectional study was performed to detect the prevalence of selected potentially periodontal pathogenic bacteria at the tongue piercing, periodontal pocket, tongue as well as cheek surface of pierced individuals and a control group without oral piercing. Moreover, correlations between the tongue piercing and the periodontal pocket should be detected in pierced participants. It was hypothesized that tongue piercings would be colonized with selected potentially periodontal pathogenic bacteria. Furthermore, these bacterial findings would be associated to the bacterial composition within the periodontal pocket.

2. Methods

This current study was part of a clinical cross sectional study, which aimed in a comprehensive examination of adults wearing tongue piercing. The clinical oral health data (part one of the cross-sectional study) have already been presented previously (Ziebolz et al. 2019). This separate part (part 2 of the cross-sectional study), which will be presented here was executed to investigate the prevalence of selected potentially pathogenic bacteria at different oral areas (piercing surface, tongue, cheek, periodontal pocket) from participants with tongue piercing. The examination has been reviewed and approved by the Ethics Committee of the University Medical Center in Goettingen, Germany (No 2/3/14).

2.1. Patients

Between 01.10.2013 and 31.12.2014, patients were recruited from two different dental practices: a) Zahnärztliche Gemeinschaftspraxis Söder (Reinfeld, Germany) and b) Zahnarztpraxis Streib & Kollegen (Obersulm-Affaltrach, Germany). All patients who visited 1 of these practices during the study period were screened for the presence of a tongue piercing. Patients suffering from such a piercing were asked for their voluntary participation. After comprehensive information about the study aim and course, performed verbally and in writing, patients provided written informed consent and received an appointment for examination. Mandatory requirements for participation were the minimum age of 18 years, as well as the presence of a tongue piercing. Furthermore, several exclusion criteria were formulated as follows: age below 18 years, impossibility to undergo microbiological examination due to worse general health, immunosuppression/immunosuppressive medication, presence of infectious diseases (e.g. tuberculosis, HIV), neurological/seizure disorders. Additionally, pregnant and lactating patients were excluded. Furthermore, the intake of any antibiotic drugs in the previous 6 months was an exclusion criterion for participation.

A matched (age, gender, smoking habits) control group without oral piercing has been recruited for comparison out of patients from the 2 dental practices. The exclusion criteria listed above were equal for both groups.

2.2. Microbiological examination

For the analysis of 11 different selected potentially periodontal pathogenic bacteria, biofilm samples were collected using sterile paper points at the deepest periodontal pockets, tongue piercing, tongue surface and cheek surface of the participants. Two paper points each area were applied. Thereby, paper points were stroked at the piercing surface, the surfaces of the tongue as well as at the cheek for 10 s. Furthermore, after removing of the supragingival biofilm from the tooth surface and draining of the area using cotton rolls, paper points were placed in deepest periodontal pocket fundi for 10 s. The samples of each area from 1 patient were placed in a transportation tube.

The microbiological analysis of selected potentially periodontal bacteria was executed using polymerase chain reaction (PCR) at the laboratory of the Dept. of Preventive Dentistry, Periodontology and Cariology, University Medical Centre Goettingen, Germany. The DNA isolation was performed using QIAamp DNA Mini Kits® (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Immediately after DNA isolation, the samples were stored at −20 °C until further processing. The commercial test kit Micro-IDent® plus (Hain Lifescience, Nehren, Germany) was used for the qualitative and semiquantitative analysis of the selected bacteria according to manufacturer's instructions. Thereby, amplification was executed using a 35-µl mixture of primers and dNTPs (Hain Lifescience, Nehren, Germany), 10.5 µl Mastermix (Qiagen, Hilden, Germany) as well as 5 µl of the DNA sample or 5 µl of water as a negative control, respectively. Amplification cycles were performed in a thermic cycler (Biometra, Goettingen, Germany). Initially, 1 cycle was conducted for 5 min at 95 °C; subsequently 10 cycles of 30 s at 95 °C and 2 min at 58 °C followed by 20 cycles of 25 s at 95 °C, 40 s at 53 °C and 40 s at 70 °C and a final cycle of 8 min at 70 °C were performed. Afterwards, samples underwent a denaturation as well as a biotinylation step. For hybridization, samples were incubated with buffer and probes containing membrane strips in a TwinCubator at 45 °C (Hain Lifescience, Nehren, Germany). Specific washing steps were performed for the removal of non-specifically bound DNA. Each membrane strip was incubated with a streptavidin-alkaline phosphatase complex containing conjugate, followed by an intensive washing step. The reaction between alkaline phosphatase and the substrate on the membrane strips with bound amplification product was displayed as color reactions and recorded accordingly. The applied membrane strips contained 2 lines as conjugate control and as amplification control, respectively. Based on the resulting color reaction, the qualitative semi-quantitative detection of the following 11 potentially periodontal pathogenic bacteria was possible: (detection threshold $>10^2$): *Aggregatibacter actinomycetemcomitans (Aa)*, (detection threshold $>10^3$): *Porphyromonas gingivalis (Pg), Tannerella forsythia (Tf), Treponema denticola (Td), Prevotella intermedia (Pi), Parvimonas micra (Pm), Fusobacterium nucleatum (Fn), Campylobacter rectus (Cr), Eubacterium nodatum (En), Eikenella corrodens* (*Ec) and Capnocytophaga* spp. *(Cs).*

2.3. Statistical analysis

All statistical tests were performed using SPSS for Windows, Version 22.0 (SPSS Inc., U.S.A.). The categorical data were analyzed with chi-square and Fisher test, respectively. The correlation of 2 variables was performed with Spearman's rho test. The significance level was set at $P < 0.05$.

3. Results

3.1. Patients

A total of 50 patients with a mean age of 28.3 ± 7.1 (TP) and 28.2 ± 7.1 (C; $P = 0.97$) were included in the current examination. The distribution of gender (80% female, 10% male participants) and smoking habits (82% smokers) was equal between TP and C group ($P = 0.99$). The dental and periodontal findings, as well as specific piercing related analyses, have been presented in the previous publication (Ziebolz et al. 2019).

3.2. Prevalence of bacteria at piercing surface

Table 1 shows the prevalence and concentration of the selected potentially periodontal pathogenic bacteria at the piercing surface of TP group. Thereby, *Fn* (96%), *Cs* (60%), *Td* (48%) and *Ec* (46%) were the most frequently detected bacteria at piercing surface.

3.3. Comparison of TP and C

In the periodontal pocket of the participants, the majority of examined bacteria including *Pg* ($P = 0.05$), *Tf* ($P = 0.04$), *Td, Pi, Pm, Fn, Cr* (pi<0.01), as well as *En* ($P = 0.02$) were more frequently detected in

Table 1
Concentration of periodontal pathogenic bacteria at tongue piercing.

Concentrations	Under detection limit	Over detection limit				Prevalence
	$<10^3$	10^3	$<10^4$	$<10^5$	$>10^6$	
Aa	47 (94%)	3 (6%)	0	0	0	6%
Concentrations	$<10^4$	10^4	$<10^5$	$<10^6$	$>10^7$	
Pg	41 (82%)	1 (2%)	5 (10%)	2 (4%)	1 (2%)	18%
Tf	46 (92%)	3 (6%)	1 (2%)	0	0	8%
Td	26 (52%)	11 (22%)	11 (22%)	2 (4%)	0	48%
Pi	38 (76%)	4 (8%)	8 (16%)	0	0	24%
Pm	39 (78%)	3 (6%)	5 (10%)	3 (6%)	0	22%
Fn	2 (4%)	5 (10%)	13 (26%)	20 (40%)	10 (20%)	96%
Cr	43 (86%)	6 (12%)	1 (2%)	0	0	14%
En	50 (100%)	0	0	0	0	0%
Ec	27 (54%)	7 (14%)	8 (16%)	8 (16%)	0	46%
Cs	20 (40%)	8 (16%)	12 (24%)	3 (6%)	7 (14%)	60%

Aa = *Aggregatibacter actinomycetemcomitans* (detection threshold $>10^3$); *Pg* = *Porphyromonas gingivalis*, *Tf* = *Tannerella forsythia*; *Td* = *Treponema denticola*; *Pi* = *Prevotella intermedia*; *Pm* = *Parvimonas micra*; *Fn* = *Fusobacterium nucleatum*; *Cr* = *Campylobacter rectus*; *En* = *Eubacterium nodatum*; *Ec* = *Eikenella corrodens*; *Cs* = *Capnocytophaga* spp.

TP compared to C group (table 2). At the tongue surface, the prevalence of *Td* ($P < 0.01$), *Pi* ($P < 0.01$) and *Pm* ($P = 0.03$) was significantly higher in TP compared to C participants (table 3). At the area of cheek, significant differences were found for *Td* ($P < 0.01$), *Pi* ($P = 0.04$) and *Ec* ($P = 0.03$) between groups (table 4).

3.4. Correlation between bacterial findings of TP with periodontal pocket

For the majority of bacteria, a significant correlation between TP surface and periodontal pocket was detected ($P < 0.05$). Thereby, the strongest correlation between TP and periodontal pocket was found for *Aa* ($r = 0.875$, $P < 0.01$), followed by *Pg* ($r = 0.793$, $P < 0.01$). The further significant bacteria, including *Tf, Td, Pi* and *Cr*, just showed quite weak correlations below $r = 0.5$ (table 5).

4. Discussion

4.1. Summary of main results

It was previously hypothesized that TP would be colonized with selected potentially periodontal pathogenic bacteria and that there would be associations to the periodontal pocket. These hypotheses could be partly confirmed, whereby several examined potentially periodontal pathogenic bacteria showed a higher prevalence at tongue, periodontal pocket and cheek of patients wearing a TP compared to unpierced control. Especially *Td* and *Pi* were more prevalent in TP at all examined areas. From the selected bacteria, *Aa* and *Pg* showed the strongest correlation between TP surface and periodontal pocket.

4.2. Comparison with existing literature

The current study is the first clinical examination which investigated the occurrence of selected potentially periodontal pathogenic bacteria at different areas of the oral cavity of patients with and without TP. Especially, the examination of correlations between periodontal pocket and tongue piercing surface is a new approach. Accordingly, the body of literature for comparison of the findings is limited. A previous study was also able to detect potentially periodontal pathogenic bacteria at the surface of TP. Thereby, increased prevalence of these bacteria was found in all of the 12 examined participants (Ziebolz et al. 2009). The low sample size, the examination of only TP surface and no comparison to an unpierced control limit the comparability to the current study.

Table 2
Comparison of bacterial concentrations at the periodontal pocket between groups.

Concentrations		Under detection limit	Over detection limit				Prevalence	*P* value
		$<10^3$	10^3	$<10^4$	$<10^5$	$>10^6$		
Aa	TP	46 (92%)	0	1 (2%)	1 (2%)	2 (4%)	8%	0.80
	C	47 (94%)	0	1 (2%)	0	2 (4%)	6%	
Concentrations		$<10^4$	10^4	$<10^5$	$<10^6$	$>10^7$		
Pg	TP	37 (74%)	2 (4%)	3 (6%)	2 (4%)	6 (12%)	26%	**0.05**
	C	46 (92%)	0	0	3 (6%)	1 (2%)	8%	
Tf	TP	38 (76%)	4 (8%)	4 (8%)	3 (6%)	1 (2%)	24%	**0.04**
	C	48 (96%)	1 (2%)	0	0	1 (2%)	4%	
Td	TP	9 (18%)	1 (2%)	3 (6%)	10 (20%)	27 (54%)	82%	**<0.01**
	C	33 (66%)	1 (2%)	2 (4%)	10 (20%)	4 (8%)	34%	
Pi	TP	17 (34%)	2 (4%)	6 (12%)	16 (32%)	9 (18%)	66%	**<0.01**
	C	36 (72%)	2 (4%)	3 (6%)	8 (16%)	1 (2%)	28%	
Pm	TP	6 (12%)	5 (10%)	14 (28%)	16 (32%)	9 (18%)	88%	**<0.01**
	C	20 (40%)	9 (18%)	16 (32%)	5 (10%)	0	60%	
Fn	TP	1 (2%)	0	3 (6%)	21 (42%)	25 (50%)	98%	**<0.01**
	C	1 (2%)	3 (6%)	9 (18%)	31 (62%)	6 (12%)	98%	
Cr	TP	19 (38%)	0	4 (8%)	12 (24%)	15 (20%)	62%	**<0.01**
	C	40 (80%)	1 (2%)	3 (6%)	2 (4%)	4 (8%)	20%	
En	TP	31 (62%)	6 (12%)	10 (20%)	2 (4%)	1 (2%)	38%	**0.02**
	C	45 (90%)	1 (2%)	3 (6%)	1 (2%)	0	10%	
Ec	TP	18 (36%)	2 (4%)	12 (24%)	18 (26%)	0	64%	0.11
	C	17 (34%)	4 (8%)	20 (40%)	8 (16%)	1 (2%)	66%	
Cs	TP	13 (26%)	10 (20%)	16 (32%)	8 (16%)	3 (6%)	74%	0.12
	C	14 (28%)	8 (16%)	7 (14%)	13 (26%)	8 (16%)	72%	

Significant values ($P < 0.05$) are highlighted in bold].
TP = tongue piercing; C = control.

Table 3
Comparison of bacterial concentrations at the tongue between groups.

Concentrations		Under detection limit	Over detection limit				Prevalence	*P* value
		$<10^3$	10^3	$<10^4$	$<10^5$	$>10^6$		
Aa	TP	47 (94%)	0	0	2 (4%)	1 (2%)	6%	0.39
	C	48 (96%)	0	1 (2%)	0	1 (2%)	4%	
Concentrations		$<10^4$	10^4	$<10^5$	$<10^6$	$>10^7$		
Pg	TP	37 (74%)	1 (2%)	4 (8%)	3 (6%)	5 (10%)	26%	0.11
	C	45 (90%)	0	2 (4%)	3 (6%)	0	10%	
Tf	TP	42 (84%)	4 (8%)	2 (4%)	1 (2%)	1 (2%)	26%	0.40
	C	47 (94%)	1 (2%)	2 (4%)	0	0	6%	
Td	TP	8 (16%)	11 (22%)	18 (36%)	12 (34%)	1 (2%)	84%	**<0.01**
	C	28 (56%)	8 (16%)	9 (18%)	4 (8%)	1 (2%)	44%	
Pi	TP	18 (36%)	14 (28%)	13 (26%)	5 (10%)	0	64%	**<0.01**
	C	36 (72%)	6 (12%)	7 (14%)	1 (2%)	0	28%	
Pm	TP	19 (38%)	15 (30%)	13 (26%)	3 (6%)	0	62%	**0.03**
	C	25 (50%)	7 (14%)	8 (16%)	10 (20%)	0	50%	
Fn	TP	1 (2%)	2 (4%)	10 (20%)	30 (60%)	7 (14%)	98%	0.75
	C	1 (2%)	5 (10%)	12 (24%)	25 (50%)	7 (14%)	98%	
Cr	TP	23 (46%)	15 (30%)	8 (16%)	3 (6%)	1 (2%)	54%	0.33
	C	30 (60%)	8 (16%)	7 (14%)	5 (10%)	0	40%	
En	TP	47 (94%)	3 (6%)	0	0	0	6%	0.62
	C	49 (98%)	1 (2%)	0	0	0	2%	
Ec	TP	24 (48%)	13 (26%)	10 (20%)	3 (6%)	0	52%	0.92
	C	23 (46%)	11 (22%)	12 (24%)	4 (8%)	0	54%	
Cs	TP	15 (30%)	12 (24%)	19 (38%)	3 (6%)	1 (2%)	70%	0.06
	C	14 (28%)	16 (32%)	10 (20%)	2 (4%)	8 (16%)	72%	

Significant values ($P < 0.05$) are highlighted in bold.

However, the occurrence of selected potentially periodontal pathogenic bacteria at TP surface is confirmed by the current study's findings. Further studies focused on bacterial findings related to different piercing materials (Borges et al. 2016; Kapferer et al. 2011). Kapferer et al. (2011) found potentially periodontal pathogens to be not commonly found at TP surface and channels, respectively (Kapferer et al. 2011). This is not in line with the current study's results as, besides *En*, all investigated potentially periodontal pathogens could be detected at TP, showing prevalence between 6 and 96%. Moreover, a recent in vitro study examined material specific bacterial colonization of piercing surfaces, showing *Ec* to be associated to differences in piercing material (Borges et al. 2016). The material specific differences raised in the available examinations can neither be confirmed nor denied. However, the current study included different piercing types without a distribution between the different materials (metal and synthetic). These potential differences must be considered in the interpretation of the results.

Furthermore, a cytopathological and chemico-physical analysis of smears surrounding oral piercings is available, showing bacterial colonization and bacterial cytolysis of epithelial cells in this area (Lupi et al. 2010). This is in line with the current investigation. Especially, the epithelial invasion might be of clinical relevance, as especially bacteria with high pathogenic bacteria like *Pg* and *Td* are able to invade epithelial cells (Tan et al. 2014). This could be similar between periodontal pocket

Table 4
Comparison of bacterial concentrations at the cheek between groups.

Concentrations		Under detection limit	Over detection limit				Prevalence	*P* value
		$<10^3$	10^3	$<10^4$	$<10^5$	$>10^6$		
Aa	TP	47 (94%)	1 (2%)	1 (2%)	1 (2%)	0	6%	0.72
	C	47 (94%)	0	2 (4%)	1 (2%)	0	6%	
Concentrations		$<10^4$	10^4	$<10^5$	$<10^6$	$>10^7$		
Pg	TP	38 (76%)	3 (6%)	4 (8%)	2 (4%)	3 (6%)	26%	0.26
	C	45 (90%)	1 (2%)	2 (4%)	2 (4%)	0	10%	
Tf	TP	46 (92%)	1 (2%)	3 (6%)	0	0	8%	0.35
	C	49 (98%)	0	1 (2%)	0	0	2%	
Td	TP	16 (32%)	9 (18%)	16 (32%)	7 (14%)	2 (4%)	68%	**<0.01**
	C	35 (70%)	7 (14%)	5 (10%)	3 (6%)	0	30%	
Pi	TP	27 (54%)	8 (16%)	11 (22%)	4 (8%)	0	46%	**0.04**
	C	38 (76%)	8 (16%)	3 (6%)	1 (2%)	0	24%	
Pm	TP	33 (66%)	10 (20%)	6 (12%)	1 (2%)	0	34%	0.11
	C	43 (86%)	5 (10%)	2 (4%)	0	0	14%	
Fn	TP	4 (8%)	12 (24%)	15 (30%)	19 (38%)	0	92%	0.44
	C	7 (14%)	14 (28%)	17 (34%)	12 (24%)	0	86%	
Cr	TP	36 (72%)	11 (22%)	2 (4%)	1 (2%)	0	28%	0.53
	C	41 (82%)	7 (14%)	2 (4%)	0	0	18%	
En	TP	48 (96%)	2 (4%)	0	0	0	4%	0.50
	C	50 (100%)	0	0	0	0	0%	
Ec	TP	39 (78%)	5 (10%)	6 (12%)	0	0	22%	**0.03**
	C	36 (72%)	13 (26%)	1 (2%)	0	0	28%	
Cs	TP	33 (66%)	11 (22%)	6 (12%)	0	0	34%	0.49
	C	28 (56%)	16 (32%)	5 (10%)	1 (2%)	0	44%	

Significant values ($P < 0.05$) are highlighted in bold].

Table 5
Correlations between findings at tongue piercing with periodontal pocket.

Bacteria	Correlation piercing with periodontal pocket	
	r	*P* value
Aa	0.875	$P < 0.01$
Pg	0.793	$P < 0.01$
Tf	0.338	$P = 0.02$
Td	0.290	$P = 0.04$
Pi	0.427	$P < 0.01$
Pm	−0.150	$P = 0.30$
Fn	0.149	$P = 0.30$
Cr	0.370	$P < 0.01$
En	-----	----
Ec	−0.011	$P = 0.93$
Cs	0.057	$P = 0.70$

Significant values ($P < 0.05$) are highlighted in bold].

and other tissues like the piercing surrounding mucosa or tongue surface. However, this might also allow another explanation for the correlation between periodontal pocket and piercing surface. After invasion of periodontal epithelial cells during periodontal inflammation, especially by *Pg*, these cells could become desquamated and matured at the piercing surface. Because the used microbiological test system does not provide information about the vitality and pathogenic activity of the detected bacteria, they could also be inactive or dead within the piercing surrounding biofilm and cell debris, which is collected with the paper tips.

Another hypothesis for the higher prevalence of selected periodontal pathogenic bacteria at different oral areas of participants with TP could be a change in the complex microbiological homeostasis of the oral cavity caused by TP and/or related factors. The human oral microbiome is highly complex and associated to oral health and disease (Costalonga and Herzberg 2014; Krishnan et al. 2017; Zhang et al. 2018). Changes in different ecological niches of the oral cavity, as well as the presence of foreign bodies like implants or piercings, can affect microbiological colonization of specific areas but also the whole oral cavity (Zhang et al. 2018). In this context, 2 aspects are of potential clinical relevance. On the one hand, the TP as a foreign body might lead to a colonization of different bacterial species at its surface and surrounding tissues. This might directly affect the microbiological symbiosis in the oral cavity. On the other hand, TPs are related to the occurrence of dental and periodontal diseases (Pires et al. 2010; Plastargias and Sakellari 2014; Plessas and Pepelassi 2012; Tomaževič et al. 2017; Ziebolz et al. 2012). Although majority of studies focused on the increased prevalence of gingival recession, a piercing related increase of periodontal inflammation seems possible. Considering the clinical findings of the previous publication showing increased periodontal burden and worse oral hygiene (Ziebolz et al. 2019), this association seems plausible. With increased severity, periodontal inflammation is potentially associated to specific bacterial species (Kinane et al. 2017). These bacteria might migrate to different areas of oral cavity, including tongue, cheek and TP surface by desquamation, gingival crevicular fluid, or salivary flow. Due to the correlations between potentially periodontal pathogenic bacteria, the joint occurrence of *Td, Pi* and *Pg* is plausible (Loozen et al. 2014; Sarkar et al. 2014). Accordingly, the increased prevalence of these bacteria in different oral areas of pierced individuals might be complex and multifactorial.

Irrespective of the mechanism of colonization and the source of bacteria, the increased prevalence of potentially periodontal bacteria as well as the correlation between periodontal pocket and TP surface is of clinical relevance. Especially the high correlation of *Pg*, as a major periodontal pathogen (Mysak et al. 2014), might be important. Thereby, the relevance of TP as a possible reservoir of these bacteria must be underlined. During periodontal therapy, TP could therefore support a fast recolonization of periodontal pocket, resulting in impaired therapy outcome. Accordingly, the consideration of TP during prevention and periodontal therapy might be important, including piercing hygiene and disinfection of piercing and surrounding tissues.

4.3. Strengths and limitations

The study question is new, innovative and of potential clinical relevance; the inclusion of 50 participants and a matched control group strengthens the findings. However, there are several limitations that must be considered in its interpretation. As 1 major point, the methodology needs to be discussed. The 11 potentially periodontal pathogenic bacteria in the current study have already been mentioned by Socransky et al. about 20 years ago (Socransky et al. 1998). In different complexes, these bacteria act in the subgingival biofilm and could be related to periodontitis (Sharma et al. 2018; Socransky et al. 1998). Therefore, these 11 bacteria are included in the applied PCR-based test system and were chosen for the current study. Although the applied test system is valid, it examines only a small extract of the complex oral microbiota. Furthermore, the presence of bacterial DNA is detected, what does not provide information about the activity of bacteria. Especially the activation of virulence factors has a major influence on the clinical relevance (Kuboniwa et al. 2012). This information would be necessary to draw meaningful conclusions about the relevance of TP in context of occurrence and progression of periodontal inflammation. In this context, the cross-sectional design is a further limitation, as it does not provide information whether increased prevalence of potentially periodontal pathogens support periodontal disease progression. Furthermore, due to its overall very low prevalence, the relevance of the correlation of *Aa* between periodontal pocket and TP remains unclear. Because the focus of the current study was set at the microbiological findings, clinical situation has not been considered and was presented previously (Ziebolz et al. 2019). This is a further limitation, as microbiological findings should be interpreted in context of the clinical condition (Untch and Schlagenhauf 2015). Moreover, the piercing material might also affect bacterial colonization (Borges et al. 2016; Kapferer et al. 2011). This could be an important influential factor in the current study. However, due to different TP materials within the study population, several subgroups with a partly very small case number would be to investigate. These small-sized subgroups would not allow robust conclusions. Therefore, the material of TP was not explicitly considered within the current study, which must be seen as a limitation and should also be recognized in future examinations.

5. Conclusion

Participants with TP suffered from an increased prevalence of potentially periodontal pathogenic bacteria, especially *Td* and *Pi*, at different areas of the oral cavity. Thereby, correlations between TP surface and periodontal pocket were found. Accordingly, the presence of a TP must be considered in prevention and therapy of periodontal diseases as a reservoir for potential periodontal pathogens.

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