Protein-losing enteropathy in Transkeian children with morbid protein-energy malnutrition

J. E. IPUTO

Abstract
A commercially available radial immunodiffusion assay was used to measure serum and faecal \( \alpha \)-antitrypsin concentrations as well as \( \alpha \)-antitrypsin clearance in 17 children with kwashiorkor, 11 children with marasmic kwashiorkor, 10 children with marasmus, and 16 normal children. Serum \( \alpha \)-antitrypsin concentrations were significantly higher than normal in the marasmus and marasmic kwashiorkor groups, and significantly lower than normal in the kwashiorkor group. The intestinal clearance of \( \alpha \)-antitrypsin was significantly higher than normal in the marasmus and marasmic kwashiorkor groups, and significantly lower than normal in the kwashiorkor group. There was a significant inverse correlation between the \( \alpha \)-antitrypsin clearance and serum albumin concentration in the marasmus and marasmic kwashiorkor groups. No such correlation was evident in the kwashiorkor group. It is concluded that protein-losing enteropathy is likely to play a significant role in the development and perpetuation of hypo-albuminaemia in children with marasmus and marasmic kwashiorkor but not in those with kwashiorkor.

Morbid protein-energy malnutrition (PEM), defined as PEM severe enough to warrant hospital admission, is a common clinical problem among children in the Transkei and is responsible for up to 9% of admissions to the children's wards in Umtata General Hospital. Kwashiorkor, the oedematous variant of PEM, is the most common form of morbid PEM seen in this hospital, and accounts for 60% of PEM admissions. It has a particularly poor short-term prognosis with a mortality rate of 40%.

Protein-losing enteropathy (PLE) has been shown to exist in PEM in conjunction with measles and diarrhoea. It is believed that PLE plays the major role in the pathogenesis of the hypo-albuminaemia and subsequent oedema of the kwashiorkor that may complicate measles. Children with PEM often present with protracted episodes of chronic or intermittent diarrhoea. The intestinal mucosa of such children has been shown to be leaky. The cause of this loss of integrity is not clear. It has been suggested that the chronic diarrhoea seen in children with PEM, rather than being the cause of the intestinal damage, is the result of that damage. If it is accepted that the leakiness of the intestinal mucosa in PEM precedes the diarrhoea, what then is responsible for the loss of integrity of the mucosa? Could it be that PEM per se can lead to PLE? It is known that PEM is associated with the histological picture of villus atrophy and inflammatory cell infiltration of the lamina propria in the intestinal mucosa. This clinical and histological picture has been associated with the malabsorption of nutrients, as well as diarrhoea, but there is a paucity of data on whether this clinical and histological picture is associated with PLE as well. It is, therefore, not clear whether the PLE seen in the chronic diarrhoea-malnutrition syndrome complex is due to the diarrhoea, or whether it is partly responsible for the diarrhoea.

This study aims to determine the presence or absence of significant protein loss in uncomplicated PEM in our environment. It is based upon the estimation of the faecal clearance of \( \alpha \)-antitrypsin, an anti-protease plasma protein that can resist the proteolytic activity of gastro-intestinal enzymes and is excreted in faeces largely undegraded. It has proved a useful naturally occurring marker of intestinal plasma protein leakage.

Subjects and methods
The study was carried out in the children's wards of Umtata General Hospital, the teaching hospital for the University of Transkei Medical School. In this hospital, PEM is classified according to the Wellcome Classification of infantile malnutrition, which is based on weight for age and presence or absence of oedema at the time of admission. The standard weight for age is the 10th percentile of the National Center for Health Statistics (NCHS) standards. The admission criteria are such that only those who fall into the kwashiorkor, the marasmus and the marasmic kwashiorkor categories of PEM (henceforth referred to as morbid PEM) are admitted into the nutrition ward.

The malnourished children were recruited into the study on the day of admission. All clinical details concerning nutritional history, past illnesses (especially diarrhoea), weight, and signs of malnutrition including oedema, hair and skin changes, were recorded. Children with concurrent illnesses such as pneumonia, tuberculosis and gastro-enteritis, or who had had these illnesses within the past week, were excluded from the study. The malnourished children were divided into the three clinical categories of morbid PEM, i.e. marasmus, marasmic kwashiorkor and kwashiorkor. A group of age-matched normal well-nourished children, comprising abandoned children housed in the hospital and hospitalised children who were being treated for orthopaedic problems, served as a control.

Stool samples were collected over a 24-hour period. Urine contamination was avoided by the use of urine bags. The stool collection was started the morning after the admission of the subjects. In each case, the fresh stool was weighed, and a 5 g sample was freeze-dried for 16 hours and then reweighed. Twenty-five milligrams of the lyophilised stool were reconstituted in 5 ml of normal saline. The mixture was centrifuged at 15 000 rpm for 15 minutes. Twenty microlitres of the supernatant were analysed for \( \alpha \)-antitrypsin by simple radial immunodiffusion with LC-PARTIGEN plates supplied by Behring. After inoculation the plates were sealed in plastic bags and incubated at room temperature for 48 hours. The plates were then coated with a fresh solution of phosphate-buffered 3,4-dihydroxyphenylalanine (DOPA) to intensify the precipitation rings. The diameter of the precipitate ring was measured by means of a dark background plate reader with side lighting. Three standard samples of known \( \alpha \)-antitrypsin concentrations, supplied by the manufacturer, were analysed along with the test samples. A reference curve was plotted using the diameters of the three standard solutions from which the amount of \( \alpha \)-antitrypsin in each test sample was read off.

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At the commencement of the stool collection, fasting venous blood samples were obtained in heparinised bottles and centrifuged at 5 000 rpm for 30 minutes. Plasma was kept frozen at -20°C until batch analysis. Analysis was performed by simple radial immunodiffusion with the NOR-PARTIGEN α-antitrypsin plates supplied by Behring, by the same method as that used in the stool analysis. Intestinal clearance of α-antitrypsin was estimated using the following formula:

\[ C = \frac{F \times W}{P}, \]

where \( C \) is the clearance of α-antitrypsin (ml/d); \( F \) is the α-antitrypsin concentration (mg/100 g dry stool); \( W \) is the dry stool weight after 24 hours; and \( P \) is the plasma concentration of α-antitrypsin (mg/dl). Student's \( t \)-test was used to compare the malnourished patients' data with control values. Statistical significance was accepted at \( P < 0.05 \).

### Results

A total of 54 children were studied, 17 with kwashiorkor, 10 with marasmus, 11 with marasmic kwashiorkor and 16 who were well-nourished. The children with marasmus and those with marasmic kwashiorkor had a longer history of illness than those with kwashiorkor. Table I summarises the anthropometric and biochemical data of the respective study groups. There was no significant age difference between the groups. The control subjects, as expected, had higher nutritional indices than the PEM subjects. The total plasma protein content among the PEM groups was similar. There was similarity in the plasma albumin content of the patients with marasmic kwashiorkor and those with kwashiorkor, and these values were significantly lower than those of the children who had marasmus (\( P < 0.05 \)).

The plasma α-antitrypsin content showed considerable variability. The kwashiorkor subjects had lower values than the control subjects. Indeed, the kwashiorkor values were below the assay normal range for plasma α-antitrypsin. The children with marasmus and marasmic kwashiorkor had values higher than the control values.

The α-antitrypsin clearance in the children with marasmus and marasmic kwashiorkor was significantly higher than in the control subjects; in those with kwashiorkor the clearance was significantly lower than that of the control subjects and those with marasmus and marasmic kwashiorkor. There was an inverse correlation between plasma albumin levels and α-antitrypsin clearance. This correlation was poor in the control subjects (\( r = 0.32, P > 0.05 \)), and in those with kwashiorkor (\( r = -0.27, P > 0.05 \)), but was significant in children with marasmus (\( r = -0.59, P < 0.05 \)) and those with marasmic kwashiorkor (\( r = -0.62, P < 0.05 \)).

### Discussion

In this study there was a marked difference in the serum α-antitrypsin concentration between the children with marasmic kwashiorkor and those with kwashiorkor. This finding contrasts with that of Schelp and Supawan, who reported increased levels of α-antitrypsin in both forms of oedematous malnutrition, and with that of Madina et al. who reported depressed levels of these protease inhibitors in all forms of morbid PEM. The elevated levels of serum α-antitrypsin in marasmic kwashiorkor and marasmus may be related to the chronic nature of these forms of PEM. Although these children showed no obvious signs of infection at the time of admission, they could have been exposed to infections during the initial stage of their illness with a consequent increase in serum α-antitrypsin levels. It is well established that serum α-antitrypsin levels take a long time to return to normal and may remain elevated long after the infection has passed. The high α-antitrypsin faecal clearance in marasmus and marasmic kwashiorkor indicates that there is a protein-losing enteropathy in these forms of PEM. In contrast the low clearance seen in kwashiorkor suggests the absence of significant protein loss in this form of PEM. This finding is similar to that of Madina et al., who reported significant intestinal protein loss in marasmus and marasmic kwashiorkor, but not in kwashiorkor. The inverse correlation between α-antitrypsin faecal clearance and hypo-albuminaemia in PEM supports the view that α-antitrypsin clearance seen in kwashiorkor is not related to infection. Indeed, the kwashiorkor clearance was significantly lower than that of the PEM groups, but the kwashiorkor group had higher nutritional indices than the PEM subjects. The total plasma protein content among the PEM groups was similar. There was similarity in the plasma albumin content of the patients with marasmic kwashiorkor and those with kwashiorkor, and these values were significantly lower than those of the children who had marasmus (\( P < 0.05 \)).

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### Table I: Laboratory data of the PEM children and the control children (means ± SEM)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>Marasmus</th>
<th>Marasmic kwashiorkor</th>
<th>Kwashiorkor</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>16</td>
<td>10</td>
<td>11</td>
<td>17</td>
</tr>
<tr>
<td>Age (mo.)</td>
<td>18 ± 4</td>
<td>15 ± 2</td>
<td>13 ± 5</td>
<td>17 ± 4</td>
</tr>
<tr>
<td>Plasma protein (g/l)</td>
<td>54 ± 3</td>
<td>49 ± 4</td>
<td>46 ± 2*</td>
<td>45 ± 5*</td>
</tr>
<tr>
<td>Plasma albumin (g/l)</td>
<td>32 ± 5</td>
<td>29 ± 3</td>
<td>19 ± 3*</td>
<td>17 ± 5*</td>
</tr>
<tr>
<td>Plasma α-antitrypsin (mg/dl)</td>
<td>235 ± 141</td>
<td>298 ± 75*</td>
<td>380 ± 104*</td>
<td>189 ± 82</td>
</tr>
<tr>
<td>α-antitrypsin clearance (ml/d)</td>
<td>1.5 ± 0.2</td>
<td>1.9 ± 0.8*</td>
<td>2.4 ± 1.0</td>
<td>0.7 ± 0.3*</td>
</tr>
</tbody>
</table>

* Significantly different when compared with control values (\( P < 0.05 \)). assay range for normal plasma α-antitrypsin levels is 200 - 300 mg/dl.

### References