The oral health status and salivary parameters of Egyptian children on haemodialysis

Abeer M. Abdellatif a,*, Salwa A. Hegazy a, Jilan M. Youssef b

a Department of Pediatric Dentistry and Dental Public Health, Faculty of Dentistry, Mansoura University, Egypt
b Department of Oral Medicine and Periodontology, Faculty of Dentistry, Mansoura University, Egypt

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Abstract This study was conducted on 28 end-stage renal disease children undergoing haemodialysis, aged 6–15 years (mean age, 11 years, 3 months) in the Pediatric Nephrology Unit of Zagazig University Hospital. The aims of the study were to monitor their oral health status, to analyse the salivary total antioxidants and protein levels and to clarify their relationship with the oral health status. The data were compared with that of a gender and age matched control group. Significantly higher prevalence of oral health parameters except debris accumulation, and lower caries prevalence, was revealed. Significant lower levels of salivary total antioxidants and proteins and a correlation between both salivary parameters and “deft” index were also reported. The results of the study suggest a need for dental advice and supervision, and indicate that dental and medical care should be closely integrated for children with renal failure.

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Introduction

Chronic renal failure (CRF) results from the progressive and chronic deterioration of nephrons, which happens over years [1]. The incidence of CRF continues to rise worldwide [2]. Patients with CRF who cannot be treated need to follow renal replacement therapy by dialysis or transplantation, and the disease is then referred as end-stage renal disease (ESRD). The prevalence of dialysis patients in Egypt is presumed to have increased from 10 per million population (pmp) in 1974 to about 165 (pmp) in 1995. A more recent study showed a further increase in CRF prevalence to 225 (pmp) in 1999 [3]. The highest prevalence was 483 (pmp) in 2004 as reported by Afifi et al. [4] in the ninth annual scientific meeting of the Egyptian Renal Registry in 2008. Most CRF patients are treated by haemodialysis, as reported in different studies [4,5].

Oral, dental and systemic complications can occur as a result of the disease or its treatment. Among the oral and dental complications are uremic stomatitis, reduced salivary flow, xerostomia, increased tendency for calculus formation, reduced caries prevalence, and enamel hypoplasia [6,7].
Advances in paediatric nephrology during the past two decades have resulted in a marked increase in the number of children surviving with CRF, allowing dentists to follow these subjects over longer periods of time [8].

As reported by Himmelfarb and Hakim 2003, renal failure is accompanied by oxidative stress, which results in damage of biological structures by reactive oxygen species [9]. These free radicals are short-lived and cannot be measured directly, but their activity can be measured by estimating the by-products and substances involved in defence against the oxidant injury [10]. Some authors found that there is direct proportion between the amount of caries in deciduous teeth and total antioxidant activity in saliva [11].

Saliva contains large numbers of proteins that participate in protection of the oral tissues, as each of them have a broad spectrum antimicrobial activity with a considerable functionality overlap [12]. Interest in saliva as a diagnostic fluid has grown in recent years and monitoring of markers in saliva instead of serum is found to be advantageous [13].

A large number of workers have investigated the salivary content and flow rate in adults with CRF. Significantly greater concentrations of salivary proteins, potassium and sodium were observed in unstimulated saliva collected from adults undergoing haemodialysis compared with healthy volunteers [14]. Others reported that the pH and buffer capacity of the unstimulated saliva was also increased in haemodialysis patients [15]. Various authors examined the relationship between the composition of saliva and cariogenic activity and the control of inflammatory processes of the mouth. A rise in the levels of free radicals (reactive oxygen species) has been found to be the common factor in these processes [16].

Because of the oral health concerns for children with CRF and ESRD, this study was designed to gain further insight into some of the oral changes seen in a sample of these patients.

In the present study, we intended to monitor the oral health status (enamel hypoplasia, dental caries, oral hygiene, gingivitis, and saliva buffering capacity) of children undergoing haemodialysis and compare them with age- and sex-matched healthy control volunteer children; and also to evaluate the total antioxidant activity (TAC), and total protein levels (TP) in their saliva in an attempt to clarify the relationship between their salivary levels and oral health status.

Subjects and methods

Sample size

In this cross-sectional study, 28 children (16 males and 12 females) aged 6-15 years (mean age, 11 years, 3 months) were studied. They were undergoing regular haemodialysis treatment in the paediatric nephrology unit at Zagazig University Hospital, Egypt.

According to the information retrieved from their files, patients were categorized according to the primary kidney disorder (Table 1). No other systemic disease was diagnosed in any of these patients. In every case, informed consent was requested and obtained from the parent of the child for their participation in the study. The study was also approved by the local ethical committee.

CRF is defined in the paediatric nephrology unit in Zagazig University Hospital as a glomerular filtration rate (GFR) of 25 ml/min. The patients had been placed on a 1 g/kg/day protein, low Na, P, and K, and high carbohydrate diet. The patients were on a medication list including phosphate binder, oral and intravenous calcium, subcutaneous erythropoietin, antihypertensive, and intravenous iron.

To examine a control group of healthy children, permission was obtained from the Department of Education of Sharkia Governorate. Informed consent forms were sent to the parents and the signed forms were collected and presented on the day of examination. A total of 28 healthy children (16 males, and 12 females), from primary and preparatory schools and according to age, gender and socio-economic status, were selected for the study. They were subjected to the same assessment procedures as those of the study group.

Clinical oral and dental examination

The study did not include any invasive technique leading to contamination to or from these patients. One examiner performed the assessment of both the study and the control children to eliminate inter-examiner variability.

Under artificial light, the examiner carried out the clinical examinations using a mouth mirror and a dental probe according to the criteria of the World Health Organization [17]. Enamel hypoplasia was assessed using the criteria determined by Alaluusua et al. [18]. Caries status was determined by recording the number of decayed (d, D), extracted/missing (e, M), and filled (f, F) teeth in the primary and permanent dentitions per patient and were referred to as deft and DMFT scores, respectively [19]. The gingival status was evaluated using the papillary, marginal and attached (PMA) index [20], and debris, and calculus status were assessed using the Simplified Oral Hygiene (OHIS) index [21]. Each subject in both groups was asked about the frequency of daily tooth brushing and dental check-ups.

Saliva sample collection and analysis

Before collecting saliva for the different proposed tests, the children were asked not to eat or drink for at least an hour. Unstimulated saliva was collected in sterilised clear containers. The containers were stored in ice for transfer and kept frozen at −80 °C until the time of assay.

Buffering capacity of saliva was calculated using the CRT Buffer Test [CRT® Buffer Test (Vivadent Ets., Lichtenstein, Australia)] using colorimetric test strips. Each strip was wetted.
with saliva using a pipette and the resulting colour change determined according to the manufacturer’s instructions. High, medium and low salivary buffer capacities are indicated by blue, green and yellow test fields, respectively.

Saliva pH was assessed using Universal pH strips (Dr. Gerhard Kloz, Berlin, Germany). The entire test field was wetted with saliva using a pipette for at least 10 s. The colour changes of the test field were compared with the chart provided by the manufacturer and the values were directly recorded.

The total salivary antioxidant capacity (TAC) and the total protein level (TP) were assessed using colorimetric method of Bio-diagnostic, 29 Tahreer St. Dokki, Giza, Egypt, with an auto-analyser.

The determination of the TAC is performed by the reaction of antioxidants in the sample with a defined amount of exogenously provided hydrogen peroxide (H₂O₂). The residual H₂O₂ is determined colorimetrically by an enzymatic reaction, which involves the conversion of 3, 5, dichloro-2-hydroxybenzen sulphonate to a coloured product [22].

TP level was measured with an auto-analyser based on the protein Biuret Method. The proteins in saliva produce an alkaline copper-protein chelate, violet in colour when combined with a specific reagent. The observed rate of chelate formation was directly proportional to the total protein in the sample [23].

**Pilot study**

A pilot study was first conducted to establish intra-examiner reliability. Five children were selected from the Pediatric Dental Clinic for that purpose. They were examined using the three dental indices (def, DMFT, PMA) and subsequently recorded by the examiner. For the OHI-S Index, five adult patients from the outpatient dental clinic were examined and scored by the same examiner. All the patients were re-examined after 24 h. Kappa values were more than 85% for all indices, indicating good reliability.

**Statistical analysis**

The statistical analysis was done using the SPSS for the Windows 13.0 statistical software package. Data were collected, tabulated and then subjected to the statistical analysis. The qualitative data were presented as numbers and percentages and the Chi square test was used for comparison between groups. The quantitative data were presented as mean ± standard deviation and the Student t-test was used for comparison between the groups. The Pearson correlation was determined between both salivary parameters, TAC and TP, and the oral health parameters. P ≤ 0.05 was considered to be statistically significant.

**Results**

A total of 28 CRF children who are undergoing haemodialysis, 57.2% (N = 16), 42.8% (N = 12) males and females, respectively, participated in the study. A control group of 28 healthy children (16 male and 12 females) matched to the study group in age and socioeconomic status was included in the study.

Past dental history of the two groups indicated that 90% of the study children never visited dentists and 85% of them never brushed their teeth, while 15% were irregularly brushing their teeth. On the other hand, 55% of the control group never visited dentists, 40% never brushed their teeth, while 60% were irregularly brushing their teeth.

The study group had significantly more enamel hypoplasia (46.4%) than the control group (7.14%) (Table 2). All ages with the disease duration of less than one year showed no clinical evidence of enamel hypoplasia, and the oldest age group, from 12 to 15 years, showed the highest number of enamel hypoplasia records (six patients) (Table 3). A significant difference between the two groups in respect of saliva buffering capacity is shown in Table 5: 50% of haemodialysed children and only 21.4% of normal children had high salivary buffer capacity. Only 17.68% of haemodialysed children had low buffering capacity compared with 28.57% of normal children.

### Table 2: Comparison between the two groups in terms of enamel hypoplasia.

<table>
<thead>
<tr>
<th>Group</th>
<th>Enamel hypoplasia</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Present</td>
<td>Absent</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Study group</td>
<td>13</td>
<td>46.42</td>
<td>15</td>
</tr>
<tr>
<td>Control group</td>
<td>2</td>
<td>7.14</td>
<td>26</td>
</tr>
<tr>
<td>χ² Test</td>
<td>9.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p-Value</td>
<td>0.002*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Highly significant difference (p ≤ 0.001).

### Table 3: Number of children with enamel hypoplasia, according to the age of onset and duration of the disease.

<table>
<thead>
<tr>
<th>Age of onset of renal disease (study group)</th>
<th>Number of children</th>
<th>Number of children with hypoplasia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration in years</td>
<td>6–9 years</td>
<td>9–12 years</td>
</tr>
<tr>
<td>&lt;1 year</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>1–3 years</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>&gt;4 years</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>10</td>
</tr>
</tbody>
</table>
The study group revealed significantly lower levels of both salivary parameters, TAC, and TP (0.707 ± 0.414 mM/L, and 0.873 ± 0.481 g/dL, respectively) compared with the control group (1.578 ± 0.621 mM/L and 1.265 ± 0.818 g/dL, respectively) (Table 6).

In the haemodialysis group, there was a correlation between both salivary TAC and TP and deft ($r = 0.36$, $p = 0.05$ and $r = 0.387$, $p = 0.042$, respectively).

Discussion

In the present study, 28 children with ESRD, on haemodialysis, clearly differed from normal children in most measured parameters; enamel hypoplasia, dental caries, gingivitis, calculus formation and saliva pH and buffering capacity. Chemical analysis revealed marked reduction in both TAC and the TP levels in the study group compared with the controls.

A significantly higher number of renal children with enamel hypoplasia were recorded. This is in accordance with other previous studies [6,7,15]. Nunn et al. [6] and Ertugrul et al. [24] reported that 83% and 47.4% of their renal patients had enamel defects. In the present study, 46.4% demonstrated enamel defects. Enamel defects noted in the study group were diffuse opacity and enamel hypoplasia as seen in patients with calcium deficiency. Renal impairment results in calcium depletion, which often leads to enamel hypoplasia if occurring during teeth mineralization [25], which may explain the cause of these defects.

In the study group, most children did not brush their teeth and had never visited a dentist. The control group children were not much different; they reported irregular teeth brushing and relatively few of them (45%) visited the dentists on demand. Children with ESRD usually consume cariogenic foods such as cakes, soft drinks and sweets to compensate for the reduction in protein intake. However, lower caries prevalence was evident in the study group in comparison with the control, which agrees with earlier studies [6,7,26], and disagrees with another study [27].

**Table 4** Comparisons between the two groups in terms of the oral health parameters and salivary pH.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Deft index mean ± SD</th>
<th>DMFT index mean ± SD</th>
<th>PMA index mean ± SD</th>
<th>OHI-S Debris index (DI) mean ± SD</th>
<th>Calculus index (CI) mean ± SD</th>
<th>Salivary pH values mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study group ($n = 28$)</td>
<td>1.333 ± 0.218</td>
<td>0.964 ± 1.267</td>
<td>1.678 ± 0.981</td>
<td>0.985 ± 0.568</td>
<td>1.050 ± 0.715</td>
<td>7.3 ± 0.947</td>
</tr>
<tr>
<td>Control group ($n = 28$)</td>
<td>2.111 ± 0.314</td>
<td>2.234 ± 1.371</td>
<td>0.964 ± 0.981</td>
<td>0.846 ± 0.600</td>
<td>0.392 ± 0.617</td>
<td>6.040 ± 0.367</td>
</tr>
<tr>
<td>Student t-test</td>
<td>5.231</td>
<td>3.600</td>
<td>2.723</td>
<td>0.890</td>
<td>3.997</td>
<td>6.565</td>
</tr>
<tr>
<td>p-value</td>
<td>0.000*</td>
<td>0.001**</td>
<td>0.009**</td>
<td>0.377</td>
<td>0.0001**</td>
<td>0.0001**</td>
</tr>
</tbody>
</table>

**Highly significant difference ($p < 0.001$).**

**Table 5** Comparison between the two groups in terms of salivary buffering capacity using the Chi-square test.

<table>
<thead>
<tr>
<th>Group</th>
<th>Salivary buffering capacity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High</td>
</tr>
<tr>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>Study group ($n = 28$)</td>
<td>14</td>
</tr>
<tr>
<td>Control group ($n = 28$)</td>
<td>6</td>
</tr>
<tr>
<td>$X^2$-Test</td>
<td>4.980</td>
</tr>
<tr>
<td>p-Value</td>
<td>0.05*</td>
</tr>
</tbody>
</table>

*Highly significant difference ($p < 0.001$).

**Table 6** Comparisons between the two groups in terms of the salivary parameters, total antioxidant and total protein levels.

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameter</th>
<th>Total antioxidant level mean ± SD</th>
<th>Total protein level mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study group ($n = 28$)</td>
<td></td>
<td>0.707 ± 0.414</td>
<td>0.873 ± 0.481</td>
</tr>
<tr>
<td>Control group ($n = 28$)</td>
<td></td>
<td>1.578 ± 0.621</td>
<td>1.265 ± 0.818</td>
</tr>
<tr>
<td>Student t-test</td>
<td></td>
<td>6.175</td>
<td>2.816</td>
</tr>
<tr>
<td>p-value</td>
<td></td>
<td>0.0001**</td>
<td>0.033*</td>
</tr>
</tbody>
</table>

*Significant difference ($p < 0.05$).

**Highly significant difference ($p < 0.001$).**
Saliva pH values of the renal children were significantly higher than the controls, which agree with another study [7]. This occurs because the salivary urea is split to form ammonia and carbon di-oxide that may raise the pH above the critical level for remineralisation of initial enamel caries [15,25]. The elevated urea levels in saliva are a complication of the disease, possibly negating the effect of any acid formation from cariogenic food intake [15]. Moreover, high salivary phosphate concentration found in patients with uraemia may facilitate remineralisation of incipient carious lesions [28].

The PMA index was used to determine the gingival condition of the study and control groups. The reason for its selection was to minimize the risk of infection, and to be faster and less invasive with exhausted children under dialysis. A significantly marked gingivitis was noted in dialysed children compared with the controls. This disagrees with the results of Jaffe et al. [25], who related the absence of gingival inflammation in their study to the paleness of the anaemic gingival tissues. The attribution of gingivitis in the present study compared to Jaffe et al. is mainly due to neglecting oral hygiene measures, the underlying uremic stomatitis, and the use of recombinant erythropoietin, which may reduce the paleness of the gingiva [29].

Although the OHI-S is a less sensitive index, it is rapid and can help in examining children on dialysis efficiently in a short time. No significant difference was reported between the two groups for debris accumulation, similar to the finding reported by Jaffe et al. [25]. This is probably due to the similar socio-economic levels and oral hygiene neglect by both groups or due to the effect of salivary uraemia and its inhibitory effect on plaque formation in the study group. However, heavier calculus was shown on the dialysis children’s teeth than the controls. In renal failure, a series of occurrences can cause an imbalance of serum calcium and phosphate ions that may sometimes accelerate the rate of calculus formation [28].

Saliva contains serum-derived components resulting from passive diffusion via gingival crevices [30]. Therefore, saliva has been proposed to be a good surrogate for blood for diagnostic purposes. Furthermore, the use of saliva samples instead of serum is advantageous because saliva collection is a non-invasive, simple, and inexpensive approach with minimal infectious risk [13]. Additionally, it was decided to take un-stimulated saliva samples, as it is preferred in the determination of antioxidant parameters to stimulated saliva [31].

The overall antioxidant capacity may provide more relevant biological information compared to that obtained by the measurement of individual components as it considers the cumulative effect of all antioxidants present in the body fluids [22]. It has been reported that an increase in the antioxidant activity of saliva has been related to an increase in the suspension of proteins and to an increase of cariogenic activity [16]. Also, a more recent study reported greater TAC among children having caries [11], which may explain the higher caries prevalence among control group with higher TAC and protein levels, compared with the study group.

Meanwhile no correlation was found between TAC and dental caries in permanent teeth in the study group; a correlation in primary dentition was detected in accordance with a previous study [11]. However, the real importance of the salivary antioxidant capacity with respect to the development of dental caries remains to be established, as other factors have been shown to influence development of dental caries to a greater or lesser degree.

Certain inflammatory processes such as periodontal diseases have been related to an increase in oxidative damage in the mouth and to a decrease in antioxidants [29]. Others reported that the antioxidant potential of saliva does not appear to be compromised in patients with periodontal diseases [32]. In this study, there was no linear relationship found between salivary TAC and gingivitis.

The protein of saliva comprises approximately 200 mg/100 ml. The concentration of a single antimicrobial protein will vary over the day in accordance with the activity of its glandular source [33]. For this reason total protein assessment was chosen for the present study. The resulting lower total protein value disagrees with the results of Shasha et al. [14]. However, the lower levels of proteins in this study may result from the young age group, representing a rapid growth phase compared with the sample of the former study. This is in addition to dietary restrictions to accommodate the reduced excretory capacity of the kidneys [34]. A correlation between total salivary protein and dental caries in deciduous teeth was shown in this study in accordance with Tulunoglu et al. [16]; however, the definite role of protein in caries incidence is not clear. On the other hand, the reduced total protein level, and thus the reduced antimicrobial activity, may play a role in gingivitis incidence. However, no correlation was found, and hence other individual and environmental factors could be important causes of that result.

Conclusion and recommendation

This sample of renal haemodialysis children revealed that enamel hypoplasia was recorded with high frequency. Although preventive measures and oral health maintenance are neglected, dental caries is less prevalent, while gingivitis and calculus formation were prevalent with possible complications contributing to morbidity and transplant rejection. A correlation was found between both salivary parameters, TAC and TP, and dental caries in primary dentition. However, further larger scale studies are needed to explore the role of these parameters on oral manifestations in children undergoing haemodialysis.

The highly prevalent enamel hypoplasia, bad oral hygiene habits, and the resultant gingival changes in the study group, suggest a need for dental advice and supervision. These children may become at slightly greater risk of developing dental caries later on in life after successful transplant. As a result the oral hygiene habits of these children should be improved and closely monitored through periodic dental check-ups. Therefore, dental and medical care should be closely integrated for children with renal failure.

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