The correlation between dental calculus and disturbed mineral metabolism in pediatric patients with chronic kidney disease

Esti Davidovich1,*, Miriam Davidovits2,3,*, Benny Peretz4, Joseph Shapira1 and Doron J. Aframian5

Abstract

Introduction

Dental calculus in healthy children is rare [1,2]; however, several studies have reported an elevated amount of calculus in the paediatric population with chronic kidney disease (CKD) [3–5].

Recently, we have reported a case with an abundant amount of dental calculus in an infant on dialysis that totally disappeared after kidney transplantation [6].

Dental calculus is a form of a calcification process in the oral cavity environment where calcium (Ca) and phosphorous (P) ions originating from saliva play a major role. This calculus is assembled through interaction with the dental plaque, which is a community of microorganisms found on the tooth surface as a biofilm [7]. In a healthy oral environment, saliva is supersaturated with Ca and P levels, however, without precipitation. Nonetheless, when this equilibrium is disturbed, dental calculus is formed enhanced by elevated salivary pH [8]. Dental calculus is formed with four different crystals of Ca–P; brushite, octa Ca–P, hydroxyapatite and whitlockite in which the most abundant crystals are the hydroxyapatite and octa Ca–P [9].

CKD is characterized by profound disturbances in Ca–P metabolism and increased Ca burden as a result of administration of Ca-based phosphate binders [10–14]. Cardiovascular complications related to abnormal Ca–P metabolism are the major cause of morbidity and mortality observed in CKD patients and particularly in end-stage renal disease (ESRD) [10–13]. Moreover, different manifestations of uraemic vasculopathy due to calcifications have been reported recently among the paediatric CKD population [14–19].

Since the quantification of dental calculus and its correlation with renal disease severity have not been previously studied in young CKD patients, our objectives were (1) to evaluate the dental calculus formation in young CKD patients, (2) to evaluate the salivary mineral changes of these patients, and (3) to explore the relationship between the two.

Methods

DCS in 74 young CKD patients were evaluated: 25 pre-dialytic (PrD), 18 on dialysis (D) and 31 with transplants (T) compared to 32 healthy participants (C). Saliva and serum analysis included creatinine (Cr), urea (U), calcium (Ca), phosphorous (P), magnesium (Mg) as well as intraoral pH levels.

Results

All patient groups presented high DCS. DCS and pH levels were higher in the D group with a positive correlation between pH and lower incisor DCS (r = 0.56, P = 0.017). The highest salivary Ca was found in the PrD group. Salivary P in the PrD group was found to be higher than in the T and C groups. The lowest salivary Mg was found in the D group while the highest salivary Ca×P product was found in the PrD group. In all patient groups, salivary U was higher than in the C group with a 2.5-fold increase in the D group. Salivary Cr resembled the U salivary concentrations.

Conclusions

Alterations in salivary Ca, P, Mg, U, Cr and intraoral pH levels were observed in the patient groups. DCS correlated with renal disease severity and therefore may be a reflection of other tissue calcification pathologies found in these patients.

Keywords: Ca × P product; chronic kidney disease; dental calculus; saliva; vascular calcifications
patients and (2) to examine salivary mineral metabolism and intraoral mucosal pH levels with correlation to dental calculus formation in different stages of kidney function impairment.

Methods

Study population

Upon approval by the Institutional Review Board for research on human subjects and informed consent, 106 children, adolescents and young adults were recruited and studied.

The CKD patients were divided as follows: 25 pre-dialytic (PrD) patients, 18 patients on maintenance dialysis (8 on haemodialysis, 10 on peritoneal dialysis) (group D), and 31 transplanted patients with a functioning graft (group T).

All patients were treated at the Institute of Paediatric Nephrology, Schneider Children’s Medical Center of Israel, Petah Tiqva, Israel. Thirty-two healthy children without previous history of impaired renal function served as the control group (group C). This group was recruited and examined in the Department of Pediatric Dentistry at the Hebrew University—Hadassah School of Dental Medicine, Jerusalem, Israel (Table 1).

Systemic variables

The glomerular filtration rate (GFR) was calculated from the serum creatinine (Cr) according to the Schwartz formula [20]. Serum Cr, urea (U), Ca, P and magnesium (Mg) concentrations were measured using the Hitachi 917 autoanalyser (Hitachi, Tokyo, Japan). Oxalate (Ox) levels in saliva were utilized in five healthy subjects (C group) and five dialysis patients (D group) using the UV method with the Boehringer Mannheim kit (Darmstadt, Germany).

Ox analysis in saliva was based on urinary analysis and was performed on Olympus 2700 analyser using an enzymatic method. The method is based on converting oxalate to CO2 and H2O2 (by the enzyme oxalate oxidase), followed by H2O2 reaction with 3-methyl-2-benzothiazolinone hydrzone (MBTH) and 3-(dimethylamino) benzoic acid (DMAB) in the presence of peroxidase, to yield an indamine dye. The intensity of the colour produced is directly proportional to the concentration of Ox in the sample. Reference intervals for 24-h urine collection are 7–45 mg/24 h for male, 4–31 mg/24 h for female and 13–38 mg/dl for children and were implemented to saliva calculation. The analytical sensitivity was 0.88 mg/dl.

Calculus composition was assessed in five dialysis patients using urinary calculus analysis kit (DiaSys Diagnostic Systems GmbH, Holzheim, Germany).

The oral surface pH was assessed with a flat, glass electrode pH meter (HANNA instruments HI 8424, Padova, Italy) as previously described [26]. Two sets of measurements were applied at seven locations: the soft and hard palate, anterior, middle, and posterior tongue, right and left buccal mucosa. Five minutes were allowed between measurements [26].

Statistical analysis

Frequencies and percentages were calculated for categorical variables. The frequencies between categorical variables were analysed by the 'chi square test' and the ‘Fisher-Exact test’. Medians, means and standard errors were calculated for continuous parameters. The results of pH parameters between subgroups of patients were compared by ‘one-way analysis of variance’ using the Bonferroni model. Due to large variability of salivary pH, the correlations between DCS and saliva pH levels were analysed by Pearson’s correlation coefficient and the correlations between saliva parameters were analysed by Spearman’s correlation coefficient. The significance level of the correlation coefficient was calculated.

Results

Dental calculus score

Calculations was not found in any individual of the C group (Table 2) as opposed in all patient groups. The highest DCS was found in the D group with a significant difference from the PrD and T groups in both upper and lower sites (P = 0.0001). The T group patients exhibited the lowest DCS; however, with no significant difference from the PrD group. The scores were higher at the lower incisor site compared to the molar site in all study groups (Table 2).
Table 3. Serum and salivary biochemistry parameters in all examined groups

<table>
<thead>
<tr>
<th>Parameter [mean ± SEM (range) median]</th>
<th>Control</th>
<th>Pre-dialysis</th>
<th>Dialysis</th>
<th>Transplanted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Blood</td>
<td>Saliva</td>
<td>Blood</td>
<td>Saliva</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>2–50</td>
<td>31.6 ± 2.8*, -</td>
<td>ND</td>
<td>80.5 ± 8.3**, x</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.5–1.1</td>
<td>0.14 ± 0.02**, x</td>
<td>2.06 ± 0.27</td>
<td>0.40 ± 0.08**, x</td>
</tr>
<tr>
<td></td>
<td>(0.06–0.44)</td>
<td>0.11</td>
<td>(0.81–5.7)</td>
<td>(0.02–2.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(1.5)</td>
<td>(0.31)</td>
</tr>
<tr>
<td>Calcium (mg/dl)</td>
<td>9.2–11</td>
<td>1.79 ± 0.17**, x</td>
<td>9.78 ± 0.2</td>
<td>4.41 ± 0.59**, x</td>
</tr>
<tr>
<td></td>
<td>(0.5–4)</td>
<td>1.55</td>
<td>(6.9–11.5)</td>
<td>(1.5–11.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(10)</td>
<td>(3.42)</td>
</tr>
<tr>
<td>Phosphorus (mg/dl)</td>
<td>3–6</td>
<td>15.82 ± 1.15**, x</td>
<td>5.0 ± 0.25</td>
<td>23.22 ± 2.10**, x</td>
</tr>
<tr>
<td></td>
<td>(7.2–41.2)</td>
<td>14.35</td>
<td>(2.7–9.4)</td>
<td>(9.3–52.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(4.6)</td>
<td>(21.5)</td>
</tr>
<tr>
<td>Magnesium (mg/dl)</td>
<td>1.6–2.4</td>
<td>0.46 ± 0.05**, x</td>
<td>ND</td>
<td>0.81 ± 0.16**, x</td>
</tr>
<tr>
<td></td>
<td>(0.12–1.1)</td>
<td>0.43</td>
<td>(0.12–1.1)</td>
<td>(0.15–3.45)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.55</td>
<td>0.25</td>
</tr>
<tr>
<td>Calcium × phosphorus product</td>
<td>30–55</td>
<td>27.9 ± 3.5**, x</td>
<td>48.45 ± 2.32</td>
<td>117.74 ± 28.07**, x</td>
</tr>
<tr>
<td></td>
<td>(7.35–107.1)</td>
<td>22.75</td>
<td>(24.84–80.84)</td>
<td>(55.86–528.13)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(67.75)</td>
<td>(67.75)</td>
</tr>
</tbody>
</table>

ND = not determined.

aNormal serum range adopted from [38].

*Significant difference from the control group.

**Significant difference from the dialysis group.

†Significant difference from the pre-dialysis group.

‡Significant difference from the transplant group.
Table 4. Intraoral mucosal pH score

<table>
<thead>
<tr>
<th>pH (mean ± SD, range)</th>
<th>Control</th>
<th>Pre-dialysis</th>
<th>Dialysis</th>
<th>Transplanted</th>
</tr>
</thead>
<tbody>
<tr>
<td>All sites</td>
<td>6.72 ± 0.06, 5.84–7.38**</td>
<td>6.82 ± 0.12, 5.60–7.85**</td>
<td>7.38 ± 0.13, 5.95–8.03</td>
<td>6.73 ± 0.07, 5.90–7.70**</td>
</tr>
<tr>
<td>Hard palate</td>
<td>7.18 ± 0.13, 5.92–7.61**</td>
<td>7.05 ± 0.15, 5.77–8.45**</td>
<td>7.73 ± 0.14, 6.46–9.6</td>
<td>6.95 ± 0.09, 6.05–8.24**</td>
</tr>
<tr>
<td>Soft palate</td>
<td>7.08 ± 0.12, 5.53–8.2**</td>
<td>7.1 ± 0.15, 5.74–8.47**</td>
<td>7.7 ± 0.16, 6.16–8.54</td>
<td>6.95 ± 0.1, 6.08–8.5**</td>
</tr>
<tr>
<td>Right mucosa</td>
<td>6.46 ± 0.06, 5.75–7.42**</td>
<td>6.58 ± 0.1, 5.39–7.88**</td>
<td>7.05 ± 0.13, 5.92–7.75</td>
<td>6.42 ± 0.06, 5.5–6.88**</td>
</tr>
<tr>
<td>Left mucosa</td>
<td>6.4 ± 0.07, 5.4–7.43**</td>
<td>6.56 ± 1.5, 5.7–7.58</td>
<td>7.00 ± 0.13, 5.78–7.71</td>
<td>6.44 ± 0.07, 5.5–7.65**</td>
</tr>
<tr>
<td>Posterior tongue</td>
<td>6.58 ± 0.09, 5.92–7.18**</td>
<td>6.80 ± 0.15, 5.36–7.95**</td>
<td>7.41 ± 0.16, 5.64–8.51</td>
<td>6.77 ± 0.08, 5.9–7.51**</td>
</tr>
<tr>
<td>Middle tongue</td>
<td>6.63 ± 0.07, 5.72–7.35**</td>
<td>6.83 ± 0.14, 5.35–7.78**</td>
<td>7.45 ± 0.15, 5.82–8.51</td>
<td>6.82 ± 0.07, 5.96–7.6**</td>
</tr>
<tr>
<td>Anterior tongue</td>
<td>6.71 ± 0.06, 5.84–7.38**</td>
<td>6.82 ± 0.12, 5.6–7.85**</td>
<td>7.35 ± 0.13, 5.90–8.37</td>
<td>6.77 ± 0.06, 6.15–7.58**</td>
</tr>
</tbody>
</table>

Significant difference from the control group (*P < 0.05; **P < 0.01).
*Significant difference from the dialysis group.
*Significant difference from the transplant group.

Serum and saliva parameters

The serum parameters including Cr, Ca, P, Mg and U (group D only) are listed in Table 3.

The salivary Ca level in the PrD group was significantly higher compared to the D, C and T groups (3.7-fold P = 0.0001, 2.5-fold P = 0.05, 1.5-fold P = 0.001, respectively). The Ca levels in the T group were found to be significantly higher than in the D group (means of 15.4 mg/dl and 1.18 mg/dl, respectively, P = 0.0001) and in the C group (means of 2.96 mg/dl and 1.78 mg/dl, respectively, P = 0.001). (Table 3).

Salivary P levels in the PrD group (mean = 21.5 mg/dl) were found to be significantly higher than in the T group (mean 15.4 mg/dl, P = 0.015) and C groups (mean 14.35 mg/dl, P = 0.001). In the D group, P levels were found to be higher than in the C group although not statistically significant (Table 3).

The salivary Mg concentrations in the D group (mean = 0.25 mg/dl) were found to be significantly lower than in the PrD (mean = 0.55 mg/dl, P = 0.0004), T (mean = 0.57 mg/dl, P = 0.002) and C groups (mean = 0.43 mg/dl, P = 0.017). The Mg values in the PrD group were found to be significantly higher than in the C group (P = 0.048) as depicted in Table 3.

The salivary Ca × P product in the PrD group was significantly higher (mean 117.74) than in the D, T and C groups (P = 0.0002, P = 0.01, P = 0.0001, respectively). The Ca × P product for the T group was also significantly higher than in the C and D groups (P = 0.002, P = 0.01, respectively) as shown in Table 3.

In all the patient groups D, PrD and T (mean = 83.9, 80.5, 52.7 mg/dl, respectively), salivary U concentration was significantly higher than in the C group (mean 31.6 mg/dl; P = 0.002, P = 0.0001, P = 0.0001, respectively) with more than a 2.5-fold increase in the D group followed by the PrD and the T groups. A significant difference was also found between the PrD group and the T group (P = 0.007) (Table 3).

A significant correlation was found between GFR and salivary U concentrations in the PrD and T groups (r = -0.66, P = 0.001, r = -0.45, P = 0.022, respectively). Also, a significantly higher salivary U concentration was found in the D group followed by the PrD and the T groups (Table 3) and corresponding to DCS (Table 2).

The Cr salivary concentration pattern resembled the urea salivary concentration with significantly higher levels in the D group (mean 0.59 mg/dl) followed by the PrD (mean 0.4 mg/dl), T (mean 0.15 mg/dl) and C groups (mean 0.14 mg/dl) (Table 3).

Oral mucosal pH scores

Table 4 represents the average of pH scores from seven intraoral mucosal sites. The average of pH values in the D group was significantly higher (mean 7.38) than in the PrD group (mean 6.82, P = 0.001), the T group (mean 6.73, P = 0.0001) and the C group (mean 6.72, P = 0.0001). No statistically significant difference was found in pH scores between the PrD, the T and the C groups. A correlation between the average pH score and the DCS at the lower incisor site was found in the D group (correlation coefficient = 0.56, P = 0.017).

Calculus composition and salivary Ox and Cit concentrations in saliva

Table 5 represents the Ca, P and Ox levels obtained from dental calculus in five D patients. High levels of Ox were found followed by P and Ca (means 19, 14 and 12%, respectively) levels. Ox and Cit were measured in saliva of five dialysis patients and five healthy age-matched children (Table 5). More than a 3-fold increase in Ox levels was found in the D group with only slight increase in the Cit levels compared to the C group.

Discussion

To our knowledge, this study is the first to examine the dental calculus formation in young patients with different stages of chronic kidney disease (CKD) including pre-dialytic (PrD) patients on dialysis (D) and patients after kidney transplantation (T) compared to control healthy children. Considering the mechanism of the calcification process and
the metabolic disturbances resulting from kidney dysfunction, we explored the Ca, P, U, Cr and Mg concentrations in the secreted saliva of these patients and the pH levels in intraoral mucosal sites.

DCS were found to be higher in the lingual aspect of the lower incisors in all the study groups (Table 2). This finding is consistent with previous observations of preferential calculus formation on the lingual surface of the six lower incisors due to their proximity to the orifices of the submandibular glands, which act as the reservoir of Ca and P ions [8]. Interestingly, when we further explored the calculus composition in the most affected D patients (Table 5), we found that the Ox component was observed. To our knowledge, no previous studies have explored Ca–Ox as part of dental calculus specifically in CKD patients. The fact that an elevated concentration of Ox in saliva was observed is probably due to the decrease in GFR and urinary excretion in these patients. Higher oral pH levels were established in the D group patients versus the C group (pH = 7.38, pH = 6.72, respectively, Table 3). In this condition, Ca–Ox saturation product becomes extremely high due to an equimolar increase in Ox as compared to Ca. Consequently, it will lead to a 10-fold higher increase in the saturation process and hence to higher risk in crystal deposition.

Dental calculus was not found in control subjects (Table 2) similar to pervious studies [1,2]. Interestingly, the most abundant amount of calculus was found in the D patient group, followed by the PrD group and the T group. The changes in dental calculus scores in this group. Moreover, a correlation between average pH measurements and lower incisor DCS was found in this group (correlation coefficient = 0.56, P = 0.017). This observation may be explained by the abundant supply of U from the salivary gland secretion hydrolysed by bacteria with the release of ammonia, leading to an elevation of pH in the dental plaque and further promoting Ca and P precipitation [29,8].

Blood U and Cr levels serve as indicators for the severity of kidney disease. Previous studies have shown elevated levels of salivary U concentrations in patients with ESRD [27,28]. These observations correlate with our study where both salivary U and Cr levels changed in saliva according to the renal function, with the highest levels found in the D group (Table 3). U has been shown to facilitate dental plaque alkalinization [8], and therefore may contribute to the highest rate of calculus formation in the D group (Figure 1). In the PrD and T groups, a significant correlation was found between the GFR and the salivary U concentrations (results are not shown). This observation may help in the future to assess renal function in these two groups (i.e. PrD and T) by measuring salivary U concentration.

The highest measurement of oral mucosal pH was found in the D group corresponding to the salivary U concentrations in this group. Moreover, a correlation between average pH measurements and lower incisor DCS was found in this group (correlation coefficient = 0.56, P = 0.017). This observation can be explained by the abundant supply of U from the salivary gland secretion hydrolysed by bacteria with the release of ammonia, leading to an elevation of pH in the dental plaque and further promoting Ca and P precipitation [29,8].

The most significantly higher concentration of salivary Ca was found in the PrD group. This finding may be a reflection of the disturbed Ca metabolism in this patients’ group manifested by the high Ca burden followed by compensatory secretion in saliva. Salivary Ca levels during dialysis were reduced, probably in parallel to the reduction of Ca levels in the serum at this stage. A further decrease in salivary Ca secretion in the T group may be attributed to a decrease in the Ca burden due to the improved renal

### Table 5. Calculus composition in dialysis patients and salivary oxalate and citrate concentrations in dialysis group versus controls

<table>
<thead>
<tr>
<th>Calculus composition (%)</th>
<th>Saliva composition (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D group</td>
</tr>
<tr>
<td></td>
<td>Ox</td>
</tr>
<tr>
<td># 1</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>12 ± 8.4</td>
</tr>
</tbody>
</table>

Calcium, oxalate and phosphate composition was obtained from calculus. Oxalate and citrate were measured in saliva of five dialysis patients and five healthy age-matched children. Ca = calcium; P = phosphorus; Ox = oxalate; Cit = citrate; ND = not detected; D = dialysis group; C = control group.
function and cessation of Ca-containing P-binder medications. Interestingly, Ca levels were still higher in the T group compared to controls, which is probably related to the still existing Ca overload state (Table 2).

P concentrations in the saliva in all groups were higher than in the serum reaching almost a 5-fold increase in the PrD group corresponding to the active mechanism of P secretion from the salivary glands [30]. Recently, Savica et al. [31] have found a significant increase in salivary P levels in adult haemodialysis patients compared to healthy controls; serum phosphorus was the only predictor of increased excretion in saliva. The authors suggested that increased salivary P secretion might be interpreted as being compensatory in the presence of renal failure [31]. Consequently, upon renal function deterioration, more P is secreted in the saliva leading to precipitation with Ca and dental calculus formation as observed in this study (Figure 1).

Elevated plasma P levels and high Ca × P ion product play a key role in vascular calcifications, increasing the prevalence of morbidity and mortality in CKD patients [13,16,17,32–34]. Furthermore, several studies have shown that long-term exposure to disturbed mineral metabolism in paediatric CKD patients appears to contribute to the development of coronary artery calcifications in young adults with ESRD [13,19,35,36]. Vascular abnormalities are already present in children and adolescents during early stages of CKD. These abnormalities are more severe in children on dialysis, improve after kidney transplantation and are related to abnormal Ca–P metabolism. In recent years, attention has focused on the mechanism of the calcification process, its detection and monitoring as well as the role of Ca–P as potentially modifiable risk factor in the development and progression of vascular calcifications [16–18]. In this study, the salivary Ca × P product was significantly higher in the PrD group followed by the T group (Table 3). Interestingly, Ca × P product levels in the serum did not significantly change between the groups. This discrepancy between serum and saliva may be attributed to higher salivary P secretion as previously discussed, emphasizing the key role of P in dental calculus formation.

The lowest salivary Mg concentration was found in the D group (Table 1). Mg acts as an inhibitor ion of the calcification process further explaining the amplification of dental calculus formation in the D group. Interestingly, Grases et al. [37] showed previously less sialolithiasis formation in patients with higher salivary Mg concentrations [36].

In conclusion, our study clearly demonstrates a possible association between the severity of renal dysfunction in young patients and the formation of dental calculus—an additional manifestation of disturbed Ca–P homeostasis.

The combination of several components in saliva including Ca, P, U and Mg plays an important role in this process. The elevated Ox content finding in the D group patients’ saliva as well as a major component in their dental calculi may also contribute to the understanding of calculus formation process in CKD patients.

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References

21. WHO oral hygiene indices http://www.whocollab.od.mah.se/exp/ohigv60.html

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