Oral infection, periodontal disease and cytokine production in adults with Down syndrome

Authors:
Miki Kosaka1,3, DDS
Hidenobu Senpuku2, DDS, PhD
Asami Hagiwara3, DDS, PhD
Yoshiaki Nomura1, Associate professor, DDS, PhD
Nobuhiro Hanada1, Professor, DDS, PhD

Affiliations:
1 Department of Translational Research, Tsurumi University School of Dental Medicine, Japan
2 Department of Bacteriology, National Institute of Infectious Disease, Japan
3 Department of Dentistry, Tokyo Children’s Rehabilitation Hospital, Japan

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Abstract
Individuals with Down syndrome (DS) are known to be highly susceptible to periodontal disease, exhibiting a rapid progression and increased severity in younger age. They are also at high risk for Alzheimer’s disease (AD) with certain risk derived from amyloid-β (Aβ) accumulation. Periodontal disease in DS individuals is related to an impaired immune system, poor oral hygiene, gingival tissue abnormalities, salivary factors, microbial factors and oxidative stress with high levels of radical oxygen resulting in genetic abnormalities. However, simultaneous assessments of these factors were not performed to clear risk factors to periodontal disease in DS individuals. This study investigated relationships among various parameters in oral and systemic diseases in DS and non-DS subjects.

Thirty DS subjects and 38 non-DS subjects were enrolled in this study and their oral hygiene and oral disease status were examined. Unstimulated whole saliva and blood samples were collected to investigate the presence of periodontal bacteria, cariogenic bacteria and opportunistic pathogens; interleukin (IL)-6, IL-8 and tumor necrosis factor (TNF)-α saliva concentrations; and Aβ42 plasma concentrations. Among tested parameters, Aβ42 plasma concentrations, development of periodontal diseases, S. mutans rate, lactobacilli per total streptococci ratio, numbers of Candida and IL-6 and IL-8 saliva concentrations were significantly higher in DS subjects than in control subjects. Additionally, oral disease parameters, except for the decay-missing-filled index, were significantly higher in DS subjects than control subjects. However, no significant difference was observed in periodontal bacteria ratios between DS and control subjects.

Our results demonstrate that DS subjects are more likely to develop periodontal diseases, produce inflammatory cytokines and become infected by opportunistic pathogens in the oral cavity than control subjects. This is likely due to poor oral hygiene and decreased host defense responses rather than infection of pathogenic bacteria or Aβ accumulation.
1. Introduction

Down syndrome (DS) is one of the most frequent genetic diseases and affects approximately 1 in 700 live births (Parker et al., 2010). DS is caused by an aberration of chromosome 21 and its major symptoms include intellectual disability, characteristic facial features, congenital heart defects, poor muscle tone, short stature, hearing loss, leukemia, immunity deficits, thyroid disease, epilepsy, premature aging and Alzheimer’s disease (AD) (Head et al., 2012, Hill et al., 2003). Cohort studies have indicated that the survival of DS individuals has dramatically increased over the past 50 years (Glasson et al., 2002). In particular, early childhood survival of DS has drastically improved due largely to advances in cardiac surgery and general health management (Glasson et al., 2002). A recent report showed that 75% of adults with DS survived to 50 years of age and 25% over 60 years of age (Glasson et al., 2002). Consequently, the expected lifespan of DS individuals is now approaching 60 years. However, DS individuals have an increased risk of AD due to amyloid-β (Aβ) accumulation by 40 years of age and a very high risk of dementia (Margallo-Lana et al., 2007, Schupf et al., 2002, Webb et al., 2012, Wisniewski et al., 1985). Symptoms such as bradykinesia, homeboundness and sleep disorder in adults with DS significantly reduce their quality of life. Adults with DS are also at a higher risk for early onset AD than healthy individuals (Mehta et al., 2007, Schupf et al., 2002, 2010, Wisniewski et al., 1994). AD is caused by an accumulation of misfolded proteins in the brain resulting in oxidative and inflammatory damage, which in turn leads to synaptic dysfunction (Querfurth et al., 2010). The main protein is Aβ cleaved from amyloid precursor protein (APP) (Zhou et al., 2011). There are two major isoforms of Aβ: Aβ40 and Aβ42. Aβ42 is known to have high aggregation capacity and neuropathogenicity and is frequently detected in senile plaques in brains of AD patients (Iwatsubo et al., 1994). The Aβ gene is located on the long arm of chromosome 21, which exists in triplicate form in DS, and overexpression of this gene may lead to increased APP levels, Aβ deposition and early onset Aβ in DS individuals (Rumble et al., 1989). Therefore, DS individuals are at high risk for early onset AD.

DS individuals are also considered to be highly susceptible to periodontal diseases, exhibiting a rapid progression and increased severity in younger age (Amano et al., 2008, Orner et al., 1976, Reuland-Bosma et al., 1986). Approximately 90% or more DS individuals 30 years of age have periodontal disease (Amano et al., 2008, Orner et al., 1976). Additionally, periodontal bacteria were detected from children with DS (Amano et al., 2000, Faria Carrada et al., 2016). Periodontal disease in DS individuals is related to an impaired immune system, poor oral hygiene, tooth and gingival tissue abnormalities, salivary factors, microbial factors and oxidative stress with high levels of radical oxygen resulting in genetic abnormalities, such as gene overexpression (Desai et al., 1997, Ugazio, 1981). Additionally, immunodeficiencies resulting in decreased chemotaxis of neutrophils and monocytes and defects in T-cell maturation and B-cell function were reported in DS individuals (Amano et al., 2008, Ram et al., 2011). Moreover, DS individuals are considered to be at greater risk of colonizing Candida species or having oral microbial imbalance because of immune system abnormalities, poor oral hygiene and oxidative stress (Mohiddin et al., 2015). Additionally, recent research indicates that...
Candida albicans protein and DNA were detected from the brain and peripheral blood of AD patients (Alonso et al., 2014; 41, 2014; 33, Olsen et al., 2015). Thus, DS individuals, who have congenitally weakened immune systems, are at increased risk of oral yeast infection (Carlstedt et al., 1996).

Periodontal pathogens and the host response elevate pro-inflammatory cytokines levels. Cytokines are then released into systemic circulation adding to the systemic inflammatory burden. These pro-inflammatory molecules can compromise the blood-brain barrier and gain access to cerebral regions, subsequently priming/activating microglial cells and Aβ deposition in the brain, leading to neuronal damage (Engelhart et al., 2004, Lossinsky et al., 2004). Cytokine production can also occur in response to oral inflammation and may induce Aβ deposition in the brain.

Several reports about periodontal disease in DS individuals have been published (Cichon et al., 1998, Feria Carrada et al., 2016, Orner et al., 1976, Sakellari et al., 2005). Because DS individuals readily develop periodontal disease, dentists recommend dental intervention from childhood and that DS individuals receive regular dental checkups. However, DS individuals still have poor oral hygiene and dental self-efficacy, particularly tooth brushing, because of intellectual deficits, tooth alignment and oral habits such as mouth opening and oral respiration. Previous studies have evaluated relationships in inflammation levels during periodontal disease, oral cavity status and bacterial infection, however, simultaneous assessment of these factors is necessary to understand their relationships in DS individuals who continuously receive oral care from dentists and dental hygienists. In this study, we investigated the relationships among Aβ plasma concentrations, periodontal disease status, numbers of oral pathogenic bacteria and total bacteria, total streptococci rates and cytokine saliva concentration in DS subjects compared with control subjects. Significant correlations and parameters were identified as potential risk factors in DS subjects.

2. Material and Methods

2-1. Subjects

The study population consisted of 30 DS individuals who attended Tokyo Children’s Rehabilitation Hospital as outpatients. Thirty-eight subjects, who were recruited from the staff of Tokyo Children’s Rehabilitation Hospital, were included as a control group. The mean ages were 28.0 ± 9.8 for DS subjects and 31.8 ± 6.4 for control subjects. For DS individuals, trisomy of chromosome 21 was diagnosed by chromosomal examination. Individuals with severe systemic disease or infectious diseases, those who had taken antibiotics a month before starting the study and smokers were excluded. Informed consent was obtained from all participants. This study was approved by the Ethics Committee of Tsurumi University (approval number: 1213) and Tokyo Children’s Rehabilitation Hospital (approval number: 26-003).

2-2. Questionnaire

Activity of daily living and willingness of activities and motivation for life were evaluated by the Barthel Index and Vitality Index, respectively (Mahoney et al., 1965, Toba et al., 2002). Additionally, the questionnaire evaluated whether subjects received regular dental checkups at least
once a year, the number of tooth brushings per day, systemic disease and medication history. The questionnaires were filled out by a family member for DS individuals or by control subjects themselves.

2-3. Blood sampling and Aβ42 measurement

When blood sampling was necessary for diagnosis, monitoring of other diseases or medical checkup, an additional 2 mL of venous blood were collected in blood collection tubes containing EDTA to measure Aβ42 concentrations. Collected blood was then centrifuged at 4,000 rpm for 15 min to obtain plasma. The plasma was kept at -50°C and transported to a medical examination company (LSI Medience Corporation, Tokyo, Japan). Aβ42 concentration was measured using a Human beta Amyloid ELISA Kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan) at LSI.

2-4. Oral examination

One dentist performed all oral examinations using a mirror and pocket probe at the dental clinic. Dental caries were evaluated using the decay-missing-filled (DMF) index (Larmas et al., 2015) and oral hygiene was evaluated using O'Leary’s plaque control records, Debris Index (DI), Calculus Index (CI) and Oral Hygiene Index (OHI) (Wei et al., 1982). Pocket probing depth was evaluated and recorded for 6 sites per tooth (mesiobuccal, buccal, distobuccal, mesiolingual, lingual and distolingual). The following clinical parameters were recorded: probing depth (PD), Gingival Index (GI) and Gingival Bleeding Index (GBI) (Wei et al., 1981).

2-5. Saliva collection

Saliva was collected within 1 month after blood sample collection. Whole saliva samples were collected prior to oral clinical examinations at least 2 h after eating, drinking or tooth brushing. Numbers of periodontal bacteria, cariogenic bacteria and opportunistic pathogens and interleukin (IL)-6, IL-8 and tumor necrosis factor (TNF)-α saliva concentrations were measured. Two milliliters of resting saliva were collected using a portable aspirator and a transtracheal aspiration kit (Sumitomo Bakelite Co., Ltd., Tokyo, Japan). Immediately after collection, a sterilized cotton swab was immersed into the sample for measurement of cariogenic bacteria and kept in transport media. The rest of the collected saliva was centrifuged at 4,000 rpm for 15 min. The supernatant was subsequently kept at -50°C until further analysis to measure IL-6, IL-8 and TNF-α concentrations. The pelleted samples were used to measure periodontal bacteria.

2-5-1. Measurement of periodontal bacteria numbers

Bacterial counts were determined by GC Corporation (GC Corporation, Tokyo, Japan). Numbers of salivary periodontal bacteria, Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, Tannerella forsythia, Treponema denticola and Prevotella intermedia, were determined by a real-time PCR assay using DNA from sample pellets. All data were calculated as copy numbers of periodontal bacteria per total bacteria × 100.
2-5-2. Measurement of cariogenic bacterial numbers

Numbers of total streptococci, mutans streptococci (MS) and lactobacilli in saliva were determined by BML (BML, Inc., Tokyo, Japan). Each sample was plated onto Mitis-Salivarius agar plates (Nippon Becton Dickinson Co. Ltd, Tokyo, Japan) for culture of total streptococci, Mitis-Salivarius agar plates containing 0.2 U/ml of bacitracin (Wako Pure Chemicals) for selective culture of MS and Rogosa SL agar plates (Nippon Becton Dickinson Co. Ltd, Tokyo, Japan) for selective culture of lactobacilli. Plating was performed using an EDDY JET spiral plating system (IUL, S.A., Barcelona, Spain) and plates were incubated at 37°C under anaerobic conditions for 48 h. Following anaerobic incubation, the number of total streptococci, MS and lactobacilli was counted and expressed in colony-forming units (CFU). The MS rate was calculated as follows: (number of MS in CFU/number of total streptococci in CFU) × 100. The lactobacilli ratio was calculated as follows: (number of lactobacilli in CFU/number of total streptococci in CFU) × 100. Measurement of causative bacteria of oral diseases was also performed by BML.

2-5-3. Measurement of opportunistic pathogens numbers

Oral mucosal samples were obtained to rub 5 times from the both buccal mucosa of each individual using a sterile cotton swab (Eiken Chemical Co., Ltd., Tokyo, Japan). The samples were inoculated onto OPA Staphylococcus aureus agar plates (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) and CHROMagar™ Candida agar plates (CHROMagar, Paris, France) using a stick. The plates were incubated in atmosphere at 37°C for 24–48 h. S. aureus and Candida colonies were counted by one examiner.

2-5-4. Determination of IL-6, IL-8 and TNF-α concentrations

IL-6, IL-8 and TNF-α concentrations in saliva samples were measured using commercially available ELISA kits: IL-6 (Salimetrics LLC, State College, PA, USA), IL-8 (Thermo Fisher Scientific K.K., Yokohama, Japan) and TNF-α (Aviva Systems Biology Corporation, San Diego, CA, USA).

2-6. Statistical analysis

Student’s t-test was used to evaluate differences in questionnaire responses, Aβ42 plasma concentrations, oral status (DMF, plaque control records, DI, CI, OHI, PD, GI and GBI), periodontal bacteria rates, numbers of MS, lactobacilli and total streptococci, MS rate and lactobacilli ratio per total streptococci, numbers of opportunistic pathogens, and IL-6, IL-8 and TNF-α concentrations between DS and control subjects. Data are presented as the mean ± standard deviation (SD) unless otherwise indicated. The detection rate of opportunistic pathogens was analyzed using Pearson's chi-square test. Correlations were expressed using Pearson’s correlation coefficient. Statistical significance was set at a p-value of <0.05. Statistical analyses of Pearson's chi-square test and Pearson’s correlation coefficient were performed using IBM SPSS Statistics (Version 22.0; IBM SPSS, Tokyo, Japan).

3. Results

3-1. Subject characteristics

The subject characteristics are shown in Table 1. DS subjects tended to be younger than control subjects, however, this
difference was not significant. Significantly more DS subjects (80%) had chronic complications, including congenital heart disease, thyroid gland malfunction, epilepsy, congenital enteropathy, psychiatric disorder, gout, hepatic dysfunction and obesity, compared with control subjects (15.8%) (p<0.001). Consequently, the number of DS subjects receiving medicine was significantly higher than that of control subjects (p<0.001). Control subjects received medication for allergic diseases such as asthma. Regarding oral care, the number of tooth brushings per day was significantly lower in DS subjects than in control subjects (p=0.013). However, 76.7% of DS subjects received regular dental checkups at least once a year in contrast to 23.7% of control subjects (p<0.001). Furthermore, Barthel Index levels were lower in DS subjects; similarly, activities of daily living levels were significantly decreased in DS subjects compared with control subjects (p<0.001). Vitality Index levels, which indicates the will of significant activity, was also significantly decreased in DS subjects compared with control subjects (p<0.001). Aβ42 plasma concentrations were significantly higher in DS subjects than in control subjects (p<0.001) (Figure 1).

Table 1. Characteristics of DS and control subjects.

<table>
<thead>
<tr>
<th></th>
<th>DS (n=30)</th>
<th>Control (n=38)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (M/F)</td>
<td>21/9</td>
<td>11/27</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>28.0 ± 9.8</td>
<td>31.8 ± 6.4</td>
<td>0.060</td>
</tr>
<tr>
<td>Chronic disease (%)</td>
<td>80.0</td>
<td>15.8</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Medication (%)</td>
<td>60.0</td>
<td>10.5</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Number of tooth brushings/day</td>
<td>2.3 ± 0.6</td>
<td>2.6 ± 0.5</td>
<td>0.013*</td>
</tr>
<tr>
<td>Dental visits (once or more/year)(%) a</td>
<td>76.7</td>
<td>23.7</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Barthel Index b</td>
<td>87.1 ± 14.2</td>
<td>100.0 ± 0.0</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Vitality Index c</td>
<td>8.5 ± 1.8</td>
<td>10.0 ± 0.0</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

* p<0.05

a Percentage (%) of people who visited a dental clinic at least once a year.

b The Barthel Index evaluates 10 items: diet, transfer, conditioning, toilet action, bathing, moving, stair climbing, changing clothes, defecation self-control and urination self-control with self-reliance degree. The score is set according to the degree of self-reliance with a maximum score of 100 points indicating complete self-reliance.

c The Vitality Index evaluates activities in everyday life in five items: awakening, communication, diet, excretion and activity with a maximum score of 10 points.
Figure 1. Aβ42 plasma concentration in DS and control subjects. Aβ42 plasma concentrations were measured by ELISA. Data are presented as a scatter plot. Bars indicate the median. DS subjects had significantly higher Aβ42 plasma concentrations than control subjects (*p<0.001).

3-2. Oral examination and evaluation of saliva samples

The DMF index, plaque control records and periodontal status of DS and control subjects are shown in Table 2. Parameters associated with periodontal disease, plaque control records, DI, CI, OHI, 3 mm < PD, GI and GBI, were significantly higher in DS subjects compared with control subjects (p<0.001, p<0.001, p=0.021, p<0.001, p=0.038, p<0.001, p=0.001). Interestingly, DMF index values were significantly lower in DS subjects compared with control subjects (p<0.001). Ratios (%) of periodontal bacteria in saliva are shown in Table 3. No significant differences were observed among the ratios of periodontal bacteria between the two groups. We next evaluated numbers of total streptococci, MS and lactobacilli in DS and control subjects. Although bacterial counts were higher in DS subjects compared with control subjects, no significant difference was observed. The MS rate and lactobacilli ratio were then calculated and found to be significantly higher in DS subjects compared with control subjects (p<0.001, p<0.001) (Table 4). Moreover, the number of Candida (35.2 ± 93.8 CFU) was significantly higher in DS subjects than in control subjects (1.1 ± 3.7 CFU) (p<0.001) (Table 5) and the isolation frequency of Candida in DS subjects (73.3%) was significantly higher than that in control subjects (13.2%) (p<0.001). C. albicans comprised most of the cultured Candida. Candida tropicalis was detected in one subject from each group. C. tropicalis and Candida parapsilosis were detected in one subject from the DS group. No significant difference was observed in the number of S. aureus and S. aureus was detected at a similar frequency in DS...
subjects (60%) and control subjects (52.6%). To evaluate inflammation levels in the oral cavity, salivary levels of inflammatory cytokines were measured (Figure 2). Salivary levels of IL-6 and IL-8 were significantly higher in DS subjects compared with control subjects ($p<0.001$, $p<0.001$) (Figure 2AB). In contrast, no significant difference was observed for TNF-α levels (Figure 2C).

![Figure 2](image_url)

**Figure 2.** Measurement of salivary cytokines. IL-6 (A), IL-8 (B) and TNF-α (C) concentrations were measured by ELISA in DS and control subjects. Data are presented as the mean ± standard deviation (SD) of three independent assays. Asterisks indicate significant differences as determined using Student’s t-test. (*$p<0.001$).
Table 2. Clinical periodontal measurements and oral characteristics in DS and control subjects.

<table>
<thead>
<tr>
<th></th>
<th>DS (n=30)</th>
<th>Control (n=38)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMF (%)</td>
<td>22.8 ± 4.9</td>
<td>35.8 ± 0.4</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Plaque control records (%)</td>
<td>49.8 ± 18.6</td>
<td>24.8 ± 13.3</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>DI</td>
<td>2.4 ± 0.5</td>
<td>1.8 ± 0.4</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>CI</td>
<td>1.0 ± 0.7</td>
<td>0.7 ± 0.6</td>
<td>0.021*</td>
</tr>
<tr>
<td>OHI</td>
<td>3.4 ± 1.0</td>
<td>2.5 ± 0.9</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>3 mm &lt; PD (%)</td>
<td>3.2 ± 5.4</td>
<td>1.4 ± 2.9</td>
<td>0.038*</td>
</tr>
<tr>
<td>GI</td>
<td>0.9 ± 0.4</td>
<td>0.4 ± 0.4</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>GBI (%)</td>
<td>21.0 ± 11.7</td>
<td>13.4 ± 7.9</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

* p<0.050

DMF: Decay-missing-filled Index, DI: Debris Index, CI: Calculus Index, OHI: Oral Hygiene Index (DI+CI), PD: pocket probing depth, GI: Gingival Index GBI: Gingival Bleeding Index.

Table 3. Rates of periodontal bacteria in saliva from DS and control subjects.

<table>
<thead>
<tr>
<th></th>
<th>DS (n=25)</th>
<th>Control (n=38)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porphyromonas gingivalis</td>
<td>0.4 ± 0.0 × E-02</td>
<td>0.3 ± 0.0 × E-02</td>
<td>0.538</td>
</tr>
<tr>
<td>Aggregatibacter actinomycetemcomitans</td>
<td>0.2 ± 0.0 × E-02</td>
<td>0.1 ± 0.0 × E-02</td>
<td>0.570</td>
</tr>
<tr>
<td>Treponema denticola</td>
<td>1.4 ± 0.0 × E-02</td>
<td>5.4 ± 0.3 × E-02</td>
<td>0.422</td>
</tr>
<tr>
<td>Tannerella forsythia</td>
<td>19.3 ± 1.1 × E-02</td>
<td>6.7 ± 0.2 × E-02</td>
<td>0.408</td>
</tr>
<tr>
<td>Prevotella intermedia</td>
<td>0.2 ± 0.0 × E-02</td>
<td>0.4 ± 0.0 × E-02</td>
<td>0.564</td>
</tr>
<tr>
<td>Red complex</td>
<td>4.5 ± 0.1 × E-02</td>
<td>1.2 ± 0.5 × E-02</td>
<td>0.423</td>
</tr>
</tbody>
</table>

Data (%) were calculated as follows: (copy numbers of periodontal bacteria/total bacteria) × 100. Red complex: P. gingivalis + T. denticola + T. forsythia.

3.3. Correlations between Aβ42 plasma concentration and oral status or salivary cytokine levels.

Correlations between Aβ42 plasma concentrations and oral status (plaque control records, OHI, PD, GI and GBI) or salivary cytokine levels (IL-6, IL-8 and TNF-α) were analyzed. No significant correlation was observed between Aβ42 plasma concentration and other parameters.
Table 4. Total streptococci, mutans streptococci and lactobacilli in saliva from DS and control subjects.

<table>
<thead>
<tr>
<th></th>
<th>DS (n=29)</th>
<th>Control (n=38)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutans streptococci (CFU)</td>
<td>3.3 ± 0.7 × E+05</td>
<td>1.4 ± 0.5 × E+05</td>
<td>0.202</td>
</tr>
<tr>
<td>Lactobacilli (CFU)</td>
<td>4.7 ± 1.4 × E+05</td>
<td>0.9 ± 0.3 × E+05</td>
<td>0.119</td>
</tr>
<tr>
<td>Total streptococci (CFU)</td>
<td>567.0 ± 34.1 × E+05</td>
<td>417.0 ± 35.0 × E+05</td>
<td>0.087</td>
</tr>
<tr>
<td>Mutans streptococci rate (%)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.4 ± 0.9</td>
<td>0.2 ± 0.5</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Lactobacilli ratio (%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.5 ± 1.4</td>
<td>0.2 ± 0.4</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

<sup>a</sup> The mutans streptococci rate was calculated as follows: (mutans streptococci in CFU/total streptococci in CFU) × 100.

<sup>b</sup> The lactobacilli ratio was calculated as follows: (lactobacilli in CFU/total streptococci in CFU) × 100.

*<i>p</i>&lt;0.05

Table 5. Number of Candida and <i>S. aureus</i> in DS and control subjects.

<table>
<thead>
<tr>
<th></th>
<th>DS (n=30)</th>
<th>Control (n=38)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida (CFU)</td>
<td>35.2 ± 93.8</td>
<td>1.1 ± 3.7</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>&lt;i&gt;S. aureus&lt;/i&gt; (CFU)</td>
<td>17.6 ± 38.9</td>
<td>10.0 ± 20.2</td>
<td>0.50</td>
</tr>
</tbody>
</table>

*<i>p</i>&lt;0.05

4. Discussion

It is well known that DS individuals readily develop periodontal disease and potentially severe inflammation (Khocht et al., 2012; 18, Morinushi et al., 1997, Reuland-Bosma et al., 1986). In this study, periodontal conditions and the oral hygiene status of DS subjects were significantly worse than that of control subjects. However, DS subjects received more dental checkups than control subjects. Generally, development of periodontal disease may be prevented if individuals receive regular dental checkups than control subjects. However, self-efficacy for tooth brushing was not sufficient for DS subjects to maintain good oral hygiene following regular checkups. Several reports have evaluated periodontal bacterial levels in samples from DS individuals (Faria Carrada et al., 2016, Morinushi et al., 1997, Sakellari et al., 2005). Cellular mobility of gingival fibroblasts was more impaired by <i>P. gingivalis</i> infection in DS individuals compared with non-DS individuals (Murakami et al., 2008). In contrast, another report indicated that salivary levels of periodontal bacteria in DS subjects did not differ from those in control subjects, although DS subjects had deep periodontal pockets (Khocht et al., 2012; 47). In the
present study, significant differences in the salivary rates of oral periodontal bacteria between DS and control subjects were not observed. Although the number of periodontal bacteria was not different between the two groups, other commensal bacteria may infect periodontal tissues and induce inflammation because DS individuals have low preventive immunity against bacterial infection. Taken together, periodontal disease likely occurs in DS individuals due to poor oral hygiene and decreased host defense responses rather than infection by periodontal bacteria.

The MS rate and lactobacilli ratio were significantly higher in DS subjects compared with control subjects. The numbers of MS and lactobacilli were also greater in DS subjects than in control subjects, although these differences were not significant. In contrast, DMF index values were significantly lower in DS subjects compared with control subjects. These results were consistent with previous studies (Areias et al., 2013, Orner et al., 1976). Moreover, plaque control records in DS subjects were significantly higher than in control subjects. We speculate that plaques containing more streptococci (including MS) did not affect development of dental caries in DS subjects. However, DS subjects visited the dental clinic more frequently than control subjects. Therefore, DS subjects received more oral checkups and dental treatment. This behavior may be important to prevent dental caries in DS subjects. One potential explanation for the discrepancy between number of cariogenic bacteria and DMF index values is inflammation according to abnormal immune reactivity. Another potential explanation may be differences in activities of antibacterial agents in salivary components and gingival crevicular fluid between DS and control subjects (Barr-Agholme et al., 1997, Tsilingaridis et al., 2012).

The average number of C. albicans and the isolation frequency was significantly higher in DS subjects compared with control subjects. This difference in the presence of C. albicans may be derived from congenital immunodeficiency in DS individuals (Carlstedt et al., 1996). Importantly, C. albicans may be a risk factor for AD (Alonso et al., 2014; 41, 2014; 33). Not only does Candida cause intraoral infectious disease, but Candida is also an opportunistic fungus that causes respiratory infectious disease (Shweihat et al., 2015). DS individuals may have a high frequency of upper respiratory tract infections characterized by increased severity and a prolonged disease course, which are attributed to immune system defects (Ram et al., 2011). Candidiasis usually affects the mouth but can spread throughout the entire body. Therefore, oral care is essential to prevent candidiasis in the oral cavity of DS subjects. C. albicans infection in the oral cavity may be useful as a marker to evaluate immune system abnormalities in DS individuals.

Salivary levels of IL-6 and IL-8 were higher in DS subjects compared with control subjects. Generally, salivary levels of IL-6 are affected by periodontal disease, sleep disorder and stress. A previous study reported that IL-6 concentrations in gingival crevicular fluid were higher in DS individuals than in non-DS individuals (Tsilingaridis et al., 2012). IL-6 induces osteoclast differentiation, which can progress to bone destruction (Iwamoto et al., 2009). Therefore, IL-6 might be induced as an oral inflammation mediator in DS subjects. The IL-8 concentration subsequently increases
due to inflammation and induces neutrophil chemotaxis during gingivitis, chronic periodontitis and aggressive periodontitis (Ertugrul et al., 2013). Periodontal disease increases salivary IL-8 levels (Ertugrul et al., 2013). Therefore, the cause of high salivary IL-8 levels may be derived from gingival tissue inflammation in DS individuals.

The Aβ42 plasma concentration was higher in DS subjects compared with control subjects. A high Aβ42 plasma concentration is a risk factor for the onset of AD (van Oijen et al., 2006). After the onset of AD, plasma Aβ42 deposits in the brain and coagulates as senile plaques; subsequently, the Aβ42 plasma concentration decreases (Schupf et al., 2010). Elevated Aβ42 plasma concentrations are associated with the progression (Mayeux et al., 2003) and survival time of AD patients (Cosentino et al., 2010). However, in this study, the Aβ42 plasma concentration did not exhibit a significant correlation with any parameters in DS subjects. Therefore, Aβ42 plasma concentrations are not likely linked to oral conditions such as development of periodontal diseases or infection of cariogenic bacteria and opportunistic bacteria.

In conclusion, we demonstrated that DS subjects are more likely to develop periodontal diseases, produce inflammatory cytokines and become infected by opportunistic pathogens in the oral cavity than control subjects. These factors are likely dependent on poor oral hygiene and decreased host defense rather than infection of pathogenic bacteria. Aβ42 plasma concentrations, although increased in DS individuals, were not associated with various oral conditions such as development of periodontal diseases or infection of cariogenic bacteria and opportunistic bacteria. It is necessary to improve self-efficacy and oral care of gingival tissues to prevent inflammation in DS individuals. Therefore, oral care education should be provided to DS individuals and caregivers to improve the quality of life of DS individuals. Detection of salivary bacteria, including cariogenic bacteria and opportunistic pathogens, and cytokines such as IL-6 and IL-8 might be useful parameters to understand oral cavity abnormalities in DS individuals.

Conflicts of interest

The authors have no conflicts of interest to declare in regard to this study.

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