Salivary Electrolytes, Focused on Salivary Calcium Level and the Periodontal State in Healthy Smoking and Non-Smoking Women

PhD Thesis

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My scientific work is based on a hypothesis put forward by Finnish authors: L.A. Sevón, K.K. Mäkinen (1996) “Dietary Shifts May Explain the Incidence of Periodontitis in Industrialized Countries”. This hypothesis postulates that

1. Hypothesis

“It has been known for 30 years that gingivitis (inflammation of the gingivae) can be experimentally induced by allowing dental plaque to accumulate. All gingivitis does not, however, lead to periodontitis. The onset of the latter seems to require gram-negative, anaerobic infection. Experimental periodontitis in laboratory animals has been established by silk ligatures and by a soft diet. The only mechanism rendering subgingival plaque formation possible operates via **gingival sulcus formation**. The sulcus, together with the calcifying plaque, manifests the subgingival space and deepens it, converting the sulcus eventually to a **gingival pocket**. Considering this type of favorable circumstances, it is only a matter of time until each pocket gets **secondarily infected** with potently virulent bacterial species which accelerate the overall pathologic process. The presence of a gingival sulcus is certainly common in modern humans; one may speak even about the so-called '**anatomical sulcus**'. The presence of a gingival sulcus may, however, result from the nature of **modern diet**. It is indeed conceivable that the existence of a sulcus is linked to altered nutrition. It is only the existence of the sulcus that explains why - nowadays - **calcifying plaque** is detrimental to the periodontium. This view is supported by clinical experience - even if the patient uses the toothbrush properly. The calcifying plaque can be considered a factor that maintains the integrity of the teeth. In the past, when there was no professional periodontal care, it would not have been biologically sensible that the same factor that protects the teeth would on the other bring about their loosening. It can be assumed that the plaque that calcified in the past times never had access to the subgingival space to form **subgingival** calculus. Instead, it is conceivable that all calculus and all plaque were **supragingival**. It has been seen in ancient skeletal material that supragingival calculus has just grown over the bone (and naturally over the gingivae), instead of becoming wedged between the bone and the tooth to form subgingival calculus.

Some mechanism must have kept the gingivae so tightly adhered to the tooth surface that bacteria did not have much access to the subgingival space. It is our opinion that **continuous tooth eruption** constitutes such a mechanism. Although human teeth do differ from rodents' incisors (which represent true continuously erupting teeth), it is nevertheless possible that the compensatory eruption demanded by the wear caused by coarse food, was sufficient to eliminate sulcus formation. We assume that the former humans had **calcifying plaque; the caries-free dentitions** of ancient skulls support this view. If these humans had had a similar anatomical sulcus as that in modem humans, all of them should have suffered from destructive periodontitis associated with pocket formation. This would have led to loosening of the teeth. However, studies on ancient skeletal material suggest that, although the former humans did have so-called **attachment loss**, it most likely resulted from eruption, i.e. the tooth **'grew out'** from the jaw bone without the alveolar bone following the tooth in this eruption process. The calcification of plaque in the modern human constitutes a problem of the minority since most people have **soft plaque** owing to **altered diet**. According to our clinical experience, those who nowadays have calcifying plaque are susceptible to periodontitis. One can, of course, avoid the disease by relying on **professional care**; it is not easy to remove calcifying plaque from the sulcus based on **home care** only. Calcifying plaque is not the same as calculus; the former is still vital, active plaque which, however, turns within a short period of time harder than non-calcified plaque. Because soft plaque can be controlled by means of home care, one has observed during the past decades that periodontitis has somewhat decreased along with improved oral hygiene. The importance of food in the development of periodontitis is accentuated by studies where the first experimental periodontitis cases in laboratory animals were induced by means of soft diet. In other words, after receiving food that did not wear teeth, only those animals whose teeth were cleaned twice daily were saved from periodontitis. Calcifying plaque, rich in calcium and phosphorus, seems to be associated with caries-free teeth and with periodontitis. We have shown higher calcium content in periodontal subjects not only in **dental plaque** but also in **saliva**. In young subjects with no periodontal problems, a **high plaque calcium** level was associated with a **high number of intact teeth** present. A significant positive correlation was observed between saliva and plaque regarding their calcium and phosphate contents. The possible role of calcium in periodontal disease has been discussed by Aleo et al from another point of view: they studied the uptake of Ca** by endotoxin-challenged fibroblasts in vitro and correlated alterations in calcium homeostasis with the pathogenesis of periodontitis. The main source of calcium present in stimulated saliva is serum, the calcium level of which is relatively

constant, and any changes in serum calcium are balanced out by dietary uptake or by mobilization of the calcium deposits of mineralized tissues. The saliva of the modern human may also be regarded as a source of Ca ++. According to recent reports, dietary calcium is lower and the sugar content is higher in today's average diet than earlier. Consequently, calcifying plaque can no longer be regarded as a common clinical finding in most industrialized countries. This assertion is in full congruence with the low prevalence of periodontitis. The idea of the gingival sulcus as being a part of the physiologic anatomy of the periodontium can be disputed. The formation of the **sulcus** may, de facto, constitute the very **first step towards periodontitis**. We would thus like to suggest as an alternative hypothesis that gingival sulcus formation does not occur at all provided that the diet consumed is sufficiently hard and coarse, leading to tooth attrition and compensatory tooth eruption.

Several studies have indeed discovered a close relationship between **attrition** and **eruption**, suggesting that the **occlusal wear** of the teeth is compensated by the eruption of the dentition. With a hard and gritty diet resulting in moderate or heavy occlusal attrition, the attachment loss of the teeth is suggested to mostly result from compensatory eruption and not from bone resorption. In industrialized countries, the diet has changed from hard to soft during the past centuries and, hence, occlusal attrition is a rare finding in **industrialized populations**. However, both sulcus formation and subgingival plaque retention are common. What caused the absence of the sulcus in ancient humans? The marginal gingival epithelium can be regarded as a fusion of two cell populations, i.e. the **oral epithelium** and the **reduced enamel epithelium**, According to our hypothesis, both maintain their own basal cell layers. It has been demonstrated previously that an internal basement lamina exists between enamel and the adjacent epithelial cells. It was recently shown that these cells divide as basal cells do. Consequently, it is obvious that the junctional epithelium has two basal cell layers, one adjacent to the connective tissue, forming the so-called external basement lamina, and one adjacent to the tooth. Maintaining the ability - even after tooth eruption - of the latter cells (so-called 'DAT' cells) to divide, was in ancient humans an unconditional prerequisite for the integrity of the epithelial attachment, since the very foundation of the attached cells (i.e. the tooth) was constantly erupting to prevent reduced occlusal vertical dimension caused by the wearing of the occlusal surface".

2. Introduction

There is no general agreement on the pathogenesis of periodontitis. However, there is increasing evidence that a **variation in salivary calcium** concentrations is an important factor in the **development of periodontitis** and overall **dental health** in industrialized countries. A high level of salivary calcium is closely related to rapidly mineralizing plaque and an increased susceptibility to periodontitis. It has been known for 30 years that gingivitis can be experimentally induced by simply allowing dental plaque to accumulate at the gingival margin. It was earlier believed that the disease progresses from gingivitis to periodontitis in a linear fashion. New epidemiological evidence suggests, however, that generalized destructive periodontitis is relatively rare even in the adult populations where gingivitis is much more common and severe. On the other hand, even patients with an overall healthy periodontal situation can sometimes reveal a severe loss of attachment at isolated sites. This discrepancy has been explained by the hypothesis that periodontal diseases are due to specific bacterial infections. According to this; periodontal infections are suggested to resemble other specific infections in that certain, so called periodontopathogen species would initiate various gingival or periodontal diseases. However, it is impossible to induce chronic adult periodontitis-like diseases experimentally by infecting animals with any of the known periodontopathogens. Subcutaneous injection of these pathogens causes acute abscesses, but not chronic periodontitis. Chronic periodontitis-like diseases can be experimentally induced by placing **sub-gingival ligatures** around the crevical area of a tooth. Due to sub-gingival ligature the plaque starts to accumulate under the gingival margin and the bacterial biofilm becomes predominantly gram-negative and anaerobic. Additionally, microbial plaque starts to migrate deeper in the sub-gingival direction and soon an inflammatory connective tissue lesions, followed by evident epithelial accretion. The existence of gingival sulcus is a predisposing factor for periodontitis. If the plaque is not rapidly calcifying, it is possible to eliminate sub-gingival plaque deposits from shallow sulci simply with good oral hygiene. However, in cases of rapidly calcifying plaque, a sub-gingival "ligature" is soon formed from the calcifying biofilm, which is impossible to eliminate by home-care methods alone. Professional cleaning is needed to eliminate this mineralizing plaque from the crevices. Despite the fact that only sub-gingival plaque retention can induce chronic periodontitis, as of yet there is no common agreement for accepting this concept as the pathogenesis of the disease.

Subgingival plaque behaves similarly to a subgingival ligature in predisposing to periodontal disease. Rapid plaque formation has been proved to promote a higher calcium content of the

saliva particularly in smokers. Studies of the oral bacteria in **smokers** and **non-smokers** have led to inconclusive results, some investigations indicating significant differences in the numbers and compositions of these bacteria between smokers and non-smokers, whereas others did not.

Only part-result has been published on the interleukin-1 level, gene polymorphism, leukocyte activity and phagocytosis in relationship to smoking and periodontal disease.

3. The aim and question to be answered

The aim of this study therefore was to assess the possibility of differences in the calcium concentrations of the saliva and whether or not it is an aggravating factor in periodontal disease for smokers.

4. Methods and materials

4.1. Studies

4.1.1. Salivary calcium concentration in relation of periodontal health of female tobacco smokers.

Adult patients were selected to permit a comparison of female smokers and non-smokers on the basis of the periodontal status. Exclusion criteria were severe general health problems (for example: diabetes, or high blood pressure) the prescription of medication (e.g. Ca antagonist), and fewer than 16 remaining teeth.

A total of 51 female patients were screened for this study. Seven women were excluded due to of systemic health problems. Following screening, a total of 44 women 24 smokers (4 periodontitis-free, 16 with chronic, and 4 with aggressive periodontitis, mean age of 50.2 years ± 6.9) and 20 non-smokers (mean age 54.7±15.6, 10 periodontitis-free, 9 with chronic and 1with aggressive periodontitis) were included in this study.

The study protocol was approved by the ethical committee of the University of Szeged. The following parameters were recorded from all existing teeth: radiographic bone loss, plaque index, gingival index and calculus index.

A single P score for the horizontal and/ or vertical periodontal bone loss, determined from an orthopantomogram, was given for each subject via the criteria of Sevón & Parvinen (1988):

. P0 = no marginal bone loss.

P1 = mild marginal bone loss throughout the dentition or at several sites, but not exceeding 30% of the root length anywhere in the dentition.

. P2 = moderate marginal alveolar bone loss, involving at least 30% of the root length throughout the dentition or at several sites, but not exceeding 50% bone loss anywhere in the dentition.

P3 = advanced marginal alveolar bone loss, involving at least 50% of the root length throughout the dentition, or at several sites.

Pocket depth was probed to the nearest millimeter with a 2 mm graduated Williams-probe at six sites per tooth. The numbers of fillings, crowns, etc. were also recorded, as were caries-free and missing tooth data.

Participants were asked not to eat or drink, and to restrain from tooth brushing and smoking for one hour before the clinical examination and saliva collection. A piece of paraffin-wax (1 g) was used to stimulate salivary secretion. The saliva secreted during the first 60 sec. was swallowed; that secreted during the next 10 min. was expectorated into graduated test-tubes and the flow-rate was measured. To standardize salivary calcium recovery, **both soluble and protein-bound, non-centrifuged whole saliva was used**, 2ml of samples of which were frozen and stored at -20° C for calcium measurements. The calcium concentration of the saliva was analyzed using atomic absorption spectrophotometry (Perkín-Elmer Atomic Absorption Spectrophotometer Model 303), as described earlier (Sevón, Söderling, Karjalainen 1990).

Clinical examinations and saliva sampling were carried out at the Department of Periodontology in Szeged Hungary, and measurements of salivary calcium at the Department of Periodontology, in Turku Finland.

The significance of the differences between groups was analyzed with the Multivariate Analysis of Variance-test.

4.1.2. THE EFFECT OF AGE ON FLOW-RATE, PROTEIN AND ELECTROLYTE COMPOSITION OF STIMULATED WHOLE SALIVA IN HEALTHY, NON-SMOKING WOMEN

The aim was to study the effect of **age** on salivary **flow rate**, the level of **calcium**, **phosphate**, **magnesium**, **sodium** and **potassium** in healthy women. These results can be used as reference values for 30-59-year-old women.

Participants

Originally our study group consisted of 1030 women (age range 30-62 years) participating in a pre-screen referral program for osteoporosis. The screening was carried out by the Public Health Centre of Raisio, a South-Western Finnish community with a population of 23 000 inhabitants. The age cohorts invited by the pre-screen program in 1999 included all women living in the community and born in the years 1940, 1941, 1943, 1945, 1949, 1954, 1957, 1959, 1964 and 1969. There was one subject born in 1937 who participated in the screening but was excluded from the present study. Women with verified (N=12) and uncertain pregnancies (N=3) were excluded. A brief medical history including medications and smoking habits were recorded by a questionnaire filled out by all consenting participants before screening. All participants having one or several systemic diseases or using medications including hormone replacement therapy were excluded. All women who reported of smoking habits were also excluded. The age distribution of the remaining healthy, non-medicated, non-smoking subjects (N=255, 30-59 years) is presented. The women were further divided in subgroups at five-year intervals.

Ethics

The study was approved by the ethics committee of the municipality of Raisio. The subjects were volunteers and informed consent was obtained from all participants.

Electrolyte and protein analysis

Calcium, magnesium, potassium and sodium concentrations were measured by atomic absorption spectrophotometer (Perkin-Elmer Atomic Absorption Spectrophotometer Model 303, Norwalk, USA). Due to the strong affinity of calcium to form complexes with salivary proteins, non-centrifuged whole saliva containing both protein-bound and soluble calcium was

used for the assay. A total of 200 µl saliva was mixed with 1760 µl of water and 40 µl of 5% lanthanum oxide. The analyses of magnesium, potassium and sodium were made from centrifuged saliva (12 000 *g*, 10 min, $+4^0$C) after dilution with ion-exchanged water. Inorganic phosphate was analyzed according to Kallner and total protein according to Lowry et al. both from centrifuged saliva. Bovine serum albumin was used as a standard for protein determinations.

Statistical analysis

The normality of distributions of the response variables were controlled by the **Kolmogorov-Smirnov test.** Before statistical analyses, logarithmic transformations of the salivary variables were made due to the skewed distributions. The statistical evaluations were performed by one-way analysis of variance. Correlations between flow rate and salivary constituents were measured by Pearson's correlation coefficients. A commercial software program (Statistical Package for Social Sciences for Windows, version 9.0, SPSS Inc., Chicago, Illinois, USA) was used to run the statistical analyses.

4.1.3. SALIVARY CALCIUM IN RELATION TO ORAL HEALTH OF TOBACCO SMOKERS

Adult patient were selected as to compare female smokers and non-smokers on the basis of sex, age, and periodontal state. Exclusion criteria were severe general health problems (for example: diabetes, or high blood pressure) and/or medication (for example: Ca antagonist), and fewer than 16 remaining teeth.

Altogether 38 women were examined, 24 of whom were smokers, and 14 were non-smokers.

Clinical data and clinical examinations were performed in the same way as it was in the first study. Clinical examinations and saliva sampling were carried out at the private practice of the principal author (E.K.) in Kecskemét, Hungary. Measurements of salivary calcium were done at the University of Turku’s Institute of Dentistry, Department of Periodontology, in Finland.

5. Results

5.1. Studies

5.1.1. Salivary calcium concentration in relation of periodontal health of female tobacco smokers.

The examination on the 44 women revealed that the **mean calcium content** of the saliva was **significantly higher** (57.76 ug/ml±18.8) for the **smokers**, than for the non-smokers (44.6 ug/ml±7.8 $p<0.05$).

The periodontal examinations demonstrated a statistically **significantly greater degree of bone loss** in the **smokers** than in the non-smokers, and the **mean P score** for the **non-smokers** P=0.75 was **lower** than that for the smokers P=1.67. The **mean probing depths** and the extent of **calculus** were **higher in smokers** ($p<0.05$), but the **plaque index** and the **bleeding**-upon probing values **did not differ** in the two groups.

5.1.2. The effect of age on flow-rate, protein and electrolyte composition of stimulated whole saliva in healthy, non-smoking women

Salivary flow rate

Flow rate of paraffin-stimulated saliva varied between 0- 4.0 ml/min and **did not show** any **age-related changes** during the time span of nearly three decades.

The effect of age on salivary composition

Salivary calcium and **phosphate** concentrations showed a clear **increase** with increasing age. Calcium and phosphate increased about 12 % at menopause as compared to the age period between 45 and 49 years.

The effect of flow rate on salivary composition

Salivary **flow rate** correlated **negatively** with **magnesium**, **potassium**, **phosphate** and **protein** level and **positively** with **sodium**. **Calcium** was the only electrolyte which did **not** show correlation with **flow rate**. Means and standard deviations (SD) of salivary flow rate, sodium, potassium, magnesium concentrations and protein content and 97.5% and 2.5% percentiles of all age groups are given.

5.1.3. SALIVARY CALCIUM IN RELATION TO ORAL HEALTH OF TOBACCO SMOKERS

10 matched groups were created, in the **9** of which the **salivary calcium** level of the **smokers** was significantly **higher** (p<0, 02, two-sample T-test). Periodontal examination revealed a **higher bone loss**, a **greater probing depth** and **fewer remaining teeth**, **less bleeding** on probing in **smoker** patients.

6. Discussion

Smoking is considered a major risk factor for the development and progression of periodontitis, the mechanisms not fully understood yet. Poorer oral hygiene in smokers, compared with non-smokers has been a consistent finding in earlier studies. It was explained by the fact that increased severity of chronic inflammatory periodontal disease in smokers is most probably due to increased amounts of plaque and calculus. However, when smokers and non-smokers with a similar level of oral cleanliness were compared for severity of periodontal disease, the differences were not statistically significant. It has also been found that smoking does not significantly increase the rate of plaque formation but it does **increase the calcium concentration** of plaque. As mentioned earlier, plaque with high calcium content rapidly hardens and is therefore an indirect cause of poor oral hygiene. It has been shown previously that smokers have difficulty in performing effective tooth brushing. Bergström et al. found that supragingival calculus was strongly associated with smoking. The influence of smoking on the amount of supragingival calculus was dose-dependent. Supra-and subgingival calculus is known to be especially favorable for microbial growth and retention.

According to Sevón (1966), subgingivally retained, rapidly mineralizing plaque may be an important reason for periodontitis susceptibility.

Thus it seems that one of the main oral **side effects** of **smoking** is more rapidly **mineralizing plaque** and **disease progression** as compared with non-smokers.

The present results are in agreement with the findings of Sevón et al (2000) and Macgregor et al (1986), that the **calcium concentration** of the **stimulated saliva** of **tobacco smokers** is **higher** than that of non-smokers. Zuabi et al (1996). found, however, reduced calcium concentration of non-stimulated saliva of tobacco smokers. According to Sevón (2004), decrease of skeletal bone density, a known side effect of smoking, may be reflected in increased levels of salivary calcium.

To our knowledge this is the first time when salivary composition has been studied for reference purposes in non-medicated and non-smoking women to this extent. In this study, apart from smoking, we wanted to exclude all possible salivary effects of medications as well. Flow-rate correlated positively with sodium and negatively with phosphate, potassium, magnesium and protein, which is partly in line with the most recent text-book data. However, some of our findings are controversial as compared to earlier reports: we found that salivary **potassium** was **negatively correlated** with **flow rate** contrary to Dawes who showed that potassium was independent of salivary flow rate. This may be due to two reasons: firstly, we studied whole saliva in contrast to Dawes whose results apply to sublingual or parotid saliva, and secondly we made inter-individual comparisons as opposed to Dawes who presented intra-individual comparisons. **Calcium** was the only electrolyte in our study, which did **not correlate** with **salivary flow-rate**. This is in contrast to earlier studies showing an increase in salivary calcium with short-term citric acid stimulation of parotid saliva.

According to our study, salivary **calcium** and **phosphate** concentrations **increase** with **age** showing peak values around **menopause**. Therefore we suggest that menopause is reflected in saliva as elevated levels of calcium and phosphate. This result is well in accordance with our earlier findings. The reason why salivary calcium seems to increase with age may be explained by the hypothesis we have presented earlier with smokers: a **decrease** in **skeletal bone** density, seen often in elderly people, may **increase** the amount of **calcium** in saliva. However, this phenomenon is not completely clear and needs further studies. We have data on salivary calcium of different study populations with **decreasing bone mineral density**, such as patients with **rheumatoid arthritis**, **heavy smokers** and women in **menopausal ages**. They all have higher means of salivary calcium level when compared to age-matched counterparts. We have also found that hormone replacement therapy, which has a stabilizing effect on calcium content of bone, has a similar effect on salivary calcium.

Earlier it was generally believed that salivary flow rate decreases with age, but increasing number of studies are showing that **aging** does **not affect** the **rate** of **stimulated whole saliva**. Our current finding of no correlation between age and salivary flow-rate is well in line with the works of Parvinen and Larmas, Tylenda et al., and with the more recent studies of Närhi et al., Percival et al., and Yeh et al.

7. Conclusion

1. Salivary **potassium** was **negatively** correlated with **flow rate.**
2. **Age** had **no** effect on the **flow-rate** of stimulated saliva.
3. Salivary **calcium** and **phosphate** concentrations **increased** with **age** showing peak values around menopause.
4. In addition, normal **reference values** of salivary electrolytes for **women** of **different age groups** are provided to enable future diagnostic use of salivary electrolytes.
5. Within their limits, the present findings seem to indicate that **smoker female periodontitis** patients exhibit **higher salivary calcium** levels compared to non smokers. The clinical significance of these findings needs, however, to be determined in further, large scale controlled studies.

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PUBLICATIONS RELATED TO THE THESIS

Articles:

1. Sevón L, Laine MA, Karjalainen S, Doroguinskaia A, Helenius H, **Kiss E**, Lehtonen-Veromaa M. Effect of age on flow-rate, protein and electrolyte composition of stimulated whole saliva in healthy, non-smoking women. Open Dent J. 2008;2:89-92. Epub 2008 Jun 11.
2. Barabasi Z., **Kiss E**., Balaton G., Vajo Z. Cutaneous granuloma and uveitis caused by a tattoo. Wien Klin Wochenschr 2008;120(1-2):18. **IF:0.857**
3. Nagy K., **Kiss E**., Erdei C., Oberna F., Fejérdy P., Márton K., Vajo Z. Complex care by multiple medical and dental specialists of a patient with agressive Gorlin-Goltz syndrome. Postgrad Med J 2008;000:1-4. **IF:1.587**
4. **Kiss E.,** Sewon L., Gorzo I., Nagy K. Salivary Calcium Concentration in Relation to Periodontal Health of Female Tobacco Smokers. A Pilot Study.
   Quintessence International 2010; accepted. **IF: 0.811**

Published Abstract:

1. **Kiss E**, Gorzo I, Sewon L. Salivary calcium in relation to oral health of tobacco smokers.
   P 3605 ; IADR 2004, Honolulu