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Persistent protein losing enteropathy in post measles diarrhoea

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SUMMARY Faecal α_1 antitrypsin was measured in two groups of children with diarrhoea aged 6 months to 6 years during the acute and recovery stages of the illness. Group 1 comprised 19 children with a history of measles in the two weeks preceding admission to hospital. In this group there were six cases of *Shigella* species, six enterotoxigenic *Escherichia coli,* and five rotavirus, and two did not yield an aetiologic agent. Group 2 comprised 15 children with diarrhoea only. In this group there were five cases of *Shigella* species, five enterotoxigenic *Escherichia coli* , and five rotavirus. Children with rotavirus diarrhoea belonging to both groups showed a transient high faecal clearance of α_1 antitrypsin during the acute stage. Post measles cases of diarrhoea showed significantly higher faecal clearance of α_1 antitrypsin than group 2 subjects in both the acute and recovery stages. The faecal clearance of α_1 antitrypsin in both groups was significantly higher during the acute stage compared with the recovery stage. Highest faecal clearances of α_1 antitrypsin were observed in children with post measles shigellosis in the acute stage and they also had persistently raised concentrations, thus suggesting prolonged protein losing enteropathy.

It is widely recognised that direct loss of nutrients is an important mechanism through which diarrhoea causes malnutrition.[1] Various gastrointestinal disorders, including diarrhoeal illness, have been associated with abnormal transmucosal protein loss from the gut.[2 3] Using spot faecal α_1 antitrypsin measurements, one recent investigation showed that 87% of patients with shigellosis, 63% with enterotoxigenic *Escherichia coli,* and 42% with rotavirus diarrhoea had significant loss of protein.[4] Another study also showed evidence of transient protein loss in rotavirus diarrhoea.[5] Measles is recognised to be one of the common precipitating factors in the development of diarrhoea, particularly of the shigellosis type.[6] Kwashiorkor in many developing countries,[7–9] including Bangladesh,[10] has been found to be associated with measles; protein loss from the intestine is considered to be one of the causes.[11 12] The duration and magnitude of protein loss, however, has not been documented in patients with post measles diarrhoea. This study was designed to obtain quantitative assessment of enteric protein loss occurring in post measles diarrhoea of known aetiology.

Patients and methods

The International Centre for Diarrhoeal Disease Research, Dhaka, Bangladesh, consisting of an outpatient rehydration centre and indoor hospital, treats about 100 000 patients annually.[13]

Study subjects were selected from patients attending the Centre during the period March 1982 to December 1983, and there were 63 children aged 6 months to 6 years. All the children had diarrhoea of less than 5 days' duration and showed a mild to moderate degree of dehydration on admission. Clinical history, physical examination, and naked eye stool examination were carried out to screen diarrhoea of specific aetiologies. Group 1 consisted of 36 children who had measles (fever, cough, coryza, and conjunctivitis followed by eruption of a maculopapular skin rash) in the two weeks preceding the diarrhoeal attack and were diagnosed as post measles diarrhoea. Group 2 consisted of the remaining 27 children who had diarrhoea only and gave no history of measles during the preceding six months. Children with complications such as meningitis, pneumonia, high fever (> 102°F), high respiratory rate (> 48 breaths/min), otitis media, severe malnutrition (<60% weight for age of National Centre for Health Statistics), and a history of consumption of antibiotics before admission were not included in the study.

All children were admitted to the clinical study ward. The study was approved by the ethical review

and research review committees of the Centre. Written consent was obtained from the parents or guardian for participation in the study.

Children were rehydrated and maintained with oral rehydration solution (Na^+90 mmol/l, K^+20 mmol/l, Cl^-80 mmol/l, HCO_3^- 30 mmol/l, Glucose 111 mmol/l). Children were kept in the hospital for roughly seven days or until they passed formed or soft stool. This period was termed the 'acute stage'. During admission to hospital paediatric urine bags were used to collect urine separately from stools. Eight hourly stool samples were kept in a refrigerator (4°C) until completion of 24 hour collections. These collections were carried out on days 1, 3, and 5 of admission to hospital. Stool specimens were then pooled and homogenised in a blender. Aliquots from the homogenised samples were kept in a deep freeze (−20°C) before lyophilisation. Complete blood count and electrolyte estimations were carried out on admission. Blood was drawn on the second day for determination of antitrypsin and albumin concentrations and packed cell volume. Serum for antitrypsin was stored deep frozen until assayed. The usual hospital diet of rice and chicken curry was provided. Treatment with antibiotics was started in children with bacteriologically confirmed shigellosis. At the time of discharge the patients or guardians were advised to bring the child for re-admission after three weeks for a further three days of observation termed the 'recovery stage'. Attempts were made to follow up those children who had positive bacteriological isolation in the acute stage. The regimen for sample collection was similar to that in the acute stage. Nineteen children in group 1 and 15 in group 2 were successfully followed up during the recovery stage.

Freeze dried faecal samples were extracted in 1 ml normal saline/100 mg of dry faeces. The mixture was centrifuged at 1500 *g* for five minutes and 5 µl supernatant were loaded to wells of radial immunodiffusion plates (Boehringwerke AG, Marburg, West Germany) containing monospecific antibody for antitrypsin. The precipitation ring was measured after 72 hours. A reference curve was established by using standard antitrypsin for each plate. Precision was checked by replicate analysis (coefficient of variation of <5%).

For serum antitrypsin concentration 5 µl of diluted serum samples were used in the same manner. Antitrypsin concentrations for both faeces and serum from the same patient were assayed using the same immunoplate. Faecal contents were expressed as mg/g lyophilised faeces. Faecal clearance was calculated using the formula C=FXW/P (where C=clearance, F=faecal concentration mg/g, W=daily faecal weight, and P=serum concentration mg/dl)[14 15] and expressed as ml serum/day. Serum albumin concentration was estimated by standard technique.[16]

Bacteriology. Rectal swabs were obtained on admission and cultured for enterotoxigenic *Escherichia coli*, *Vibrio cholerae*, *Shigella* species, *Salmonella* species, and *Campylobacter jejuni*. Suspected *E. coli* colonies were tested for heat labile enterotoxin using Chinese hamster ovary cells[18] and for heat stable enterotoxin by the infant mouse assay.[19] Presence of rotavirus antigen in stool was tested using enzyme linked immunosorbent assay.[20]

Statistical analysis. Mean value of faecal clearance of α_1 antitrypsin and serum albumin concentrations of the two groups during acute and recovery stages were compared using the two tailed Student's *t* test. One way analyses of variance was done to see the difference of faecal α_1 antitrypsin clearance over time.

Results

On admission patients with post measles diarrhoea (group 1) and patients with diarrhoea only (group 2) were similar for age, weight, weight for age, weight for height, plasma protein concentrations, and packed cell volume. Children in group 1 had a somewhat longer duration of symptoms preceding their admission to hospital (Table 1).

Children with shigellosis in group 2 showed higher weight gain during the recovery stage (Table 2), while the post measles group did not. None of the children with *E. coli* diarrhoea showed improvement in body weight during recovery. The decrease

Table 1 *Characteristics of the study children on admission to hospital. Values are No or mean (SD)*

	Group 1 (Post measles diarrhoea)	*Group 2 (Diarrhoea only)*
No of children	19	15
Organism isolated:		
Shigella species	6	5
Enterotoxigenic *Escherichia coli*	6	5
Rotavirus	5	5
Organism not detected	2	0
Age (months)	28·8 (23·6)	28·8 (24·5)
Weight (kg)	8·6 (3·2)	8·7 (2·9)
Weight for age (%)	69 (10)	70 (9)
Height (cm)	83 (20)	80 (15)
Weight for height (%)	76 (13)	80 (13)
Packed cell volume (%)	33·8 (3·5)	33·0 (3·9)
Total serum albumin (g/l)	40·1 (10·0)	39·0 (7·7)
Duration of diarrhoea (h)	102 (45)	82 (32)
Days of measles preceding admission to hospital	9.3 (4·3)	Not applicable

Table 2 *Nutritional state of children with shigellosis and* E. coli *diarrhoea in both groups in acute and recovery stages. Values are mean (SD)*

	Shigellosis				*E. coli*			
	Group 1		*Group 2*		*Group 1*		*Group 2*	
	Acute	*Recovery*	*Acute*	*Recovery*	*Acute*	*Recovery*	*Acute*	*Recovery*
Weight (kg)	11·6 (4·4)	11·6 (4·3)	7·2 (0·3)	7·3 (0·3)	8·6 (2·5)	8·6 (2·6)	10·3 (2·9)	10·4 (2·8)
Total serum protein (g/l)	68 (13)	64·2 (7·2)	64 (30)	62·2 (4·3)	63 (7·1)	62·6 (10·8)	70 (9)	66 (3·4)
Serum albumin (g/l)	39 (9·5)*	31 (5·6)*	36 (5·3)	35 (2·1)	36·6 (7·5)	32·3 (6·0)	44 (9·5)	44·2 (5·7)
Packed cell volume (%)	36·7 (2·0)	34 (2·0)	31·8 (2·7)	31·7 (2·6)	31·6 (5·6)	31·6 (5·6)	36·6 (2·0)	30 (2·0)

Conversion: SI to traditional units—Total protein: 1 g/l=0·1 g/100 ml.
*p<0·01.

Table 3 *Daily faecal α_1 antitrypsin clearance (ml/day) and its ratio between the two groups. Values are mean (SEM)*

Day	*Group 1 (n=19)* (Post measles diarrhoea)*	*Group 2 (n=15)* (Diarrhoea only)*	*Group 1: Group 2*
1	129 (98)	59 (40)	2·2
3	99 (93)	45 (26)	2·3
5	64 (80)	35 (30)	1·8
21	50 (45)	21 (13)	2·4
22	47 (47)	15 (9)	3·0
23	53 (44)	13 (9)	4·1

*p<0·01 for all days.

in serum protein concentration during the recovery stages was not significant in either group. Serum albumin concentrations fell in subjects with shigellosis in group 1 during the recovery stage ($p<0·05$).

Table 3 presents the ratio of faecal clearance of α_1 antitrypsin between the groups on specified days. Clearance in group 1 was significantly higher than in group 2 ($p<0·01$) and the ratio tended to increase with time.

The Figure shows how faecal α_1 antitrypsin clearance changed during the acute and recovery stages of diarrhoea of different aetiology. In group 1 the children with shigellosis persistently showed the highest faecal α_1 antitrypsin excretion. Prolonged magnitude of α_1 antitrypsin clearance steadily decreased during the recovery period. By contrast children with shigellosis in group 2 had normal clearance values (≤20 ml/day) during recovery. The difference in faecal clearance of α_1 antitrypsin between the two groups was always significant ($p<0·01$).

Among the children with enterotoxigenic *Escherichia coli* the clearance was higher in group 1 during the acute stage only ($p<0·01$). In both groups the loss tended to decline but did not return to normal during the study period. Among the children with rotavirus diarrhoea there was a transient loss of lower magnitude of α_1 antitrypsin up to the third day

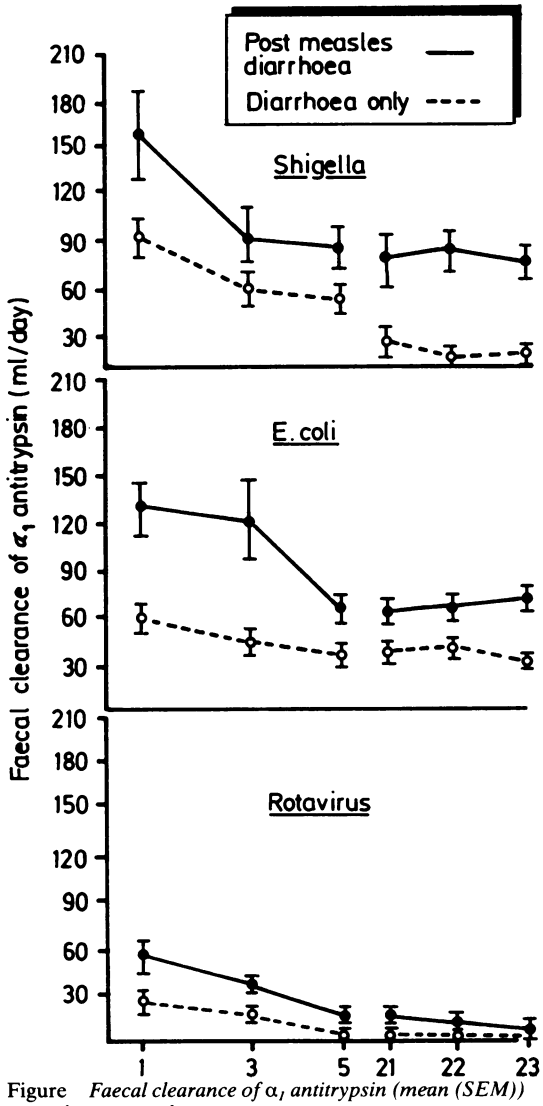

Figure *Faecal clearance of α_1 antitrypsin (mean (SEM)) according to aetiology.*

of admission to hospital. On the first day group 1 showed significantly higher clearance of α_1 antitrypsin compared with group 2 ($p<0{\cdot}01$) and both groups had normal faecal clearance by the end of the acute stage.

Discussion

Our data show that protein losing enteropathy occurred during diarrhoea with or without measles but was more severe and prolonged in cases with post measles diarrhoea. It was most severe in children with both post measles shigellosis and diarrhoea caused by enterotoxigenic *Escherichia coli*, perhaps explaining the well known association between diarrhoea and malnutrition. This also corroborates previous observations of prolonged protein malabsorption[21] and growth faltering in children with enterotoxigenic *Escherichia coli*.[22]

The use of α_1 antitrypsin as an endogenous marker[23] and the determination of faecal clearance of α_1 antitrypsin enabled the diagnosis of protein losing enteropathy.[3 14 24 25] The test is simple and non-invasive and does not require radioactive isotope and is therefore a suitable test for children in circumstances where more sophisticated techniques are not readily available. There was some controversy about the validity of the test, which has now been resolved.[3 26]

When faecal clearance of this protease inhibitor is greater than normal ($\leq$20 ml/day) it reflects loss of plasma protein into the gut lumen as food does not contain α_1 antitrypsin. This may lead to hypoproteinaemia.[23] Intestinal lymphangiectasia, oedema of the intestinal mucosa, and inflammation causing disruption of the intercellular tight junctions might be involved in the mechanism of protein leakage.[27] Increased intestinal protein loss due to bacterial overgrowth in hypomotile loops of intestine cannot be ruled out.[28]

In post measles shigellosis protein losing enteropathy was associated with hypoalbuminaemia during the recovery stage, suggesting a relation between serum albumin concentration or intestinal loss of serum protein concentration and extent of severity of the disease process.[27] The other group of children, although having raised faecal clearance of α_1 antitrypsin, were not hypoalbuminaemic, suggesting that hepatic protein synthesis could compensate.

The higher clearance of α_1 antitrypsin in post measles diarrhoea may be attributed to direct action of measles virus on the intestinal mucosa not unlike those seen on the skin.[29] Giant cell formation is known to occur in the mucosa of the intestine,[30] and severe necrotising gastroenteritis has been reported in measles. The high faecal clearance of α_1 antitrypsin in patients with shigellosis in both groups is indicative of abnormal enteric protein loss through gastrointestinal leakage,[31] in addition to the intestinal loss of red blood cell as found in shigellosis.[32]

Persistent higher loss of α_1 antitrypsin during the recovery stage in post measles shigellosis may reflect prolonged disease activity. The explanation of prolonged protein losing enteropathy in *E. coli* diarrhoea is still unclear. Our finding, however, supports the previous observation of protein losing enteropathy in measles enteritis[11] where specific aetiologies have not been looked for. We conclude that faecal α_1 antitrypsin can provide a valid estimate of enteric protein loss in childhood diarrhoea. The extent and duration of protein losing enteropathy, however, varies with aetiology. Persistent protein losing enteropathy may be an important factor in the development of malnutrition after post measles diarrhoea.

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