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Urinary Retinol Excretion in Children with Acute Watery Diarrhoea

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ABSTRACT

Children with diarrhoea due to rotavirus infection excrete retinol in urine. This study aimed at demonstrating the extent and mechanism of urinary retinol excretion in children with acute watery diarrhoea caused by pathogens other than rotavirus. Thirty-two children, aged five months to five years, hospitalized with watery diarrhoea predominantly due to enterotoxigenic *Escherichia coli* in Bangladesh, were studied. Their serum retinol and retinol-binding protein (RBP) were low at admission and increased significantly after recovery from illness. The mean hospital stay of these patients was four days. Forty-seven percent of the children excreted retinol in urine on day 1, and about 38% continued excreting retinol on day 3. The estimated urinary retinol loss of 3.44 μmol for the illness episode represented more than 40% of liver retinol reserve (8.25 μmol) in malnourished children. A conservative estimate of the loss would represent at least 20% of the liver reserve in relatively better-nourished children. Kidney tubular dysfunctions of increased RBP excretion significantly predicted urinary retinol excretion in children with watery diarrhoea.

Key words: Diarrhoea, Acute; Diarrhoea, Infantile; Rotavirus; Rotavirus infections; Vitamin A; Vitamin A deficiency; Retinol excretion; Retinol-binding protein; *Escherichia coli*; Bangladesh

INTRODUCTION

Vitamin A deficiency is a major public-health problem in children with infection in developing countries (1). Even in developed countries where vitamin A status of population is supposed to be adequate, acute illnesses, such as measles, respiratory syncytial infection, and other respiratory infections, can decrease serum concentrations of vitamin A (retinol) significantly (2,3). Mechanisms of vitamin A deficiency during infection are not conclusive. Previous studies have focused on mechanisms, including inadequate intake (4), decreased absorption (5,6), and increased demand of vitamin A during infection (7). More recently, urinary excretion of retinol has been shown to be one of the major routes of vitamin A loss during infection (8-11).

Results of an earlier study by Lawrie *et al*. showed evidence of frequent urinary excretion of vitamin A in subjects with respiratory diseases (12). Later, results of studies by Stephensen *et al*. in adults with septicaemia and pneumonia showed that urinary retinol excretion is positively associated with fever and with severity of infection (8). Alvarez *et al*. showed significant amounts of urinary retinol excretion in Peruvian children with watery diarrhoea, particularly due to rotavirus infection (9). We observed previously that urinary retinol excretion could be a significant mechanism of vitamin A loss in

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children with shigellosis (11). We also showed that impaired kidney tubular re-absorption of low-molecular weight proteins, such as retinol-binding protein (RBP) might cause urinary retinol loss in case of shigellosis. However, more data are needed to demonstrate if urinary retinol loss is a significant mechanism of vitamin A deficiency during some other common childhood infections and to understand the pathophysiology of such losses. The aims of this study were: (i) to determine the extent of urinary retinol loss in children with acute watery diarrhoea due to pathogens other than rotavirus infection (some results from these subjects have been previously reported) (10) and (ii) to identify the predictors of retinol excretion in children with acute watery diarrhoea.

MATERIALS AND METHODS

The study was conducted at ICDDR,B: Centre for Health and Population Research at Dhaka, Bangladesh, during May-December 1995. Male children, aged five months to five years, hospitalized with history of acute watery diarrhoea, were eligible. The study excluded children with any other complications, including known liver or kidney diseases and congenital illnesses. An informed written consent was obtained from parents before enrolling children in the study. The Ethical Review Committee of ICDDR,B and the University of Alabama at Birmingham approved the study.

After initial enrollment, children were observed in a study ward for about 4-6 hours. Depending on the status of dehydration, the children were rehydrated using either an oral rehydration solution (ORS) or an intravenous polyelectrolyte solution. Stool samples were examined for rotavirus antigen using a rapid latex agglutination test (Slidex Rota kit, Biomerieux, France) (13). Children were enrolled in this study if their stools were negative for rotavirus by this agglutination test.

Stool samples on admission were cultured for *Vibrio cholerae*, *Shigella*, and *Salmonella* using standard methods (14). Stool samples were also confirmed for rotavirus by enzyme-linked immunosorbent assay (ELISA), using the Dakopatts kit (Glostrup, Denmark). Stool microscopy was performed for parasites, ova, and blood cells. After initial rehydration, blood samples were obtained by venipuncture and kept in containers wrapped with aluminum foil to prevent degradation of vitamin A by light. The blood samples were tested for retinol, RBP, transthyretin (TTR) (previously known as pre-albumin), electrolytes, creatinine, glucose, albumin, and phosphate. Urine samples were collected for 24 hours on day 1 and day 3 using single-use, sterile, paediatric urine-collectors (PUC, Kobayashi Shoji K.K., Tokyo, Japan). Older children were asked to void in plastic containers. Mothers were trained to use PUC bags. Trained health workers also monitored urine collection. To avoid spillage or any faecal contaminations, PUC bags were changed as soon as the child voided, and the bags were half-full. We did not encounter any leakage of bags. The urine samples were measured in a dimly-lit room and kept in a nearby freezer at –20 ˚C until these were assayed. All samples were analyzed within two weeks after collection at the ICDDR,B lab. The urine samples were tested for retinol, RBP, electrolytes, creatinine, glucose, albumin, and phosphate. Laboratory methods for these tests were reported elsewhere (11).

For the measurement of retinol in urine, 1.0 mL of urine was mixed with 1.0 mL of methanol containing retinyl acetate (950 µL methanol plus 50 µL retinyl acetate), vortexed for 10 seconds, and extracted with 2 mL of hexane. The extract was evaporated under nitrogen in a dry block (40–45 ºC). Retinol concentration was measured by high-performance liquid chromatography (HPLC) using a 95:5 solution of methanol and water as mobile phase, Waters Bondapak C_{18} column, and Waters (Model 481) detector at 325 nm. The precision of the measurements was 5%.

Fractional excretion of a solute, e.g. glucose, was calculated using the following formula (15):

$$\frac{\text{(Urinary solute) x (Plasma creatinine)}}{\text{(Plasma solute) x (Urinary creatinine)}}$$

Body weights of children were measured to the nearest 0.01 kg using an electrical scale (Model 727; Seca Corporation, Columbia, MD) at admission and every morning. Body height or recumbent length was measured to the nearest 1 cm using standard height or length boards at the time of discharge. Z-scores for weight-for-age, weight-for-height, and height-for-age were calculated using the National Center for Health Statistics (NCHS) standards (16,17). The children were supplemented with a large dose of vitamin A at discharge. Children aged less than one year received 100,000 IU and those aged one year or more received 200,000 IU of vitamin A.

Statistical analyses

Data were entered and analyzed using SPSS for Windows (version 10.0; SPSS Inc., Chicago, IL). Descriptive

statistics, including mean, median, standard deviation, and 25th and 75th percentiles were calculated for important outcome variables. Serum concentrations of retinol, RBP, and TTR at admission and discharge were compared using paired sample *t*-test. Having skewed distribution of 24-hour urine, urinary retinol, and fractional excretion of retinol, data of day 1 and day 3 were compared using non-parametric tests. The amount of urinary retinol excretion was categorized based on the FAO/WHO-recommended basal intake of vitamin A: 0.7-0.87 μmol (200-250 μg) for children aged 1-5 year(s) (18). In this case, 0.7 μmol (200 μg) of retinol was considered standard daily requirement. Predictors of urinary retinol excretion were determined by stepwise linear regression analysis. Skewed data were log-transformed prior to analysis. Probability levels of ≤0.05 were considered to be statistically significant.

Table 1. Pathogens isolated from stool culture of children with acute watery diarrhoea, May-December 1995, ICDDR,B Hospital, Dhaka, Bangladesh

Pathogen	No.	%
Enterotoxigenic *Escherichia coli*	17	53
Vibrio cholerae	4	13
Aeromonas	3	9
Rotavirus	1	3
Not identified	7	22
Total	32	100

Table 2. Serum retinol, retinol-binding protein (RBP), and transthyretin (TTR) at admission and discharge in 32 children with acute watery diarrhoea

Variable (μmol/L)	Admission	Discharge	p value
Serum retinol	0.58±0.2	0.95±0.3	<0.001
Serum RBP	0.90±0.2	1.4±0.3	0.01
Serum TTR	1.6±0.6	2.2±0.3	0.25

Data are mean±SD

RESULTS

In total, 32 children were enrolled, having a median age of 12 months. They were severely malnourished, as indicated by the median weight-for-age z-score of -2.18. On an average, they had a history of diarrhoea for four days before admission and stayed in the hospital for another four days until recovery. The majority (53%) of them had enterotoxigenic *Escherichia coli* (ETEC) infection, followed by *V. cholerae* (Table 1). Only one had rotavirus detected by ELISA test, although none had rotavirus by rapid agglutination test.

Serum retinol concentrations were low at admission (0.58±0.2 μmol/L) and increased significantly at discharge (0.95±0.3 μmol/L), although no supplemental vitamin A was administered during the illness (Table 2). Serum RBP concentrations also increased significantly at discharge compared to those at admission.

The amount of urine decreased significantly at admission compared to that at discharge, indicating presence of dehydration at admission. However, the blood samples were collected after rehydration. Forty-seven percent of the children excreted any amount of retinol in urine on day 1. Interestingly, 12 (38%) children continued to lose retinol in urine on day 3 (Table 3 and Fig.). The urinary loss of retinol ranged from 0.004 μmol to 0.43 μmol in 24 hours. Taking 0.7 μmol (200 μg) as the minimum daily metabolic requirement for children, three (9%) children had a urinary retinol loss greater than 20% of daily requirements on day 1, and two (6%) had a loss greater than 50% of requirements on day 3 (Fig.).

In stepwise regression analyses, urinary retinol was used as a dependent variable. Fever, z-scores for weight-for-age, weight-for-height, and height-for-age, and fractional excretion of RBP, electrolytes, glucose, albumin, and inorganic phosphate were used as predictor variables. Fractional excretion of RBP was the significant predictor of urinary retinol excretion (R^2=0.89; p<0.001) (Table 4).

Table 3. Urine output, urinary retinol, and fractional excretion of retinol on day 1 and day 3 (24 hours in 32 children with acute watery diarrhoea)

Variable	Day 1	Day 3	p value
Urine output (mL/24 hours)	360 (105 – 850)	565 (95–1,325)	0.006*
Urinary retinol (μmol/24 hours)	0.003 (0 – 0.18)	0.001 (0–0.43)	0.48*
Fractional excretion of RBP (μmol/24 hours)	0.001 (0 – 0.09)	0.001 (0–0.42)	0.40*
No (%) having urinary retinol	15 (47)	12 (38)	0.50†

Data are median (range) or number (%)
* Wilcoxon test
† Sign test

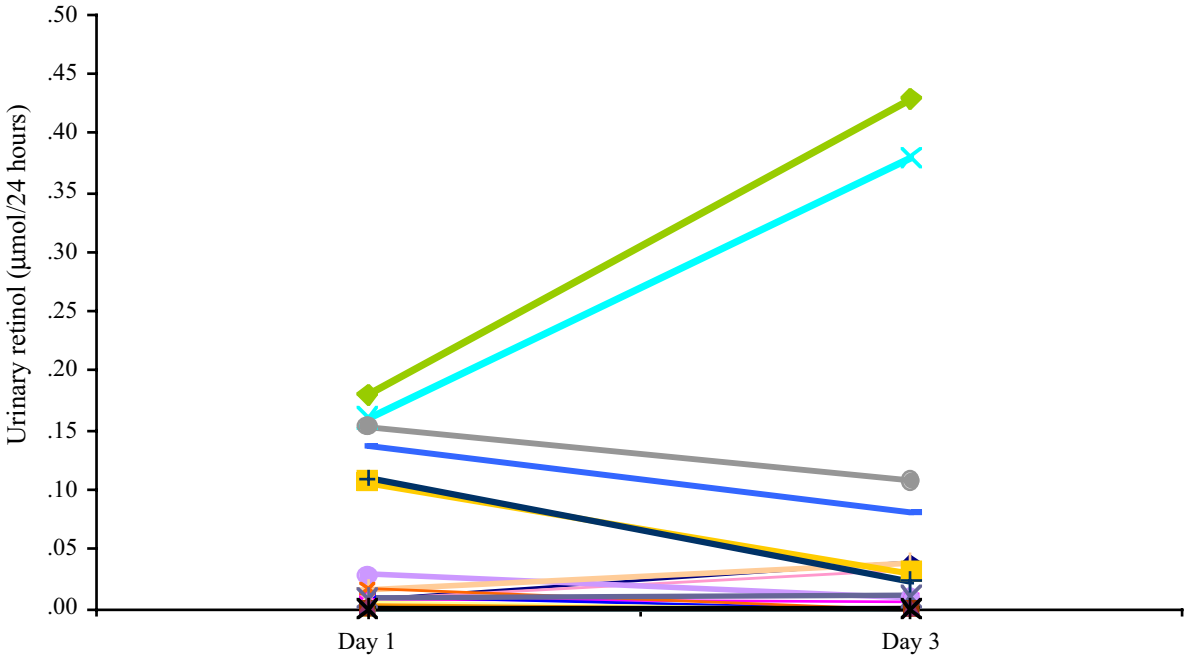

Fig. Urinary retinol loss on day 1 and day 3 in 32 children with acute watery diarrhoea

Table 4. Predictor of urinary retinol excretion in children with acute watery diarrhoea

Variable	ß-coefficient	SE (ß)	p value
Fractional excretion of RBP (µmol/24 hours)	33.322	4.074	< 0.001
Constant	0.001	0.001	0.43
R^2	0.893		

SE=Standard error

Variable excluded (p>0.05): fever; z-scores for weight-for-age, weight-for-height, and height-for-age; and fractional excretion of sodium, potassium, chloride, glucose, albumin, and inorganic phosphate

DISCUSSION

This study demonstrated that urinary excretion of retinol is a significant route of vitamin A loss during acute watery diarrhoea, predominantly due to ETEC. The maximum urinary loss of retinol observed in the study subjects was 0.43 µmol in 24 hours, which represents 61% of the minimum daily requirement of vitamin A (0.7 µmol) for a child. The estimated maximum urinary loss of retinol in a child for the entire illness, lasting for about eight days, is 3.44 µmol, assuming that the amount of retinol loss remains constant throughout the period of illness. Can such a loss of retinol decrease the liver reserve?

Olson reported that 29% of Brazilian children aged less than four years had liver reserves no more than 0.017 µmol/g, a level associated with a risk of xerophthalmia (19). A low liver reserve of retinol was also confirmed by indirect biochemical tests, including relative dose response (RDR) and modified relative dose response (MRDR) in Bangladesh and in other developing countries (20-22). In our previous report in children who died at this hospital due to complications of diarrhoea and malnutrition, 79% had liver retinol reserves less than 0.07 µmol/g, a level considered to be inadequate (23). The median concentration of retinol in liver was 0.033 µmol/g (range: 0.001-0.35 µmol/g), and the mean liver weight was 246±96 g. The total liver reserve of retinol was estimated to be only 8.25 µmol. The maximum urinary retinol loss observed in a child in the present study (3.44 µmol) represents 42% of the estimated liver reserve if the child is malnourished.

Let us now consider another scenario where the child is relatively better-nourished. This is a Bangladeshi child aged about two years, weighing 12.5 kg. For such a child, liver weight would be approximately 500 g (24), and the total liver reserve would be 16.5 µmol. In the later

case, the maximum retinol loss observed would represent 21% of the reserve. Thus, urinary retinol loss could significantly decrease liver vitamin A reserve in selected population.

The mechanism of urinary loss of retinol observed in the study children with watery diarrhoea was consistent with our previous findings in children with shigellosis (11). Both the studies have shown that excretion of low-molecular-weight proteins, such as RBP, predicts the urinary loss of retinol. Since retinol is not soluble in water, it is likely that most urinary retinol was excreted as the RBP-retinol complex. Several studies have indicated earlier that urinary excretion of low-molecular-weight proteins, including RBP, can be used as an index of proximal kidney tubular dysfunction in subjects with several conditions, including febrile illnesses, renal disease, diabetes mellitus, hypertension, and cadmium poisoning (25-27). However, re-absorption of other low-molecular-weight proteins may also be impaired during an acute illness (11). It would have been useful to monitor excretion of another small protein, such as, ß2-microglobulin, in addition to RBP.

We further observed a transient decrease of serum retinol in subjects at admission in this study. Again, this finding confirmed previous evidence of decreased serum retinol during illness (28). It is likely that serum concentrations of inflammatory cytokines, including interleukin-1 (IL-1), IL-6, and tumor necrosis factor (TNF-α), increase during acute infection (29). These cytokines down-regulate the synthesis of RBP and TTR in liver, and thereby decrease serum concentrations of retinol (29). It is likely that, upon recovery from illness, these cytokines are not sufficient to depress carrier proteins of retinol (RBP and TTR), and serum retinol concentrations are, thus, increased. Further studies are needed to determine the effects of cytokines in regulating vitamin A status during and after illnesses.

This study demonstrated that retinol loss in urine could be a substantial route of vitamin A deficiency during acute watery diarrhoea and that such urinary loss of retinol is due to kidney tubular dysfunctions of increased RBP excretion.

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