

Original Article

Unsuitability of Fecal Alpha 1-Antitrypsin as a Marker for Differentiation of Microbial and Non-Microbial Diarrhea

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ABSTRACT

Objectives: Alpha 1 - antitrypsin (AAT), an acute phase protein of human serum, is eliminated in the feces in some intestinal disorders, especially diarrhea and its estimation has been used as a marker for protein loss. In this study, we have investigated the possibility of using the determination of fecal AAT in the differential diagnosis of microbial and non-microbial diarrhea, which is ordinarily done by stool culture.

Methods: In this case-control study, fecal AAT concentration was estimated in children hospitalized in the Pediatric department of Hajar hospital, Shahrekord, Iran. Group 1 consisted of 30 children with microbial diarrhea. Group 2 consisted of 30 children with non-microbial diarrhea and the control group consisted of 30 children without diarrhea. Stool samples were collected

from all children. Fecal samples were subjected to stool culture and examination. Fecal AAT was estimated using radial immunodiffusion technique.

Results: The mean fecal AAT concentration was 50.0 ± 46.2 mg/dl in group 1, 25.8 ± 38.3 mg/dl in group 2 and 1.1 ± 3.4 mg/dl in the control group. There was a significant difference in fecal AAT values in case of diarrhea when compared with the control group.

Conclusion: Fecal levels of AAT were significantly higher in microbial diarrhea than in non-microbial diarrhea and levels in both were much higher than in controls. However, AAT levels were low in some individual microbial cases making this measurement unhelpful for differential diagnosis.

KEYWORDS: Alpha-1 antitrypsin, diarrhea, radial immunodiffusion, pediatrics

INTRODUCTION

Alpha 1 - antitrypsin (AAT) is one of the acute phase protein^[1] with protease inhibitor activity and with a normal concentration of 0.02 - 0.4 mg/dl in human serum^[2]. This normal concentration is increased following cancers^[3,4] and some hepatic^[5] and infectious disease^[6]. Determination of fecal AAT has been considered as a suitable, reliable and cheap method for estimation of enteric protein loss^[7,8] without the use of radioactive tracers^[9]. It has been also shown that random fecal AAT concentration is a valuable screening test for mucosal disorders associated with abnormal transmucosal serum protein loss^[10]. The concentration of AAT in random fecal samples from 68 infants with acute diarrhea and 32 healthy controls was determined by Fonata *et al* (1988). The mean for AAT in infants with diarrhea was 2.07 mg/g dry stool compared with 1.29 mg/g in controls. They showed that fecal AAT was significantly greater than that of controls only for

diarrhea caused by Rotavirus or Salmonella^[11]. Lisowska *et al* (1998) also studied the concentration of AAT in random fecal samples in 32 children with acute and chronic diarrhea and in 23 healthy children. They suggested that determination of fecal AAT could give clinically useful information about the difference between infectious and non-infectious diarrhea^[12]. Differential diagnosis of microbial from non-microbial diarrhea is usually performed by using culture techniques and these methods are long lasting. Therefore in this study, the suitability of fecal AAT concentration as a marker to differentiate between microbial and non-microbial diarrhea has been investigated.

MATERIALS AND METHODS

In this case-control research, the study population consisted of all children aged 1-42 months, hospitalized in the Pediatrics department of Hajar hospital in Shahrekord, Iran, from October 2001 - March 2002. Fecal samples were collected for

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all the above children. These samples were subjected to microbial culture using selective and differential mediums including MacConkey and Salmonella and Shigella, EMB and Preston mediums according to the method described in the Diagnostic Microbiology Textbook^[13]. According to the consistency of the fecal samples and results of stool culture, three groups of children were established as follows:

1. Group 1 consisted of 30 children with diarrhea and positive microbial culture.
2. Group 2 consisted of 30 children with diarrhea and negative microbial culture.
3. Control group consisted of 30 children with normal fecal consistency (without diarrhea).

All groups were matched according to the age, sex, signs and symptoms of individuals.

Stool examination using wet smear was performed on all samples according to standard techniques^[14]. AAT was purchased from BETA Company in Mashhad, Iran. Anti AAT antibody was raised by subcutaneous immunization of rabbits with AAT and Freund's adjuvant as we had described previously for other antigens^[15]. The AAT concentration in fecal samples was estimated using radial immunodiffusion technique. For this purpose, two milliliters of rabbit anti AAT serum was incorporated into 12 ml 1.5% molten agar at 56°C. Templates were used to cut the pattern of wells. Stool samples were diluted with normal saline and applied into the wells. Three dilutions of AAT with pre-determined concentration were also applied into wells in the same plate. The plates were kept in a humid chamber at 37°C overnight. Since the antibody is uniformly distributed throughout the plate, the distance of diffusion of antigen and hence the ring diameter was proportional to the concentration of the AAT. Therefore, the AAT concentration for each stool sample was calculated from the radial diameter of that sample in mg/dl.

The concentrations of AAT were expressed as mean \pm SD. Statistical analysis was performed using student's t-test. In all cases, p value less than 0.05 was considered significant.

RESULTS

In this study, 50% (45) of the children were male and the rest were female. The mean age of the children in cases and control group is shown in Table 1. In 31% of the children, the only sign was diarrhea and in the remaining 69%, at least one more sign was observed. These signs included infirmity (28%), fever (22%), vomiting (9%), coughing (6%) and epilepsy (2%). Dysentery was observed in only one child. The mean of fecal AAT concentration for children from group 1 (with

Table 1

Mean of age of the children with microbial diarrhea (Group 1), with non-microbial diarrhea (Group 2) and without diarrhea (Group 3).

Groups	Mean (month)	SD
Group 1	12.9	7.7
Group 2	11.6	6.4
Group 3	9.4	8.3

diarrhea and positive microbial stool culture) was 50.0 + 46.2 mg/dl, in group 2 (with diarrhea and negative microbial stool culture) was 25.8 + 38.3mg/dl and in control group (without diarrhea) was 1.1 + 3.4 mg/dl. A significant difference in fecal AAT concentration was observed between group 1 and 2 ($p < 0.05$), group 1 and group 3 ($p < 0.05$) and group 2 and group3 ($p < 0.05$). However, there was no significant difference among the three groups when they were compared for number of leukocytes (WBC), erythrocyte (RBC) and fatty bodies observed in stool examination. Isolated bacteria in positive microbial stool culture were *E.coli* enteropathogen in 28 cases (93.3%) and *Shigella* in two cases (6.7%). No *Campylobacter* was isolated.

DISCUSSION

In this research, fecal ATT concentration was estimated in three groups of matched children hospitalized in the Pediatrics department of Hajar hospital in Shahrekord Iran. The fecal ATT concentration in children with diarrhea was much higher than that of children without diarrhea. Also fecal ATT concentration in children with microbial diarrhea was higher than that of children with non-microbial diarrhea. Fonata *et al* (1988) studied concentration of AAT in random fecal samples of infants with acute diarrhea and some healthy controls. They showed that in cases where diarrhea was caused by some microbial agents, the fecal AAT concentration was significantly greater than that in the healthy control group^[11]. Weizman *et al* (2002) also showed that children with acute diarrhea and those with persistent diarrhea had significantly higher fecal AAT levels as compared with the control group^[16]. Lisowska *et al* (1998) studied fecal AAT concentration in children with acute and chronic diarrhea and control groups. They suggested that determination of fecal AAT in patients with diarrhea might give clinically useful information about the differences between infectious and non-infectious diarrhea^[12]. Dassinger *et al* (1996) also showed that protein losing enteropathy is associated with *Clostridium difficile* diarrhea^[17]. The results of our study also revealed that fecal concentration of AAT was significantly

different among groups with diarrhea (either microbial or non-microbial) and the control group. However, not all individuals with microbial diarrhea had high concentrations of AAT.

In conclusion, it appears that determination of fecal AAT concentration would not be a valuable marker for differentiation between microbial and non-microbial diarrhea. This problem may be due to variations in the concentration of serum AAT in different individuals. Therefore, in continuing the work, we would like to examine if simultaneous determination of the serum and fecal AAT concentration in those individuals with microbial diarrhea, but low fecal AAT, would show them to also be those with low serum AAT.

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