Scientific Research Report

Differences Between Vietnamese Living in Germany and German Periodontitis Patients in Periodontal Conditions and Subgingival Microbiota

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ARTICLE INFO

Article history:
Received 13 June 2024
Received in revised form
4 September 2024
Accepted 15 September 2024
Available online 5 October 2024

Key words:
Periodontitis
Next-generation sequencing
Microbiota
Ethnic groups
Porphyromonas gingivalis
Fusobacteria

ABSTRACT

Introduction and aims: A number of studies have reported ethnic differences in the prevalence and severity of periodontitis. Such discrepancies could be attributed to disparities in periodontal risk factors, as well as variations in the composition of the subgingival microbiota. Given the substantial Vietnamese population residing in the former German Democratic Republic, the present study aimed to compare the clinical and microbial characteristics of periodontitis patients of Vietnamese Asian origin living in Germany with those of German Caucasian periodontitis patients.

Methods: A total of 60 patients with a minimum stage II periodontitis diagnosis were included in the study. Of these, 30 were of Vietnamese origin, with an average age of 55 years and a male prevalence of 33.3%. The remaining 30 patients were of German origin, with an average age of 54.5 years and a male prevalence of 40%. The periodontal diagnosis was made in accordance with the recently revised classification of periodontal disease. The pooled subgingival plaque samples were subjected to next-generation sequencing on the MiSeq platform (Illumina).

Results: The German patients were significantly more likely to be smokers (56.7% vs 13.3%), had significantly higher body mass index (26 vs 22.6 kg/m^2), probing depth (4.1 vs 3.6 mm), and clinical attachment loss (5 vs 4.1 mm). In terms of microbiota, the Vietnamese patients exhibited significantly lower beta diversity compared to the German patients, and smokers demonstrated a significantly higher beta diversity compared to nonsmokers. The microbiota of both groups differed most significantly in the relative abundance of *Porphyromonas gingivalis* (Vietnamese) and *Fusobacteriia* (German).

Conclusions: German patients with periodontitis showed more severe periodontal symptoms and more pronounced periodontal risk factors compared to Vietnamese patients. Both patient groups also showed significant differences in the subgingival microbiota.

Clinical relevance: Compared to Vietnamese living in Germany, German patients have a higher need for periodontal treatment and at the same time the risk factors of smoking and obesity should be reduced. More research is needed before the differences in oral microbiota between the two groups can lead to individualised therapeutic approaches.

Introduction

Periodontitis is one of the most common chronic inflammatory noncommunicable diseases with a global prevalence of 7.4% for the sever form.[1] The prevalence of milder forms is estimated to be over 50%.[2]

There are ethnic differences in the prevalence of periodontitis. Periodontal disease is most prevalent among Africans, followed by Hispanics and Asians.[3] Dietary habits, socio-economic conditions, smoking behaviour and genetic background in immune defence against putative periodontal pathogens have been discussed as possible causes.[4] In addition, the oral microbiota itself may play a role, as differences in the abundance of

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https://doi.org/10.1016/j.identj.2024.09.021

putative periodontal pathogens or specific serotypes of these bacteria have been found in different ethnic groups.[5-8]

According to the Central Register of Foreigners in (2020) Asians represented 21.5% of the foreign population in Germany (2.45 million people), including 0.9% Vietnamese. The Vietnamese population lives mainly in the former East Germany and is part of the largest Southeast Asian community in Berlin.

Few studies have compared the periodontal status of Vietnamese living in European countries with that of Caucasians of European origin. In most cases, only children or adolescents who came to these countries as refugees have been included. A Swedish study[9] revealed poorer periodontal findings in Vietnamese migrant children than in Swedish children. The Vietnamese children had probing depths ≥4 mm on several tooth surfaces and significantly more tooth surfaces reacted with bleeding on probing (BOP). In a Norwegian study[10] a group of 200 Vietnamese refugees aged from 12 to 40+ years were analysed. Values for subgingival calculus and gingivitis were above those of comparable Norwegian subject groups. A further Finish study[11] compared clinical conditions between a group of 16 Vietnamese and 16 Finnish children. The Vietnamese also had a refugee background. They had worse periodontal conditions, such as a higher BOP index, more calculus, and greater probing depths. The study is particularly interesting because it also included microbiological testing of saliva and plaque. The Vietnamese test group was significantly more affected by *Streptococcus mutans* (100% vs 62%) and *Aggregatibacter actinomycetemcomitans* (A.a.) (72% vs 13%). Given the described ethnic differences in the prevalence and severity of periodontitis and the opportunity to study a group of adult Vietnamese patients with periodontitis living in Germany, the aim of this study was to evaluate putative differences in periodontal conditions between Vietnamese and German patients with periodontitis. As the composition of subgingival plaque plays an important role in the aetiology of periodontitis, possible differences in the subgingival microbiota should also be investigated using next-generation sequencing (NGS).

Material and methods

Enrolment of the patients

This study was approved by our university's human research ethics committee (registration number 2019-091) and was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2013. In addition, the study was registered in the German Clinical Trials Register (DRKS) under the registration number 00019827. All subjects gave written informed consent to participate in the study after being informed of its aims and possible risks.

Of 69 patients with periodontitis who visited two dental practices between December 2019 and October 2021, 60 were eligible for inclusion in the study. Seven patients did not meet the inclusion criteria, and two patients refused to participate in the study. A total of 30 patients were of Vietnamese Asian origin and 30 were of German Caucasian origin. All Vietnamese patients were born in Vietnam and were of Asian descent.

The following criteria were required for inclusion in the study: All study participants should live in Germany, have health insurance and approximately equal access to the health care system, should have periodontitis at least stage II periodontitis according to the latest Classification of Periodontal and Peri-Implant Diseases and Conditions,[12] age ≥35 years, and at least 10 natural teeth. Exclusion criteria were pregnancy or lactation, diabetes mellitus, subgingival scaling, and root planing in the previous 6 months, use of antibiotics 3 months prior to the baseline examination, use of any medication that could have a significant effect on the inflammatory or immune response of the periodontal tissues.

Demographic and clinical conditions

Baseline variables such as age, body mass index (BMI), smoking status were assessed as part of the patient's medical history. BMI was calculated as weight in kilograms divided by height in meters squared. To define smokers and nonsmokers, we considered the results of Antonello et al[13] who showed that former smokers who quit more than 5 years ago had microbiota profiles comparable to those who had never smoked. Therefore smokers were defined as people who currently smoked at least one cigarette per day or who had quit smoking less than 5 years ago. Nonsmokers were people who had never smoked or who had quit smoking more than 5 years ago. Prior to the dental examination, patients were asked how often they brushed their teeth per day and whether they used dental floss or interdental brushes for interdental hygiene. They were also asked if they had ever had periodontal treatment. A distinction was made between nonsurgical, surgical, and antibiotic treatment. The clinical dental assessment included the decayed missing filled teeth index,[14] plaque index (PI),[15] and BOP.[16] For determination PI and BOP six surfaces around each tooth (mesio-buccal, mid-buccal, disto-buccal and midlingual) were examined. For the calculation of PI, each tooth surface was assigned a code of 0 to 3. Code 0 means no plaque; code 1 means thin plaque, not visible, only detectable with a dental probe; code 2 means clinically well-visible plaque in the sulcus and areas close to the gingiva, but no plaque accumulation in the interdental space; code 3 means thick plaque, visible to the naked eye, filling the interdental spaces. The codes were then summed and divided by the number of tooth surfaces assessed. To determine BOP, the number of sites bleeding 30 seconds after probing was divided by the total number of sites evaluated. Measurements of both, clinical probing depth (PD = distance between the gingival margin and the apical stop of the probe) and clinical attachment loss (CAL = distance between the cementoenamel junction and the apical stop of the probe) were also taken at six sites around each tooth. Mean values were calculated by adding the PD and CAL values and dividing the sum by the number of tooth sites examined. To obtain reproducible results for PD and CAL, the examiner (H.S.) was specially trained in the use of a pressure-sensitive dental probe (UNC 15 0.2 N Aesculap) and clinically calibrated. Readings were taken to the nearest millimetre. If a measurement point (gingival margin or cementoenamel junction) was between two marks on the scale, the measurement was estimated to be 0.5 mm. For clinical calibration, the investigator measured PD and CAL twice in 10 patients. The

reproducibility of the double measurements was assessed using the Bland–Altman method.[17] The difference (d) between the two measurements was calculated and plotted against the mean of the two measurements. Measurements were considered sufficiently reproducible if 95% of the differences (d) were in the range $d \pm 2 \times s$, where s is the standard deviation of the differences. For our rater, the differences between the two measurements for PD and CAL were 100% in the range $d \pm 2 \times s$. Thus, the evaluator was able to produce reproducible measurements.

Periodontitis was diagnosed according to the new classification of Periodontal and Peri-Implant Diseases and Conditions.[12] The assessment of periodontitis stage was based on the degree of radiographic bone loss, the amount of proximal CAL, tooth loss due to periodontitis, and the amount of probing depth. If less than 30% of teeth were affected at the highest stage, localised periodontitis was present. If at least 30% of the teeth were affected, generalised periodontitis was diagnosed. The bone loss index (bone loss (%)/age) and the presence of the risk factors smoking and/or diabetes were used to determine the grading.

Collection and DNA isolation of subgingival microbial samples

Microbial samples were taken from the site with the greatest probing depth in each quadrant. A sterile paper point (Paper Points, Size 50, Hain Lifescience) was inserted for 20 seconds. All 4 bacterial plaque samples from an individual were pooled in one tube. The mean values for PD and CAL at the microbial plaque sample sites were calculated for each patient. After drying, the paper tips were stored at −20°C until DNA isolation. Microbial DNA was extracted from the paper tips using a professional DNA extraction kit (QIAamp DNA mini kit, Qiagen) according to the manufacturer's protocol.

After quality and quantity control with a spectrophotometer (Nanodrop Thermo Fisher Scientific), the DNA was sent to a commercial NGS laboratory (Novogene Co, Ltd.) for further analysis on dry ice.

Analysis of subgingival plaque samples with NGS

DNA concentration and quality were assessed on a 1% agarose gel. Microbial DNA primers targeting the 16S V3 and V4 regions were used to generate amplicons. A commercially available PCR master mix (Phusion High-Fidelity PCR Master Mix New England Biolabs) was used for amplification. PCR products were purified using a commercially available gel extraction kit (Qiagen Gel Extraction Kit Qiagen) according to the manufacturer's protocol. Library preparation and indexing were performed using a DNA library pre-kit (NEBNext UltraTM DNA Library Pre Kit Illumina) according to the manufacturer's recommendations. For library quantification and normalisation, DNA was analysed using a (Qubit 2.0 Fluorometer Qubit 2.0 Fluorometer Thermo Scientific) and a bioanalyzer system (Agilent Bioanalyzer 2100 system Agilent). The Illumina Novaseq X Plus platform (Illumina) was used for sequencing analysis and 250 bp paired-end reads were generated.

Data analysis

Our study was the first to investigate the microbiota of Vietnamese and German periodontitis patients using NGS. As the expected differences between the two ethnic groups were not known, it was not possible to collect the required number of cases in each cohort before the start of the study. Therefore, we based the sample size on a study that showed significant differences in the microbiota between Vietnamese and Finnish individuals.[11] Our study included 30 subjects in each group, which was more than in the Finnish study. We got significant results in terms of the microbiota. Therefore, we assume that the number of patients in each group is sufficient to compare the microbiota.

Statistical analyses were performed using commercially available software (SPSS v.22.0 package, IBM). Values $P \leq .05$ were considered significant. Metric demographic, clinical, and microbiological data were tested for normal distribution using the Kolmogorov–Smirnov test and the Shapiro–Wilk test. Normally distributed metric data were presented as mean ± standard deviation and compared using the t test. Nonnormally distributed variables were presented as median and 25th/75th percentiles. The Mann–Whitney U test was used for statistical evaluation. Categorical variables were reported as number/percentage. Chi-squared test was used for comparison. If the expected number of cases in any field of the four-field table was less than 5, Fisher's exact test was used for correction. Linear regression was used to assess several factors influencing the severity of periodontitis measured as CAL.

The microbiological data were evaluated according to our specifications by Novogene, Europe, Cambridge, UK. The statistical methods used were taken from the results report provided by the company.

Paired-end read assembly: Reads were assembled using FLASH software.[18] Using the QIIME workflow, the raw tags were preprocessed and quality filtered.[19] Tags were compared to a reference database for chimaera depletion using the UCHIME algorithm.[20]

Operational taxonomic unit (OTU) clustering and species annotation: Uparse software was used for sequence analysis. Sequences with >97% similarity were assigned to the same OTU.

The GreenGene database was used for species annotation based on the RDP classifier algorithm.[21] Normalisation of OTU abundance information was performed to a standard sequence corresponding to the sample with the fewest sequences. The normalised data were used for all subsequent analyses.

Alpha diversity: Alpha diversity was calculated using QIIME (V1.7.0) and evaluated using R software (V2.15.3). The Shannon index was used to determine community diversity (http://mothur.org.wiki/Shannon, accessed 22 March 2023). Differences in alpha diversity (Shannon indices) were assessed using the Wilcoxon signed-ranks test and corrected for multiple comparisons using a Benjamini–Hochberg false discovery rate of 5%.

Beta diversity: Beta diversity was also calculated using QIIME software (V1.7.0). The general distribution of the resulting bacterial community composition was evaluated using Principal Coordinates Analysis with the R package ggplots2 (V2.15.3). The linear discriminant analysis (LDA) and effect size (LEfSe) pipeline was applied using the Galaxy software

Table 1 – Demographic and clinical data of Vietnamese periodontitis patients compared with German-Caucasians.

Variable	Vietnamese periodontitis patients (*N* = 30), median (25th/75th percentiles), mean ± SD or *n* (%)	Caucasian periodontitis patients (*N* = 30), median (25th/75th percentiles), mean ± SD or *n* (%)	*P* value
Age (y)	55 (49.8/60)	54.5 (41.5/58.3)	.297*
Gender, male	10 (33.3)	12 (40)	.592†
BMI (kg/m^2)	22.6 (21.5/24.7)	26 (23.9/28.8)	.001*
Smoking			
Never or quit smoking >5 y	26 (86.7)	13 (43.3)	
Current or quit smoking <5 y	4 (13.3)	17 (56.7)	<.001‡
Periodontal conditions			
Frequency of dental care/d			.105†
1	4 (13.3)	0	
2	25 (83.3)	28 (93.3)	
3	1 (3.3)	2 (6.7)	
Interdental cleaning	20 (66.7)	22 (73.3)	.317†
Bleeding on probing	76.9 ± 13.4	79.5 ± 13.8	.458§
Plaque index	1.6 (1/1.8)	1.5 (1.1/1.8)	.057*
Tooth loss probably due to periodontitis	4.7 ± 3.0	9.2 ± 6.5	.036§
DMFT index	10.3 ± 6.7	17.3 ± 6.3	<.001§
The onset of periodontitis was known	5 (16.7)	16 (53.3)	.003†
Onset of periodontitis (y)	46 (39/48.5)	34.5 (26.3/43.0)	.006*
Possible tooth loss due to periodontitis in 1st-degree relatives	14 (46.7)	15 (50.0)	.796†
Previous periodontitis therapy			
Nonsurgical (scaling, root planing)	6 (20.0)	23 (76.7)	<.001†
Surgical (flap surgery)	0	0	
Antibiotics	0	0	
Probing depth (mm)	3.6 (3.2/3.9)	4.1 (3.5/4.4)	.019*
Attachment loss (mm)	4.1 (3.6/4.7)	5.0 (3.9/5.8)	.039*
Plaque collecting sites			
Probing depth (mm)	5.9 (5.2/6.9)	6.5 (5.9/7.7)	.197*
Attachment loss (mm)	6.5 (5.8/7.8)	7.9 (6.5/9.0)	.039*
Staging			
II generalised	6 (20.0)	1 (3.3)	
III localised	13 (43.3)	10 (33.3)	
III generalised	10 (33.3)	15 (50.0)	
IV	1 (3.3)	4 (13.3)	.080†
Grading			
B	19 (63.3)	12 (40.0)	
C	11 (36.7)	18 (60.0)	.071†

BMI, body mass index; DMFT, decayed missing filled teeth; SD, standard deviation.

* Mann–Whitney *U* test.
† Chi^2-test.
‡ Fisher's exact test.
§ *t* test.

provided by Dr. Huttenhower (https://huttenhower.sph.harvard.edu/galaxy, accessed 22 March 2023). Differences in means between the two ethnic periodontitis groups (at all phylogenetic levels) were evaluated using the *t* test, including false discovery rate analysis.

Results

Clinical conditions bivariate comparisons

Caucasian periodontitis patients had a significantly higher BMI and were significantly more likely to be current smokers or past smoker who quite smoking <5 years ago. The Vietnamese patients reported in comparison to Caucasians a 10-year delay in the onset of periodontitis and only 20% had ever received systematic periodontal treatment.

Considering clinical measurements Caucasians had significantly higher decayed missing filled teeth index and levels of CAL (general and microbial test sites), PD, and periodontitis-related tooth loss. There was a trend for an increased PI among the Vietnamese patients. Using the new classification, more patients in the German Caucasian group had severe periodontitis (stage III or IV) with a high rate of progression (grade C). However, these differences were not statistically significant (Table 1).

Clinical conditions – multivariate analysis

In addition to ethnicity, the influence of other factors such as age, gender, BMI, PI, smoking, and previous periodontal treatment on the severity of periodontitis, measured as CAL was analysed using linear regression. Only the PI was found to be a significant adjusted risk factor for CAL. For the smoking

Table 2 – Linear regression to assess various factors influencing the severity of periodontitis, measured as clinical attachment loss.

Variable	Regression coefficient beta	Standard error	Significance	95% confidence interval for beta	
				Lower	Upper
Constant	−0.14	2.2	0.95	−4.62	4.33
Age	0.03	0.02	0.18	−0.02	0.08
Gender	−0.34	0.38	0.38	−1.11	0.43
Body mass index	−0.001	0.07	0.99	−0.13	0.13
Smoking status	0.85	0.46	0.07	−0.08	1.77
Ethnic affiliation	0.45	0.55	0.36	−0.60	1.59
Plaque index	1.01	0.39	0.01	0.22	1.80
Previous periodontal therapy*	0.20	0.44	0.65	−0.68	1.08

Patients who had undergone subgingival scaling and root planing in the previous 6 months or who had received antibiotics in the 3 months prior to baseline were excluded from the study.

* Only nonsurgical periodontal therapy was used, not surgery or antibiotics.

status, only a trend for an association with CAL was identified (Table 2).

Subgingival microbiota

The relative abundance of the top 10 OTUs was calculated at the phylum, class, order, family, and genus levels. The presentation of the results is restricted to the species level. The data for the other taxonomic classifications are available from the authors on request. There were no significant differences in the relative bacterial count between Germans and Vietnamese, both in the overall groups (Figure 1) and in the groups separated for smokers and nonsmokers (Figure 2). In both groups, *Porphyromonas gingivalis* (*P.g.*), *Treponema denticola*, *Porphyromonas endodontalis*, and *Prevotella intermedia* (*P.i.*) were the most common bacteria. *P.g.* and *Aggregatibacter aphrophilus* were more frequently in Vietnamese patients with periodontitis, while *A.a.*, *Alloprevotella tannerae* (A.t.), and *Haemophilus parainfluenzae* were more common in German patients. Even when smokers and nonsmokers were considered separately, the same bacteria as in the overall groups were identified as the most common. In addition, *P.g.* was more common in the Vietnamese group and generally more common in nonsmokers than in smokers. *A.a.* was only conspicuous among German patients with positive smoking

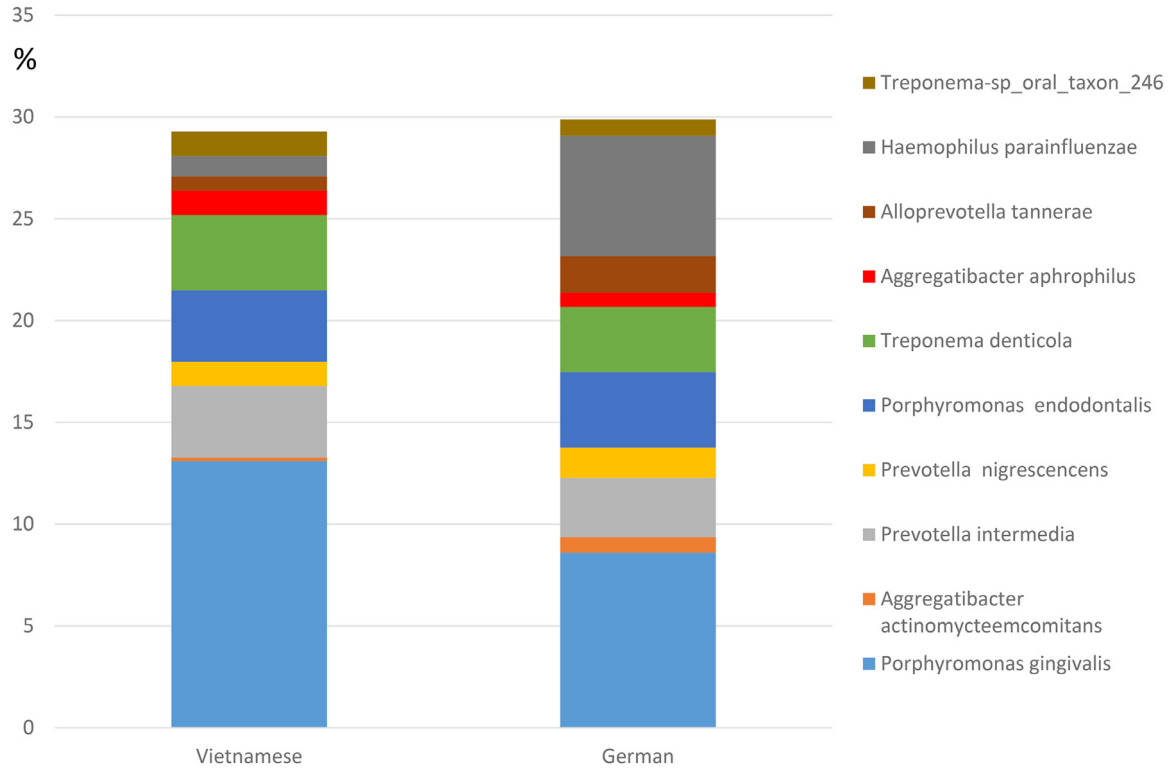

Fig. 1 – Microbial community composition at the species level in Vietnamese-Asian and German-Caucasian periodontitis patients.

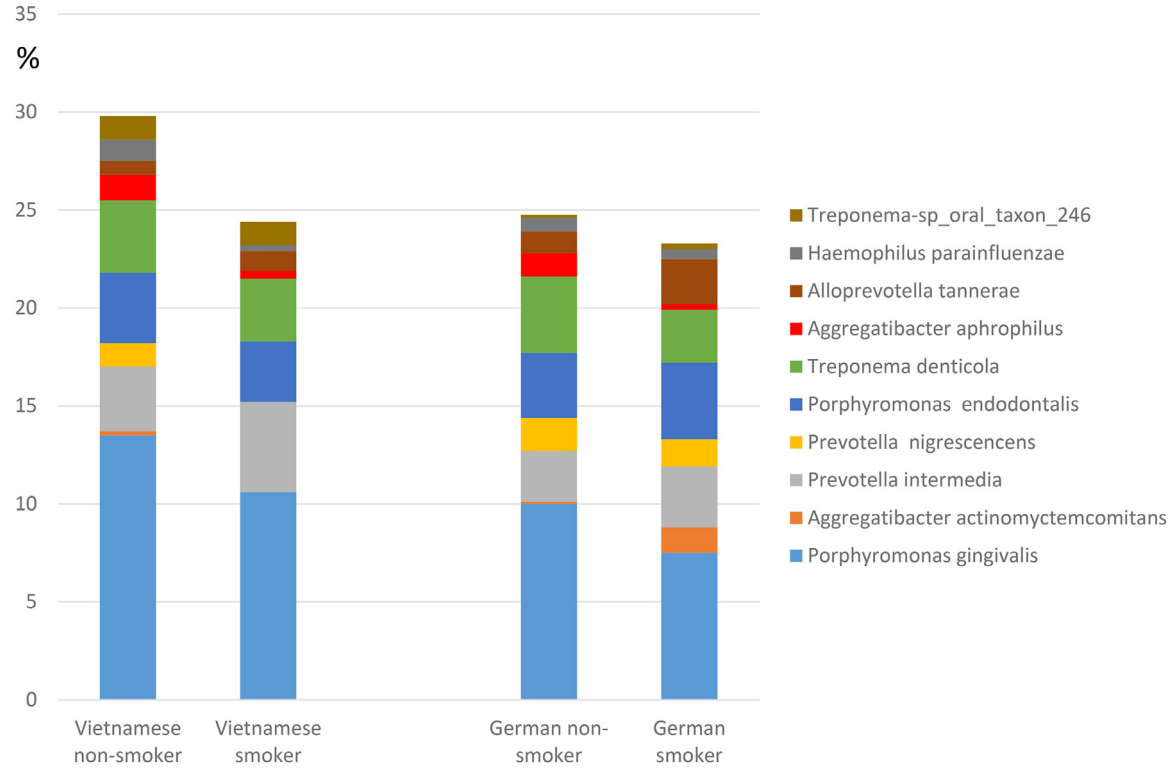

Fig. 2 – Composition of the microbial community at the species level in periodontitis patients of Vietnamese and German Caucasian origin according to smoking status.

status (Figure 2). German patients showed a tendency for an increased alpha diversity (Figure 3), measured as the Shannon index. However, smokers in both groups tended to have a higher Shannon index (Figure 4). Vietnamese patients had significantly lower beta diversity than patients of German Caucasian origin (Figure 5). This difference remained significant when smokers and nonsmokers were considered separately, with smokers having significantly lower beta diversity in both groups (Figure 6).

LDA with LEfSe was used to calculate the taxa that best distinguished the microbiotas of the two groups studied. The periodontitis pathogen *P.g.* (including the parent taxonomic categories family: *Porphyromonadaceae* and genus: *Porphyromonas*) was characteristic of Vietnamese periodontitis patients. In contrast, bacteria from the phylum *Fusobacteriota*, class *Fusobacteriia*, order *Fusebacteriales* were characteristic of the German Caucasian patients.

Finally, all species that differ significantly in absolute frequency between the two groups are listed. The prevalence of *P.g.* was markedly higher in patients with Vietnamese periodontitis, whereas *A.t.* was more prevalent in German-Caucasian patients (Figure 7). For *Fusobacterium nucleatum* (*F.n.*), only a trend for an increased occurrence among German patients was found (bacterial count: 1.3e-04 vs 2.3e-04).

Discussion

In our study, we found more pronounced periodontal and cariological symptoms in German periodontitis patients compared to Vietnamese periodontitis patients. In terms of clinical findings, our results do not confirm those of other studies that have found poorer periodontal health in Vietnamese compared to Caucasians of Northern European origin.[4,9,11] The possible reason for this is as follows. Most previous studies have examined children and adolescents, many of whom were refugees. A meta-analysis showed poorer oral health among refugees because of limited access to dental care due to low financial resources, fear of dental treatment, or cultural and language barriers.[22] Participants in our study were adults aged from 40 to 60 years. In general, prevalence and severity of periodontitis are increasing with age.[23] Many of the Vietnamese patients studied had lived and worked in Germany for a long time, had health insurance and access to the health care system. This may explain why the differences in periodontal status between the two groups were significant but rather small. In the multivariate analysis, we show that PI and smoking (borderline significance) but not ethnicity or BMI were associated with the severity of periodontitis assessed as CAL. A recent study confirms the influence of supragingival plaque on the severity of periodontitis. Individuals with higher PI scores (>30%) had a less healthy periodontal status, a higher risk of recurrent periodontitis, and a lower likelihood of achieving ≤4 sites with PD ≥ 5 mm after active periodontal therapy.[24] Smoking is an established risk factor for periodontitis and was found associated with a 1.85-fold increase in the risk of developing periodontitis.[25] It is assumed that smoking may delay the recruitment and migration of neutrophils into the periodontal tissues, thereby impairing the immune response.[26] The increase in the concentration of the proinflammatory cytokines such as IL-1 and IL-6 found in smokers may promote periodontal bone

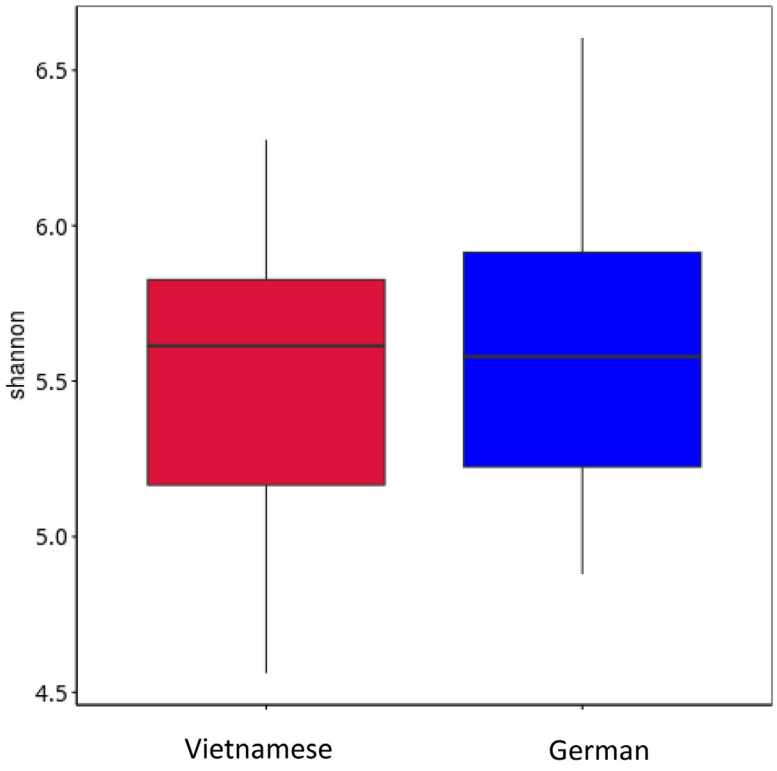

Fig. 3 – Box plots of the Shannon index to visualise alpha diversity. There was a trend for a lower Shannon index among German periodontitis patients.

resorption.[27] In addition, increased levels of elastase[26] and matrix metalloproteinase-8 and matrix metalloproteinase-9 with proteolytic activity[28,29] have been described, while levels of protease inhibitors such as alpha-2-macroglobulin and alpha-1-antitrypsin were reduced. Increased proteolytic activity combined with reduced gingival blood vessels may impair periodontal healing.[30,31]

Before sequencing techniques were used to analyse the oral microbiota, the results on the influence of smoking on the oral microbiota were inconsistent and contradictory. New studies

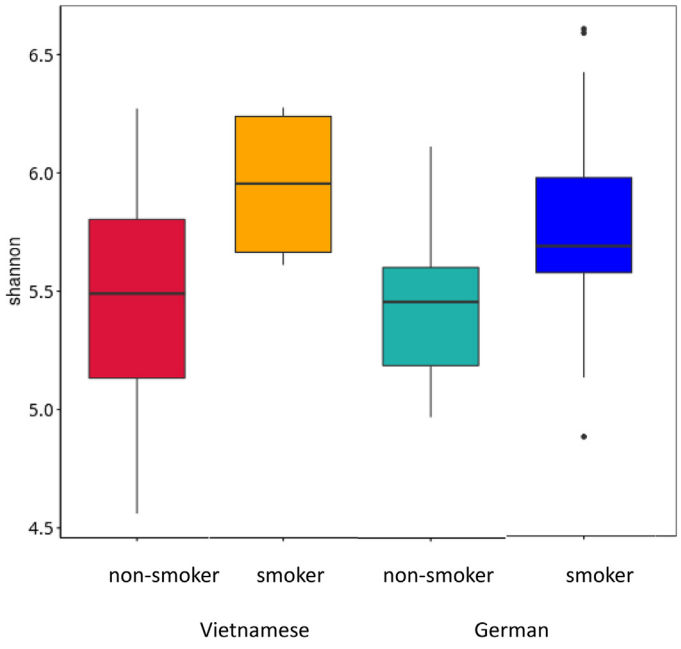

Fig. 4 – Shannon indices in periodontitis patients of Vietnamese and German Caucasian origin according to smoking status. There was a trend for increased Shannon indices in smokers.

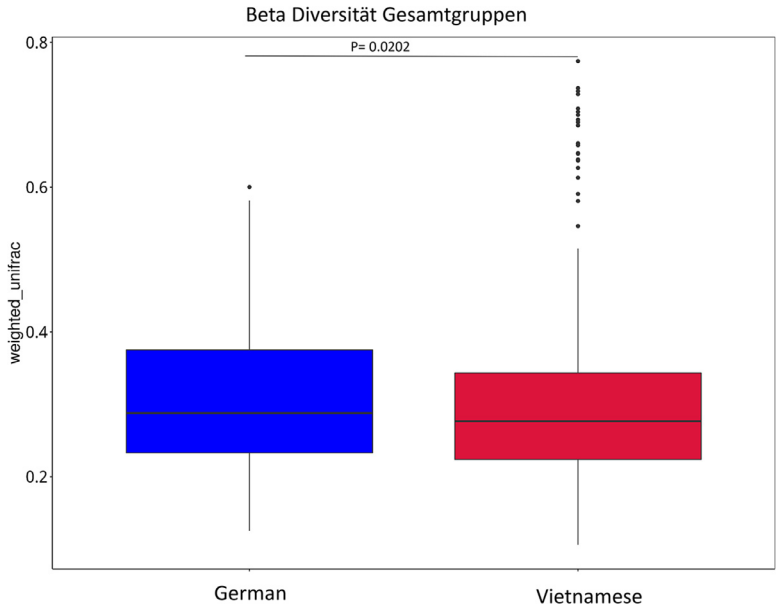

Fig. 5 – Comparison of beta diversity in periodontitis patients of German and Vietnamese origin. Beta diversity was significantly reduced in Vietnamese patients (weighted Unifrac analysis, comparison of paired groups using Wilcoxon signed-rank test).

using sequencing techniques showed that smoking may influence the composition of the oral microbiota. For instance, *Fusobacterium*, *Prevotella*, and *Selenomonas* were more abundant in periodontitis patients who were smokers, while the genera *Peptococcus* and *Capnocytophaga* were more abundant in nonsmokering periodontitis patients.[32] Another study revealed that the microbial community of smoker-associated periodontitis was found less diverse and the relative abundance of the TM7 candidate division was three-fold increase in comparison to nonsmokers.[33] Smoking has been observed to increase the pathogenicity of putative periodontal pathogens, especially the red complex microorganisms, by promoting their colonisation and infection and by regulating the expression and function of various virulence factors.[34]

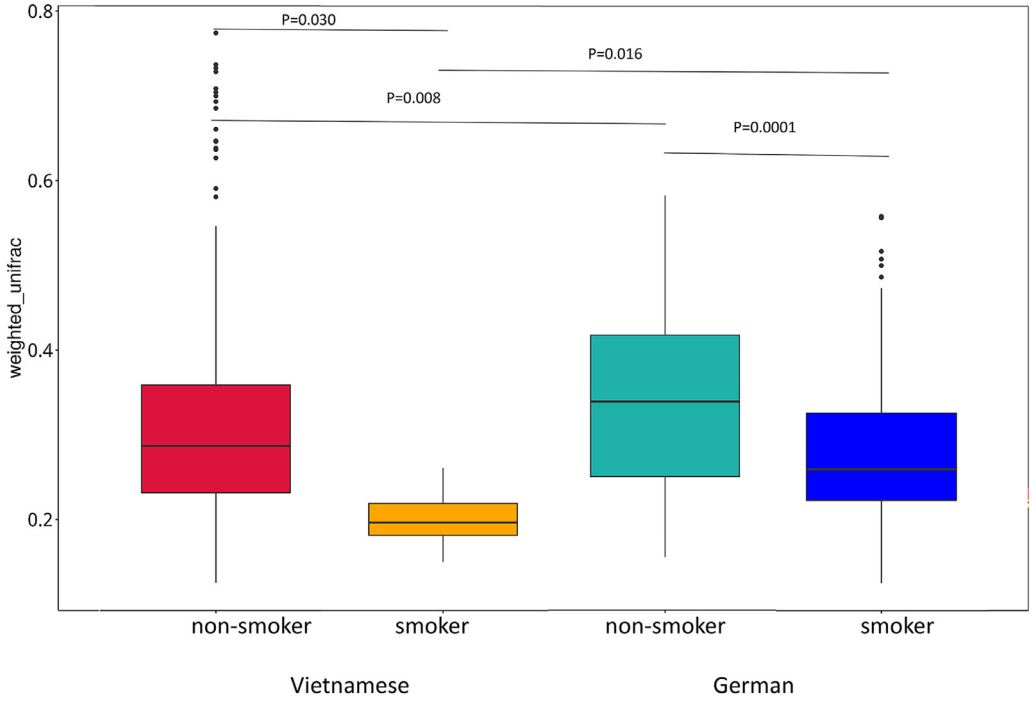

Fig. 6 – Beta diversity was significantly reduced in Vietnamese patients compared to German patients and in smokers in both groups.

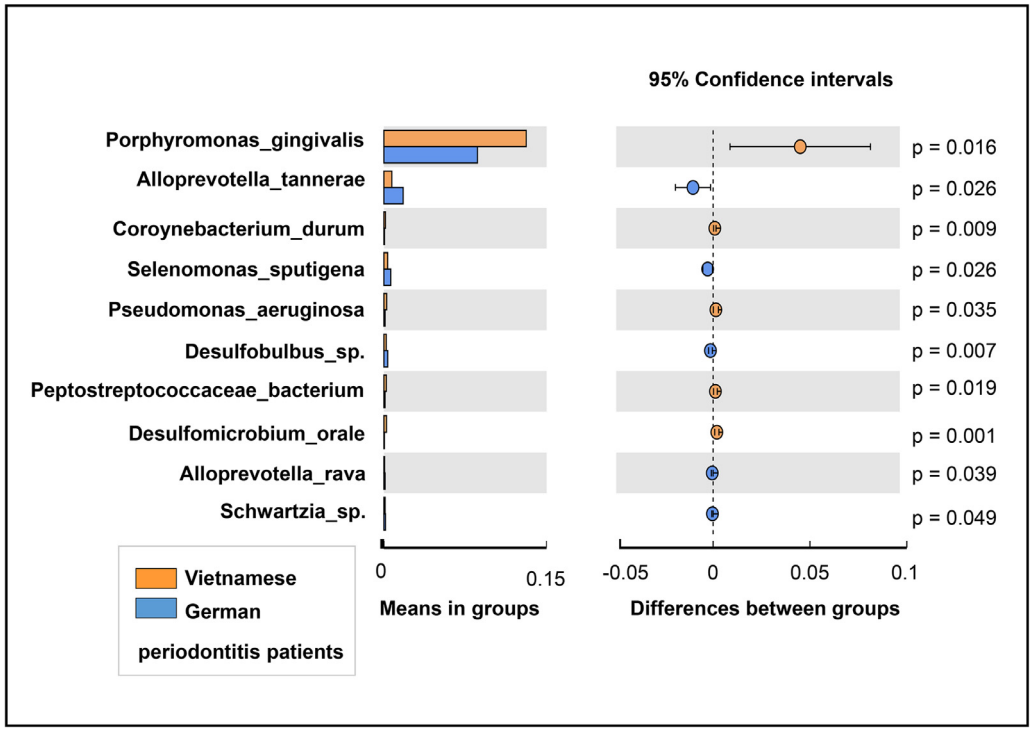

Fig. 7 – Mean differences at species level in Vietnamese and German periodontitis patients.

In our study, the influence of nicotine consumption on the subgingival microbiota was particularly evident when analysing alpha and beta diversity in both groups. The alpha diversity assessed by Shannon index reflecting both richness and evenness of the microbiota in one habitat. In contrast, beta diversity describes the differences in microbiota composition between different habitats or communities. In both study groups smoking appeared to increase alpha diversity (Figure 4) and decrease beta diversity (Figure 6). However, the trend of lower alpha diversity in German patients and significantly lower beta diversity in Vietnamese patients is also evident in the smoker/nonsmoker groups. On these two variables, therefore, there appear to be real ethnic differences.

The importance of alpha diversity in relation to the presence of periodontitis is controversially discussed. One hypothesis is that the richness and diversity of the oral microbiota is increased in subjects with periodontitis compared to those without the disease. This could be explained by the fact that the loss of periodontal support tissue with pocket formation provides better ecological growth conditions for several bacteria, while at the same time limiting the immune response to the bacteria organised in the biofilm. In line with this hypothesis, two previous papers[35,36] reported higher bacterial richness and Shannon diversity in patients with chronic periodontitis compared to healthy controls. A second hypothesis is that greater community diversity is associated with increased ecosystem resilience and a healthier state.[37] Two other studies have indeed demonstrated a disease-related decrease in alpha diversity in patients with periodontitis compared to periodontally healthy individuals.[38,39] Moreover, it is assumed that disease severity increases, beta diversity appears to decrease as the microbiota becomes increasingly similar due to the dominance of putative pathobionts associated with periodontitis.[38] In the LDA/LefSe analysis, the overall groups were not divided into smokers and nonsmokers, as the number of cases in the individual groups was too small to be able to draw reliable conclusions. Vietnamese and German microbiotas differed mainly in the relative distribution of *P.g.* (Vietnamese) and *Fusobacterium* (Caucasians). *P.g.* is a Gram-negative, anaerobic bacterium belonging to the red complex of Socransky et al[41] and is one of the most potent putative periodontal pathogens. The bacterial count was associated with both probing depth and disease activity. In addition, *P.g.* may be involved in the pathogenesis of several diseases such as atherosclerosis, rheumatoid arthritis or Alzheimer's disease.[40] The class *Fusobacteria* includes the periodontal pathogen *F.n.* with the subspecies *F. n. vincentii, F. n. nucleatum, F. n. polymorphum* and *F. periodonticum*, which assigned to the orange complex according.[41] *F.n.* is a Gram-negative, anaerobic bacterium and is classified as a moderate periodontal pathogen.[42] In biofilm formation, *F.n.* is thought to play a bridging role, mediating the aggregation and interaction of primary colonisers, such as *Streptococcus* species, with anaerobic secondary colonisers, such as *P.g.* and *A.a.*[43] *F.n.* increases the infectivity of *P.g.*, suggesting that these bacteria act cooperatively to counteract the host immune response.[44]

Comparison of absolute mean values at the species level shows a significantly higher incidence of *A.t.* (formerly *Prevotella tannerae*) in Germans compared to Vietnamese patients. *A.t.* is a Gram-negative, obligately anaerobic, nonmotile bacterium from the genus *Alloprevotella* and family *Prevotellaceae*.[45] Two species of this family, *P.i.* and *Prevotella nigrescens*, belong to the orange complex of putative periodontal pathogens. *P.i.* is a highly and *P.n.* a moderately pathogenic

bacterium. A.t. may be important also in aetiology of peri-implantitis, early childhood caries, dentinal caries, endodontic infections, halitosis, and oral squamous cell carcinoma.[46]

Other previous studies using NGS, like ours, also suggest ethnic differences in the oral microbiota. Li et al[8] examined the salivary microbiota of healthy individuals from Alaska, Germany, and Africa and found differences in alpha and beta diversity. Mason et al[6] studied plaque and saliva samples from 192 individuals from four major ethnic groups in the US (non-Hispanic blacks, non-Hispanic whites, Chinese, and Latinos). They found that the Shannon index was significantly lower in non-Hispanic blacks than in the other groups. Using an automated classification system, a person's ethnicity could be determined by their subgingival microbiological signature. Another study showed differences in alpha diversity and bacterial composition according to disease severity and ethnicity (Spain, Colombia). In Colombians, but not in Hispanics, a significant increase in alpha diversity was found with disease severity.[7]

The differences in both, periodontal conditions and the microbiota that have been highlighted in this study may underline the need for individualised medicine, especially in an increasingly multicultural society. German patients have a greater need for periodontal treatment and should be motivated to change their diet and give up smoking. Only 20% of Vietnamese periodontitis patients lived in Germany have received periodontal treatment to date (76% in German patients). Thus, Vietnamese patients need to be examined more for periodontitis. Analysis of the microbiota may be one necessary prerequisite for more targeted therapy of periodontitis. Thus, a first commercial test (PadoBiom, IAI AG) was developed to analyse the subgingival microbiota using NGS (https://www.institut-iai.ch, accessed 2 October 2023). Whether this test can actually answer the question of whether a patient will actually benefit from adjuvant antibiotic therapy and which antibiotics are the most suitable has to be investigated in further studies.

In order to reduce the use of antibiotics in treatment of periodontitis, alternative therapeutic strategies are increasingly being proposed. For instance, probiotics and parabiotics added to toothpastes, chewing gums, or mouthwashes for daily oral hygiene may selectively influence the microbiota.[47] Toothpaste with probiotics, alone or in combination with a chewing gum with probiotics lead to reduction in orange complex bacteria, including *P.i.* and *F.n.*[48] In contrast, daily use of a toothpaste and mouthwash containing parabiotics led to a reduction in red complex bacteria.[49] Further research is also needed on this topic.

Limitations of the study

The results of the study are limited to adult Vietnamese with periodontitis living in Germany and are not representative of the entire Vietnamese population. Based on the data collected in our study, it was not possible to investigate the reasons for the lower caries experience of the Vietnamese patients. This would have required dietary analysis and examination of supragingival plaque. We suspect that many Vietnamese patients, although living in Germany, have maintained their traditional diet, which may be less cariogenic.

The results regarding the subgingival microbiota generated in the present study should be verified in larger cohorts and with multivariate study design.

Conclusions

German-Caucasian patients with periodontitis had more severe periodontal symptoms, more periodontal risk factors (smoking, obesity), and a higher caries prevalence than Vietnamese-Asian patients living in Germany. Only one-fifth of Vietnamese patients with periodontitis have received periodontal therapy to date. The demonstrated differences in oral microbiota between the two groups do not yet justify an individualised anti-infective treatment approach.

Conflict of interest

The authors declare that they have no conflict of interest. No external funding, apart from the support of the author's institution, was available for this study.

Funding

No.

Data availability statement

The comprehensive study protocol is available from the German Clinical Trials Register (DRKS) under the registration number 00019827. The sequencing data have been published in the SRA database 'https://dataview.ncbi.nlm.nih.gov/object/PRJNA1077902?reviewer=2nhtme7gvam8e7938lno6o2anu' under accession number PRJNA1077902.

Author contributions

All authors have made substantial contributions to conception and design of the study. Conceptualization, St.R., H.S., S.S.; methodology, St.R., H.S., S.S.; software; validation; formal analysis; investigation; resources; data curation, St.R., H.S., S.S.; writing – original draft preparation, St.R.; writing – review and editing, St.R., S.S., H.S. visualization, St.R., S.S.; supervision, St.R.; project administration, St.R., S.S. All authors have read and agreed to the published version of the manuscript.

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