

**PROTEIN-LOSING ENTEROPATHY IN DIARRHOEA :
APPLICATION OF α_1 -ANTITRYPSIN ASSAY**

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PREFACE

The International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) is an autonomous, international, philanthropic and non-profit centre for research, education and training as well as clinical service. The Centre is derived from the Cholera Research Laboratory (CRL). The activities of the institution are to undertake and promote study, research and dissemination of knowledge in diarrhoeal diseases and directly related subjects of nutrition and fertility with a view to develop improved methods of health care and for the prevention and control of diarrhoeal diseases and improvement of public health programmes with special relevance to developing countries. ICDDR,B issues two types of papers: scientific reports and working papers which demonstrate the type of research activity currently in progress at ICDDR,B. The views expressed in these papers are those of authors and do not necessarily represent views of International Centre for Diarrhoeal Disease Research, Bangladesh. They should not be quoted without the permission of the authors.

ABSTRACT

Protein losing enteropathy (PLE) has been documented in several disorders, but little is known about it in diarrhoeal diseases of various aetiologies. As diarrhoea and malnutrition are closely interrelated, determination of the role of direct loss of plasma proteins in diarrhoeal diseases was considered to be important. By assaying α_1 -antitrypsin in both stool and serum and using the ratio we have been able to document that 87% of patients with shigellosis, 63% with infection due to enterotoxigenic *E. coli* and 42% with rotavirus were associated with PLE. None with cholera or healthy subjects without diarrhoea were positive for PLE. In this population it was noted that presence of pus cell and red blood cells in the stools was strong evidence of both PLE and shigellosis.

INTRODUCTION

Diarrhoea and malnutrition are closely interrelated (1). Reduced intake of food coupled with increased catabolism and malabsorption combine to produce malnutrition and diarrhoea (2). Direct loss of plasma proteins in diarrhoea can occur but has received little attention although there has been several reports on such protein loss from the gastrointestinal tract in non-diarrhoeal diseases (3,4,5). Cholera, a non-inflammatory disease, does not lead to gastrointestinal protein loss (6,7,8). Loss of plasma proteins in shigellosis and amoebiasis which are inflammatory diseases of the gut should occur but have not been documented to our knowledge. Recently established pathogens such as rotavirus, enterotoxigenic *E.coli* or *V.parahaemolyticus* are important causes of diarrhoea in the developing countries, where malnutrition among the children are commonly found. Documentation of protein loss in diarrhoea caused by these agents are therefore important.

Here, we report our findings on loss of protein in diarrhoea of several aetiologies using a simple, safe and non-invasive technique.

PATIENTS AND METHODS

The Dacca treatment centre of the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) takes care of over 100,000 diarrhoeal patients a year, most of whom are children. Seventy patients aged eight months to 55 years, with a clinical history of watery or bloody mucoid diarrhoea (dysentery) of less than five days duration were selected for the study. Patients who had received antibiotics prior to admission or those with complications like pneumonia, high fever and malnutrition were excluded. Informed consent was obtained from the patients or their guardians.

The patients were rehydrated with intravenous diarrhoea replacement solution containing Na = 133, K = 13, Cl = 98 and Acetate 48 mEq/L. Antibiotics were used when needed. After obtaining history and carrying out physical examination, duplicate catheter stool specimens were collected and immediately frozen at -60°C until lyophilized. Simultaneous serum samples were also obtained and frozen at -60°C until assayed. Stools were tested to determine the aetiology of diarrhoea. Toxigenicity testing of *E. coli* was done by Chinese Hamster Ovary cell and the infant mouse assay (9,10). Rotavirus was diagnosed by an enzyme-linked immunosorbent technique (11).

Specimens of stools and blood were obtained daily for 3 days from 3 healthy Bangladeshi physicians and from 6 healthy non-Bangladeshi volunteers without history of any intestinal illness.

Stool samples were processed according to Crossley *et al.* (12). α_1 -antitrypsin was measured in both stools and serum by using M-partigen plate (Behringwerke). For each batch of plates, a reference curve was established with a standard solution. Protein losing enteropathy (PLE) was defined as those where the ratio of faecal α_1 -antitrypsin (mg/gm dry weight) over α_1 -antitrypsin of serum (gm/L) was 1.0 or more.

To test the stability of α_1 -antitrypsin in faeces, 8 different portions of stool from the same patient were kept at room temperature for 4 hours and at $+4^{\circ}\text{C}$ overnight and then transferred to a deep freezer. These stool samples were subsequently lyophilized and assayed blindly and there was agreement within 5%.

RESULTS

Figure 1 shows the distribution of cases according to microbiological diagnosis and α_1 -antitrypsin ratio. It may be seen that 87% of shigella, 63% enterotoxigenic *E.coli* (ETEC) and 42% rotavirus diarrhoea were associated with PLE. There was one patient with *E.histolytica* who had α_1 -antitrypsin ratio of 4.3. The other 44% of the patients having no confirmed aetiology also showed evidence of protein loss. No proven cholera cases or any of the normal subjects were positive according to the criteria used.

Table I shows that 38% of patients who had complained of watery diarrhoea and 82% of patients complaining of mucoid and/or bloody diarrhoea had α_1 -antitrypsin ratio 1.0 or more (Chi square = 13.90. $P < .002$).

Figure 2 shows the mean and distribution of α_1 -antitrypsin ratio according to microscopic examination of stool. The mean and standard errors were respectively 2.19 ± 0.47 , 1.52 ± 0.36 and 0.58 ± 0.15 for the patients with both WBC and RBC, only WBC and no cells in their stool. Protein loss was seen in 88% of patients having both WBC and RBC in their stool and 63% of those who had WBC only in their stool. Among patients showing neither white blood cell (WBC) nor red blood cell (RBC) in the stool, 22% were positive for PLE.

DISCUSSION

Shigellosis, diarrhoea caused by enterotoxigenic *E.coli* (ETEC) and rotavirus were found to be associated with significant loss of protein from plasma into the gut lumen. *Shigella bacilli* attack the epithelial lining cells of the large intestine, producing

FIGURE 1

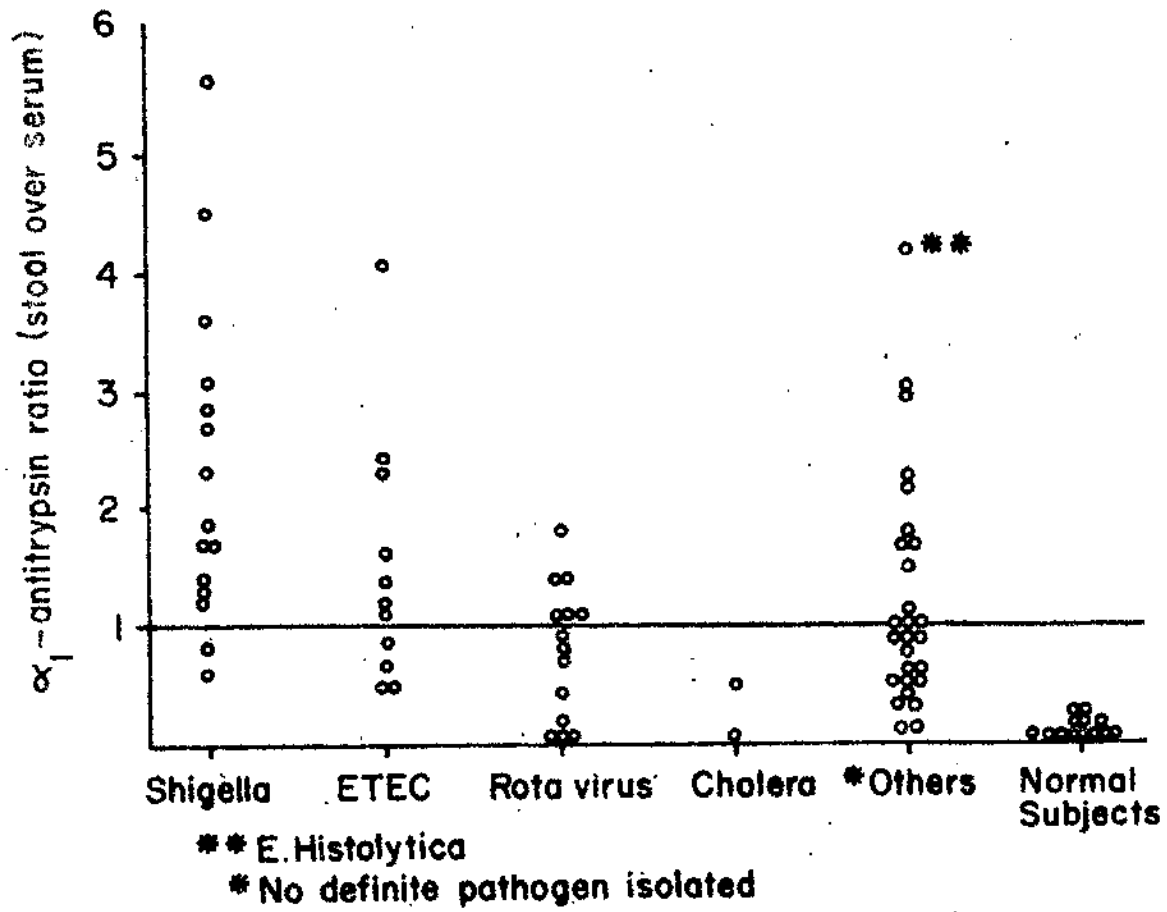
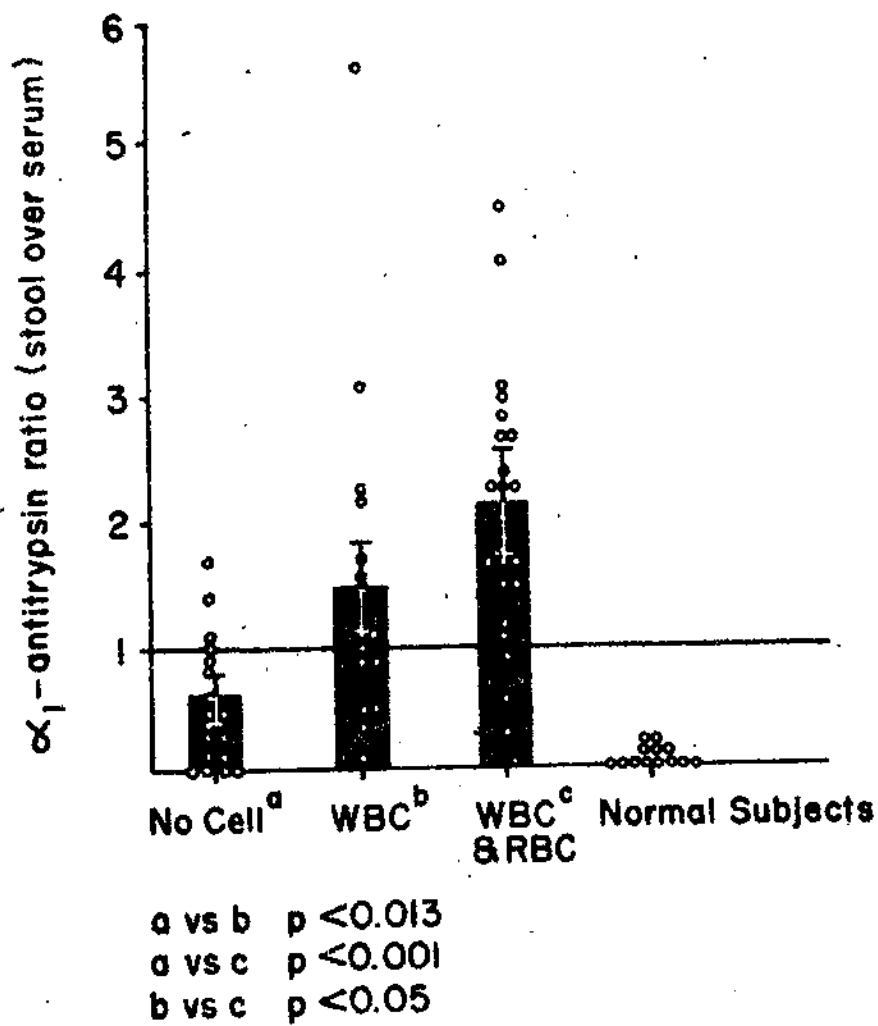


TABLE I--DISTRIBUTION OF CASES ACCORDING TO DIARRHOEAL COMPLAINTS
AND NUMBER OF POSITIVE CASE (α_1 AT RATIO > 1.0)

Diarrhoeal complaints	Number	Number positive	Percentage
Watery	37	14	38
Mucoid and/or bloody	33	27	82

$p < 0.002$

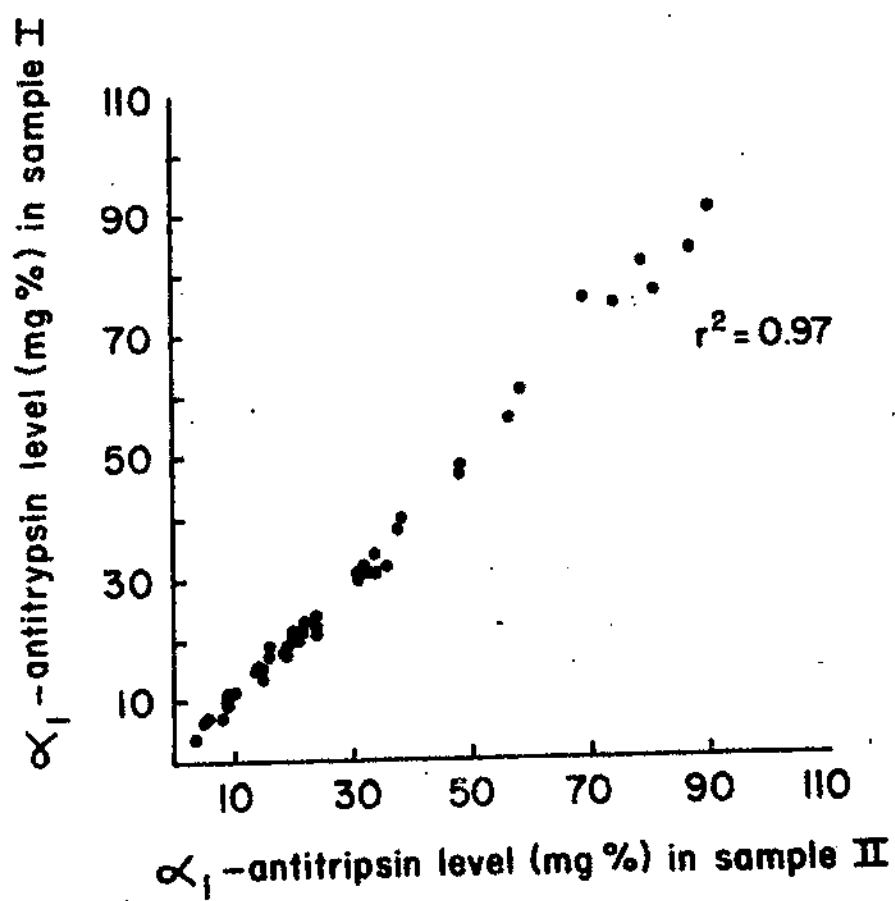
FIGURE 2



intense inflammation and ulceration (13). Rotavirus diarrhoea also produces inflammatory changes in the small intestine (14) which may explain the loss of protein. Tissue destruction is not currently thought to be a part of ETEC diarrhoea, but in view of these findings may need to be investigated further. In this series we had only 2 cholera patients, neither showed protein loss. Previous studies however had documented that cholera is not one of the protein losing enteropathies (6,7,8). Among the patients having no confirmed aetiology there were 44% losing protein. They had inflammatory exudates in their stools. This suggests that there are a group of patients with a dysentery-like picture but from whom no known pathogens could be isolated. Presence of WBC alone or WBC with RBC were consistently associated with protein loss as shown in Figure 2. However, even when WBC or RBC were absent as many as 22% of patients were PLE positive. Thirtythree out of 59 patients had ova or intestinal helminths like ascaris, hookworm and trichuris trichuria present in their stool. As helminthiasis may damage intestinal mucosa through their physical presence, excretion of toxins and allergens (15), their role in causing protein losing enteropathy could not be ruled out. The same explanation could be applied to patients having watery diarrhoea but showing a high ratio of α_1 -antitrypsin.

Methods previously used for studying gastrointestinal protein loss required radio-isotopes, which in developing country settings, are technically difficult, time consuming, expensive and associated with potential health hazards. The development of α_1 -antitrypsin method (12) using an endogenous marker is safe and non-invasive. Moreover, the results of duplicate samples (Figure 3) illustrate the reproducibility of the method and its simplicity leads us to conclude that it is a useful screen for gastrointestinal protein loss.

FIGURE 3



LEGENDS FOR FIGURES

- FIGURE 1 - Distribution of α_1 -antitrypsin ratios according to aetiology.
- FIGURE 2 - Mean and distribution of α_1 -antitrypsin ratios according to presence of red blood cells and white blood cells in stool.
- FIGURE 3 - Relationships between the levels of α_1 -antitrypsin (mg%) in duplicate samples.

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