Prostaglandin E_2 level in gingival crevicular fluid from patients with Down syndrome

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Barr-Agholme M, Krekmanova L, Yucel-Lindberg T, Shinoda K, Modéer T. Prostaglandin E_2 level in gingival crevicular fluid from patients with Down syndrome. Acta Odontol Scand 1997;55:101–105. Oslo. ISSN 0001–6357.

The levels of prostaglandin E_2 (PGE_2) and interleukin-1β (IL-1β) were determined in gingival crevicular fluid (GCF) collected from patients with gingivitis: 15 Down syndrome children and 15 controls. The mean level of PGE_2 in GCF was significantly higher ($P < 0.05$) in the Down syndrome group (10.0 pg/µl GCF) than in the control group (4.6 pg/µl GCF). In GCF samples collected from sites characterized as noninflamed, the mean level of PGE_2 was significantly higher ($P < 0.001$) in the Down syndrome group than in the controls. The mean level of PGE_2 in samples from inflamed sites, on the other hand, did not differ between the two groups. The mean level of IL-1β was not significantly higher in the Down syndrome group than in the controls. This study shows that the level of PGE_2 detected in GCF from Down syndrome patients is increased, a fact that may be of importance in the pathogenesis of the periodontal disease frequently seen in these patients. □ *Children; gingivitis; interleukin-1β; periodontal disease*

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Down syndrome is a genetic abnormality resulting from trisomy of the 21st chromosome (1). A decrease in the number of mature T lymphocytes and functional defects of polymorphonuclear leukocytes (PMN cells), resulting in reduced chemotaxis (2, 3) and impaired phagocytosis (4), are characteristic features of the syndrome. Thus, an increased susceptibility to infections is frequently seen among Down syndrome patients (for a review, see Ref. 5).

Patients with Down syndrome have a high prevalence of periodontal disease (6–9). Alveolar bone loss, occurring primarily in the mandibular anterior region, can be seen as early as at 11 years of age (9). In addition, it has been reported that Down syndrome patients develop more extensive gingival inflammation than controls during a period when all oral hygiene procedures are discontinued (10).

The inflammatory mediators interleukin-1 (IL-1), tumor necrosis factor α (TNFα), and prostaglandin E_2 (PGE_2) have been highlighted in the pathogenesis of periodontal disease (for a review, see Ref. 11). During the development of gingivitis, the concentrations of interleukin-1β (IL-1β) and PGE_2 in gingival crevicular fluid (GCF) have been reported to increase (12, 13). Furthermore, a positive correlation between the level of PGE_2 in GCF and the progression of the periodontal disease has been reported (14–17).

We have previously reported that the microbial composition of subgingival plaque is altered in Down syndrome patients with a higher frequency of *Actinobacillus actinomycetemcomitans* than controls (18). In the light of this finding and the fact that bacterial lipopolysaccharides from *A. actinomycetemcomitans* have been reported to induce the production of IL-1β in gingival fibroblasts (19) and in monocytes (20) and the production of PGE_2 in monocytes (21), the present investigation was undertaken. The aim was therefore to study whether the levels of PGE_2 and IL-1β in GCF, collected from sites with and without gingivitis, are increased in children with Down syndrome as compared with healthy controls.

Materials and methods

The patients with Down syndrome ($n = 19$) and the subjects in the control group ($n = 19$) chosen to match the Down syndrome patients with regard to age and sex, received regular dental treatment at the Department of Pediatric Dentistry, Karolinska Institutet, Huddinge, Sweden. Bitewing and periapical radiographs were taken using a standardized long-cone technique to examine the alveolar bone on the mesial and distal surfaces of fully erupted first permanent molars and central incisors. Alveolar bone loss was diagnosed when the distance from the cementoenamel junction to the alveolar crest, as measured on the radiograph, exceeded 2 mm (22). In addition, the clinical examination of the patients also included determination of the probing depth of the periodontal pockets on the mesial and distal surfaces of all first molars and central incisors. Patients who had one or more sites with an increased probing depth (>4 mm) or alveolar bone loss were excluded from the study. Four

Table 1. Distribution of gingival inflammation in Down syndrome subjects and in the control group

	Controls (n = 15)	Down syndrome (n = 15)	Significance
Gingival bleeding index			
<25%	8 (53%)	1 (6%)	
25%–50%	0	3 (20%)	
>50%	7 (47%)	11 (73%)	
No. of sites with bleeding on probing at sample site	42/94 (45%)	74/106 (70%)	**

Chi-square: $^{**}P < 0.01$.

patients in the Down syndrome group were therefore excluded. Among the controls, four patients were being treated orthodontically with fixed appliances and were excluded. The mean age of the final group of Down syndrome subjects (n = 15) was 12.1 years (range, 7–18 years) and in the control group (n = 15) 13.8 years (range, 8–18 years). The study was approved by the Ethical Committee of Karolinska Institutet.

Interview

The patients, together with their parents or guardians, answered a questionnaire concerning medical diseases, present medication, and frequency of infections. Patients reporting more than 10 infections per years were classified as susceptible to infections.

Crevicular fluid

GCF was collected from the mesial surfaces of first molars and/or central incisors: 110 samples in the Down syndrome group and 103 in the control group. Before the collection of GCF, supragingival plaque was carefully removed with a curette and cotton pellets without touching the marginal gingiva. The site was isolated with cotton rolls, and the surface was gently dried. A prefabricated paper strip (Periopaper, Pro Flow, Amityville, N.Y., USA) was inserted atraumatically into each sulcus and left for 15 sec. Paper strips contaminated with blood during GCF collection were excluded. A total of 106 samples in the Down syndrome group and 94 samples in the control group were analyzed. The volume of the GCF sample was quantified using a Periotron 6000 (Pro Flow). Thereafter the paper strip was placed in a tube with 125 µl phosphate buffer, pH 6.8, containing 0.15 mM indomethacin (Sigma Chemical Co., St. Louis, Mo., USA) and stored at −70 °C until analyzed.

Clinical examination

Gingival inflammation was assessed as bleeding on probing at six points around each tooth, in accordance with the gingival bleeding index (GBI) (23). The probing was performed by sweeping the probe (type LM; LIC, Finland; pressure, 25–30 g) around the tooth. The percentage of surfaces with gingivitis was estimated for each individual.

PGE_2 and IL-1β analysis

The level of PGE_2 was determined by radioimmunoassay with a commercially available kit with ^{125}I-PGE_2 (Du Pont/NEN, Dreieich, Germany) as tracer. The GCF samples were diluted between 1 and 10 times before the analysis of PGE_2 and IL-1β. The level of IL-1β was determined using a commercially available ELISA kit in accordance with the manufacturer's instructions (Quantikine, R&D systems, Minneapolis, Minn., USA). The detection limit for IL-1β with the ELISA kit was 2.0 pg/ml.

Statistical analysis

The Student's t test (two-tailed) was used to compare the means between the two groups. To adjust the skewed distribution with regard to the levels of GCF, PGE_2, and IL-1β, logarithmic values of the observations were used. Geometric means and 95% confidence intervals were calculated from the antilogarithms (24).

To compare frequencies between the two groups, the chi-square test was used. Analysis of variance (nonparametric test, Kruskal–Wallis) was performed to evaluate differences within the Down syndrome group with regard to the volume of GCF, the levels of PGE_2 and IL-1β when subgrouping for medical diseases, the present medication, and the frequency of infections.

Results

Questionnaire

On the basis of the questionnaire, the medical diseases noted among the Down syndrome patients were as follows: four subjects had an increased susceptibility to infections, primarily respiratory tract infections; three patients had thyroid deficiencies and were treated with sodium levothyroxine; one had diabetes mellitus; and three had congenital cardiovas-

Table 2. The mean volume of gingival crevicular fluid (GCF) and the levels of prostaglandin E_2 (PGE_2) and interleukin 1β (IL-1β) in Down syndrome subjects and in controls

Variables	Controls (n = 15), mean†	Down syndrome (n = 15), mean	Significance
GCF (µl)	0.23 (0.16–0.32)	0.32 (0.27–0.35)	NS
PGE_2 (pg/µl GCF)	4.6 (2.7–8.0)	10.0 (6.6–15.0)	*
(pg/0.1 ml)	0.6 (0.4–1.4)	2.2 (1.3–3.8)	*
IL-1β (pg/µl GCF)	89.1 (60.1–134.9)	135.9 (98.6–187.5)	NS
(pg/0.1 ml)	5.3 (10.0–23.6)	20.1 (14.0–28.9)	NS

Student's t test: *P <0.05.
† The results are presented as geometric mean and 95% confidence intervals.

cular malformation. None of these were of clinical significance at the time of the study. Among the controls, no medical diseases were diagnosed.

PGE_2 and IL-1β analysis

The clinical condition of the patients studied is shown in Table 1. The number of inflamed sites, on the basis of bleeding on probing, was significantly higher (P <0.01) in the Down syndrome group than in controls (Table 1). The mean volume of GCF and the levels of PGE_2 and IL-1β are shown in Table 2. There was no significant difference in the mean volume of GCF between the Down syndrome group (0.32 µl) and the controls (0.23 µl). The mean level of PGE_2 was significantly higher (P <0.05) in the Down syndrome group (10.0 pg/µl GCF) than in the control group (4.6 pg/µl GCF) (Table 2). The level of significance was the same, regardless of whether the concentration of PGE_2 in GCF was expressed as pg/µl GCF or as pg/0.1 ml. In contrast to PGE_2, the mean level of IL-1β did not differ significantly between the Down syndrome group (135.9 pg/µl GCF) and the controls (89.1 pg/µl GCF).

The levels of PGE_2 and IL-1β, in relation to the condition of the gingiva at the sample site, are presented in Table 3. In GCF samples collected from sites characterized as noninflamed, the mean level of PGE_2 was significantly higher (P <0.001) in the Down syndrome group (5.1 pg/µl GCF) than in the control group (2.1 pg/µl GCF). The mean volume of GCF collected from noninflamed sites was also significantly higher (P <0.05) in the Down syndrome group (0.23 µl) than in the control group (0.14 µl). In inflamed sites neither the mean volume of GCF nor the level of PGE_2 differed significantly between the two groups (Table 3).

In contrast to the inflamed sites, the mean concentration of IL-1β in GCF samples from the noninflamed

Table 3. The mean volume of gingival crevicular fluid (GCF) and the concentrations of prostaglandin E_2 (PGE_2) and interleukin 1β (IL-1β) at sample sites in relation to gingival condition at the sample site in Down syndrome patients and in the control group

	Noninflamed sites		Inflamed sites	
Variables	Controls (n = 52), mean†	Down syndrome (n = 35), mean	Controls (n = 42), mean	Down syndrome (n = 71), mean
GCF (µl)	0.14 (0.11–0.17)	0.23* (0.17–0.31)	0.30 (0.24–0.36)	0.26 (0.22–0.31)
PGE_2 (pg/µl GCF)	2.1 (1.6–2.9)	5.1*** (3.4–7.3)	4.6 0(3.1–7.2)	6.0 (4.5–8.3)
(pg/0.1 ml)	2.4 (1.8–3.2)	9.1*** (5.6–14.7)	11.3 (7.2–17.6)	12.8 (9.1–18.1)
IL-1β (pg/µl GCF)	63.1 (56.5–99.5)	79.4 (54.6–130.0)	63.1 (49.3–95.9)	93.3 (71.0–135.9)
(pg/0.1 ml)	8.2 (6.2–11.0)	15.6** (11.0–22.0)	15.9 (10.8–23.4)	19.4 (14.9–25.3)

Student's t test: *P <0.05; **P <0.01; ***P <0.001.
† The results are presented as geometric mean and 95% confidence intervals.

sites was significantly higher ($P < 0.01$) in the Down syndrome group (15.6 pg/0.1 ml) than in the control group (8.2 pg/0.1 ml). However, when the concentration of IL-1β was expressed as pg/µl GCF, there was no significant difference between the two groups in either the samples from the inflamed sites or the samples from the noninflamed sites.

Analysis of variance within the Down syndrome group showed no significant difference in the volume of GCF or in the levels of PGE_2 and IL-1β between the different medical diseases, the present medication, and the frequency of infections noted in the group.

Discussion

The novel finding in this study is that the level of PGE_2 in GCF is enhanced in Down syndrome patients as compared with controls. The higher PGE_2 concentrations in these patients as compared with the controls were found in GCF samples collected from noninflamed sites but not from inflamed sites. In contrast to PGE_2, the level of IL-1β did not differ significantly between the two groups.

In accordance with previous findings (9, 10, 18) we found that Down syndrome patients have more extensive gingival inflammation since the number of sites with bleeding on probing was significantly higher in the Down syndrome group than in the control group. The variable plaque, however, was not assessed in the patients owing to the limited extent of cooperation in the clinical examination among the Down syndrome patients. All subjects included in the study had gingivitis, but none of the patients had any site with an increased probing depth (>4 mm) or alveolar bone loss. GCF was collected from noninflamed and/or inflamed sites in both Down syndrome patients and controls. The mean volume of GCF, on the basis of the individual level, did not differ between the two groups even though the volume of GCF from noninflamed sites was higher in the Down syndrome patients than in the controls. However, one must consider that the classification of the sites, especially in Down syndrome patients, may be incorrect because of the patient's insufficient cooperation, and therefore the number of inflamed sites found may be too low. To minimize this source of error and the influence of the variation in sampling techniques used to collect GCF, the levels of PGE_2 and IL-1β were expressed not only as the concentration pg/0.1 ml but also as pg/µl GCF.

The greater volume of gingival exudate collected in noninflamed sites among the Down syndrome patients as compared with controls is compatible with previous findings of a more pronounced outflow of PMN cells in the tissue during a period of experimental gingivitis in Down syndrome patients than in the controls (25, 26). In addition, children with Down syndrome also had more gingival inflammation than controls, although the plaque accumulation was similar in the two groups (10). The enhanced level of PGE_2 found in GCF collected from noninflamed sites in Down syndrome patients is compatible with the view that prostanoids have the ability to induce vasodilatation (27), resulting in an increase in vasopermeability and an enhanced volume of gingival exudate. Whether there is a causal relationship between the enhanced PGE_2 level and the increased volume of GCF found in noninflamed sites in Down syndrome is so far unclear.

We have previously reported that the microbial composition of subgingival plaque in Down syndrome subjects is altered compared with controls, with higher levels of *A. actinomycetemcomitans* (18). The enhanced level of PGE_2 found in GCF collected from Down syndrome patients may be related to the altered composition of the subgingival microflora in Down syndrome patients. This suggestion is based on the fact that lipopolysaccharides from *A. actinomycetemcomitans* have been reported to stimulate the production of PGE_2 in monocytes (21). In the light of this finding and the fact that the number of mononuclear cells increases during inflammation, one would also expect the level of PGE_2 in samples from inflamed sites to be higher in Down syndrome patients than in controls.

The enhanced level of PGE_2 in GCF in Down syndrome patients, however, can be related to the trisomy state. This assumption is based on the observation that the enzyme Cu/Zn superoxide dismutase (CuZnSOD), which has been reported to be enhanced in Down syndrome cells (28, 29), converts superoxide anion radicals to hydrogen peroxide. In addition, hydrogen peroxide has been reported to stimulate prostaglandin endoperoxide (PGH) synthase (30), a key enzyme involved in PGE_2 formation and which results in an enhancement of the basal production of prostanoids (31). There is, however, one report suggesting that the increased gene dosage of CuZnSOD causes a reduction of PGE_2 formation in trisomy cells (amniotic cells) (32). It is so far unclear whether the effect of hydrogen peroxide on the metabolism of arachidonic acid differs in various types of cells.

We also determined the level of the cytokine IL-1β in GCF, since its level has been reported to be increased in GCF collected from sites with gingival inflammation (12, 13). In contrast to PGE_2, the level of IL-1β was not significantly higher in the Down syndrome group than in the controls. The reason why the level of IL-1β did not differ between the two groups, even though gingival inflammation was more pronounced among the Down syndrome subjects, may be related to the fact that the inflammatory mediator PGE_2 has been reported to downregulate the production of IL-1β in various cell types, such as fibroblasts and monocytes (33), which all contribute to the level of IL-1β detected in GCF. The fact that the level of PGE_2 but not that of IL-1β is enhanced in the GCF in Down syndrome patients as compared with controls, further supports the view that Down syndrome patients may have an alteration of the

metabolism of arachidonic acid, which may contribute to the enhanced level of PGE_2 in crevicular fluid.

In conclusion, this study shows that the level of PGE_2 in GCF is enhanced in Down syndrome patients and may be of importance in the pathogenesis of the periodontal disease frequently seen among these patients.

Acknowledgements.—This study was supported by grants from the Swedish Medical Research Council (Project 7211), the Swedish Patent Revenue Research Fund, and the Faculty of Odontology, Karolinska Institutet.

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Received for publication 4 June 1996
Accepted 31 October 1996