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**Department of Dental Medicine**

# Inflammatory response in periodontal tissue in children with Down syndrome

**AKADEMISK AVHANDLING**

som för avläggande av medicine doktorexamen vid Karolinska Institutet offentligen försvaras i sal 4U, plan 4, Institutionen för Odontologi, Huddinge

**Onsdagen den 5 juni, 2013 , kl 09.00**

av

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**Stockholm 2013**

## ABSTRACT

Periodontal diseases are inflammatory diseases affecting the supporting tissues of the teeth. Subjects with Down syndrome have a higher prevalence of periodontal disease compared to healthy controls. Periodontal disease in Down syndrome is considered to be multifactorial, although the aetiology is uncertain. The aim of this thesis was to study the inflammatory response in periodontal tissue in terms of cytokines, prostaglandins, matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) in children with Down syndrome as well as in healthy controls.

In **study I**, 18 subjects with Down syndrome and 14 controls were clinically and radiographically examined and matched for age and degree of gingival inflammation expressed as percentage of bleeding on probing (BOP%). In all subjects, gingival crevicular fluid (GCF) was collected from six sites with paper strips, and levels of prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), leukotriene B<sub>4</sub> (LTB<sub>4</sub>), and MMP-9 were analysed using RIA and ELISA kits. BOP% and volume of GCF (μL) were similar in both groups while Down syndrome patients had significantly higher ( $p < 0.05$ ) mean levels of PGE<sub>2</sub>, LTB<sub>4</sub>, and MMP-9 in GCF than controls.

In **study II**, PD and BOP% were clinically assessed in subjects with Down syndrome ( $n=24$ ) and controls ( $n=29$ ) (both groups, mean age 16.4 yr). The controls were matched for age and BOP% to subjects with Down syndrome. GCF was collected and Bio-Plex cytokine multiplex assays were used to determine levels of interferon- $\gamma$  (IFN- $\gamma$ ), tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), and interleukin (IL)-1 $\beta$ , IL-4, -6, -10, -12, and -17. GCF volume (μL) was significantly higher in subjects with Down syndrome ( $p < 0.001$ ) than controls. Mean levels of IL-1 $\beta$ , IL-4, IL-6, IL-10, IL-12, IFN- $\gamma$ , and TNF- $\alpha$  in GCF were significantly ( $p \leq 0.005$ ) increased in subjects with Down syndrome compared with controls. The correlation between IFN- $\gamma$  and IL-4 in GCF in subjects with Down syndrome differed significantly from controls ( $p < 0.01$ ).

In **study III**, 21 adolescents with Down syndrome exhibiting gingivitis (DS-G), 12 subjects with Down syndrome exhibiting periodontitis (DS-P), 26 controls with gingivitis (HC-G), and 8 controls with periodontitis (HC-P) were clinically and radiographically examined. All patients were between ages 11 and 20 yr. GCF was collected from each subject and the amounts of MMP-2, -3, -8, -9 and -13 and of TIMP-1, -2 and -3 were determined with R&D multianalyte kits. The amounts of MMP-2, -3, -8, and -9 and of TIMP-2 in GCF were significantly higher ( $p < 0.005$ ) in the DS-G than the HC-G group. The correlation coefficient between MMP-8 and TIMP-2 also differed significantly ( $p < 0.01$ ) between the DS-G and HC-G groups. In contrast, the correlation coefficients between the MMPs and TIMPs did not differ significantly between the DS-P and the HC-P groups. The DS-P group, however, exhibited significantly ( $p < 0.005$ ) lower amounts of TIMP-2 in GCF compared to the HC-P group.

In **study IV**, children with Down syndrome ( $n=10$ ) and controls ( $n=10$ ) were clinically and radiographically examined during dental treatment under general anaesthesia. Peripheral blood and GCF were gathered from each patient and levels of MMP-2, -3, -8 and -9, of TIMP-1, -2 and -3 in serum, and of GCF were determined. Peripheral blood leukocytes were isolated, and the relative amounts (%) of the various cells were determined with flow cytometry. Peripheral blood cells were stimulated with lipopolysaccharide (LPS) from *Porphyromonas gingivalis* (Pg) and MMP and TIMP levels were measured. Levels of MMP-3 and -8 and TIMP-1 in serum were significantly enhanced ( $p < 0.05$ ) in subjects with Down syndrome compared to controls. When peripheral blood leukocytes were cultured in the presence or absence of *Porphyromonas gingivalis* lipopolysaccharide, MMP-8 levels were significantly ( $p < 0.05$ ) higher in the Down syndrome group compared to controls. Children with Down syndrome exhibited significant positive correlations of CD8<sup>+</sup> T cells with MMP-8 ( $r=0.630$ ;  $p=0.050$ ) and MMP-9 ( $r=0.648$ ;  $p < 0.05$ ) and of CD56<sup>+</sup> NK cells with MMP-3 ( $r=0.828$ ;  $p < 0.005$ ) compared to controls.

### Conclusions

Subjects with Down syndrome had increased levels of the arachidonic acid metabolites PGE<sub>2</sub> and LTB<sub>4</sub>, the cytokines IL-1 $\beta$ , IL-4, IL-6, IL-10, IL-12, IFN- $\gamma$  and TNF- $\alpha$ , and of MMP-2, -3, -8 and -9 and TIMP-2 in GCF compared to controls. In addition, the balance between pro- and anti-inflammatory cytokines and between MMPs and TIMPs was altered in subjects with Down syndrome but not in controls. Furthermore, in contrast with controls, no significant differences in MMP and TIMP levels in GCF were observed between Down syndrome patients with gingivitis and periodontitis. This finding might indicate that the inflammatory response in Down syndrome is already upregulated during early stages of periodontal disease. We also demonstrate an association between MMPs and lymphocyte subpopulations (CD8<sup>+</sup> T-cells and CD56<sup>+</sup> NK-cells), which may facilitate the migration of immune cells into the periodontal tissue. This assumption is well compatible with the higher levels of MMPs in GCF found in Down syndrome subjects. These findings, may contribute to the increased periodontal inflammation demonstrated in this current cohort of Down syndrome subjects.