PERIODONTAL DISEASE MEASURES AND GINGIVAL CREVICULAR FLUID INTERLEUKIN - 1β IN DOWN SYNDROME

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This thesis is submitted by Josean Martinez and has been examined and approved by an appointed committee of the faculty of the School of Graduate Studies of the Medical College of Georgia.

The signatures which appear below verify the fact that all required changes have been incorporated and that the thesis has received final approval with reference to content, form and accuracy of presentation.

This thesis is therefore accepted in partial fulfillment of the requirements for the degree of Master of Science.

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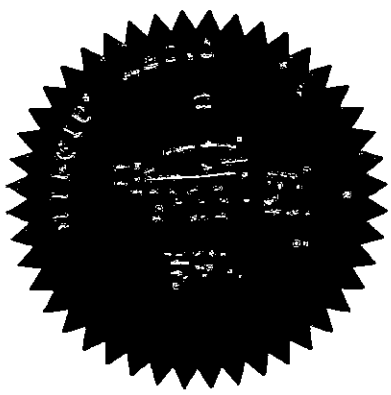

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I. Introduction

A. Statement of the problem

Down syndrome (DS) affects millions of Americans. Several reports, mostly from Europe, indicate extensive, but unexplained, periodontal disease and tooth loss among patients with DS. This lack of understanding of the pathogenesis of periodontitis in this patient population may lead to their inappropriate and inefficient care. Recent evidence shows enhanced matrix metalloproteinase (MMP) activity in gingival tissues from these patients (Komatsu, Kubota, & Sakai, 2001). This enhanced activity, as well as the extensive gingival inflammation and progressive alveolar bone loss, all suggest abundant interleukin-1 beta (IL-1β) activity. IL-1β is a well- documented biological factor in the pathogenesis of periodontitis. The role of IL-1β in relation to periodontitis in patients with DS is not known.

B. Significance

Understanding the role of IL-1β in the loss of periodontal tissues in patients with DS will generally enhance our understanding of the pathogenesis of periodontal disease and will provide information to plan and deliver better oral care to these patients.

C. Review of Literature

1. Down syndrome

Down syndrome, also known as trisomy 21, is a genetic disease associated with gross chromosomal abnormalities. The affected individual is born with an extra chromosome number 21. The extra copy of this chromosome results from abnormal segregation of chromosomes during meiotic cell division. The condition occurs in approximately 1 out of 800 births (Amano et al., 2000). Characteristics of this condition include mental retardation, altered facial features, congenital heart defects, and altered immune responses.

2. Periodontitis and Down Syndrome

Periodontitis

Periodontitis is a bacterial infection resulting in inflammation within the supporting tissues of the teeth, and leading to progressive loss of periodontal attachment and bone loss. The condition is characterized by pocket formation and/or gingival recession and is common among patients with DS. In response to bacterial plaque, patients with DS develop an overly intense gingival inflammatory response and rapid destruction of alveolar bone. Periodontal disease among children with DS and their unaffected siblings has been examined using the periodontal index of Russell. The results showed that the prevalence and severity of periodontitis were greatest in the children with

DS (Orner, 1976). Periodontal disease associated with patients with DS has also been evaluated using an orthopanographic technique. Using that technique eighty-four percent of patients with DS showed advanced alveolar bone loss (Saxen, Aula, & Westermarck, 1977). In a 5 year follow up study of the same subjects, it was reported that the percentage of teeth affected with bone loss in patients with DS significantly increased from 25% to 47% (Saxen & Aula, 1982). Gingival inflammation in children with DS has been shown to start early, and is characterized by a decrease in collagen fiber density of about 35-40% (Reuland-Bosma, Liem, Jansen, van Dijk, & van der Weele, 1988a). Patients with DS have been followed clinically and radiographically for a 7-year period (Agholme, Dahllof, &.Modeer, 1999). Those results showed that the frequency of periodontitis markedly increased during the 7-year follow up period. Patient populations evaluated in these studies were predominantly white. Periodontal disease, specifically in African American patients with DS, has not been assessed.

Despite similar levels of supragingival plaque, patients with DS usually develop a more rapid and severe inflammatory response than do patients without DS. The presence of *Actinobacillus actinomycetemcomitans*, *Capnocytophaga*, and *Porphyromonas gingivalis* has been noted in subgingival plaque of adolescents with DS (Barr-Agholme, Dahllof, Linder, & Modeer, 1992). Various periodontopathic bacteria also can colonize the mouth in the very early childhood of patients with DS (Amano et al., 2000). The subgingival microflora in patients with DS has been compared to other mentally retarded individuals (Reuland-Bosma, van der Reijden, & van Winkelhoff, 2001). Despite

advanced periodontitis in patients with DS, no differences in the prevalence of distinct suspected periodontopathic bacteria were established between the patients with DS and the control group. The authors concluded that host factors are the most likely explanation for the advanced periodontal disease associated with patients with DS compared to the mentally retarded group.

Impaired PMN and lymphocyte responses in a child with DS have been observed (Reuland-Bosma, Liem, Jansen, van Dijk, & van der Weele, 1988b). Additionally, it has been reported that an altered early response of PMN cells in patients with DS was associated with a decrease in gingival collagen fiber density (Reuland-Bosma et al., 1988). Defective neutrophil chemotaxis in patients with DS also has been found (Izumi et al., 1989). In addition, a strong association between the occurrence of *Actinobacillus actinomycetemcomitans* and neutrophil defense activity in patients with DS has been demonstrated (Sreedevi & Munshi, 1998). However these reports of altered PMN function do not fully explain the extensive gingival inflammatory response and rapid destruction of periodontal tissues seen in patients with DS.

Interleukin-1

Production of inflammatory mediators and cytokines such as tumor necrosis factor alpha (TNFα), interleukin-1 beta (IL-1β), and prostaglandin E_2 (PGE_2) by inflammatory and native gingival cells, play an important role in initiating and propagating gingival inflammation and loss of periodontal tissues. Many studies showed that IL-1β is prominent in gingival sites with periodontal disease (Ebersole, Singer,

Steffensen, Filloon, & Kornman, 1993; Feldner, Reinhardt, Garbin, Seymour, & Casey, 1994; Hou, Liu, & Chang, 1994; Hou, Liu, & Rossomando, 1995; Jandinski et al., 1991; Liu, Hou, Wong, & Rossomando, 1996; Matsuki, Yamamoto, & Hara, 1992, , 1993; Stashenko et al., 1991; Stashenko, Jandinski, Fujiyoshi, Rynar, & Socransky, 1991; Yavuzyilmaz et al., 1995). The most important sources of IL-1β in the gingival tissues are fibroblasts (Dongari-Bagtzoglou & Ebersole, 1996; Okamatsu, Kobayashi, Nishihara, & Hasegawa, 1996) and resident macrophages(Ebersole, Singer, Steffensen, Filloon, & Kornman, 1993; Feldner, Reinhardt, Garbin, Seymour, & Casey, 1994; Jandinski et al., 1991; Matsuki, Yamamoto, & Hara, 1992, , 1993). IL-1β is a powerful inflammatory and immune response mediator, as well as a potent mediator of bone resorption. This mediator promotes destruction of gingival tissues through activation of matrix metalloproteinases and mediates bone resorption by inducing osteoclastic bone activity. Recent reports suggest that the carriage of certain alleles of IL-1α and IL-ß is associated with increased severity of periodontal disease. Allele 2 of the IL-1α gene at position -889 is associated with elevated levels of IL-1α in the gingival tissues of periodontitis patients (Shirodaria, Smith, McKay, Kennett, & Hughes, 2000). An association between allele 2 and the IL-1ß +3953 polymorphism results in increased production of IL-1ß and periodontitis (Gore, Sanders, Pandey, Palesch, & Galbraith, 1998). The combined presence of alleles IL-1α (-889 or +4845) and IL-1ß (+3954) (formerly +3953) also is associated with increased incidence and severity of periodontitis (Kornman et al., 1997).

Mechanisms similar to those previously mentioned may lead to the overproduction of IL-1β in gingival tissues of patients with DS. Elevated IL-1ß levels in gingival crevicular fluid (GCF) sampled from non-inflamed sites of patients with DS have been reported (Barr-Agholme, Krekmanova, Yucel-Lindberg, Shinoda, & Modeer, 1997). Enhanced matrix metalloproteinase (MMP) activity in the gingival tissues of the patients with DS was found, and suggests elevated IL-1β activity, since IL-1β is a primary activator of some MMP genes (Komatsu, Kubota, & Sakai, 2001). The reports documenting extensive gingival inflammatory response, advanced periodontal clinical attachment loss, and progressive radiographic bone loss all suggest IL-1β activity in the gingival tissues of patients with DS.

D. Purpose

The purpose of this study was to investigate the level of IL-1ß activity in the gingival crevicular fluid of patients with DS in comparison to patients without Down syndrome.

E. Hypothesis

In presence of bacterial plaque, the gingival tissues are exposed to bacterial irritants initiating an intense inflammatory response characterized by the production of IL-1β. IL-1β is an important mediator of tissue destruction in the periodontal lesion. Patients with DS are known to develop advanced periodontitis. We hypothesize that increased production of IL-1β in the gingival tissues of patients with DS may account for their advanced periodontits.

We tested this hypothesis by comparing the GCF levels of IL-1β in patients with DS and a comparison group of patients without DS. We examined the relationship between GCF IL-1β levels and periodontitis in both groups. We compared the size of the regression coefficients relating indices of gingival inflammation and periodontal status to the GCF IL-1β levels. These latter analyses address the question of whether the mechanism of periodontitis differs between the patients with and without DS.

According to this hypothesis, patients with DS would show higher levels of GCF IL-1β than the patients without DS. These patients also would show a higher association between GCF IL-1β levels and periodontitis.

II. Materials and Methods

A. Overview

Levels of GCF IL-1ß were measured in patients with DS and in patients without DS. Clinical periodontal measures were taken to determine the severity of the periodontal disease in each group. The results were analyzed to determine the activity of IL-1ß in each group, and to examine the relationship between the clinical periodontal measures and the GCF levels of IL-1ß.

B. Subject recruitment and characteristics

This study was submitted to and approved by the MCG Human Assurance Committee (03-03-239). Two groups of subjects were recruited for this study: a group of subjects with DS and a control group of subjects without DS.

Number of subjects

A total of 61 patients with DS and 124 patients without DS were recruited. Subjects were balanced by race, gender, age, and socioeconomic status. Subjects with DS

were recruited first, and comparison subjects were selected to balance the group proportion on race, gender, age and socioeconomic status. In this way, similar risk factors were present, and allowed for a more reliable statistical analysis of putative risk factors. Since smoking is rare in persons with DS only non smoking controls were eligible for this study.

The non-Down syndrome group allowed the comparison of the periodontal status of Down syndrome patients to a systemically normal population while controlling for age, sex, and racial status of the index cases. It also permitted the results to be generalized to the main population. Further, these data were used to isolate potential differences in mechanisms of periodontal disease development between subjects with DS and clinic controls. The control group provided a check on the consistency of laboratory findings with past research. With a clinical control, laboratory errors were obvious, and therefore, conclusions were clearer.

Recruitment

The Down Syndrome patients were recruited from the Gracewood State Hospital, Augusta, GA. The Gracewood State Hospital (collaborating institution) has a dental clinic dedicated to serve mentally challenged individuals. The database includes over 1600 individuals with a diagnosis of Mental Retardation, and a sizable percentage of those have DS. Most of the individuals there have mild mental disability and live in group homes, although some of them live with their families. In addition, access to other

populations of DS and mentally handicapped patients was possible through project LINK (for families of patients with mental disabilities) at the Medical College of Georgia in Augusta, GA. The total number of accessible patients with DS exceeded 500. The study was announced to parents and guardians of patients seeking care at the collaborating facilities. A pamphlet and flyers describing the study were available and posted in public waiting areas. The parent and/or guardian responsible for the patient was contacted either directly at time of visit or by mailed letter and follow up phone call. If participation in the study was agreed upon, a screening evaluation was scheduled. The non-Down subjects were recruited from the MCG dental clinic so as to control for care-seeking behavior.

Composition of the proposed study population

The various locations of collaborating clinics ensured demographic diversity with respect to women and minorities being well-represented. The following strategy was utilized for recruitment. A categorized list of the number of subjects needed to fill the various categories (race, age and gender) was determined. Charts of patients with DS records were checked, and names grouped by race, age, and gender category. Patients in all listed categories were contacted as described above to satisfy the number of subjects needed per category. When the available patient pools did not fulfill the subject needs, the help of other local handicapped centers was enlisted. Similar recruitment methods were used for the clinic group controls.

Enrollment

Individuals interested in participating in the study were given a consent form to read and sign. The parent/guardian of mentally handicapped individuals and children signed for the subject. Completion of a medical/dental history questionnaire followed this step. An oral examination and screening for study inclusion/exclusion criteria was performed.

Inclusion/Exclusion Criteria

Patients with DS met the following inclusion criteria:

1. Signed informed consent form.
2. Subject must have an established diagnosis of Down syndrome.
3. Subject must have a minimum of 10 permanent teeth.
4. Age range: 18 years to older (no maximum age limitation).
5. No antibiotic treatment in the last three months prior to entry the study.

Patients with DS who met the inclusion criteria listed above were enrolled in the experimental (DS) group. Subjects recruited for the control groups were selected to balance the group characteristics of the patients with DS and satisfied inclusion criteria 1, 3, 4 and 5.

Individuals with the following conditions did not participate in the study

1. Any contra-indications to dental evaluation and treatment.

2. Intraoral conditions present which may interfere with accuracy of periodontal probing e.g gingival overgrowth, orthodontic wires, excessive calculus.

3. Less than 10 permanent teeth present.

4. Age less than 18 years.

5. Antibiotic treatment in the past three months prior to enrollment in the study.

C. Clinical evaluations

Gingival Index (GI)

The Loe gingival index (Loe, 1967) was used to determine status of marginal gingival tissues. A periodontal probe was used for stimulation of gingival margins. Four sites were scored around each tooth: mesiobuccal, mesiopalatal/ lingual, distobuccal, and distopalatal/lingual. Scoring was as follows: 0 = healthy gingiva, 1 = change in color, 2 = bleeding upon stimulation, 3 = spontaneous bleeding.

Periodontal Probing Measurements

The examiner performed the periodontal probing measurements. All fully erupted teeth present, excluding third molars, were measured. A periodontal probe with Williams markings (Hu-Friedy PQ-OW) was used to determine probing depth (PD) at four sites per

tooth: mesiobuccal, distobuccal, mesiolingual, distolingual. The probe was placed interproximally as close to the contact area as possible. A reading to the nearest mm was taken at the gingival margin. A light grasp was utilized while probing however, no attempt was made to standardize the probing force. The position of the gingival margin in relation to the cementoenamel junction also was recorded at the same four sites per tooth to the nearest mm. A negative sign was given when the gingival margin was coronal to the cementoenamel junction and a positive sign was given when it was apical. Attachment level was calculated by adding probing depth to position of gingival margin.

Bleeding upon probing (BOP)

To evaluate gingival inflammatory status after insertion of a probe to the base of the sulcus/pocket, gingival bleeding upon probing (BOP) was recorded for each site as (1) if present and (0) if absent.

Plaque Index (PI)

Teeth were disclosed with FD & C Red #3 dye, and plaque scores on all natural tooth surfaces were determined using a modification of the Quigley & Hein plaque index (PI) (Khocht, Spindel, & Person, 1992). Four surfaces were scored on each tooth: mesiobuccal, mesiopalatal/lingual, distobuccal, and distopalatal/lingual. Plaque levels were scored as follows: 0 = no plaque, 1 = discontinuous plaque less than 1 mm in thickness, 2 = 1 mm plaque confined to gingival margin, 3 = band of plaque wider than 1

mm but less than 1/3 of surface, 4 = plaque covering 1/3 or more but less than 2/3 of surface, 5 = plaque covering at least 2/3 of surface.

The examiner was calibrated by Dr. Khocht before data collection was begun, and a random sample of subjects was evaluated by Dr. Khocht and the examiner every month thereafter, to assess inter-rater reliability of probing depth, BOP and PI, and prevent bias introduced by 'examiner drift'.

Subject questionnaire interview

All subjects were interviewed regarding their living conditions, their practice of oral hygiene, access to professional dental care, parent education and family income. The general population subjects answered for themselves. The Down subjects came with a family member or a caretaker who answered the questionnaire for them.

D. Gingival crevicular fluid (GCF) sample collection for IL-1β level analysis

GCF was collected from 6 sites: 3 non-diseased (probing depth $\leq$ 3mm) and 3 diseased sites (probing depth $\geq$ 4mm) when present. Sites with obvious bleeding were avoided. GCF was collected on filter paper strips (Schein). The selected sites were isolated, and the tooth surface was air-dried prior to sample collection. The strip was inserted into the gingival crevice until mild resistance was encountered, and then left for 30 seconds. Sample volume was assessed with an oral fluid volume detector device

(Periotron 8000, Pro Flow, Amityville New York). Samples from diseased sites were pooled in a single Eppendorf centrifuge tube; the samples from non-diseased sites were pooled in a similar manner. Following sample collection, the centrifuge tubes were immediately placed on ice and subsequently frozen at -70 °C until analyzed for IL-1ß levels.

E. Determination of GCF interleukin-1ß levels

The levels of IL-1ß for each patient were determined with a commercially available kit that employs a sandwich enzyme immunoassay technique. (Quantikine Human IL-1β Immunoassay, R & D Systems Minneapolis, MN). This assay was a sequential test performed in a polystyrene 96 well microtiter plate. A monoclonal antibody specific for IL-1β was pre-coated onto a microplate. The test is able to detect 3.9 – 250 pg/ml in 4.5 hours. This assay detects more than 90% of the mature, biologically active form of IL-1β. Less than 10% of detected amounts are in the pro-form. Each kit supplies enough reagents for determination of 42 patient samples, and a standard curve performed in duplicate.

The IL-1ß present on the three pooled stripes in each vial was diluted by immersion in 1 ml of phosphate buffered saline containing 0.1% Triton 100 and 0.1% Bovine Serum Albumin (BSA). The elution process was allowed to occur overnight under refrigeration. A set of controls containing known amounts of this substance was used to mimic the elution of IL-1ß from filter paper strips, which allowed for the

calculation of the percent recovery. A standard curve was generated in the same elution buffer, thus maintaining matrix integrity. After the samples were eluted and centrifuged for 5 minutes at 18,000 rpm, 200 µl of each sample was added in duplicate to microtitration wells, which had been coated with a mouse monoclonal antibody specific for the mature form of IL-1β. The remaining eluate was reserved by freezing at -20°C, in the event the original ELISA test did not meet testing standards. The microplate was covered with the adhesive strip provided and incubated for 2 hours at room temperature. After the incubation, each sample was aspirated and the wells were washed with buffer for a total of three washes. The wash was performed by filling each well with wash buffer (400 µl) using a squirt bottle. After the last wash, any remaining wash buffer was removed by aspirating and decanting. The plate was inverted and blotted against clean paper towels. 200 µl of anti IL-1β conjugate was added to each well. The microplate was covered with a new adhesive strip and incubated for 1 hour at room temperature. The aspiration/wash process was repeated. Substrate solution (200 µl) was added to each well, and the plate was incubated for 20 minutes at room temperature, protected from light. Then 50 µl of Stop Solution was added to each well, changing the color in the wells from blue to yellow. The optical density of each well was determined within 30 minutes, using an ELISA microplate reader (Versamax, Molecular Devices, Sunnyvale, California) set to 540 nm. From the standard curve, and control strip recoveries, the concentration (pg/ml) of IL-1ß in the eluate was determined. For the purpose of this study we reported IL-1 β concentration as pg/ml collected per 30 seconds.

F. Data Analysis

Overview

The first hypothesis was tested by comparing the GCF IL-1β levels in patients with DS with the GCF IL-1β levels in patients without DS. The second hypothesis was tested by comparing the size of the regression coefficients relating indices of gingival inflammation and periodontal status to the GCF IL-1β levels.

Statistical analysis

Student's t-tests were used to compare population means of the groups for continuous variables. Chi-square tests were used to compare population proportions of the groups for variables measured at the nominal level. Pearson correlations were calculated to assess the degree of linear association between continuous variables. Multiple linear regression and analysis of covariance (ANCOVA) were used to assess differences in the conditional means of GCF IL-1β while adjusting for group effects and the periodontal disease measures. JMP Version 6 (SAS Institute, Cary, NC) software was used for all statistical analyses. All statistical tests were conducted at the p 0.05 significance level.

III. Results

A total of 185 subjects consented to participate in the study. Of these 163 (88 %) provided GCF samples for determination of IL-1β levels. Table 1 summarizes the demographic characteristics of both subject groups, the Down and non-Down; it shows that both groups were distributed similarly with regard to sex, race and age. Table 2 summarizes the oral evaluations of each group. These data show that the Down subjects have higher levels of plaque ($p = 0.01$), gingival inflammation ($p = 0.0001$), bleeding on probing ($p = 0.0001$), periodontal attachment loss (as calculated from probing depth and position of gingival margin to cementoenamel junction) ($p = 0.0007$), and a higher number of missing teeth ($p = 0.0001$). Thus the Down subjects have higher levels of periodontal disease than the non-Down subjects.

Table 3 summarizes IL-1β levels in GCF from both diseased and non-diseased sites by group. In the non-diseased sites, the Down subjects showed higher levels of IL-1β than the non-Down subjects ($p = 0.0001$). In the diseased sites the IL-1β levels were comparable in both groups. Table 4 summarizes the correlation between GCF IL-1β levels from non-diseased sites with clinical periodontal parameters for all subjects

grouped together and separated by group. For all subjects, GCF levels from non-diseased sites correlated with PI ($p = 0.0001$), GI ($p = 0.006$), BOP ($p = 0.04$), AL ($p = 0.0006$) and number of missing teeth ($p = 0.025$).

The degree of association of IL-1β from non-diseased sites with periodontal clinical parameters varied between the two groups. In the non-Down group, relevant associations were noted with PI ($p = 0.01$), BOP ($p = 0.06$) and AL ($p = 0.001$). In the Down group, only PI showed association with IL-1β GCF levels ($p = 0.002$). Further analysis showed that the difference in association between clinical parameters and GCF levels between the two groups is non-relevant. GCF levels from diseased sites showed no significant association with any of the clinical parameters (data not shown).

The multiple regression analysis in table 5 examines the association of IL-1β GCF levels from non-diseased sites on selected clinical parameters, while adjusting for group effect. For GI, BOP and number of missing teeth no relevant associations were found. However, regardless of group effect, IL-1β levels influenced AL (std. beta = 0.20, $p = 0.01$). A relevant group X IL-1β interaction was noted ($p = 0.09$); ANCOVA indicated that the effect of IL-1β on AL was stronger in the DS group than non-DS group. Thus IL-1β GCF levels seem to influence periodontal loss of attachment in all subjects in general and the Down group in particular.

Table 1: Demographics

	Non-Down N=124	Down N=61	Chi-square with 1 df	p-value
Gender (males)	42.74 %	50.82 %	1.08	0.30
Race (white)	72.58 %	78.69 %	0.80	0.37

	Non-Down N =124 mean (SD)	Down N =61 mean (SD)	df	t-test	p-value
Age	38.70 (12.57)	36.09 (10.15)	144.55	-1.52	0.13

Table 2: Clinical Data

	Non-Down N=124 mean (SD)	Down N=61 mean (SD)	df	t-test	p-value
Plaque index	1.21 (0.71)	1.55 (0.90)	94.64	2.58	0.01
Gingival index	0.64 (0.38)	0.91 (0.30)	145.23	5.22	0.0001
Bleeding on probing	21.69 (22.88)	42.46 (31.20)	92.79	4.62	0.0001
Gingival margin	-0.12 (0.45)	0.07 (0.62)	92.34	2.14	0.03
Probing depth	2.34 (0.61)	2.61 (0.47)	151.46	3.35	0.001
Attachment loss	2.22 (0.78)	2.69 (0.90)	105.58	3.49	0.0007
Miss. Teeth	1.67 (2.55)	4.42 (4.37)	80.70	4.54	0.0001

Table 3: IL-1β Levels (reported as pg/ml/30 sec collection time)

	N	Non-Down mean (SD)	N	Down mean (SD)	df	t-test	p-value
Non diseased	113	23.38 (21.61)	50	39.42 (29.78)	70.84	3.40	0.0001
Diseased	42	72.59 (86.29)	24	84.10 (75.86)	53.25	0.56	0.29

Table 4: Correlations of IL-1β by Clinical Measures on Non-diseased sites

	All Subjects N=163 r (p-value)	Non-Down N=113 r (p-value)	Down N=50 r (p-value)	Non-Down r vs. Down r p-value
Plaque index	0.38 (0.00001)	0.24 (0.01)	0.44 (0.002)	0.12
Gingival index	0.21 (0.0065)	0.09 (0.34)	0.24 (0.11)	0.85
Bleeding on probing	0.16 (0.04)	0.18 (0.06)	-0.05 (0.73)	0.14
Gingival margin	0.08 (0.31)	0.12 (0.22)	-0.06 (0.67)	0.26
Probing depth	0.31 (0.0001)	0.30 (0.001)	0.24 (0.09)	0.16
Attachment loss	0.27 (0.0006)	0.30 (0.001)	0.08 (0.6)	0.09
Miss. Teeth	0.18 (0.025)	0.07 (0.43)	0.09 (0.54)	0.85

Table 5: Regression analysis examining the association of IL-1β on clinical measures after adjusting for group effect.

Dependent Variable	Independent Variable	Standardized Beta	p-value
Gingival index	IL1β	0.12	0.11
	Group	-0.30	0.0001 >Down Group
	IL1β*Group	-0.01	0.85
Bleeding on probing	IL1β	0.06	0.40
	Group	-0.30	0.0002 > Down Group
	IL1β*Group	0.11	0.14
Attachment loss	IL-1β	0.20	0.01
	Group	-0.20	0.01 > Down Group
	IL1β*Group	0.12	0.09
Miss. Teeth	IL1β	0.08	0.29
	Group	-0.32	0.0001 > Down Group
	IL1β*Group	-0.01	0.85

> Down Group = higher in Down Group

IV. Discussion

The primary objective of this study was to investigate the IL-1ß levels in the gingival crevicular fluid of individuals with DS in comparison to non-DS individuals. Our results showed no significant differences in IL-1 β levels in periodontally diseased gingival sites between DS and non-DS subjects. In contrast, IL-1 β levels in non-diseased gingival sites was significantly elevated in DS subjects compared to non-DS subjects. Our data analysis also identified an important association between IL-1 β GCF levels in non-diseased sites and the average loss of periodontal attachment in all subjects in general, and the DS subjects in particular.

The lack of difference between the DS and non-DS subjects in IL-1 β GCF levels from diseased sites may be due to a masking effect, whereby excessive production of IL-1 β in diseased sites masks the differences in cytokine production between the groups. In association with ongoing inflammation, IL-1 β production in diseased sites is at peak levels. The higher production levels may obscure any differences between the groups. This finding suggests that sampling diseased sites may not be an ideal means to compare production potential of inflammatory mediators such as IL-1 β between different groups.

Sampling non-diseased sites allows a better comparison of production potential of inflammatory mediators, such as IL-1 β, among different groups. In non-diseased sites, production of inflammatory mediators is at sub-peak levels that allow greater sensitivity in detecting differences between groups. As previously stated, in non-diseased samples, IL-1β GCF levels in DS patients were significantly higher than non-DS subjects. The higher IL-1β GCF levels in DS patients may be related to extrinsic or intrinsic factors. Extrinsic factors such as bacterial plaque could play an important role in IL-1 β production. Our data showed a relevant association between IL-1 β levels in non-diseased sites and plaque levels in both groups. The higher plaque levels noted in the DS group may explain in part the elevated IL-1β levels in this group. Bacterial plaque induces inflammatory responses in the gingival tissues (Honig, Rordorf-Adam, Siegmund, Wiedemann, & Erard, 1989). IL-1β is one of the important inflammatory cytokines produced in inflamed gingival tissues. Intrinsic factors, such as a genetic predisposition to greater IL-1β production, also may account for the elevated levels of IL-1β in GCF samples from non-diseased sites in DS patients. This supports the notion that intrinsic factors also may contribute to higher IL-1β production in DS patients.

For the purposes of this research we elected to report IL-1β levels as pg/ml per 30-second collection time. Since standardized collection of equal amounts of crevicular fluid was not possible, we chose to standardize collection time and report IL-1β levels as

the total amount collected within a 30 second time interval. Another way of reporting IL-1β is to use crevicular fluid volume collected to calculate IL-1β concentration. This option of data reporting might yield additional information and may be pursued in a future reanalysis of the data.

Previously, a single report investigated IL-1β production in gingival crevicular fluid of DS subjects (Barr-Agholme, Krekmanova, Yucel-Lindberg, Shinoda, & Modeer, 1997). In this study 19 children with DS were studied. All showed signs of gingival inflammation; however none had periodontitis. No increased probing depth or radiographic alveolar bone loss was present in any of the subjects. They also reported elevated IL-1β levels in non-inflamed gingival sites in DS individuals. We extend the finding of this early report by including a larger, more diverse, and older group of DS subjects with various levels of periodontal problems, including increased probing depth and loss of periodontal attachment. We also investigated the relationship between IL-1β production and loss of periodontal attachment.

Our clinical data confirmed earlier reports of increased periodontal disease in DS subjects. Several investigations attempted to explain the periodontal problems in DS patients. Factors previously investigated include subgingival bacterial composition, immune dysfunction, and inflammatory dysfunction.

A. Subgingival bacterial composition:

Microbiological studies investigating the role of bacteria and other pathogens in the initiation and development of periodontitis in DS patients suggest that elevated proportions of periodontopathic bacterial species and other pathogens present are important contributing factors to periodontitis in this patient group.

Barr-Agholme (1992) showed the presence of *Actinobacillus actinomycetemcomitans*, *Capnocytophaga* and *Porphyromonas gingivalis* in subgingival plaque of adolescents with DS. *A. actinomycetemcomitans* was detected in 35% of patients with DS compared to 5% in the healthy, age and sex matched controls. This increased frequency of *A. actinomycetemcomitans* indicated an altered microbial composition in the subgingival plaque of DS patients as compared to healthy controls.

Amano et al. (2000) found various periodontal disease-causing bacteria present in very young DS patients. Pathogens in DS patient's subgingival plaque were detected with far greater frequency than in the age-matched controls. This may be why these DS patients have such intense gingival inflammation. They concluded that periodontopathic pathogens establish a presence at a very early age, and that certain bacteria, like *P. gingivalis*, play a key role in the initiation of gingival inflammation.

In contrast to the previously cited studies, a recent study did not find differences in subgingival microflora of DS patients in comparison to mentally retarded patients. Reuland-Bosma et al. (2001) compared subgingival microflora in DS patients to other mentally retarded individuals. Despite the advanced periodontitis in DS patients, no differences in the prevalence of distinct suspected periodontopathic bacteria were established between the DS patients and the control group. The authors concluded that host factors were the most likely explanation for the advanced periodontal disease associated with DS patients.

B. Immune Dysfunction

Various studies investigated different components of the immune system in relation to periodontitis in DS patients. These studies suggest that immune dysfunction is a contributing factor to periodontitis in DS patients.

1. Polymorphonuclear Leukocyte (PMNs) Alterations:

Reuland-Bosma et al. (1988) created experimental gingivitis conditions in children with DS and healthy controls and studied the cellular aspects and effects on their gingiva. The start of the inflammation was marked by an early and pronounced response of PMNs in the DS children as compared to the normal children. However, the DS children then showed an impaired leukocyte migration, whereas the control group experienced an increase in the number of leukocytes at affected sites.

Izumi et al. (1989) found faulty neutrophil chemotaxis in these patients as well. They reported that DS patients had significantly lower chemotaxis compared to healthy controls. Since the neutrophils are the major cell type involved in the first line of host defense in bacterial invasion, having a defective neutrophil chemotaxis can lead to the progression of periodontitis. Significant correlations were identified among the amount of bone loss and the age and chemotactic index of the DS patients. The authors found that the rate of periodontal destruction was dependent on the degree of defective chemotaxis.

Sreedevi and Munshi (1988) examined neutrophil chemotaxis in DS children and normal children toward the bacteria *Actinobacillus actinomycetemcomitans*. They found that neutrophil chemotaxis was significantly impaired in the DS children compared to the controls. Their study also suggested that the higher prevalence of periodontitis in DS patients was more related to this impaired host defense rather than to the occurrence of specific bacteria like *A. actinomycetemcomitans*. They described a strong association between the occurrence of *A. actinomycetemcomitans* and neutrophil defense activity in DS patients.

2. Lymphocyte/Monocyte Alterations:

In 1992, Sohoel et al. studied the composition of mononuclear cells in the gingival inflammatory infiltrate in DS patients with marginal periodontitis. They discovered that DS patients had a larger number of cells in the cellular infiltrate of chronic marginal periodontitis (CMP) compared to normal patients. There were

increased numbers of CD22+ cells (B lymphocytes), CD3+ cells, CD4+ cells, CD8+ cells, and CD11+ cells (macrophages). There also was a significantly higher CD4+/CD8+ ratio in DS patients when compared to the normal controls that could indicate active tissue destruction. This study concluded that DS patients had a more pronounced and altered immune response when compared to controls.

In 1995, Sohoel et al. published another study investigating the expression of HLA class II antigens in chronic marginal periodontitis (CMP) in patients with DS. They found that variations in this expression on antigen-presenting cells play an important role in immune regulation. The results of this study indicated an increased frequency of HLA class II antigens in the CMP of DS patients when compared to controls. They concluded that there was a highly activated immune response in DS patients with CMP , as well as a more pronounced inflammatory response in their gingival tissue.

3. Changes in Immunoglobulin Production:

Another possible contributor to increased susceptibility to periodontitis in DS patients is an alteration in salivary immunoglobulins. Barr-Agholme (1998) studied the periodontal conditions and salivary immunoglobulins in patients with DS. The results showed an altered distribution of IgG subclasses in saliva, with an increased amount of IgG1 in DS patients compared to controls. Also, it was noted that in DS patients with

bone loss, there was an increased level of IgA, compared to those DS patients without bone loss. The fact that these immunoglobulins were increased in periodontal disease indicated that they play a crucial role in oral mucosal defense against invading bacteria.

B. Altered Inflammatory Responses:

Studies investigating inflammatory responses in the gingival tissues of DS patients are sparse. Barr-Agholme et al. (1997) investigated the levels of prostaglandin E_2 (PGE_2) and IL-1β in gingival crevicular fluid (GCF), which was collected from both DS patients and healthy controls. They found the mean level of PGE_2 in the GCF was significantly higher in the DS patients as compared to the controls. This finding suggests an alteration in arachidonic acid metabolism in DS patients. As previously mentioned, higher levels of IL-1β in non-inflamed gingival sites also were reported.

Komatsu et al. (2001) examined both the amount of matrix metalloproteinase (MMP-2) present and the specific enzyme activity in the gingival tissues of Down syndrome patients and controls. The authors reported that there was a significantly higher production of MMP-2 in the cultured gingival fibroblasts from the Down syndrome patients when compared with the controls. In addition, the mRNA expressions of membrane-type I metalloproteinases (MTI-MMP) and MMP-2 were markedly different when the cultured fibroblasts of the DS patients were compared to the controls. This

would indicate that the increased amount of active MMP-2 produced in DS could be linked to the simultaneous expression of MTI-MMP, which could also be connected to the cause of periodontal disease that is seen in a majority of DS patients.

Results of this study support the notion that altered inflammatory responses in the gingival tissues of DS subject are relevant to their periodontal problems. Our results indicate that inflammatory responses in the gingival tissues of DS subjects, as measured by IL-1β GCF levels, are associated with loss of periodontal attachment. The higher production of IL-1β in the gingival tissue of DS individuals may also explain the elevated MMP activity in gingival tissues of DS individuals reported by Komatsu et al. (2001).

In conclusion, Down syndrome patients have greater susceptibility to periodontitis than non-Down subjects. Also Down syndrome patients have worse oral hygiene and more gingival inflammation than non-Down syndrome subjects. IL-1β production by gingival tissues of DS patients is higher than non-Down syndrome subjects. These higher levels of IL-1β apparently associate with loss of periodontal attachment in all subjects and stronger in DS subjects and may explain, in part, the greater susceptibility of Down syndrome patients to periodontal diseases. It would be interesting to compare these parameters in a group of patients with mental disabilities of different origin.

V. Summary

Periodontitis is a bacterial infection resulting in inflammation within the supporting tissues of the teeth, and leading to progressive loss of periodontal attachment and bone loss. Periodontal disease is very common among patients with Down syndrome (DS). In response to bacterial plaque, patients with DS develop an overly intense gingival inflammatory response and rapid destruction of alveolar bone. Several studies have demonstrated a positive correlation between IL- 1β and periodontitis. We investigated the level of IL-1β activity in the gingival crevicular fluid of patients with DS in comparison to patients without Down syndrome.

A total of 61 patients with DS and 124 patients without DS were recruited. Subjects were balanced by race, gender, age, and socioeconomic status. Plaque index, gingival index, bleeding on probing, attachment loss and missing teeth were measured. Gingival crevicular fluid was obtained from 3 non-diseased and 3 diseased sites from both groups using filter paper strips. The paper strips were left in the gingival sulcus or pocket for 30 seconds. The 3 non-diseased sites were pooled together, as were the samples from diseased sites, and the GCF was eluted into a single sample. The levels of IL-1β for each patient were determined using an ELISA technique and the data presented as pg/ml per 30 seconds.

Our data show that both groups were distributed similarly with regard to sex, race and age. The Down subjects had higher levels of plaque ($p = 0.01$), gingival inflammation ($p = 0.0001$), bleeding on probing ($p = 0.0001$), periodontal attachment loss ($p = 0.0007$), and a higher number of missing teeth ($p = 0.0001$). In the non-diseased sites, the Down subjects showed higher levels of IL-1β than the non-Down subjects ($p = 0.0001$). In the diseased sites, the IL-1β levels were comparable in both groups. GCF IL-1 β levels from non-diseased sites correlated with PI ($p = 0.0001$), GI ($p = 0.006$), BOP ($p = 0.04$), AL ($p = 0.0006$) and number of missing teeth ($p = 0.025$). The degree of association of IL-1β from non-diseased sites with periodontal clinical parameters varied between the two groups. In the non-Down group, relevant associations were noted with PI ($p = 0.01$), BOP ($p = 0.06$) and AL ($p = 0.001$). In the Down group, only PI showed association with IL-1β GCF levels ($p = 0.002$). Regression analysis adjusting for group effect indicated a relevant association between GCF IL-1β levels and loss of periodontal attachment ($p = 0.01$); the association was stronger in the DS group than non-DS group, but the result was not quite significant ($p = 0.09$).

Down patients have greater susceptibility to periodontitis than non-Down subjects. In addition, Down patients have worse oral hygiene and more gingival inflammation than non-Down subjects. IL-1β production by gingival tissues of DS patients is higher than non-Down subjects. These higher levels of IL-1β apparently associate with loss of periodontal attachment in all subjects and stronger in DS subjects and may explain, in part, the greater susceptibility of Down patients to periodontal diseases.

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