Enhanced levels of prostaglandin E_2, leukotriene B_4, and matrix metalloproteinase-9 in gingival crevicular fluid from patients with Down syndrome

Georgios Tsilingaridis, Tülay Yucel-Lindberg and Thomas Modéer

Department of Pediatric Dentistry, Institute of Odontology, Karolinska Institutet, Huddinge, Sweden

Tsilingaridis G, Yucel-Lindberg T, Modéer T. Enhanced levels of prostaglandin E_2, leukotriene B_4, and matrix metalloproteinase-9 in gingival crevicular fluid from patients with Down syndrome. Acta Odontol Scand 2003;61:154–158. Oslo. ISSN 0001-6357.

The aim was to study the levels of prostaglandin E_2 (PGE_2), leukotriene B_4 (LTB_4), and matrix metalloproteinase-9 (MMP-9) in gingival crevicular fluid (GCF) from Down syndrome patients exhibiting gingival inflammation. The levels of PGE_2, LTB_4, and MMP-9 were determined in GCF from 18 Down syndrome patients and from 14 controls matched with respect to age and degree of gingival inflammation. Probing depth (PD) and gingival inflammation, assessed by bleeding on probing (BOP), were determined around all teeth. In each patient, GCF was collected from 6 sites (16m, 26m, 36m, 46m, 41m, 11d) using periopaper, and the volume was determined using Peritron 8000. The PGE_2 and LTB_4 levels were determined using RIA kits and MMP-9 level using ELISA kits. The degree of gingival inflammation, expressed as mean value of BOP (%) as well as the volume of GCF, was similar between Down syndrome patients and controls. The mean levels of PGE_2, LTB_4, and MMP-9 were significantly ($P < 0.05$) higher in GCF from Down syndrome patients compared to controls. When comparing the two groups, the correlation coefficients for LTB_4 to BOP and PD, respectively, as well as for MMP-9 to BOP significantly differed between Down syndrome and controls ($P < 0.05$). The study supports the concept of an altered host response in periodontal tissue in Down syndrome subjects. □ *Crevicular fluid; Down syndrome; LTB_4; MMP-9; periodontal disease; PGE_2*

Thomas Modéer, Department of Pediatric Dentistry, Institute of Odontology, Karolinska Institutet, Box 4064, SE-141 04 Huddinge, Sweden. Tel. +46 8 728 83 21, e-mail. thomas.modeer@ofa.ki.se

Down syndrome is the most common chromosomal aberration resulting from trisomy of the 21st chromosome (1, 2). Several functional disorders and physical stigmata, such as mental abnormalities, susceptibility to infections, and hypotonic muscle function associated with the syndrome, have been described (3). Furthermore, Down syndrome patients also exhibit a decreased number of mature T-lymphocytes, functional defects of polymorphonuclear leukocytes, and decreased levels of serum IgG_2 and IgG_4, resulting in impaired phagocytosis and reduced chemotaxis (4–7).

Patients with Down syndrome are more prone to periodontal disease than are healthy subjects and other groups of mentally handicapped patients (8–10). In addition, children with Down syndrome develop more extensive gingival inflammation than controls when oral hygiene procedures are discontinued (9). Moreover, early signs of alveolar bone loss, mainly localized around incisors in the lower front region, can be seen as early as 11 years of age in Down syndrome children (2, 10).

In general, the host response in the dento-gingival area, induced by bacteria in the oral biofilm, leads to a cascade of inflammatory mediators including cytokines, chemokines, and prostaglandins (11). The inflammatory mediators interleukin-1 (IL-1), tumor necrosis factor α (TNFα), and the arachidonic acid metabolite prostaglandin E_2 (PGE_2) are highlighted in the pathogenesis of periodontal disease (12). In addition, the mediator leukotriene B_4 (LTB_4), another arachidonic acid metabolite, is enhanced in gingival crevicular fluid (GCF) collected from patients during development of experimental gingivitis (13). To our knowledge, no reports regarding GCF levels of LTB_4 in Down syndrome patients have been published previously. We reported earlier that PGE_2 level in GCF in Down syndrome patients is enhanced compared with that in age-matched controls (14). The enhanced PGE_2 levels in GCF in Down syndrome children may contribute to development of periodontal disease in light of the positive correlation between the level of PGE_2 in GCF and progression of periodontal disease (15–17).

The matrix metalloproteinases (MMPs), enzymes with the capacity to degrade the extracellular matrix (18), are also suggested to play an important role in degradation of the periodontal tissue. Furthermore, enhanced GCF levels of MMP-1, MMP-2, MMP-3, MMP-8, and MMP-9 have been demonstrated in patients with periodontitis (19). According to Halinen et al. 1996 (20), Down syndrome patients exhibit higher MMP-8 and MMP-9 immunoreactivity in saliva as well as in GCF, compared to age-matched healthy individuals.

In this study we test the hypothesis whether PGE_2, LTB_4, or MMP-9 levels are enhanced in GCF from Down syndrome patients compared to controls matched with respect to age and degree of gingival inflammation.

Taylor & Francis healthsciences

DOI 10.1080/00016350310002270 © 2003 Taylor & Francis

Table 1. The mean value of clinical and biochemical variables studied in Down syndrome and control subjects

	Down syndrome ($n = 18$)		Control ($n = 14$)		
	Mean	*s*	Mean	*s*	*P*
Clinical					
Age	16.8	4.0	16.4	2.7	ns
BOP (%)	75.9	23.9	76.1	30.3	ns
PD (mm)	2.6	0.5	3.2	0.3	**
Biochemical					
GCF (µl)	0.23 (0.07–0.42)	0.1	0.29 (0.12–0.46)	0.1	ns
PGE_2 (pg/ml)	335 (75.6–721.3)	191	185 (50.2–710)	169	*
LTB_4 (pg/ml)	916 (528.2–1376)	230	760 (447.3–1010)	178	*
MMP-9 (ng/ml)	94.8 (9.8–246.3)	68.2	37.3 (14.2–164.6)	37.9	**

Mean values between the groups were analyzed using Student's *t* test.
$P < 0.05$; ** $P < 0.01$; ns = not significant.
s = standard deviation.

Materials and methods

Down syndrome patients and controls, chosen to match the Down syndrome patients with regard to age and degree of gingival inflammation, received their regular dental treatment at the Department of Pediatric Dentistry, Karolinska Institutet, Huddinge, Sweden. The patients were examined clinically and radiographically. All the patients and/or their parents filled in a questionnaire regarding health conditions. The Ethics Committee at Huddinge University Hospital, Karolinska Institutet approved the study.

Patients exhibiting one or more sites with a probing depth more than 4 mm or alveolar bone loss were excluded from the study. From the original group of Down syndrome patients ($n = 26$), 3 were excluded owing to the occurrence of alveolar bone loss or attachment loss and 5 were excluded owing to lack of compliance during the clinical examination. The final group of Down syndrome patients ($n = 18$) were between the ages of 12 and 23, with a mean age of 16.8 years. The mean percentage of BOP in Down syndrome patients was 75.9% and varied between 17% and 100%. Of the Down syndrome patients, 6 had congenital cardiac malformations, 7 had increased susceptibility to upper respiratory tract infections, 1 had diabetes mellitus, and 3 had thyroid deficiencies treated with Levothyroxine (Levaxin®).

Out of 90 healthy (no medical disorders) clinically examined controls, 23 were found to match the final group of Down syndrome patients with respect to age and degree of gingival inflammation expressed as percentage of sites positive for bleeding on probing (BOP). Nine patients of the controls were excluded because of the occurrence of attachment loss or early signs of alveolar bone loss. The final control group ($n = 14$) exhibited a mean age of 16.4 years. The mean percentage of BOP was 76.1% and varied between 23% and 100%.

Clinical examination

The patients were examined clinically and radiographically. Gingival inflammation was assessed by sweeping the probe (type PCPUNC15, Hu-Fridey, Chicago, Il., USA) in the gingival sulcus at 6 points around each tooth (mesio-buccal, mid-buccal, mesio-lingual, disto-buccal, disto-lingual, and mid-lingual) according to Bleeding Index (21). The percentage of surfaces with BOP was estimated for each individual. Probing depth (PD) was measured to the nearest millimeter on mesial, distal, buccal, and lingual surfaces of all teeth using a graded probe (type PCPUNC15, Hu-Fridey, Chicago, Il., USA) with a pressure of 25–30 g. In addition, bitewing and periapical radiographs were taken using a standardized long-cone technique to examine the alveolar bone height on mesial and distal surfaces of fully erupted first permanent molars and central incisors. Alveolar bone loss was classified when the distance from the cemento-enamel junction to the alveolar crest on the radiograph exceeded 2.0 mm (22).

Sampling of gingival crevicular fluid

GCF was collected from the mesial surfaces of teeth 16, 26, 36, 46, 41 and the distal surface of 11. Supragingival plaque was carefully removed using cotton pellets and a curette before taking the sample. Each site was isolated with cotton rolls and gently dried with air. The paper strip (Periopaper, Pro Flow, Amityville, NY, USA) was inserted into each sulcus and left for 15 s. Paper strips contaminated with blood during the GCF collection were excluded. In total, 108 samples from the DS group and 80 samples from the control group were analyzed. Of the 108 samples from Down syndrome patients, 17 were taken from sites negative for BOP, whereas 91 were positive, i.e. inflamed sites. In the controls, the number of samples from non-inflamed sites was 10, and 70 samples were taken from inflamed sites. The volume of the GCF sample was

Table 2. The mean value of GCF, PGE_2, LTB_4, and MMP-9 in relation to gingival condition at the sample site in Down syndrome and control subjects

	Non-inflamed sites		Inflamed sites	
Variables	Control ($n = 10$) Mean (s)	Down syndrome ($n = 17$) Mean (s)	Control ($n = 70$) Mean (s)	Down syndrome ($n = 91$) Mean (s)
GCF (μl)	0.28 (0.19)	0.23 (0.23)	0.30 (0.19)	0.27 (0.17)
PGE_2 (pg/ml)	102 (51)	252 (201)	199 (195)	359 (244)
LTB_4 (pg/ml)	703 (171)	883 (241)	760 (278)	923 (338)
MMP-9 (ng/ml)	20.7 (16.1)	126.8 (108.0)	43.9 (54.6)	95.7 (79.9)

s = standard deviation.

quantified using a Peritron 8000 (Pro Flow). Thereafter the paper strip was placed in a tube containing 125 μl phosphate buffer (pH 6.8) and 0.15 mM indomethacin (Sigma Chemical Co., St. Louis, Mo., USA) and stored at $-70°C$ until analyzed.

Biochemical analysis

The levels of PGE_2, LTB_4, and MMP-9 were determined in GCF. The levels of arachidonic acid metabolites, PGE_2, and LTB_4 were determined using commercially available radioimmunoassay (RIA) kits (NEN Life Science products, Belgium) with ^{125}I- PGE_2 as tracer for PGE_2 and ^{3}H-tracer for LTB_4, respectively. The levels of total MMP-9 (active and pro-MMP-9) were determined using enzyme-linked immunosorbent assay (ELISA) kits from R&D systems, United Kingdom.

Statistical analysis

Student's independent t test (two-tailed) was used to compare the means between the groups as well as the correlations within the group. A general linear model ANOVA test was used when testing the difference in correlation coefficients between Down syndrome patients and controls. The Statistical Package for the Social Sciences (SPSS 10.0) was used as statistics program.

Table 3. Correlation coefficients between PD, BOP, GCF and the biochemical variables PGE_2, LTB_4, MMP-9 within Down syndrome and control subjects

	Down syndrome				Control		
	PD	BOP	GCF		PD	BOP	GCF
PGE_2	−0.03	0.096	0.23	*	0.160	0.416	−0.05
MMP-9	−0.11	−0.51	−0.11	*	0.601	0.287	−0.05
LTB_4	−0.27	−0.128	0.56	*	0.594	0.515	0.09

Difference in correlation coefficients between the groups was analyzed by General Linear Model ANOVA test, * $P < 0.05$.

Results

The clinical and biochemical variables studied in Down syndrome subjects and controls are given in Table 1. The degree of gingival inflammation, expressed as BOP (%), as well as the volume of GCF (μl) were in the same magnitude in Down syndrome patients as in matched controls. The mean value of PD (mm) was significantly ($P < 0.01$) lower in the Down syndrome group compared to the controls (Table 1). Levels of the biochemical variables PGE_2 (pg/ml), LTB_4 (pg/ml), and MMP-9 (ng/ml) were significantly ($P < 0.05$) higher in Down syndrome patients compared to controls (Table 1). Even though the mediators were expressed as pg/μl GCF, the levels of PGE_2, LTB_4, and MMP-9 were significantly higher in Down syndrome (data not shown).

The mean level of PGE_2, LTB_4, and MMP-9 in relation to gingival inflammation at the sample site is demonstrated in Table 2. The levels of PGE_2, LTB_4, and MMP-9 were higher in Down syndrome compared to controls, regardless of whether the GCF samples were obtained from sites characterized as non-inflamed or inflamed (Table 2).

The correlation coefficients between the clinical and biochemical variables within controls and the Down syndrome group are demonstrated in Table 3. The correlation coefficients for LTB_4 to BOP, as well as LTB_4 to PD, significantly ($P < 0.05$) differed between Down syndrome subjects and controls. In addition, MMP-9 in relation to BOP also significantly ($P < 0.05$) differed between Down syndrome subjects and controls.

Discussion

We demonstrate that the GCF levels of PGE_2, MMP-9, and LTB_4 are enhanced in Down syndrome compared to

controls matched with respect to age and degree of gingival inflammation. Moreover, the correlation coefficients for LTB_4 to BOP and to PD, respectively, as well as for MMP-9 to BOP ($P < 0.05$) differed significantly between Down syndrome subjects and controls.

In the present study the degree of gingival inflammation in terms of BOP as well as the volume of GCF were similar between Down syndrome patients and the matched controls. Thus, both groups consisted of patients exhibiting gingivitis with a similar degree of gingival inflammation. The Down syndrome subjects, however, exhibited a significantly lower mean value of PD compared to the control subjects. It is likely that the determination of PD among the Down syndrome patients might be underestimated compared to controls, because of insufficient cooperation during the probe penetration of periodontal pockets.

The level of PGE_2 was enhanced in GCF from Down syndrome compared to controls matched with respect to degree of gingival inflammation. The enhanced level of PGE_2 in Down syndrome subjects is in agreement with a previous study (14), although the controls in that study were not matched with respect to degree of gingival inflammation. Together with the previous finding, the results in this study further support the concept that the level of PGE_2 is enhanced in GCF in Down syndrome regardless of degree of gingival inflammation. This is compatible with the finding that PGE_2 is higher in Down syndrome patients, compared to controls, regardless of whether GCF samples were obtained from non-inflamed or inflamed sites, although the number of non-inflamed sites is low. However, one has to take into consideration that the number of subjects in this study is limited. Whether the enhanced level of PGE_2 in GCF in Down syndrome is associated with the chromosomal aberration is therefore a question which has to be further addressed.

Interestingly, we can demonstrate that not only PGE_2 but also the inflammatory mediator LTB_4 level is enhanced in GCF from Down syndrome subjects. Owing to the study design, however, we cannot rule out whether the enhanced level of LTB_4 is influenced by gingival inflammation, since the majority of the sample sites were characterized as inflamed. The enhanced levels of PGE_2 and LTB_4 in GCF reflect an upregulation of arachidonic acid metabolism, since both prostaglandins and leukotrienes are derived from arachidonic acid by action of the enzymes cyclooxygenase (COX) and lipooxygenase (23). The enhanced level of PGE_2 in GCF in Down syndrome might be caused by an alteration in the subgingival microflora (24). This assumption is based on the finding that the periodontopathogen *A. actinomycetemcomitans* (Aa) is more frequently found in subgingival plaque from Down syndrome patients compared to controls, and that LPS from Aa stimulates the production of PGE_2 in monocytes (25) and thereby contributes to enhanced GCF level of PGE_2.

In this study we also found that MMP-9 in GCF was enhanced in Down syndrome patients. The enhanced level of MMP-9 might be related to an upregulation of PGE_2 since it has been reported that PGE_2 stimulates MMP-9 production in monocytes/macrophages via the cAMP-signal dependent pathway (26, 27). In addition, the tissue inhibitor of matrix metalloproteinase-1, TIMP-1, reported to be decreased in GCF samples from periodontitis-affected patients compared to healthy controls (28), may also be of importance for the enhanced level of MMP-9 found in Down syndrome patients.

When comparing the correlations between clinical and biochemical variables (Table 3), the relationship between LTB_4 and the clinical condition in terms of BOP and PD differed significantly between Down syndrome and the controls. In the controls, LTB_4 and MMP-9 GCF levels were positively correlated to BOP, which is compatible with the view of an accumulation of neutrophils in the dento-gingival area during gingival inflammation and that the neutrophils are the major source of their production (13, 20). The mediator LTB_4 plays an important role in the recruitment of neutrophils during inflammation and consequently is strongly associated with gingival inflammation (13). In the Down syndrome patients, however, the LTB_4 was negatively associated with BOP, which may be related to a decreased chemotaxis, which has previously been reported in Down syndrome (4, 5, 29). The altered relationship between LTB_4 and BOP in the Down syndrome subjects, compared to controls, supports the concept of an altered host response in periodontal tissue in Down syndrome subjects. The altered correlation for MMP-9 to BOP between Down syndrome patients and controls may also be a consequence of the impaired chemotaxis in Down syndrome subjects, since MMP-9 is predominantly derived from degranulated triggered neutrophils in gingival crevice (30). However, to a minor extent, MMP-9 can also be derived from resident cells such as fibroblasts, endothelial cells, and epithelial cells (31, 32).

Regarding the relationship between the mediator PGE_2 to BOP, there was no statistical difference between Down syndrome and the controls. This is compatible with the finding that PGE_2 is enhanced in Down syndrome independently of degree of gingival inflammation and PGE_2 is mainly produced by cells that are not primarily affected by chemotaxis like neutrophils.

In conclusion, we have demonstrated that the levels of PGE_2, LTB_4, and MMP-9 in GCF are enhanced in Down syndrome compared to controls matched with respect to gingival inflammation and age. The altered correlation for the mediators LTB_4 and MMP-9 to BOP between Down syndrome and controls supports the concept of an altered host response in periodontal tissue in Down syndrome subjects.

Acknowledgements.—We are grateful to Mrs Agneta Gustavsson and Lena Johansson for skilful technical assistance and we thank the Jerome Lejeune foundation and the Swedish Patent Revenue Research Fund for financial support.

References

1. Lejeune J, Gautier M, Turpin R. Etude des chromosomes somatique de neuf enfante mongolien. C R Acad Sci 1959;248: 1721–22.
2. Ulseth JD, Hestnes A, Stavner LJ, Storhaug K. Dental caries and periodontitis in persons with Down's syndrome. Spec Care Dent 199;11:71–3.
3. Pueschel SM, Annerén G, Durlach R, Flores J, Sustrova M, Verma IC. Medical care in Down syndrome. Acta Paediatr Scand 1995;84:823–7.
4. Barkin RM, Weston WL, Humbert JR, Maine F. Phagocytic function in Down's syndrome. I. Chemotaxis. J Ment Defic Res 1980;24:243–9.
5. Annerén G, Björksten B. Low superoxide levels in blood phagocytic cells in Down's syndrome. Acta Paediatr Scand 1984;73:345–8.
6. Levin S. The immune system and susceptibility to infections in Down's syndrome. Prog Clin Biol Res 1987;246:143–62.
7. Annerén G, Magnusson CGM, Nordvall SL. Increase in serum concentrations of IgG_2 and IgG_4 by selenium supplementation in children with Down's syndrome. Arch Dis Child 1990;65:1353–5.
8. Barnett ML, Press KP, Friedman D, Sonnenberg EM. The prevalence of periodontitis and dental caries in a Down's syndrome population. J Periodontol 1986;57:288–93.
9. Reuland Bosma W, van Dijk LJ. Periodontal disease in Down's syndrome: A review. J Clin Periodontol 1986;13:64–73.
10. Modéer T, Barr M, Dahllöf G. Periodontal disease in children with Down's syndrome. Scand J Dent Res 1990;98:228–34.
11. Birkedal-Hansen H. Role of cytokines and inflammatory mediators in tissue destruction. J Periodont Res 1993;28:500–10.
12. Page RC. The role of inflammatory mediators in the pathogenesis of periodontal disease. J Periodontal Res 1991;26:230–42.
13. Heasman PA, Collins JG, Offenbacher S. Changes in crevicular fluid levels of interleukin 1 beta, leukotriene B_4, prostaglandin E_2, thromboxane B2 and tumor necrosis factor alpha in experimental gingivitis in human. J Periodont Res 1993; 28: 241–7.
14. Barr-Agholme M, Krekmanova L, Yucel-Lindberg T, Shinoda K, Modéer T. Prostaglandin E_2 level in gingival crevicular fluid from patients with Down's syndrome. Acta Odontol Scand 1997; 55:101–5.
15. Offenbacher S, Odle BM, Van Dyke TE. The use of crevicular fluid prostaglandin E_2 levels as a predictor of periodontal attachment loss. J Periodont Res 1986;21:101–12.
16. Offenbacher S, Odle BM, Braswell LD, Johnson HG, Hall CM, McClure H, et al. Changes in cyclooxygenase metabolites in experimental periodontitis in *Macaca mulatta*. J Periodont Res 1989;24:63–74.
17. Offenbacher S, Heasman PA, Collins JG. Modulation of host PGE_2 secretion as a determinant of periodontal disease expression. J Periodontol 1993;64:432–44.
18. Reynolds JJ. Collagenases and tissue inhibitors of metalloproteinases: a functional balance in tissue degradation. Oral Dis 1996; 2:70–6.
19. Nomura T, Ishii A, Oishi Y, Kohma H, Hara K. Tissue inhibitors of metalloproteinases level and collagenase activity in gingival crevicular fluid: the relevance to periodontal diseases. Oral Dis 1998;4:231–40.
20. Halinen S, Sorsa T, Ding Y, Ingman T, Salo T, Konttinen YJ, et al. Characterization of matrix metalloproteinase (MMP-8 and -9). Activities in the saliva and in gingival crevicular fluid of children with Down's syndrome. J Periodontol 1996;67:748–54.
21. Axelsson P, Lindhe J. Effect of fluoride on gingivitis and dental caries in preventive program based on plaque control. Community Dent Oral Epidemiol 1975;3:56–60.
22. Källestål C, Matsson L. Criteria for assessment of interproximal bone loss on bitewing radiographs in adolescents. J Clin Periodontol 1989;16:300–4.
23. Granström E. The arachidonic acid cascade. The prostaglandins, thromboxanes and leukotrienes. Inflammation 1984;8:15–25.
24. Barr-Agholme M, Dahllöf G, Linder L, Modéer T. Actinobacillus actinomycetemcomitans, Capnocytophaga and Phorphyromonas gingivalis in subgingival plaque of adolescents with Down's syndome. Oral Microbiol Immunol 1992;7:244–48.
25. Garrison SW, Nichols FC. LPS-elicited secretory responses in monocytes: altered release of PGE_2 but not IL-1 beta in patients with adult periodontitis. J Periodontal Res 1989;24:88–95.
26. McLaren J, Taylor DJ, Bell SC. Prostaglandin E (2)-dependent production of latent matrix metalloproteinase-9 in cultures of human fetal membranes. Mol Hum Reprod 2000;6:1033–40.
27. Wahl LM, Corcoran ML. Regulation of monocyte/macrophage metalloproteinase production by cytokines. J Periodontol 1993; 64:467–73.
28. Soell M, Elkaim R, Tenenbaum H. Cathepsin C, matrix metalloproteinases, and their tissue inhibitors in gingiva and gingival crevicular fluid from periodontitis-affected patients. J Dent Res 2002;81:174–8.
29. Yavuzyilmaz E, Ersoy F, Sanal O, Tezcan I, Ercal D. Neutrophil chemotaxis and periodontal status in Down's syndrome patients. J Nihon Univ Sch Dent 1993;35:91–5.
30. Makela M, Salo T, Uitto VJ, Larjava H. Matrix metalloproteinases (MMP-2 and MMP-9) of the oral cavity: cellular origin and relationship to periodontal status. J Dent Res 1994;73:1397–406.
31. Smolian H, Aurer A, Sittinger M, Zacher J, Bernimoulin JP, Burmester GR, et al.. Secretion of gelatinases and activation of gelatinase A (MMP-2) by human rheumatoid synovial fibroblasts. Biol Chem 2001;382:1491–9.
32. Lechapt-Zalcman E, Escudier E. Implication of extracellular matrix metalloproteinases in the course of chronic inflammatory airway diseases. Morphologie 2000;84:45–9.

Received for publication 10 December 2002
Accepted 11 March 2003