EVALUATION OF PROTEIN CREATININE RATIO AS A MARKER FOR PROTEINURIA AMONG CHILDREN RECOVERED FROM HYPERNATREMIA: A LONGITUDINAL OBSERVATIONAL STUDY

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ABSTRACT

Background: Proteinuria is one of the many markers of kidney damage. An increased protein creatinine ratio (Pr/Cr ratio) is known to have both diagnostic and prognostic value in detecting renal damage. The previous observational study revealed that children with hypernatremia are at risk of developing acute kidney injury (AKI), which positively correlates with recovery from hypernatremia. Detection of high Pr/Cr ratio in the spot urine samples is a predictor for proteinuria. However, there is lack of evidence for whether or not renal damage persists in children who recovered from hypernatremia. Therefore, we aimed to evaluate the long-term renal function of the children who had recovered from hypernatremia. **Methodology:** The study was an observational longitudinal study conducted in Dhaka Hospital of icddr,b from March 2016 to March 2017. The objective of this study was to evaluate the renal function of children 12 months after recovering from hypernatremia. All these children in this cohort study were brought to Dhaka Hospital of icddr,b for a spot urine analysis to evaluate their kidney function. **Results:** Among the 224 children who recovered from hypernatremia 143 (64%) children's mothers gave consent to participate in follow-up study. Half of the study children demonstrated to have a raised urinary Pr/Cr ratio. However, majority of them did not have any evidence associated with urinary problems. Proteinuria was comparable in different types of hypernatremic children. After adjusting for potential co-variates like age, sex, any form of malnutrition, severity of hypernatremia and co-morbidity, 86% of children from better socioeconomic family are less likely to have high urinary Pr/Cr ratio in comparison with those from poorest households. Regression analysis also revealed that children who were less than 36 months of age are 2 times more likely to have raised urinary Pr/Cr ratio than those more than 36 months old (Odds Ratio 2.31(1.06, 5.02); p 0.035). **Conclusion:** Children from low socioeconomic households and below 36 months of age are more likely to have proteinuria after recovering from hypernatremia. However further study is required to correlate the high Pr/Cr ratio with other parameters to rule out the long-term effect of hypernatremia on renal function.

KEYWORDS: Protein Creatinine Ratio, Proteinuria, Hypernatremia, Under Five Children.

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Introduction

Protein in urine in excess amount is defined as proteinuria. Healthy kidney doesn't let albumin pass from the blood into the urine. A damaged kidney lets some albumin pass into the urine. Literature shows that proteinuria is documented as an independent risk factor for renal disease. Measuring protein in the urine is, therefore, one way to check the health of the kidneys. However, children may have only temporary proteinuria without any evidence of kidney disease. increased protein excretion is considered as a valuable indicator for the initial detection and confirmation of renal disease as observed by Ruggenti and colleagues (Ruggenenti et al. 1998). The study revealed that Pr/Cr ratio in a random urine specimen provides evidence of significant proteinuria as detected in a 24-hour urine sample. Research findings show that spot urine Pr/Cr ratio has a wide range of sensitivity and specificity ranging between 69% and 96%, and 41% and 97%, respectively (Price, et al. 2005). Although 24-hour protein-creatinine measurement is used as a reference, there is an issue of poor compliance. Moreover, this test is expensive (Ruggenenti et al. 1998). US Food and Drug Administration (USFDA) pointed proteinuria as a surrogate indicator of chronic kidney disease. Spot (random) urine Pr/Cr ratio is also noted as an alternative fast and simple method for detecting proteinuria. A history, a physical examination, and laboratory tests help to determine the causes of temporary kidney disorders. In some disease conditions, proteinuria may occur but resolves when there are no stimulating factors such as fever, exercise stress, and cold exposure. Orthostatic proteinuria is the most common type of proteinuria in children. The urine dipstick test is the most widely used screening method for proteinuria. For quantitative determination of 24-hour urine, a protein excretion test is usually recommended. However, it is difficult to collect 24-

hour urine from children. It is suggested that when urinary Pr/Cr ratio is more than 0.2 or urinalysis results are abnormal, additional investigations are required to rule out significant renal disease in children (Leung et al. 2017). In predicting the progression of renal disease in the long term Pr/Cr ratio is a reliable indicator (Ruggenenti et al. 1998).

The increasing incidence of chronic kidney disease (CKD) among both children and adults is a global health problem. Urinalysis is an important screening test to find out CKD subjects. Serious renal diseases may be present without any symptoms. Proteinuria as well as hematuria may be the only early signs of renal disease (membranous nephropathy, membranoproliferative glomerulonephritis, post-infectious glomerulonephritis, IgA nephropathy and others) (Hajar et al. 2011). Proteinuria may induce renal insult through many pathways such as initiation of chemokines expression and complimentary activation which eventually lead to inflammatory cell infiltration and fibrogenesis (Abbate et al. 2006).

CKD is defined as urinary albumin to creatinine ratio (ACR) equal or >30mg/g, or estimated glomerular filtration rate (eGFR) lower than 60mL/min/1.73 m^2 for 3 months (Levin, Stevens et al. 2013). One study in Bangladesh demonstrated an incidence of CKD of 7.9% and prevalence of renal disease 4.4% among children (Roy et al. 2015). Hypernatremia (serum sodium level >150 mmol/L) which is a serious complication of diarrheal diseases in children and is caused by a water deficit. Cases with hypernatremia and dehydration appear to cluster among children and elderly persons with altered level of consciousness. There is a link between hypernatremia and acute kidney injury, defined as an abrupt decline in renal function.

It is evident that acute kidney injury (AKI) is not uncommon among children with severe dehydrating diarrhea. A prospective observational case control study has been conducted in icddr,b from August 2013 to October 2015 on hypernatremic children with diarrhea. This hypernatremic cohort were found to have a linear relation of serum creatinine level with increasing serum sodium. Among the cohort, 28% had acute kidney injury (personal communication, unpublished data). About half of them required renal replacement therapy (dialysis). In this cohort, infants with AKI had an independent association with hypernatremia (odds ratio (OR) = 8.66, 95% confidence interval (CI) = 3.88–19.22) (Shahrin et al. 2020). However, it was not known to what extent hypernatremia contributes to the subsequent morbidities and physical development in later age of those children. The purpose of the present study was to evaluate long term renal function of the children who recovered from hypernatremia.

Methodology

Study Design

The study was an observational longitudinal study conducted in Dhaka Hospital of icddr,b from March 2016 to March 2017.

Study Subject

The study participants were enrolled from a cohort of under 5 years who were enrolled in a previous prospective observational study conducted during August 2013 to October 2015, presented with hypernatremia (serum Na ≥150 mmol/l). Two hundred twenty-Four under-5 children who had recovered from hypernatremia from the previous study were contacted. However, 143 were enrolled in the present study after consent from their parents or legal guardian. All these children were brought to Dhaka Hospital of icddr,b and an analysis was done on spot urine samples for evaluation of kidney function.

Previously diagnosed hypernatremic children were grouped as mild hypernatremia (serum Na less than 160 mmol/L), moderate hypernatremia (Serum Na 160 mmol/L to 169 mmol/L) and severe hypernatremia (Serum Na ≥ 170 mmol/L).

Study Methodology

Nutrition and morbidity assessment*:*

On arrival every child's weight, height and mid upper arm circumference (MUAC) was recorded using standard procedure. Height was measured by stadiometer (Model SECA 213) and length (for children less than two years old) by infantometer (to nearest 1 mm), mid-upper arm circumference (MUAC) was measured by standard MUAC measuring tape (nearest 1 mm). Head circumferences were also measured according to standard procedure. Data was collected on pre-designed and pre-tested questionnaires, which included the child's medical records address, socio-demographic information including family status, living conditions, breastfeeding history, history of any illness, immunization status and so on. Socioeconomic information of the study subject was collected by pre-structured questionnaire on occupation, education, income, and residential area.

Biochemical Test

Spot urine sample obtained from all children and microscopic examination performed for the detection of Pr/Cr ratio for detection of glomerular damage. Urine routine examination from morning spot samples was performed by Automated urine analyzer (Sysmex UX-2000) and the underline principle of test in analyzer was based on:

a) Photometry method to analyze chemistry of urine
b) Refractometry method to analyze Specific Gravity
c) Flow Cytometry method to analyze urine sediment such as WBC (pus cells), RBC, epithelial cells, fungi.

Urine protein was measured photometrically in the automated chemistry analyzers of the Beckman Coulter AU series using reagents from the manufacturer. Urine creatinine was measured in the same analyzers with enzymatic method using reagents from the same manufacturer. Urinary Pr/Cr ratio was calculated by Laboratory Information System (LIS) software using the following formula:

Total Protein:Creatinine ratio = Total Protein in g/L ÷ Creatinine in g/L

Data Management and Analysis

All data was entered into a database and analyzed using SPSS version 20 (SPSS Inc., Chicago, IL) and STATA version13. For dichotomous factors, a normal approximation test (Chi squared test) or Fisher's exact test used; for continuous variables, either a t-test or Mann-Whitney test used. Logistic regression) used to identify independent predictor of proteinuria. This analysis used to model the relationship between a binary outcome and one or more predictor variables. Linear regression used to model the relationship between a quantitative outcome and one or more predictor variables. Strength of association determined by estimating odds ratios (OR) or standardized regression coefficients and their 95% confidence intervals (CI). p-values

<0.05 were considered significant. In univariate models, we compared sociodemographic characteristics and different anthropometric indices of children presented with and without proteinuria. Variable included in this model were age, anthropometry, hypernatremia group, family income, parent's education, any co-morbidity and asset score. We performed logistic regression analysis to identify characteristics that were significantly associated with proteinuria after adjusting for the covariates.

Ethical Consideration

Ethical approval for this study was obtained from the Institutional Review Boards (IRB) of icddr,b. The study did not involve any invasive procedures like blood specimen collection. Data was collected by interviewing mothers using questionnaires and testing children through age-appropriate tasks. Before enrolment, signed informed consent was obtained from the parents/guardians of the children. The consent forms were written in Bangla in a format so that it would be easily understood by the legal guardians of study subjects even with little or no educational background. The consent form was read out to the legal guardian/parent of the study subject if he/she was unable to read. Signed consent or the left thumb impression will be obtained from the care-giver/legal guardian/parent for the participation of the children in the study.

Results

Among the hypernatremic children in this follow up study 143 (64%) children's mother gave consent to participate in present follow-up study. At the time of discharge, they all became normonatremic (serum Na <150 mmol/l). More than half of the study children came from outside of Dhaka city. About 40% of the children belonged to poorer household. Mean age of the children was 35 ± 8 months; male participants were predominant among the children less than 36 months age (Table-1). Mean±SD of weight and length of the study participants were 12.3 ±3.2 kg and 89.1 ±8.5 cm respectively. After stratifying the study participants with urinary Pr/Cr ratio ≥ 0.2) and without proteinuria (urinary Pr/Cr ratio < 0.2), family income was comparable. Maternal education was comparable among two groups. However, level of paternal education was greater among the children who had normal Pr/Cr ratio. One third of the study children presented with upper respiratory infections (Table 1).

Table 1. Sociodemographic characteristics of children with and without proteinuria

Parameter	Child with Proteinuria n=70	Child without Proteinuria n= 73	p-value
Sex; n (%)			
Male			
Upto 36 months	28 (71.8)	23 (50.0)	0.048
>36 months	11 (28.2)	23 (50.0)	
Female			
Upto 36 months	20 (64.5)	13 (48.1)	0.289*
>36 months	11 (35.5)	14 (51.9)	
Low Birth weight, n (%)	19 (27.1)	9(12.3)	0.021*
Place of Residence n (%)			
Dhaka city	15 (21.4)	11 (15.3)	0.467
Within Dhaka district	11 (15.7)	16 (22.2)	
Outside Dhaka	44 (62.9)	45 (62.5)	
Any Co-morbidity	38 (55.1)	37 (51.4)	0.661
Mother's Education n (%)			
Illiterate	12 (17.1)	8 (11.0)	0.518
Up to Primary	19 (27.1)	19 (26.0)	
Above Primary	39 (55.7)	46 (63.0)	
Father's Education n (%)			
Illiterate	20 (28.6)	12 (16.4)	0.022*
Upto Primary	22 (31.4)	15 (20.5)	
Above Primary	28 (40.0)	46(63.0)	
Asset Quintile n (%)			
Poorest	23 (32.9)	6 (8.3)	<0.001*
Poorer	11 (15.7)	17 23.6)	
Middle	19 (27.1)	10 (27.1)	
Rich	7 (10.0)	21 (29.2)	
Richest	10 14.3)	18 (25.0)	

Although there is statistical difference in mean weight and length among two groups, mean WHZ (-0.43± 1.44 vs -0.54± 1.63; p=0.665), WAZ (, -1.11± 1.4 vs -1.05± 1.6; p=0.824) and LAZ score (-1.41± 1.39 vs -1.24± 1.58; p=0.490) were comparable (Table-2). There was lack of associated urinary problem and glomerular damage among these children with high urinary Pr/Cr ratio. Proteinuria (Pr/Cr ≥0.2 gm/L) was comparable in different groups of hypernatremic children. After adjusting for potential co-variates like age, sex, any form of malnutrition, different forms of hypernatremia and co-morbidity regression analysis showed that 86% of the children from better socioeconomic status were less likely to have high Pr/Cr ratio in comparison with the poorest counterpart (p=<0.001). Children who had mild hypernatremia were more likely to have raised urinary Pr/Cr ratio although it was not statistically significant. Children below 36 months age are 2 times more likely to have raised urinary Pr/Cr in comparison with those more than 36 months old ;2.31(1.06, 5.02); p-0.035. (Table-3).

Table 2. Presenting characteristics of children with and without proteinuria

Parameter	Children with Proteinuria n-70	Children without Proteinuria n=73	p-value
Age (month)	32.9 ±7.9	36.2± 7.40	0.012
Weight in Kg	11.7 ± 3.5	12.8± 2.7	0.041*
Length in cm	87.1±9.2	90.9 ±7.4	0.006*
MUAC in cm	156.8 ±28.95	164.19 ±16.52	0.064
Weight for Height (WHZ) score	-0.43± 1.44	-0.54± 1.63	0.665
Weight for Age (WAZ) score	-1.11± 1.4	-1.05 ±1.6	0.824
Height for Age (HAZ) score	-1.41± 1.39	-1.24 ±1.58	0.490

All parameters are in Mean ±SD

Table 3. Results of logistic regression to explore independent predictor of raised protein creatinine ratio in children who recovered from Hypernatremia

Parameter	Unadjusted		Adjusted	
	Odds ratio (95% CI)	p- value	Odds ratio (95% CI)	p-value
Hypernatremia group				
Severe HN	Ref		Ref	
Mild HN	1.48 (0.82-2.68)	0.360	1.84(0.66-5.10)	0.243
Moderate HN	1.05 (0.41-2.68)	0.916	0.94(0.31-2.85)	0.912
Age				
≥36 months	Ref		Ref	
Up to 36 months	2.24(1.13-4.44)	0.020*	2.31(1.06-5.02)	0.035*
Sex				
Male	Ref		Ref	
Female	1.35(0.69-2.65)	0.375	1.13(0.52- 2.46)	0.756
Any form of Malnutrition	1.14(0.59 -2.21)	0.696	1.25(0.58-2.69)	0.562
Asset Index				
Poorest	Ref		Ref	
Poorer	0.17 (0.05-0.55)	0.003	0.14(0.04-0.49)	0.002*
Middle	0.49(0.15-1.61)	0.244	0.45 (0.13-1.59)	0.216
Rich	0.09(0.03-0.30)	<0.001	0.06 (0.02-0.24)	<0.001*
Richest	0.14(0.04-0.47)	0.001	0.13(0.04-0.46)	0.001*

Discussion

Increased urinary Pr/Cr ratio is an early detection tool for diagnosis and management of renal disease who are at risk (Price C.P. et al. 2005). The present study revealed that about half of the study children in their follow up visits at 12 months presented with increased Pr/Cr ratio recovering from hypernatremia. There is lack of evidence in literature that relates hypernatremia to raised Pr/Cr ratio. Our study revealed that asset index which is an indicator of socioeconomic status has relation with raised Pr/Cr ratio as one of the markers of Choric Kidney Disease (CKD). Probable explanation might be that children from low socio-economic conditions are exposed to more unhygienic conditions leading to possibility of urinary infection. The findings of our present study corroborate well with other cross-sectional studies among rural residence in China showing that socioeconomic status is partly related to CKD (Shen Q et al. 2019). In this study, the author revealed that farmers and those with a lower educational level individual are more likely to develop CKD due to less access to healthcare facilities with benefits. Several other studies also explored the association between socioeconomic status and the prevalence of CKD (Martins et al. 2006, Judge et al. 2012, Fraser et al. 2014, Barreto et al. 2016). Malnutrition is also recognized to be a serious and common complication of chronic kidney disease (CKD). It is associated with increased morbidity and mortality in children (Hossain et al. 2022). However, the present study did not detect malnutrition as a predictor of proteinuria or CKD. Children below 36 months were more likely to present with raised Pr/Cr ratio. This might be the possibility of less effectual kidney function. On the other hand, 27% of the children who presented with proteinuria had history of low birth weight, which may indicate the association of children with LBW have smaller size of kidneys and less access to protein intake causing high protein and creatinine ratio. This is also supplemented by previous study which showed that individuals with low birth weight could relate to micro proteinuria. Low nephron number at birth, or postnatal or genetic influences could be the etiological factor (Yudkin, J., et al. 1997).

We found that hypernatremia is not a potential predictor for the development of proteinuria in children. Probable mechanism may be that these children had temporary renal insufficiency when they had hypernatremia which resolves spontaneously when they become normonatremic. Our study however revealed that for children from poorer household, paternal literacy level and low birthweight had association with raised Pr/Cr ratio. from spot urine analysis. One of the limitations of this study is that we did not perform serum creatinine during the follow up to calculate Glomerular Filtration Rate (GFR) to measure the extent of damage.

We found spot urine analysis is useful for detection of proteinuria and therefore to rule out renal damage as a consequence of hypernatremia in post hypernatremic children. Although progression of renal disease in long term can be defined by Pr/Cr ratio in spot urine analysis, there are limitations. Spot urine protein creatinine ratio possibly did not reflect the diurnal variation of protein excretion in children of the present study. Further well controlled studies employing 24-hour urine analysis versus spot urine samples with more appropriate biomarkers for detection of proteinuria are warranted to rule out renal damage as a consequence of hypernatremia in children from developing countries.

However, more study is required to confirm the spot urine analysis with 24-hour urine analysis for detection of proteinuria in post hypernatremic children to rule out renal damage as a consequence of hypernatremia.

Conclusion

The present study revealed that about half of the study children in follow up presented with increase Pr/Cr ratio recovering from hypernatremia. Moreover, asset index which is an indicator of socioeconomic status has relation with increased Pr/Cr ratio which denote as one of the markers of Choric Kidney Disease (CKD). Long term evaluation of renal function of the children that recovered from hypernatremia did not show any clinical abnormalities like associated abnormal urine analysis. Further study is warranted to correlate the higher Pr/Cr ratio with other parameters to rule out the long-term effect of hypernatremia on kidney function.

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