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Urinary secretory IgA after nutritional rehabilitation

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Abstract

We studied the secretory IgA (sIgA) response of the mucosal urinary tract of malnourished children before and after nutritional rehabilitation. sIgA concentration (mg/l) was determined by ELISA in 187 children aged 3 months to 5 years. The children, who frequented a day care center, were divided into four groups, according to nutritional status: 57 were eutrophic, 49 were undergrown, 57 were moderately malnourished and 24 were severely malnourished. In addition, dip slide (Urotube, Roche) and dip-stick (Combur 9-Boehringer) tests showed that children had no bacteriuria or any other urinary abnormalities. Plasma albumin concentration (g/dl) was significantly lower ($P<0.005$) in the severely malnourished group (mean 3.0 ± 0.3 SD) than in the eutrophic group (mean 4.0 ± 0.5 SD). When each nutritional state was analyzed, no significant differences in the sIgA were found between the 0 ⊢1 and 1 ⊣5 year age range. In the moderately and severely malnourished groups, sIgA (0.36 and 0.45, respectively) was significantly lower than in the eutrophic (0.69) and undergrown (0.75) groups. Ninety-five children were included in the 8-month follow-up study; 30 children were excluded from the follow-up because 4 had bacteriuria, 11 had leukocyturia, 8 had proteinuria and 7 had hematuria. Among the malnourished children, 40% showed nutritional improvement ($P<0.05$) and significantly increased sIgA as compared to reference values for the eutrophic and undergrown groups. These data suggest that malnourished children have a significantly lower urinary sIgA than eutrophic children. After nutritional rehabilitation, they develop local immunity with a significant increase in sIgA.

Key words
• Urinary tract
• Local immune response
• Secretory IgA
• Nutritional status

Introduction

The immune system of the mucous membranes is under the influence of many factors such as diet (1), microbial flora and changes in the hormonal levels of the organism (2). Secretory IgA (sIgA) is the predominant immunoglobulin in mucosal immunity and probably exerts an important protective effect on urinary tract infection by preventing bacteria from adhering to uroepithelial cells (2-4).

The effect of protein-energy malnutrition on the sIgA response has been studied in duodenal fluid, saliva, nasal secretions, urine and tears of malnourished children (5-11). However, no reports on the effect of renutrition on urinary sIgA are available. The aim of this study was to determine the sIgA re-

sponse in the mucosal urinary tract of malnourished children before and after nutritional rehabilitation.

Subjects and Methods

Subjects

The study involved a group of 187 children aged 3 months to 5 years frequenting day-care centers in low-income communities of the metropolitan Recife area, Brazil. Nutritional status was determined by weight and height measurements and expressed as percentile (P) for age and sex according to the National Center for Health Statistics (12). Four groups were considered: eutrophic with weight and height equal to or above P10; undergrown with normal weight and height below P10; chronic moderately malnourished with weight and height between P10 and P3; chronic severely malnourished with weight and height below P3. Children with hereditary disorders, nephropathies, obesity or urinary tract infection were excluded from the study.

The study was approved by the Ethics Committee of the Universidade Federal de Pernambuco and informed consent was obtained from the parents of the children.

Methods

Children were weighed and measured for height on a monthly basis on the day of urine collection. A 16-kg capacity scale (Filizola model) was used to weigh children under 2 years of age; older children were weighed to the nearest milligram on a 160-kg capacity scale (Filizola model). Heights for children under 2 years of age were measured using an anthropometric scale; older children were measured to the nearest millimeter using the Stanley somatometric tape.

A midstream urine specimen was collected into a sterile tube between 8:00 and 10:00 a.m., after appropriate hygiene. Bacteriuria was determined by the dip-slide method (Urotube, Roche). In addition, the dip-stick (Combur 9, Boehringer) was used to assess the presence of leukocytes, nitrite, red blood cells or protein. The results were interpreted visually according to standard color charts.

Urinary sIgA concentration (mg/l) was measured by ELISA, according to a procedure previously developed at the Nephrology Laboratory, "Escola Paulista de Medicina", Universidade Federal de São Paulo, Brazil (13). Polystyrene microplates (Hemobag Produtos Químicos Ltda., Campinas, SP, Brazil) were coated with sheep anti-human SC antibodies purified by affinity chromatography at a concentration of 5 µg/ml, in a 0.1 M sodium bicarbonate buffer, pH 9.0, and incubated for 24 h at 4°C. After washing (3x) with 0.05% PBS Tween, 0.5% bovine serum albumin in PBS solution was added, and incubated for 1 h at 37°C to coat sites not covered by the antibody. After washing, triplicates of the samples (100 µl per well) diluted in 0.05% 20 Tween 0.5 M PBS NaCl were added to the microplates and incubated for 24 h at 4°C. After washing, peroxidase-conjugated sheep anti-human IgA antibodies were added, diluted to 1/1,500 in 0.05% 20 Tween 0.5 M PBS NaCl, and incubated for 2 h at 37°C. Washing was repeated and 0.4 mg/ml orthophenylenediamine in 0.1 M citrate and 0.2 M phosphate buffer, pH 5.0, with H_2O_2 at a 0.01% final concentration was added and incubated for 30 min at room temperature. The reaction was stopped by adding 25 µl 1.0 M H_2SO_4 to each well, and the color developed was measured on a #307C microplate reader, at the 492 nm wavelength. The color of three microplate wells to which no samples were added was used as blank. IgA semipurified in the laboratory itself was used for constructing the standard curve. With this methodology the confidence interval (5th and 95th percentiles) for urinary sIgA concentration was 0.26 to 2.30 mg/dl (14).

Urinary creatinine (mg/dl) was measured

by the alkaline picrate method. Plasma total protein was determined by the biuret method (Biolab, Rio de Janeiro, RJ, Brazil) and the protidogram by electrophoresis using cellulose (Cellogel, Milano, Italy).

Follow-up

Ninety-five children who attended four day-care centers 5 days per week (Monday through Friday) were included in the follow-up. All of them received the same daily care from a pediatrician, a nutritionist and two health care assistants. Body weight was determined every 15 days and height was determined 2, 4, 6 and 8 months after the beginning of the study. During an 8-month period the children were checked monthly for urinary sIgA and creatinine. In addition, a urine specimen was collected to determine bacteriuria or other urinary abnormalities.

Nutritional rehabilitation was associated with cure from malnutrition, i.e., malnourished children at the beginning of the 8-month follow-up were considered eutrophic at the final evaluation.

Statistical analysis

Secretory IgA concentration is reported as median. Urinary creatinine and plasma albumin are reported as mean ± SD. The Mann-Whitney test was used to evaluate the differences in sIgA between equally nourished children aged 0⊢1 and 1⊣5 years. The differences in sIgA in different nutritional conditions were analyzed by the Kruskal-Wallis test. The Wilcoxon rank-sum test was used to analyze sIgA differences at the beginning and at the end of the study. Differences in creatinine concentration were analyzed by the Mann-Whitney test since values did not fit a Gaussian distribution. The differences in plasma albumin concentration between different nutritional conditions were analyzed by the Student *t*-test. The significance in the percentage of nutritional improvement or rehabilitation during the 8-month period was analyzed by the MacNemar test.

A P value <0.05 was taken as significant.

Results

Of the 187 children, 57 were eutrophic, 49 were undergrown and 81 had malnutrition (Table 1). Ninety-five children were included in the 8-month follow-up study; 30 children were excluded from the follow-up because 4 had bacteriuria, 11 had leukocyturia, 8 had proteinuria and 7 had hematuria. Of the remaining 65 children who were followed up, 38 were eutrophic and 27 were malnourished.

Table 1 - Nutritional status of 187 children who participated in the study.

Nutritional status was assigned on the basis of weight and height for age and sex using National Center for Health Statistics (12).

Age (years)	Nutritional status								Total
	Eutrophic		Undergrown		Malnourished				
					Moderately		Severely		
	Male	Female	Male	Female	Male	Female	Male	Female	
0⊢1	4	5	7	3	8	6	1	10	44
1⊣5	30	18	18	21	17	26	4	9	143
Total	34	23	25	24	25	32	5	19	187

Plasma albumin concentration was significantly lower (P<0.005) in the severely malnourished group than in the eutrophic group (Table 2). Urinary creatinine (mg/dl) was significantly lower in the moderately and severely malnourished children (38.8 ± 27.8 and 35.3 ± 22.0, respectively) than in eutrophic children (60.0 ± 35.6) of the same age range.

When analyzed for each type of nutritional status, differences in sIgA were not significant between 0⊢1 and 1⊣5 years of age. sIgA was significantly lower in the moderately and severely malnourished children than in eutrophic and undergrown children (Table 3). Among the 95 children followed up, a significant percentage (40%; P<0.05) showed nutritional improvement. The urinary sIgA concentration of 27 malnourished children with nutritional rehabilitation at the end of the study was significantly increased compared to reference values for the eutrophic or undergrown groups (Figures 1 and 2).

Table 2 – Plasma total protein, albumin (A) and γ-globulin (γ) of the children in the study.

Data are reported as mean ± SD. *Albumin and A/γ significantly lower than in the eutrophic group (P<0.05; Student *t*-test); **γ-globulin significantly higher than in the eutrophic group (P<0.05; Student *t*-test).

Nutritional status	Total protein (g/dl)	Albumin (g/dl)	γ-globulin (g/dl)	A/γ
Eutrophic (N = 57)	7.3 ± 0.8	4.0 ± 0.5	1.4 ± 0.3	1.2 ± 0.3
Undergrown (N = 49)	7.3 ± 0.6	3.6 ± 0.4*	1.6 ± 0.4**	1.0 ± 0.3*
Moderately malnourished (N = 57)	7.0 ± 0.7	3.1 ± 0.4*	1.7 ± 0.6**	0.9 ± 0.2*
Severely malnourished (N = 24)	6.7 ± 0.9*	3.0 ± 0.3*	1.5 ± 0.5	0.9 ± 0.2*

Table 3 - Median and range of urinary sIgA concentration and excretion rate in children of different nutritional status.

*Significantly lower than in the eutrophic and undergrown groups (P<0.05; Mann-Whitney test).

Nutritional status	Age (years)	sIgA (mg/l)			sIgA excretion rate (mg/g creatinine)		
		N	Median	Range	N	Median	Range
Eutrophic	0⊢1	9	0.63	(0.23-1.80)	9	1.14	(0.60-2.20)
	1⊣5	48	0.73	(0.17-1.90)	44	1.60	(0.25-3.91)
	0⊢5	57	0.69	(0.17-1.90)	53	1.47	(0.25-3.91)
Undergrown	0⊢1	10	0.46	(0.17-1.80)	8	1.57	(1.10-3.25)
	1⊣5	39	0.85	(0.18-2.20)	33	1.43	(0.53-3.00)
	0⊢5	49	0.75	(0.17-2.20)	41	1.45	(0.58-3.25)
Moderately malnourished	0⊢1	14	0.45*	(0.15-1.60)	14	1.78	(0.44-2.66)
	1⊣5	43	0.35*	(0.06-1.90)	41	1.03	(0.16-3.64)
	0⊢5	57	0.36*	(0.06-1.90)	55	1.31	(0.16-3.64)
Severely malnourished	0⊢1	11	0.31*	(0.04-0.83)	8	0.96	(0.30-1.50)
	1⊣5	13	0.74*	(0.03-1.50)	12	1.21	(0.15-3.16)
	0⊢5	24	0.45*	(0.03-1.50)	20	1.08	(0.15-3.16)

Discussion

The children in the age range studied did not show any sign of sIgA increase with age, nor was any significant difference in sIgA concentration detected during the early months of life. These results do not agree with data in the literature concerning local antibody ontogenesis. Fliedner et al. (15) failed to detect sIgA in the urine of eight infants under 6 months of age.

Urinary secretory IgA has been studied in the duodenal fluid, saliva, tears and urine of malnourished children, with conflicting results (6,8,9). In the present study, urinary creatinine was significantly lower in malnourished children than in eutrophic children. This finding must be considered in the evaluation of the sIgA excretion rate (mg sIgA/g creatinine), which did not differ significantly between malnutrition and normal nutritional status. Urinary creatinine has been used to predict muscle mass. When dietary creatinine is negligible and kidney function is normal, the amount formed and excreted depends on the creatine concentration in muscle and muscle mass. In malnourished children the low levels of urinary creatinine reflect a direct deleterious effect of protein-calorie malnutrition on the kidney and represent a physiologic response to the nutritional insult (16). This adaptation by the kidney to a deficient diet limits the use of the mg sIgA/g creatinine index. This finding must be considered in the evaluation of urinary sIgA excretion rate in malnourished children.

As is the case for all immunoglobulins, sIgA, which is predominant in mucosal immunity, has a double function, i.e., to recognize and eliminate the antigen. Secretory IgA antibodies exert their function by binding to antigenic epitopes on the invading microorganisms, limiting their mobility and adhesion to the epithelium of the mucous membrane (2,3,17). There are few references to epidemiologic studies on urinary tract infection in Brazilian children. Teodósio

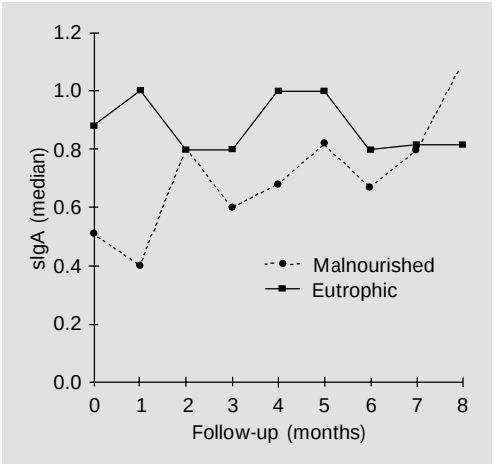

Figure 1 - Median urinary sIgA concentration of children aged 3 months to 5 years who were eutrophic or malnourished at the beginning of the 8-month follow-up, and with nutritional rehabilitation at the final evaluation. Data are reported as median of urinary sIgA for 27 malnourished and 38 eutrophic children.

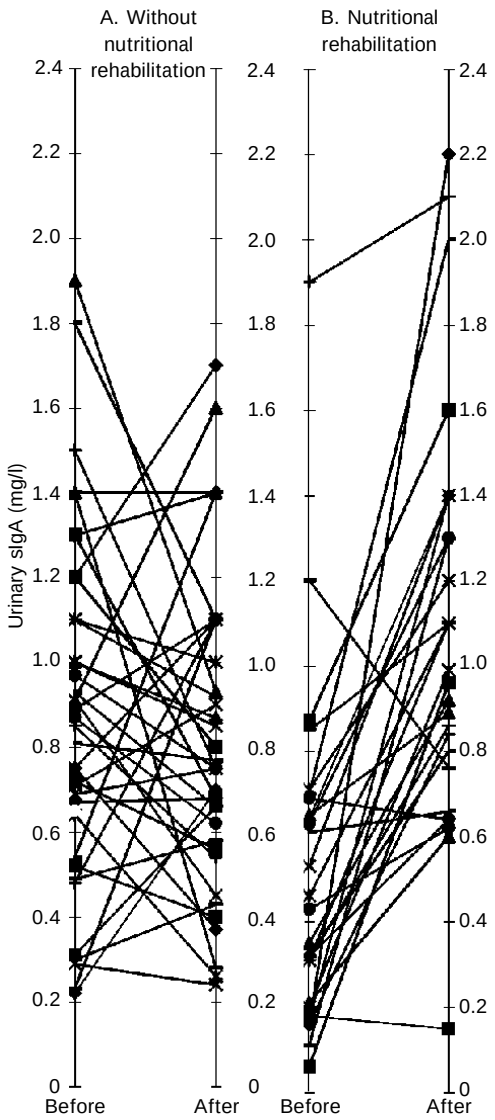

Figure 2 – Effect of nutritional rehabilitation on urinary sIgA of malnourished children who received (N = 27), or not (N = 38), nutritional rehabilitation for 8 months. Data are reported as sIgA mg/l urine. *A*, Without nutritional rehabilitation: median before 0.88 mg/l, median after 0.82 mg/l. *B*, With nutritional rehabilitation: median before 0.51 mg/l, median after 1.09 mg/l ($P<0.05$ compared to before rehabilitation, Wilcoxon test).

et al. (5) and Leite et al. (18) reported a high prevalence of urinary tract infection in children from low-income communities of Great Recife, PE and Salvador, BA, Brazil, respectively. In children with chronic protein-energy malnutrition the abnormal local immune response of the urinary tract may be a factor contributing to the genesis of non-obstructive urinary tract infection (19).

Prospective and long-term studies will be needed, however, to identify specific local antibodies that oppose the antigens of the infecting microorganism and to provide a measure of the intensity and specificity of the local immune response in malnourished children.

Our results indicate that malnourished children have significantly lower urinary sIgA than eutrophic children. After nutritional rehabilitation they develop the local immunity with a significant increase in sIgA.

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