

Mammary epithelial paracellular permeability in atopic and non-atopic mothers versus childhood atopy

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Sodium/potassium (Na/K) ratios are considered to be a marker of mammary epithelial paracellular permeability. The aim of the present study was to investigate the association between maternal atopy and Na/K ratios in breast milk and the association between Na/K ratios in breast milk and the development of atopy in the offspring. Early and mature milk samples were obtained from 30 atopic and 43 non-atopic women. We found no differences in the Na/K ratios between atopic and non-atopic women. At 18 months of age, 22 (30%) of the children had a positive skin prick test (SPT) and 26 (36%) had symptoms of atopic diseases. Overall, high levels of Na/K compared with low and slightly raised levels of Na/K in the maternal milk tended to be associated with a positive SPT and atopic disease. However, if the mother was atopic, high levels of Na/K in early or mature milk were associated with a significantly increased risk of a positive SPT or atopic disease in the offspring [RR = 4.8 (1.9–12)] whereas no such association was observed in non-atopic mothers [RR = 0.8 (0.4–1.7), *p* for interaction = 0.001]. Thus, high Na/K levels in the breast milk may be associated with the development of atopy and atopic diseases in the offspring of atopic mothers.

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Sodium/potassium (Na/K) ratios are considered to be a marker of mammary epithelial paracellular permeability (1). In the mammary gland, the tight junctions of the alveolar epithelial cells are leaky during gestation and then undergo closure starting after parturition to become impermeable. As a consequence, the sodium level in the milk is high during the first 1–2 days postpartum and then decreases rapidly (1). The sodium content in the milk is normally low during exclusive lactation and until weaning, but milk stasis and mastitis are accompanied by local inflammation and increased epithelial paracellular permeability resulting in increased sodium levels in the milk (1). The potassium content is in comparison relatively stable.

Atopy has been suggested to be associated with increased epithelial leakiness (2, 3). We hypothesized that atopic mothers have increased mammary epithelial paracellular permeability

and thereby increased allergen load in the milk and increased risk of sensitization of their breastfed children. Using breast milk samples from a previously conducted study, we studied the association between maternal atopy and Na/K ratios in breast milk and the association between Na/K ratios in breast milk and the development of atopy in the offspring. *Post hoc* we also decided to study the effect of high Na/K ratios on the development of atopy in the offspring according to maternal atopy. In addition we studied the associations between Na/K ratios and the levels of interleukin-8 (IL-8) and total secretory immunoglobulin A (s-IgA).

Methods

Early milk, obtained within 4 days after delivery, and mature milk samples, obtained at 1 month, were available from 73 women. Thirty (41%) of

the women were defined as atopic and 43 (59%) as non-atopic, based on a history of typical atopic symptoms and circulating allergen-specific IgE antibodies. Among the atopic mothers, 14 had allergic rhinoconjunctivitis against inhalant allergens, three presented symptoms of asthma and three had symptoms of atopic eczema or food allergy during childhood. The remaining atopic mothers presented a combination of several atopic diseases. All the women were exclusively breastfeeding their babies for at least 1 month. The milk samples were taken at the mid of the second meal of the day. Breast milk samples were centrifuged at 680 *g* for 15 min at 5°C. The whey was removed and centrifuged at 10 000 *g* for 20 min at 5°C. The translucent whey was stored at -20°C until analysed. Sodium and potassium levels in breast milk were measured at the Clinical Chemistry Department at Linköping University Hospital according to in-house routines (ion selective electrodes). Data on both sodium and potassium were available from 66 samples of early milk and 72 samples of mature milk. As performed by others (4), we categorized Na/K ratios into normal (≤ 0.6), slightly raised (>0.6 to ≤ 1.0), and high (> 1.0).

The levels of total s-IgA antibodies were determined as previously described (5). Data were available from 66 and 69 samples of early and mature milk, respectively. The levels of IL-8 in breast milk were analysed by means of a commercial enzyme-linked immunosorbent assay kit (R & D, Abingdon, UK) according to the manufacturer's instructions. Data were available from 46 and 55 samples of early and mature milk, respectively.

The children were followed until 18 months of age. Skin prick tests (SPT) for egg, milk, cat and peanut were considered positive if at least one of the mean diameters was equal to 3 mm. Atopic symptoms were considered if a child suffered from one or more of the following: 'atopic eczema' defined as pruritic chronic or chronically relapsing dermatitis with typical morphology and distribution. 'Asthma bronchiale' defined as bronchial obstruction three times or more, and at least once verified by a paediatrician. 'Food allergy' defined as a positive SPT, combined with a positive clinical history of immediate symptoms upon exposure to a certain food and clinical remission of atopic symptoms on an exclusion diet. The Local Ethical Committee at Linköping University Hospital approved the study.

The association between the three groups of Na/K ratio and maternal atopy and the association between Na/K ratio and the development of positive SPT or atopic disease were

investigated by comparing binary outcomes. Relative risks (RR) were obtained by using a log-link in a generalized linear model. The 95% confidence intervals were based on Wald's tests. The correlations between maternal atopy and IL-8 and s-IgA were tested in chi-square tests and Mann-Whitney tests. The correlations between Na/K and IL-8 and total s-IgA antibodies were assessed by means of the Spearman correlation coefficients. All analyses were performed in SAS 8.2.

Results

Elevated Na/K ratios above 0.6 were more common in early milk than in mature milk (Fig. 1). Atopic and non-atopic women did not differ with regard to Na/K ratios in either early or mature milk (Table 1). In early milk, the median (25th to 75th percentiles) s-IgA levels were 940 (460–1968) in atopic women vs. 1360 (572–2071) in non-atopic women ($p = 0.57$). In mature milk the levels were 348 (245–788) and 322 (219–534), respectively ($p = 0.48$). In early milk, IL-8 was detected from 16 (70%) of 23 atopic women vs. 17 (74%) of 23 non-atopic women ($p = 0.74$), and in mature milk from 10

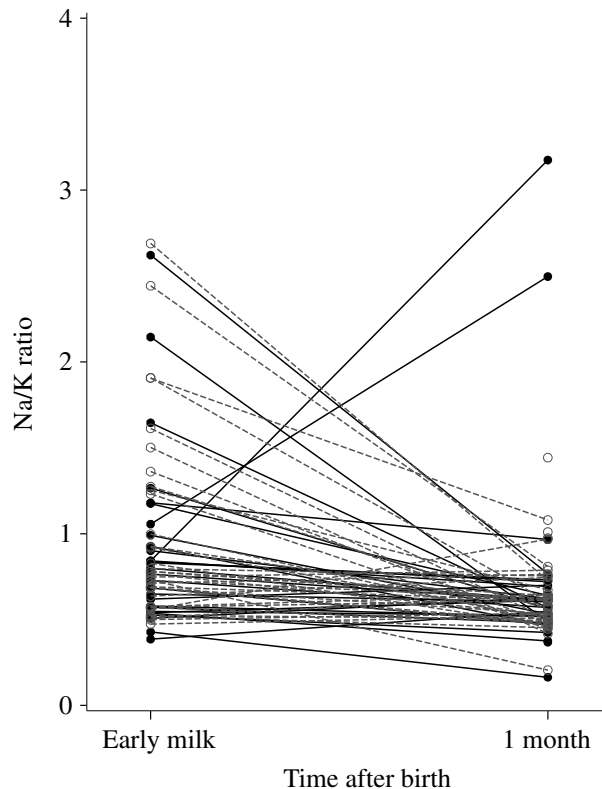


Fig. 1. Na/K ratios in early milk and at 1 month in atopic (—) and non-atopic (---) women.

Table 1. Associations between Na/K ratios, maternal atopy, and atopy and atopic disease in the offspring

Na/K ratios	Mothers	Number	Child skin prick test		Child atopic disease	
			Positive (%)	Negative (%)	Yes (%)	No (%)
Early milk						
>1.0	Atopic	7	4 (57)	3 (43)	5 (71)	2 (29)
	Non-atopic	10	3 (30)	7 (70)	3 (30)	7 (70)
>0.6, ≤ 1.0	Atopic	13	1 (8)	12 (72)	2 (15)	11 (85)
	Non-atopic	17	3 (18)	14 (82)	6 (35)	11 (65)
≤ 0.6	Atopic	9	1 (11)	8 (89)	2 (22)	7 (78)
	Non-atopic	10	5 (50)	5 (50)	5 (50)	5 (50)
Mature milk						
>1.0	Atopic	2	1 (50)	1 (50)	2 (100)	0 (0)
	Non-atopic	3	1 (33)	2 (67)	1 (33)	2 (67)
>0.6, ≤ 1.0	Atopic	13	1 (8)	12 (92)	1 (8)	12 (92)
	Non-atopic	18	7 (39)	11 (61)	6 (33)	12 (67)
≤ 0.6	Atopic	15	5 (33)	10 (67)	7 (47)	8 (53)
	Non-atopic	21	6 (29)	15 (71)	8 (38)	13 (62)

(42%) of 24 atopic women vs. seven (23%) of 31 non-atopic women ($p = 0.12$). The Na/K ratios correlated with IL-8 levels in early milk [Spearman correlation coefficient 0.36 ($p = 0.02$)] and in mature milk [0.35 ($p < 0.01$)], as well as with total s-IgA in early milk [0.42 ($p < 0.01$)], and tended to do so in mature milk [0.20 ($p = 0.10$)]. These correlations did not differ between atopic and non-atopic mothers (data not shown).

At 18 months of age, 22 (30%) of the children had at least one positive SPT and 26 (36%) had symptoms of atopic disease. Among the latter, 16 (62%) had a positive SPT. Overall, high levels of Na/K (> 1) as compared with lower levels (≤ 1) tended to be associated with a positive SPT at 18 months of age for early milk [RR = 2.0 (0.9–4.5)] and mature milk [RR = 1.4 (0.5–4.4)]. The findings were similar for clinical symptoms of atopic disease at 18 months of age, RR = 1.5 (0.8–3.0) in early milk and RR = 1.8 (0.8–4.0) in mature milk.

The effects of high levels of Na/K differed between atopic and non-atopic mothers. Having high levels in early and/or mature milk was associated with an increased risk of a positive SPT in offspring of atopic mothers [RR = 3.7 (1.0–13)], but not non-atopic mothers [RR = 0.9 (0.4–2.4)]. The same was observed with regard to atopic disease in the offspring of atopic mothers [RR = 4.1 (1.6–11)] and non-atopic mothers [RR = 0.9 (0.3–2.1)]. Summing up the information, having high levels of Na/K in early and/or mature milk was associated with an increased risk of either a positive SPT or atopic disease in the offspring of atopic mothers

[RR = 4.8 (1.9–12)] but not in non-atopic mothers [RR = 0.8 (0.4–1.7)], p for interaction = 0.001). Two atopic mothers had particularly high Na/K ratios in mature milk (Fig. 1). One of these women also had Na/K ratio > 1 in early milk; the child had a positive SPT and symptoms of atopic disease. The other child had symptoms of atopic disease.

Discussion

Na/K ratios, which are markers of mammary epithelial paracellular permeability, were similar in atopic and non-atopic mothers in early milk and mature milk. Secretory IgA levels were similar in the two groups as also reported previously (5). There was a tendency for increased IL-8 in mature milk from atopic women, but the tendency was not present in early milk and the difference in mature milk did not reach statistical significance in contrast to a previous study (6). However, the levels of IL-8 in the present study were generally lower, maybe because of different methods used. In conclusion, this study does not support the hypothesis of increased mammary permeability or increased mammary gland inflammation in atopic women, but it should be noted that our sample size is small.

During the first week of lactation, higher levels of IL-8 (4) and s-IgA (5) were found in the milk. During milk stasis and mastitis, inflammatory responses induce synthesis of chemokines such as IL-8 and RANTES, which again induce the migration of neutrophils (7) and macrophages/monocytes (8). Increased levels of s-IgA have also been observed (9). The inflammatory process leads to increasing permeability and leakage of various components into the milk, sodium being among them. Accordingly, we found that IL-8 and s-IgA levels were positively correlated with Na/K ratios in both early milk and mature breast milk, as also previously reported for mature milk (4).

As expected *a priori*, high levels of Na/K in the milk tended to be associated with both a positive SPT and atopic disease at 18 months of age in the children. We found a significant interaction between high levels of Na/K and maternal atopy; the increased risk being confined to children of atopic mothers, in which high levels of Na/K in early and/or mature milk was associated with a nearly five times increased risk of atopy in the offspring. Our study population was small and the result should be interpreted with great caution. However, it seems biologically plausible that if high levels of Na/K are associated with

increased allergen load in the milk, this would be most detrimental in children with a genetic predisposition to atopic diseases. In support of an association, although there was no way of proving it, we found that the two atopic mothers who had particularly high Na/K ratios in mature milk both had children with positive SPT and/or atopic disease. In conclusion, high Na/K levels in the breast milk may be associated with the development of atopy and atopic diseases in the offspring of atopic mothers. If increased permeability is associated with increased risk of atopy, then improved counselling during breastfeeding in order to decrease the prevalence of milk stasis and mastitis could contribute to prevention of atopic disease (10).

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