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Increased oxidative stress biomarkers in the saliva of Down syndrome patients

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ABSTRACT

Objective: The DNA oxidation byproduct 8-hydroxy-2′-deoxyguanosine (8-OHdG) is a well-known biomarker used to evaluate oxidative stress. We previously reported that the generation of reactive oxygen species (ROS) is increased in cultured gingival fibroblasts (GF) from patients with Down syndrome (DS). Thus, the aim of this study was to evaluate 8-OHdG as a marker of oxidative stress in saliva of DS patients.

Materials and methods: The study group consisted of DS patients (66 patients; age range 1–62 years) and systemically healthy control subjects (71 subjects; age range 4–58 years). Periodontal status was judged based on standard measurements of probing depth (PD) and gingival index (GI). The salivary levels of 8-OHdG were determined using an enzyme-linked immunosorbent assay.

Results: The mean of PD and GI values were not significantly different between young (1–12 years) patients with DS (DS-1) and controls (C-1) or between adult (30–62 years) patients with DS (DS-2) and controls (C-2). There were statistically significant positive correlations between the salivary 8-OHdG levels and GI in the DS-1, DS-2 and C-2 groups, but not in the C-1. There were also statistically significant positive correlations between salivary 8-OHdG levels and PD in the DS-2 and C-2 groups, but not in the DS-1 or C-1 groups. The salivary levels of 8-OHdG of DS-1 and DS-2 groups were significantly higher than in the C-l and C-2 groups, respectively.

Conclusions: These results suggest that progressive oxidative stress occurred in DS patients. Oxidative stress may contribute to the clinical features of DS, particularly to the progressive periodontitis characteristic of early ageing.

1. Introduction

Down syndrome (trisomy 21; DS) is caused by a chromosomal aberration that generates peculiar physical characteristics, mental retardation, and systemic alterations. The general systemic dysfunction characteristic of DS manifests as a propensity for leukaemia,[1] thyroid dysfunction,[2] immune disorders,[3] diabetes,[4] precocious dementia of the Alzheimer type,[5] obesity,[6] and premature ageing. Conversely, the

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prevalence of coronary artery diseases such as atherosclerotic complications and cardiovascular disease beginning in adulthood[7] and solid tumors[8] is low among DS patients. The copper-zinc superoxide dismutase (SOD-1) gene is a candidate tumour suppressor gene because it is overexpressed on chromosome 21 of DS patients.

Oral manifestations of DS include high susceptibility to gingival inflammation with early onset and rapidly progressive and generalized periodontitis leading to subsequent destruction of supporting tissues[9] and loss of teeth. Among DS patients under 30 years of age, 60% to 100% suffer from periodontal disease.[10]

Several *in vitro* and *in vivo* studies have indicated that general systemic dysfunction characteristic of DS is associated with oxidative stress, mainly due to the over-expression of SOD-1,[11,12] which is encoded at chromosomal region 21q22, giving rise to an oxidative stress positive feed-back.[13] We previously reported that increased $HO^{\bullet}$ generation in cultured gingival fibroblasts with DS was strongly decreased in the presence of the hydrogen peroxide (H_2O_2) scavenger catalase, or the iron chelator desferal.[14] This may due to the enzymatic ability of over-expressed SOD-1 in DS to catalyze the formation of H_2O_2 from $O_2^{\bullet-}$.

Oxidative stress is defined as a condition occurring when the physiological balance between oxidants and antioxidants is disrupted in favour of the former with potential for damage to the organism.[15] Oxidative stress is involved in many pathologic conditions such as cancer,[16] atherosclerosis,[17] diabetes[18] and inflammatory diseases.[19] Periodontal infection is also associated with increased oxidative stress.[14,20] It is well known that proteins, lipids, and DNA are susceptible to oxidation, resulting in a wide variety of chronic diseases and acute pathologic processes. Reactive oxygen species (ROS) can attack nucleic acids in living cells. One of the byproducts of oxidative damage of DNA in the nucleus is 8-hydroxy-2′-deoxyguanosine (8-OHdG), which arises from specific enzymatic cleavage after 8-hydroxylation of guanine. Several studies have indicated that the 8-OHdG levels in body fluids are a biomarker of oxidative stress.[21] Urinary 8-OHdG has been detected in aged patients and in patients with cancer, atherosclerosis, rheumatoid arthritis, Parkinson disease, diabetes, and Down syndrome.[22,23] Recently, 8-OHdG was used as a marker for the evaluation of oxidative stress in subjects with periodontitis.[24] To date, no studies have direct assessed 8-OHdG in the saliva of patients with DS. Thus, the present study was designed to evaluate oxidative stress in saliva samples from DS patients and relationship between oxidative stress and various clinical parameters of DS patients.

2. Materials and methods

2.1. Study population

The subjects comprised 66 systemically healthy DS patients (39 males and 27 females; age range 1–66 years), who were referred to Kanagawa Dental College Hospital College Hospital or Kanagawa Children Medical Center for dental treatment, and 71 systemically healthy control subjects (C) (29 males and 42 females; age range 4–58 years).

Patients were instructed to refrain from taking antioxidant supplements during the study. Subjects were enrolled following informed consent as approved by the Ethical Committees of two independent institutions (Kanagawa Dental College, Kanagawa Children Medical Center, Kanagawa, Japan), in accordance with the Helsinki Declaration of 1975, as revised in 1983.

2.2. Clinical criteria for assessment of periodontal tissue

The clinical characteristics of periodontal tissue were assessed using standard measurements of clinical probe depth (PD) and gingival index (GI).[25] PD was measured using a PDT Sensor Probe® at 6 sites on each tooth: mesiobuccal, midbuccal, distobuccal, mesiolingual, midlingual, and distolingual. GI was measured at 4 sites; mesiobuccal, midbuccal, distobuccal, and midlingual. The respective sets of values were averaged to generate the mean PD and GI. The diagnostic criteria used have been reported previously.[26] None of the subjects showed bleeding or depth greater than 3 mm.

2.3. Saliva levels of 8-OHdG

Whole saliva samples were collected by the cotton roll method as non-stimulated saliva at least 4 h after food intake and before clinical measurements, because of the difficulty in collecting stimulated saliva in patients with mental retardation. Saliva samples were kept on ice during correction and centrifuged at $10{,}000 \times g$ for 15 min at 4 °C[27] and were filtered using an ultrafilter (cutoff molecular weight 10 kDa) to exclude interfering substances. The supernatants were stored at −80 °C for up to 1 month prior to analysis. The 8-OHdG levels were determined using an enzyme-linked immunosorbent assay (highly sensitive 8-OHdG Check™; Japan Institute for the Control of Ageing, Fukuoka, Japan). The levels of 8-OHdG were expressed relative to the amount of deoxyguanosine (dG) detected by UV absorbance at 254 nm. The amount of DNA was determined based on UV absorbance using a calibration curve generated with known amounts of 8-OHdG. The sensitivity range of the assay was from 0.125 to 200 ng/mL. Analyses were routinely run in duplicate to minimize instrumental and inter assay drift.

2.4. Statistical analyses

Descriptive data that included mean and standard deviations were determined for each clinical parameter in each group and were used for analysis. One-way ANOVA was used for multiple group comparisons followed by Scheff's test for pairwise comparisons. Correlations between salivary 8-OHdG levels and clinical parameters were analysed by Pearson' correlation test. For all tests, a P value of 0.05 or less was considered for statistical significance. The statistical analysis was done using SPSS software 15.01 version.

3. Results

3.1. Subjects

The gender composition and median ages of the study groups are shown in Table 1. The DS and C groups were subdivided

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