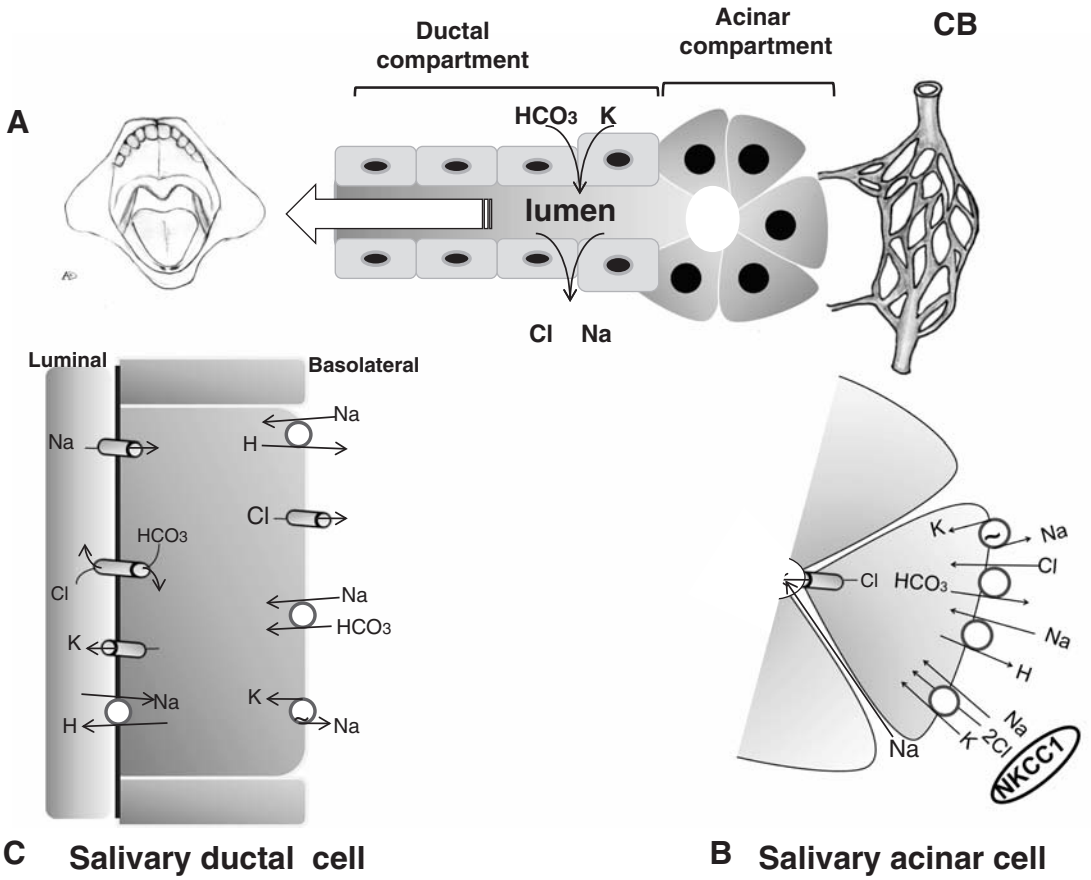

Fig. 2. Mechanism of saliva formation and K excretion pathway. Salivary glands are composed of highly differentiated epithelial cells. The salivary glands consist of two distinct functional and anatomic regions, acinar and ductal (**A**). Acinar cells are salt secreting and are considered to be the exclusive site of fluid movement in these glands. Ductal cells reabsorb Na and Cl transcellulary and secrete K and HCO_3 (**A**–**C**). For primary saliva formation generated from the enriched surrounding capillary bed (CB), the transcellular movement of Cl is a key step. Cl is taken up basolaterally via the NKCC1 (**B**) and thereafter leaves the acinar cell through Ca-activated Cl channels into the luminal pole (B). Upon Ca induced activation, of luminal and basolateral transport systems, large amount of isotonic NaCl rich fluid is collected into the acinar lumen compartment. The major task of salivary duct gland epithelia is to modify the primary isotonic saliva produced in the acinar compartment. Since the reabsorption of NaCl exceed the secretion of K and HCO_3, the final saliva becomes hypotonic (C). Relatively little is known regarding the K channels underlying luminal K secretion (for further reading, see [30]).

Saliva samples were thawed and then centrifuged (2000 *g*, 20 min, 25°C). The supernatants were analysed in a Hitachi 917 autoanalyser (Roche Ltd., Bohemia, NY), and the concentrations of K, Na, Cl and U were measured.

Decayed, missing and filled permanent teeth (DMFT)/decayed, missing and filled deciduous teeth score (dmft), [17] plaque index (PI) [18] and gingival index (GI) [19] were measured on the buccal and lingual surfaces of the maxillary first permanent molar or the right primary second molar, primary or permanent left central incisor, and the right first bicuspid or primary molar; and of the mandibular left first permanent molar or second primary molar, the right central primary or permanent incisor, and the right first bicuspid or first primary molar.

Statistical analysis

Frequencies and percentages were calculated for the categorical variables. Frequencies between categorical variables were analysed by chi-square test and Fisher–Irwin exact test. Medians, means and standard errors were calculated for continuous parameters. The pH parameters were compared between subgroups of patients by one-way analysis of variance (ANOVA) using the Bonferroni model. Due to the large variability in salivary components, the Kruskal–Wallis test was used to compare among saliva parameters. The results between pairs of saliva parameters were analysed using the Wilcoxon signal rank test. The correlation between saliva parameters was analysed by Spearman's correlation coefficient. The significance level of the correlation coefficient was calculated.

Results

Sialochemistry

The following groups of paediatric CKD patients were compared with a group of healthy children (C): pre-dialytic (PreD), on maintenance dialysis (D) and transplanted with functioning graft (T). The concentration of secreted salivary K in the D group (mean 28.86 mmol/L, median 28.42 mmol/L) was significantly higher than in the C group (mean 23.21 mmol/L, median 22.20 mmol/L, P = 0.03). The K levels in the PreD group (mean 31.6 mmol/L, median 31.10 mmol/L) were significantly higher than in the T (mean 25.65 mmol/L, median 25.0 mmol/L, P = 0.01) and C (P = 0.0004) groups (Figure 1).

No significant differences were found between the CKD groups and controls for Na levels in the saliva. However, Cl level in the D group was significantly higher (mean 22.24 mmol/L, median 18.0 mmol/L) than in the

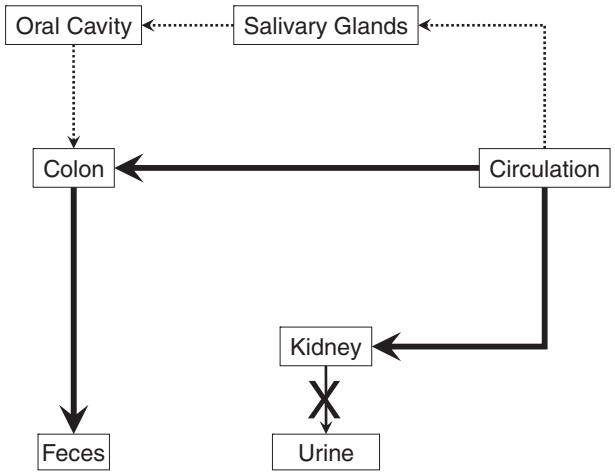

Fig. 3. Schematic chart of suggested salivary K extension of the colon excretion mechanism in hyperkalaemia. Solid arrows represent the known colon excretion mechanism. Dotted arrows represent a suggested pathway in which K, from the circulation infiltrates into the salivary gland system and via active transport into the oral cavity and swallowing, enters the gastrointestinal tract.

C group (mean 16 mmol/L, median 14.50 mmol/L, P = 0.01). The Cl level in the PreD group (mean 24.75 mmol/L, median 23.0 mmol/L) was significantly higher than in the C group (P = 0.0001). The Cl level in the T group (mean 20.86 mmol/L, median 21.0 mmol/L) was also significantly higher than in the C group (P = 0.0004) (Figure 1).

The urea (U) concentration in the D group (mean 29.95 mmol/L, median 28.02 mmol/L) was significantly higher than in the C group (mean 11.28 mmol/L, median 9.46 mmol/L, P = 0.002). The U concentration in the PreD group (mean 28.73 mmol/L, median 27.48 mmol/L) was significantly higher than in the T group (mean 18.81 mmol/L, median 14.63 mmol/L, P = 0.007) and C group (P = 0.0001). The U level in the T group was significantly higher than in the C group (P = 0.0001) (Figure 1).

A significant negative correlation was found between GFR and salivary K in PreD and T patients ($r = -0.32$, P = 0.02).

Oral health scoring

The DMFT/dmft, representing the caries status, in the C group was significantly higher than in the D group (P = 0.0001), the PreD group (P = 0.0001) and the T group (P = 0.0001) (Table 1).

The C group had a significantly lower PI score than the D group (P = 0.03, Table 1). The GI score in the C group was also significantly lower than in the D group (P = 0.037) and T group (P = 0.018), and lower with a tendency towards significance than the PreD group (P = 0.06).

Discussion

Hyperkalaemia is one of the major causes of morbidity and mortality in CKD patients. These patients possess an extra-renal K disposal mechanism which involves gastrointestinal excretion and cellular uptake of K that is not excreted via the kidneys [6]. In contrast to patients with normal renal function who only eliminate 5–10% of their daily K load through the gut, gut elimination of K in CKD patients potentially accounts for as much as 25% of daily elimination [2,3,6]. This gut K adaptation mechanism is mediated by increased colonic secretion, which is 2–3-fold higher in patients on dialysis than in patients with normal kidney function [2,3,6].

Approximately 750–1000 mL of saliva, representing ~20% of blood volume, is secreted into the oral cavity daily. The salivary system's basic unit comprised the acinar and ductal compartments, with K secretion occurring in the ductal compartment (Figure 2) [20,21].

The increased salivary K levels in all study groups compared with controls probably reflect an additional route for expelling K from the circulation via the swallowing of saliva into the gastrointestinal tract (Figure 3). We can only speculate on which of these mechanisms are involved in the adaptation process; thus, increasing plasma K concentration (total body K load) probably enhances the activity of transporters (Figure 2).

The significant negative correlation between salivary K and GFR (increase in secretion strongly related to severity of renal dysfunction) probably reflects activation of this mechanism in parallel to the decrease in renal function. Indeed, most of the oral fluids are reabsorbed through the gastrointestinal system, and the effect of compensatory secretion is therefore attenuated by the ingestion of saliva and its subsequent absorption.

Recently, the natural polymer chitosan incorporated to chewing gum was introduced by Savica *et al*. as a novel therapeutic device for salivary phosphate-binding vehicle [22]. The authors had shown a significant reduction in salivary and serum phosphate levels (55% and 31%, respectively) upon using this chitosan-loaded chewing gum twice daily for 2 weeks in haemodialysis patients.

Consequently, the question if such a novel approach is applicable to remove excess K in CKD patients by capturing it from the saliva is intriguing. However, two major drawbacks exist: (i) the amount of K excretion via the salivary glands system is not substantial enough to spare the gastrointestinal system's extra-renal K disposal mechanism, and (ii) chewing gum indeed enhances saliva secretion rate; however, by swallowing the saliva, the K will be reabsorbed in the gastrointestinal tract.

Interestingly, in the present study, we found secreted K levels in the saliva of the PreD group to be the highest, followed by the D group (30%, P = 0.0004 and 25%, P = 0.03, respectively, compared with the C group). This observation can be explained by the removal process of K during dialysis: the haemodialysis procedure leads to rapid and significant K removal from the plasma with a steep decline in plasma K level right after dialysis starts. Saliva was collected from this group during the dialysis session. Consequently, it would be interesting to examine saliva versus serum K levels before, during and after the dialysis process. It should be noted that although the rate of K removal with peritoneal dialysis is much slower than that with haemodialysis, it is still effective, resulting in a high-normal plasma K level in most patients.

We also examined the salivary biochemistry with regard to U, Cl and Na. Lower levels of Na, corresponding to high

levels of K, in the saliva relative to the plasma are a reflection of the active transport mechanism that has been reported in the literature [20,21]. Changes in Cl levels were similar to changes in K levels, probably reflecting an increase in corresponding anion secretion, due to the active secretion of cation (K). Interestingly, Na levels did not differ among groups.

We found elevated levels of U in the saliva of CKD patients, as has been previously described [23]. Our data indicated a low incidence of caries in CKD patients, corresponding to the well-established knowledge reported by us and others [23–25]. This can be explained by the fact that high amounts of U in the saliva increase its buffering capacity as a result of the high concentration of ammonia arising from urea hydrolyzation: buffering maintains higher pH levels and consequently a lower risk of caries development.

The PI score measures the accumulation of dental plaque and hence reflects oral hygiene habits. The significantly higher PI score in the D group compared with the C group is not surprising since these patients require frequent hospitalization: this decreases their quality of life, resulting in poor oral hygiene compliance [26,27].

Regarding the GI score in CKD patients, the literature is controversial: some studies [28,29] have shown less gingival inflammation, which might be a reflection of the chronic immunosuppressive state of these patients which masks the inflammatory process. However, more recent studies [23,24,27] have found gingival inflammation in CKD patients with no relation to the extent of the immunosuppressive condition. Our results favour the latter observation, and indicate that inflammation in the gingival tissue may be expressed in a different way, despite the impaired systemic condition. The GI score in the C group was significantly lower than those in the D and T groups, and showed a tendency towards being significantly lower compared with the PreD group. This provides further proof that the GI score, observed in healthy subjects, correlates with plaque and not necessarily with the impaired systemic condition.

Conclusions

Increased excretion of salivary K was found in paediatric patients with CKD, correlating with the severity of renal failure. This finding implies a never before reported extension of the gastrointestinal adaptive mechanism in paediatric CKD patients suffering from K load. Exploration of the exact mechanism of increased salivary K secretion might provide a further therapeutic approach for K removal in the hyperkalaemic state, mainly in CKD patients. Further work should address the K levels in saliva with comparison to blood levels in these patients.

Conflict of intertest statement. None declared.

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