Comparison of Oral Hygiene Status and Relative Abundance of Periodontal Pathogens Between Individuals With and Without Intellectual Disabilities: a Cross-Sectional Study

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Abstract

Background: Studies have reported a high prevalence of periodontal disease in individuals with intellectual disability (ID). The aim of this study was to compare the relative abundance of periodontal pathogens (red, orange, yellow, purple, and green complexes) between individuals with ID and healthy controls.

Methods: Of the 31 subjects enrolled in this study, 16 with severe ID were selected from the outpatient clinic of the Special Care Dentistry of Hiroshima University Hospital, and 14 healthy subjects were selected from the outpatient clinic of another department at the same hospital. Dental plaque was sampled after oral examination. Decayed, missing, and filled permanent teeth (DMFT) counts were obtained and periodontal measurements were taken using the papillary-marginal-attached (PMA) index, plaque index (PI), gingival index (GI), and probing depth (PD). Gene sequencing (16S rRNA) was performed for each sample using next-generation high-throughput sequencing methods. The relative abundance of the periodontal pathogens and the clinical parameters were compared. An unpaired t-test was used to compare the oral health status in both groups and analysis of covariance was performed to compare the relative abundance of each pathogen.

Results: No statistically significant difference in DMFT was observed between the two groups. However, significant differences in the median PMA index, PI, and GI were noted between the groups ($P < 0.0001$). In addition, the mean PD in the ID group was significantly higher than that in the control group ($P < 0.0001$). The relative abundances of *Tannerella* spp. and *Treponema* spp. were significantly higher in the ID group when compared to the control group at the genus level ($P = 0.0383$ and 0.0432, respectively); alternatively, the relative abundance of *Porphyromonas* spp. was significantly lower in the ID group ($P < 0.0001$).

Conclusions: The PMA index, PI, GI, and PD were significantly lower in the ID group than in the control group. On the other hand, no significant difference in DMFT was observed between the two groups. Furthermore, our findings indicate that *Tannerella forsythia* might be more closely associated with periodontal disease than *Porphyromonas gingivalis* in individuals with ID.

Background

The surface of the oral cavity is colonized by numerous, diverse, and indigenous microorganisms that affect the oral health and disease states in humans [1, 2]. There are an estimated 700 + different bacterial species that form a complex microbial community in the oral cavity, most of which are commensal and essential for maintaining the ecosystem [3]. Furthermore, the different ecological conditions and microbial communities form a unique community that varies depending on the location in the oral tissues [1].

Periodontal disease is one of the pathological states caused by the oral microbial communities, which include bacteria such as *Tannerella forsythia*, *Porphyromonas gingivalis*, *Prevotella intermedia*,

Fusobacterium spp., and spirochetes (such as *Treponema denticola*) [4]. It has been reported that bacterial species exist in complexes in subgingival plaque [5]. Socransky et al. reported that six major complexes, including 28 species, were consistently observed in the dental biofilms of patients with periodontal disease [5]. In addition, *P. gingivalis*, *T. forsythia*, and *T. denticola*, which form the "red complex," were found to be strongly associated with periodontal pockets and considered as the most common etiologic agents of periodontitis [5]. Those complexes were named red complex, orange complex, yellow complex, purple complex, and green complex according to the strength of periodontal pathogenicity [5].

Intellectual disability (ID) is defined as a neurodevelopmental disorder that begins during childhood and is characterized by intellectual difficulties as well as difficulties in conceptual, social, and practical areas of living [6]. Some studies have reported that the oral hygiene condition is poorer and the risk of dental disease is higher in individuals with ID when compared with those without disabilities [7–11]. The condition of the oral cavity is affected by the degree of intellectual disability and stages of development in individuals with ID [12]. In addition, other factors such as age, underlying medical conditions, physical disabilities, and the caregiver's personality as well as concern about the oral health of the person being cared for influence the condition of the oral cavity [8]. Some individuals with ID do not cooperate with the caregivers because it is difficult for them to recognize the importance of maintaining oral health [13]. It is not easy to maintain a healthy oral environment in patients with ID [14]; therefore, measures that can be used to prevent oral diseases and improve the oral health in these individuals are necessary [14, 15]. Individuals with ID have difficulty maintaining good oral hygiene despite the guardian's/caregiver's efforts. Thus, patients with ID can be models of patients whose oral hygiene condition remains poor such as those with dementia and higher brain dysfunction. It is necessary to consider the oral microbial flora to develop strategies for maintaining oral health of patients with ID. Some studies attempted to elucidate the constitution of the oral microbial flora in patients with Rett syndrome and Down syndrome [16, 17]; however, to the best of our knowledge, there are no reports on the oral microbial flora in individuals with ID.

The aim of this study was to compare the differences in the microbial compositions of the oral cavities between individuals with and without ID using 16S rRNA gene high-throughput sequencing. The relative abundance of the five complexes (red, orange, yellow, purple, and green) was compared between the two groups of individuals. Additionally, the decayed, missing, and filled permanent teeth (DMFT) counts, papillary-marginal-attached (PMA) index, plaque index (PI), gingival index (GI), and probing depth (PD) were compared between the two groups.

Methods

Study design and subjects

Thirty outpatients (16 with ID and 14 without ID) were allocated to the ID and control groups, respectively. All subjects underwent several periodontal examinations and plaque samples were collected in November

2018. The characteristics of the subjects are shown in Table 1. The inclusion criteria for the study included subjects confirmed with "severe ID" based on a nursing certificate issued by the Hiroshima City. The diagnosis was made based on the developmental age of the individuals using multiple development tests and the estimated intelligence quotient was 35–50. The other criteria for inclusion in the study were as follows: age, 20–60 years; presence of permanent dentition; and history of having visited the outpatient clinic at the Department of Special Care Dentistry, Hiroshima University Hospital for regular check-ups after active treatment for dental disease for at least 1 year. The exclusion criteria were being extremely uncooperative while collecting the dental plaque, being under medications (including antibiotics over the past 3 months), and smoking regularly. The hospital treatment records of the individuals for 2016–2018 were reviewed to clarify that the subjects did not have any disease, such as diabetes, autoimmune disease, or infectious disease, that may affect the development of periodontal disease.

Based on a similar study [12], we determined that at least 12 subjects with ID and healthy subjects were needed to ensure an adequate sample size. Initially, 20 patients who seemed to meet the criteria were provided with explanations about the study; subsequently, detailed interviews were taken, and 16 of them were included in the ID group. The control group comprised 14 age- and gender-matched subjects from the outpatient clinics of other departments at the hospital.

Ethical information

This study was approved by the Ethical Committee of the University (Epidemiology-No. E-342), and was registered at the University Hospital Medical Information Network (UMIN) Center (ID 000023503). After having explained the purpose and contents of this study according to ethical guidelines of the Helsinki Declaration (2013), written consent from each participant or guardian was obtained. The subjects were free to withdraw their consent at any time for any reason.

Table 1. Characteristics of the patients

	ID group	Control group	P-value
n	16	14	-
Gender, n, (%)			0.4572[a]
Male	11 (68.75)	7 (50.00)	
Female	5 (31.25)	7 (50.00)	
Age, years, mean ± SD	35.4 ± 8.1	38.0 ± 12.5	0.5078[b]
Disability, n, (%)			
Intellectual disability	16 (100.00)	0 (0.00)	<0.0001[a]
Physical disability	1 (6.25)	0 (0.00)	<0.0001[a]
Number of teeth, mean ± SD	26.4 ± 2.3	28.0 ± 0.00	0.0060[c]
DMFT, mean ± SD	11.1 ± 8.0	8.5 ± 5.5	0.3025[b]
PMA index, median, [IQR]	22.0 [20.5, 26.0]	2.5 [1.0, 4.0]	<0.0001[b]
PI, median, [IQR]	1.9 [1.0, 2.2]	0.4 [0.0, 0.2]	<0.0001[b]
GI, median, [IQR]	1.6 [1.3, 1.7]	0.1 [0.0, 0.4]	<0.0001[b]
PD, mean ± SD	2.8 ± 0.3	2.1 ± 0.2	<0.0001[b]

[a] Fisher's exact test, [b] unpaired t-test, and [c] Mann−Whitney U test.

Values are mean ± SD unless otherwise noted.

DMFT, Number of decayed, missing, and filled teeth; IQR, Interquartile range; PI, plaque index); GI, gingival index; PD, probing depth; SD, standard deviation; ID, intellectual disability; PMA, papillary-marginal-attached

Clinical periodontal parameters measurements

Oral and periodontal examinations were performed by a well-experienced dentist under artificial lighting with the patient lying supine on the dental chair. The DMFT score, PMA index [18], PI [19], GI [20], and PD were recorded. The DMFT score was calculated as the total number of carious (D), extracted (M), and restored (F) teeth per person. The PMA index was evaluated by visual inspection of the anterior gingival units (papillary, marginal, and attached gingiva) around the erupted teeth based on the degree of redness and swelling, without the aid of an instrument. The PMA index was graded as follows: grade 0, no

inflammation; and grade 1, presence of inflammation. The GI was graded as follows: 0, no inflammation; 1, mild inflammation; 2, moderate inflammation; and 3, severe inflammation. The GI and PD were assessed using a calibrated periodontal probe (UNC-15; Hu-Friedy, Chicago, IL, USA) on four aspects (mesiobuccal, distobuccal, mesiolingual, and distolingual) of six teeth (maxillary right first molar, maxillary right lateral incisor, maxillary left first premolar, mandibular right first premolar, mandibular left lateral incisor, and mandibular left first molar).

Intra-examiner reliability

To assess the reproducibility of the PD, 10 patients with two contralateral teeth and a periodontal pocket depth of 6 mm or more who did not participate in the study were used. The patients were evaluated by the investigators three times at three-day intervals with sufficiently reproducible PD measurements (grand mean, 2.2; change in mean, 0.07; typical error, 0.30; repeatability coefficient, 0.84; and intraclass correlation coefficient, 0.63). The reproducibility of the PMA index, PI, and GI were assessed as described for PD, showing a sufficiently high repeatability (data not shown).

Collection of oral samples and DNA extraction

Dental plaque was collected by thoroughly brushing all the teeth for 1 min using a sterile toothbrush [13,14] followed by the rinsing of the toothbrush in 15 mL of distilled water for 30 s. The obtained samples were placed in tubes and immediately sent to the J-Bio 21 Center laboratory (Nippon Steel & Sumikin Eco-tech Corp., Japan), where the genomic DNA was extracted and stored at −20°C. DNA extraction, polymerase chain reaction (PCR) analysis, next-generation sequencing, and analysis using the Quantitative Insights Into Microbial Ecology (QIIME) [21] software package were all performed in the same laboratory. Genomic DNA was extracted from a 0.5-g sediment sample using the Extrap Soil DNA Kit Plus, ver.2 (J-Bio21 Center, Tsukuba, Japan).

PCR amplification and DNA sequencing of bacterial 16S rRNA

The extracted DNA was quantified using a PicoGreen dsDNA assay kit (Invitrogen, Carlsbad, USA), and the number of 16S rRNA gene copies in the extracted DNA was determined by quantitative PCR assay using a Bac1115 Probe quenching probe and primers Bac 1055YF and Bac1392R [22]. The Bac1115Probe was labeled with boron-dipyrromethene at the 3′-end and a quenching fluorescence dye (Qprobe; J-Bio21 Center, Tsukuba, Japan) at the 5′-end. All the adenine nucleotides of the Bac1115Probe were modified with locked nucleic acids. Quantitative PCR assays were performed using a Rotor-Gene Q (Qiagen, Valencia, CA, USA) with the following parameters: initial denaturation at 93°C for 2 min, followed by 50 cycles of 15 s at 93°C and 20 s at 61°C. Thereafter, the number of 16S rRNA gene copies per gram in each sample was calculated for each sediment sample. The U515F (5′-GTGYCAGCMGCCGCGGTA-3′) and 926R (5′-CCGYCAATTCMTTTRAGTT-3′) primer sets were used to amplify the V4−V5 region of the bacterial 16S RNA gene [21]. PCR product sequencing was performed with an Illumina MiSeq instrument (Illumina KK, Tokyo, Japan) and sequence data were analyzed using QIIME [23]. Approximately 30,000 430-bp reads were obtained for each sample after filtration (minimum quality value, 25) and removal of

the chimeras. Sequences with ≥97% similarities were then clustered in operational taxonomic units (OTUs) using UCLUST with the furthest algorithm [24]. The most abundant reads from each cluster were assigned at the representative sequences. The OTU representatives were subjected to phylogenetic analysis using the Greengenes [25] and Living tree of Silva [26] databases.

The relative abundance of bacterial 16S rRNA gene copies for each OTU in the sediment samples (copies g-sediment^{-1}) were evaluated by multiplying the total number of 16S rRNA gene copies per gram of sediment by the number of target bacterial sequence read OTUs and dividing by the total number of sequence reads.

OTUs related to bacteria capable of utilizing azo dyes and aniline (as a representative aromatic amine) were analyzed against the KEGG [27] and UniProt (UniProt Consortium, 2011) databases. In order to facilitate quantitative comparisons with data for gene copy number among the samples collected over 3 years, OTU sequences related to bacteria capable of utilizing azo dye and aniline were represented as 16S rRNA gene copies per g of sediment.

Statistical analysis

The sample size was derived by power estimation as described in a previous study, which evaluated the difference in the percentage of periodontal pathogens in the subgingival biofilm between healthy individuals and patients with chronic periodontitis [28]. The values for continuous variables with non-normal distribution are expressed as median and interquartile range (IQR), while those with normal distribution are expressed as mean and standard deviation (SD); the values for categorical variables are presented as number and percentage. An unpaired *t*-test was used to compare continuous variables with normal distribution, while Mann–Whitney *U* test was used for variables with non-normal distribution and the Fisher's exact test for nominal variables. Endpoint analysis was based on an analysis of covariance model, with the test group as effect and the baseline value as a covariate. We calculated the least square mean (LSM) of the difference in the relative abundance of each bacteria between two groups using the baseline abundance rate as a covariate. The SAS 9.4 software package (SAS Institute Inc., Cary, NC, USA) was used for all statistical analyses, and *p*-values <0.05 indicated statistical significance.

Results

Participants

The characteristics of the patients are shown in Table 1. The participants in the ID group (11 men, 5 women; mean age, 35.4 years; range, 26−56 years) presented with severe ID (intelligence quotient, 35−50). The 14 participants (7 men, 7 women; mean age, 38.0 years; range, 20−56 years) in the control group did not have any disabilities. No significant differences in gender, age, and DMFT were observed between the two groups. All subjects in the ID group had IDs and one of them had physical disabilities. The mean number of teeth in the ID group (26.4) was significantly lower than that (28.0) in the control group (*P* =

0.0060). The PMA score, PI, GI, and PD were significantly higher in the ID group compared to the control group (*P* < 0.0001).

OTU analysis

The bacteriological constitution of each specimen was evaluated using the 16S rRNA gene amplicon analysis. A total of 2,669,004 high-quality leads of the 2,935,700 reads obtained were used for the analysis. The 16S rRNA gene amplicon analysis identified a total of 1,967,010 high-quality paired sequences obtained from 30 samples with 66,930.5 ± 20,454.5 (average ± SD) reads per sample, which were assigned 3,091 OTUs. The total and average of the total reads, and the total and average of the OTU reads in each group are shown in Table 2.

Table 2. OTU analysis		
	ID group	Control group
Number of samples, n	16	14
Number of total reads, n	743,620	1,223,390
Avg. number of reads, n	46,476	87,385
Number of reads in OTUs, n	3,091	1,979
Avg. number of OTUs, n	193	141
ID, intellectual disability; OTU, operational taxonomic units		

Number of carriers and relative abundance of each pathogen at the genus level

The number of carriers and the relative abundance of each pathogen at the genus level are shown in Table 3. The relative abundance of *Porphyromonas* spp., which includes *P. gingivalis*, was significantly lower in the ID group when compared to that in the control group. (*P* < 0.0001). The relative abundances of *Tannerella* spp. (including *T. forsythia*) and *Treponema* spp. in the ID group were significantly higher than those in the control group (*P* = 0.0383 and 0.0432, respectively). Alternatively, the relative abundances of five bacteria in the orange complex (*Prevotella* spp., *Fusobacterium* spp., *Streptococcus* spp., *Capnocytophaga* spp., and *Aggregatibacter* spp.) were significantly lower in the ID group when compared with those in the control group (*P* = 0.0006, *P* < 0.0001, *P* = 0.0008, *P* = 0.0236, and *P* = 0.0001, respectively).

Table 3
Number of carriers and the relative abundance of each pathogen at the genus level

	Group	Number of carriers, n (%)	Relative abundance, %, mean ± SD		LSM	Difference	95% CI			P-value	
Porphyromonas spp.	ID	16 (100)	0.35	± 0.34	0.35	−1.61	−2.07	to	−1.14	< 0.0001	*
	Control	14 (100)	1.96	± 0.80	1.96						
Tannerella spp.	ID	16 (100)	0.61	± 0.52	0.61	0.33	0.02	to	0.64	0.0383	*
	Control	14 (100)	0.28	± 0.20	0.28						
Treponema spp.	ID	16 (100)	1.17	± 1.33	1.19	0.83	0.03	to	1.64	0.0432	*
	Control	14 (100)	0.36	± 0.29	0.36						
Prevotella spp.	ID	16 (100)	1.16	± 0.78	1.17	−2.24	−3.43	to	−1.05	0.0006	*
	Control	14 (100)	3.41	± 2.08	3.41						
Fusobacterium spp.	ID	16 (100)	0.53	± 0.01	0.53	−4.10	−5.18	to	−3.02	< 0.0001	*
	Control	14 (100)	4.63	± 1.94	4.63						
Peptostreptococcus spp.	ID	13 (81)	0.02	± 0.04	0.02	−0.04	−0.07	to	−0.00	0.0433	
	Control	14 (100)	0.06	± 0.05	0.06						
Campylobacter spp.	ID	16 (100)	0.16	± 0.14	0.16	−0.12	−0.25	to	0.02	0.0851	
	Control	14 (100)	0.28	± 0.20	0.28						
Eubacterium spp.	ID	15 (94)	0.12	± 0.14	0.12	−0.01	−0.10	to	0.09	0.8375	
	Control	14 (100)	0.13	± 0.09	0.13						
Streptococcus spp.	ID	16 (100)	3.73	± 4.78	3.74	−13.48	−20.83	to	−6.12	0.0008	*
	Control	14 (100)	17.22	± 12.90	17.22						
Actinomyces spp.	ID	16 (100)	0.75	± 0.81	0.75	0.24	−0.27	to	0.75	0.3406	
	Control	14 (100)	0.51	± 0.43	0.51						
Veillonella spp.	ID	16 (100)	0.09	± 0.13	0.73	0.21	−0.34	to	0.75	0.4465	
	Control	14 (100)	2,37	± 1.27	0.53						
Eikenella spp.	ID	16 (100)	0.07	± 0.13	0.07	0.06	−0.02	to	0.14	0.1120	
	Control	14 (100)	0.01	± 0.01	0.01						
Capnocytophaga spp.	ID	16 (100)	5.45	± 3.29	5.46	−3.21	−5.96	to	−0.46	0.0236	*
	Control	14 (100)	8.67	± 3.82	8.67						
Aggregatibacter spp.	ID	2 (13)	0.00	± 0.00	0.00	−3.28	−4.77	to	−1.79	0.0001	*
	Control	14 (100)	3.28	± 0.29	3.28						

* p < 0.05
ID, intellectual disability
LSM (Least square mean): LSM is the least mean square of the difference in relative abundance of each bacterium between two groups.
95% CI: 95% Confidence interval of the difference in relative abundance of periodontal pathogens.

Number of carriers and relative abundance of each pathogen at the species level

The number of carriers and the relative abundance of each pathogen at the species level are shown in Table 4. Among the red-complex bacteria, the relative abundance of *T. forsythia* in the ID group was significantly higher than that in the control group (*P* = 0.0383). No significant differences in the relative abundances of the other two species were observed. Two bacteria from the orange complex (*C. gracilis* and *C. ochracea)* presented with significantly higher relative abundances in the ID group when compared with those in the control group (*P* = 0.0034). The relative abundances of *P. intermedia*, *F. nucleatum*, *C. gingivalis*, and *C. sputigena* in the ID group were significantly lower than those in the control group (*P* = 0.0065, *P* < 0.0001, *P* < 0.0001, *and P* < 0.0001, respectively).

Table 4

Number of carriers and relative abundance of each pathogen at the species level

	Group	Number of carriers, n (%)	Relative abundance (%) mean ± SD			LSM	Difference	95% CI			P-value	
P. gingivalis	ID	8 (50)	0.07	±	0.16	0.07	−0.04	−0.21	to	0.13	0.6430	
	Control	2 (14)	0.10	±	0.27	0.10						
T. forsythia	ID	16 (100)	0.61	±	0.52	0.61	0.33	0.02	to	0.64	0.0383	*
	Control	14 (100)	0.28	±	0.20	0.28						
T. denticola	ID	14 (88)	0.36	±	0.62	0.36	0.30	−0.05	to	0.65	0.0928	
	Control	10 (71)	0.06	±	0.06	0.06						
P. intermedia	ID	3 (19)	0.00	±	0.01	0.00	−0.43	−0.72	to	−0.13	0.0065	*
	Control	14 (100)	0.43	±	0.56	0.43						
P. nigrescens	ID	11 (69)	0.00	±	0.00	0.00	0.00	−0.00	to	0.01	0.4889	
	Control	7 (50)	0.00	±	0.01	0.00						
F. nucleatum	ID	1 (6)	0.00	±	0.00	0.00	−0.03	−0.04	to	−0.02	< 0.0001	*
	Control	14 (100)	0.03	±	0.02	0.03						
C. gracilis	ID	16 (100)	0.13	±	0.12	0.13	0.10	0.03	to	0.17	0.0045	
	Control	14 (100)	0.03	±	0.03	0.03						
S. sanguis	ID	16 (100)	0.12	±	0.23	0.12	0.00	−0.14	to	0.14	0.9934	
	Control	14 (100)	0.12	±	0.08	0.12						
S. gordonii	ID	16 (100)	0.04	±	0.05	0.04	−0.04	−0.09	to	0.01	0.0901	
	Control	14 (100)	0.09	±	0.07	0.09						
A. odontolyticus	ID	15 (94)	0.04	±	0.03	0.04	−0.03	−0.08	to	0.12	0.1922	
	Control	14 (100)	0.08	±	0.08	0.07						
E. corrodens	ID	14 (88)	0.07	±	0.13	0.07	0.06	−0.02	to	0.14	0.1120	
	Control	14 (100)	0.01	±	0.01	0.01						
C. gingivalis	ID	15 (94)	0.07	±	0.08	0.07	−3.30	−4.35	to	−2.26	< 0.0001	*
	Control	14 (100)	3.37	±	1.96	3.37						
C. sputigena	ID	16 (100)	0.31	±	0.36	0.31	−2.88	−3.90	to	−1.86	< 0.0001	*
	Control	14 (100)	3.19	±	1.89	3.19						
C. ochracea	ID	2 (13)	1.98	±	1.52	1.98	1.43	0.52	to	2.34	0.0034	*
	Control	14 (100)	0.55	±	0.57	0.55						

* p < 0.05

ID, intellectual disability

LSM (least square mean): LSM is the least mean square of the difference in the relative abundance of each bacterium between two groups.

95% CI: 95% confidence interval of the difference in the relative abundance of periodontal pathogens.

Discussion

The next-generation high-throughput sequence technique may clarify the constitution and function of the biofilm by metagenomics and metatranscriptome analysis [29]. Using next-generation high-throughput sequencing, we were able to examine the periodontal pathogens at both the genus and species levels at the same time. This study hypothesized that the relative prevalences of all five bacterial complexes was higher in the ID group when compared with those in the control group. However, the results of this study showed that the prevalence of periodontal disease bacteria in individuals with ID who had poor oral cleaning conditions was not consistently higher than that in healthy individuals. The relative abundance of *Porphyromonas* spp. was significantly lower in subjects with ID when compared with that in healthy

individuals. Though, *P. gingivalis* has been the focus of several studies due to its role in periodontitis, where it is often referred to as a "keystone pathogen" [30], the results of this study indicate the possibility that other periodontal pathogens are more deeply related to periodontal disease than *P. gingivalis*, particularly in individuals with ID.

The relative abundances of *Tannerella* spp., *T. forsythia*, and *Treponema spp.* were significantly higher in individuals with ID. Some studies have reported a strong association between *T. forsythia* and periodontitis [31]. Bloch et al. have reported the existence of an S-layer, which is the surface layer of *T. forsythia* [32]. It provides a protective coat with molecular sieving properties that aids in maintaining bacterial integrity, displaying the bacterial components, and interacting with host immune and non-immune cells [32]. Furthermore, *T. forsythia* is considered to possess certain factors that act as 'biological warfare agents', thereby supporting its pathogenicity [33]. Thus, it is possible that *T. forsythia* might play an important role during the initial stages of periodontitis by composing a "biological warfare" (mechanism wherein the bacteria support each other) for the other pathogens so that more pathogenic bacteria can live in periodontal pockets. Consequently, suppressing the proliferation of *T. forsythia* might be effective in preventing the progression of periodontal disease. However, further studies are required to understand the role of *T. forsythia* during the onset of periodontal disease in individuals with ID.

Evolution toward gum disease goes through three stages (i) formation of dental plaque; (ii) gingivitis, which is an inflammation of the gum due to the dental plaque, and (iii) periodontitis, in which alveolar bone and fibers that hold the teeth in place are irreversibly damaged [34]. The individuals in the ID group had difficulty in maintaining their oral hygiene and presented with poorer scores than the healthy individuals. However, the PD score of the ID group was < 3.0 mm, just like the control group, which indicated that there was no alveolar bone defect yet and the stage of periodontal disease was still gingivitis. On the other hand, the PMA, PI, and GI scores in the ID group were significantly higher than those in the control group. These results indicate that the progression of periodontal disease in disabled individuals was during the early stage and caused by poor oral cleaning. A previous study reported that *P. gingivalis* impairs host immune responses and appears to be necessary, but not sufficient, to cause periodontitis [35]. Hence, it is possible that *P. gingivalis* is not highly associated with the initial stages of periodontitis. In the current study, *T. forsythia*, rather than *P. gingivalis*, was found to be more deeply associated with early-onset periodontal disease in individuals with ID. However, the involvement of *P. gingivalis* may develop with the progression of the disease, loss of attachment, and deepening of the periodontal pocket. Thus, the dental plaques should be examined at an early stage of periodontitis occurrence to clarify the relative abundance of *T. forsythia*, and patients with a high relative abundance of these bacteria may be treated adequately to delay the progress of the disease via methods such as shortening the maintenance period and using probiotics that have an inhibitory effect on *T. forsythia*.

The clinical parameters of periodontal disease, such as PMA index, PI, GI, and PD, were significantly worse in the ID group when compared with those in the control group; these findings are in line with those reported previously [7−11]. On the other hand, there was not a significant difference in the DMFT score between the two groups. This may be because the ID subjects in this study experienced periodontitis

owing to poor oral hygiene. On the other hand, it is possible that regular professional oral cleaning performed in our department might have stopped or delayed the occurrence of dental caries in ID group because, unlike periodontal disease, dental caries does not develop within a few days.

Our study subjects were limited to patients with severe ID. The cleaning of the teeth using a toothbrush in such patients is difficult for the caregivers due to the patients' uncooperative attitude. Although the sample comprised individuals with ID, it should be noted that the same difficulties in providing oral care may be encountered in elderly individuals who cannot brush their teeth on their own. Nevertheless, additional studies are needed to investigate the relationship between periodontal pathogens and periodontal diseases in the elderly.

Taken together, we conclude that *T. forsythia* may be more closely associated with periodontal disease than *P. gingivalis* during the initial stages of periodontal disease in individuals with ID.

Limitations

This study has some limitations. First, the sample size was small because few patients with severe ID met the criteria for the study. This exploratory research was conducted with the minimum sample size determined by outcomes in a similar study conducted previously. Second, despite the progress in genome analysis technology, 100% accuracy is not guaranteed, especially at the species level. Therefore, new approaches and an analysis of the oral microbial flora are necessary to improve our understanding of the ecology of periodontal disease. However, the relative abundances of periodontal pathogens between individuals with IDs and healthy individuals were different.

Conclusions

In conclusion, the oral health status of patients with ID was worse than that of the healthy controls. In addition, the relative abundances of periodontal pathogens between individuals with ID and healthy individuals were significantly different. The relative abundance of *T. forsythia* was significantly higher in individuals with ID when compared with that in healthy individuals. These findings suggest that *T. forsythia* may be closely associated with periodontal disease in individuals with poor oral hygiene.

List Of Abbreviations

ID: Intellectual disability

PMA index: Papillary-marginal-attached index

PI: Plaque index

GI: Gingival Index

PD: Probing Depth

DMFT: Decayed, missing, and filled permanent teeth

Declarations

Ethics approval and consent to participate

This study was approved by the Ethical Committee of the University (Epidemiology-No. E-342), and has been registered in the UMIN Center (ID 000023503). After having explained the purpose and contents of this study according to ethic guidelines on Helsinki Declaration (2013), written consent from each patient or at least one guardian was obtained. Participants were told that they could withdraw their consent at any time for any reason.

Consent for publication

All authors agree with the publication.

Availability of data and materials

All data generated or analyzed in this study are included in this article. Any other information is available from the corresponding authors on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Author's contributions

YO, HN designed the study. CF, YM recorded and organized the data, performed for the oral examination, and contacted the patients. RK was responsible for data analysis. YO, JM, YO, HN participated in the manuscript processing and approved the final version.

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