Prevalence and associated factors of persistent diarrhoea in Iranian children admitted to a paediatric hospital

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ABSTRACT To identify the frequency and associated factors of persistent diarrhoea in a paediatric hospital in Tehran, Islamic Republic of Iran, children admitted with acute diarrhoea were followed prospectively until resolution of the episode. Persistent diarrhoea developed in 19.6% of the 424 diarrhoea cases. Children with persistent diarrhoea were significantly younger than those with acute diarrhoea (15.3 versus 29.4 months). Enteroaggregative Escherichia coli was the most prevalent pathogen in both groups, but otherwise the profile of symptoms and isolated organisms was similar in the 2 groups. History of dietary change prior to admission and use of antibiotics and anticholinergic drugs in the acute phase were significantly higher in the persistent than acute diarrhoea cases.

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Diarrhoea is still an important cause of child morbidity and mortality, causing about 2.2 million deaths annually in the paediatric population [1]. With recent improvements in rehydration therapy programmes, a decline has been observed in deaths associated with acute watery diarrhoea, and thus the problem of persistent diarrhoea has become more concerning. Persistent diarrhoea—defined by the World Health Organization as a diarrhoeal episode with an acute onset and a presumptive infective etiology lasting ≥ 14 days—comprises only 3%–20% of diarrhoea-related deaths [2]. Persistent diarrhoea also imposes a heavy economic burden on low socioeconomic status countries with its devastating effects on nutritional status, growth and development of children [3,4].

There is little information about persistent diarrhoea in the Islamic Republic of Iran. A 7.7% prevalence was reported in a study from the west of the country, and young age, non-breastfeeding, use of antibiotics and presence of mucoid or bloody diarrhoea were factors associated with it [5]. These factors, in addition to malnutrition, were also observed in other studies of Iranian children [6,7]. We conducted the present study to identify the pattern and etiology of persistent diarrhoea among children admitted to a paediatric hospital in Tehran and to compare these cases with cases of acute diarrhoea.

Methods

A cross-sectional prospective study was performed from June 2006 to June 2007 in Mofid Paediatric Hospital, a university-associated referral hospital in Tehran.

Sample size

Estimated sample size was approximately 400, considering the 7.7% prevalence of persistent diarrhoea in Iranian children reported in a study with a similar methodology [5], and 95% confidence level and 2.5% acceptable error.

Patients

The study population was all children below 12 years of age who were admitted for acute diarrhoea during the study period. Previously diagnosed cases of inflammatory bowel disease, celiac disease and cystic fibrosis were excluded. Also children whose parents were not willing to take part in the study were excluded. Finally, 537 data collection forms were completed. As we were not able to follow up 113 patients due to missing or incorrect contact details or non-response, 424 data collection forms were available for analysis.

Data collection

Data were collected from the child’s medical record and an interview with the parents. Trained nurses visited all the children admitted to the hospital with gastroenteritis. After a brief explanation of the study to one of the parents and acquiring verbal informed consent, a data collection form was completed. This included the demographic characteristics of the child and the episode of diarrhoea (duration, signs and symptoms, changes in diet and any drugs used during the episode).

Diarrhoea was defined as the passage of at least 3 non-bloody stools or 1 bloody loose stool per 24 hours. For exclusively breastfed infants, increased frequency or decreased consistency of stool compared with the previous bowel habit of the infant and noticeable by the mother was considered diarrhoea. The duration of the episode of diarrhoea was calculated from the onset of the disease as stated by the parents. Cases were defined as persistent diarrhoea (duration ≥ 14 days) or acute diarrhoea (duration < 14 days). The child’s body weight was measured after rehydration. Weight-for-age z-score was calculated using Epi-win, version 6.01, based on the National Center for Health Statistics (NCHS) tables and was used to categorize the cases as normal weight (z-score ≥ –1.0), mild underweight (–1.0 < z-score ≤ –2.0), moderate underweight (–2.0 < z-score ≤ –3.0) and severe underweight (z-score < –3.0). We analysed nutritional status based on weight-for-age z-score score (to avoid bias, we disregarded height-for-age and weight-for-height scores since we observed mistakes during height measurements).

The patients were followed up until resolution of the diarrhoea episode, even if they were discharged from hospital.

Laboratory methods

A fresh stool specimen was obtained for laboratory testing during the initial days of the diarrhoea. Each specimen was first examined by a light microscope. A saline and iodine wet-mount preparation was used for detection of amoebae species, Giardia lamblia and Blastocystis hominis, and a formol-ether concentration method for cysts. Trichrome staining and polymerase chain reaction (PCR) was performed to differentiate Entamoeba histolytica/dispar complex from other non-pathogenic intestinal amoeba and E. histolytica from E. dispar respectively. In order to distinguish Cryptosporidium spp., Cyclospora spp. and Isospora belli, a Ziehl–Neelsen acid-fast stained slide was prepared. We used modified trichrome stain for Microsporidium spp.

Bacterial cultures were done on MacConkey and xylose–lysine–deoxycholate agar plates for Salmonella spp., Shigella spp. and diarrhoeagenic Escherichia coli. PCR was used to detect isolates that carried the eae and bfp genes for enteropathogenic E. coli (EPEC), pCVD432 plasmid for enteroinvasive E. coli (EIEC), heat-labile (LT) and heat-stable (ST) genes of enterotoxigenic E. coli (ETEC), stx1 and stx2 genes for Shiga-toxin-producing E. coli (STEC), ipaH genes for enteroinvasive E. coli (EIEC) and Shigella spp., and invA gene for Salmonella spp.
Statistical analysis

For comparison of proportions between acute and persistent diarrhoea cases, the $\chi^2$ or Fisher exact tests were used and for comparison of means the Student $t$-test was used. We considered a $P$-value < 0.05 as statistically significant. Data were analysed using SPSS, version 13.0.

Results

Patient characteristics

A total of 424 children were enrolled in the study. Persistent diarrhoea developed in 83 (19.6%) cases, both hospitalized and discharged patients. The mean age of the study population was 26.6 months, and 57.3% of the patients were male. The prevalence of persistent diarrhoea reached 24.4% in the 291 children aged < 2 years and 21.8% in the 372 aged < 5 years. The male to female ratio was not significantly different between the acute diarrhoea and persistent diarrhoea group (1.4:1 and 1.1:1, respectively). More than 80% of the persistent diarrhoea cases were aged < 18 months. The mean age in the persistent diarrhoea group was 15.3 months compared with 29.4 months in the acute diarrhoea group ($P < 0.001$). There was also a significant difference in mean age between the 2 groups, when we considered children < 2 years: 12.1 months in the 220 acute diarrhoea cases versus 9.5 months in the 71 persistent diarrhoea cases ($P = 0.002$).

Symptoms and characteristics of the diarrhoeal episodes

The mean duration of the diarrhoea episode in the acute diarrhoea group was 5.9 (SD 2.7) days; median 6 days, and in the persistent diarrhoea group 24.9 (SD 18.6) days, median 19 days.

Data regarding related symptoms and some characteristics of the diarrhoeal episodes are summarized in Table 1. Among the symptoms related to diarrhoeal diseases (abdominal pain, nausea and vomiting, loss of appetite and fever) only fever was significantly different between the 2 groups (86.2% in the acute versus 74.7% in the persistent diarrhoea group). Defecation times per 24 hours and frequency of bloody or mucoid stool (which may indicate severity of the disease) were not statistically significant between the groups.

The frequency of concurrent diseases, including other infections and immunodeficiency and gastrointestinal disorders, was similar in acute diarrhoea and persistent diarrhoea cases (21.4% and 20.5%, respectively $P = 0.853$) (Table 1). A history of major diet change (i.e. introducing complementary food or animal milk, changing the formula brand or introducing wheat to the child) 2 weeks prior to admission was found in 10.6% of acute diarrhoea and 20.5% of persistent diarrhoea cases ($P = 0.014$).

|Table 1| Demographic data, related symptoms and some characteristics of children hospitalized with acute and persistent (acute phase) diarrhoea |
|---|---|---|---|
|Symptoms and characteristics| Acute diarrhoea ($n = 341$) | Persistent diarrhoea ($n = 83$) | $P$-value |
|Mean (SD) age (months)| 29.4 (29.6) | 15.3 (17.4) | < 0.001 |
|Male:female ratio| 1.4 | 1.1 | 0.258 |
|Signs and symptoms| | | |
|Abdominal pain| 166 | 31 | 0.063 |
|Nausea and vomiting| 256 | 57 | 0.234 |
|Loss of appetite| 261 | 58 | 0.207 |
|Fever| 294 | 62 | 0.010 |
|Defecation ≥ 6 times per 24 hours| 210 | 60 | 0.069 |
|Bloody stool| 80 | 21 | 0.724 |
|Mucoid stool| 13 | 6 | 0.177 |
|Clinical history| | | |
|Concurrent diseases present| 73 | 17 | 0.853 |
|History of previous persistent diarrhoea| 18 | 7 | 0.298 |
|History of major diet change| 36 | 17 | 0.014 |
|Antibiotics| 284 | 78 | 0.013 |
|Anticholinergic drugs| 18 | 10 | 0.026 |
|Breastfed| 144 | 46 | 0.513 |

$^a$Infections, immunodeficiency and gastrointestinal disorders.

$^b$Data for children aged < 2 years only ($n = 220$ for acute diarrhoea and $n = 71$ for persistent diarrhoea group).

SD = standard deviation.
Antibiotic and anticholinergic drug use during the acute phase was significantly higher in the persistent diarrhoea than the acute diarrhoea group (Table 1). A summary of medication administered during the acute phase of the diarrhoeal disease (including drugs administered in the hospital before and after admission by parents or other physicians) is shown in Figure 1.

The mean of weight-for-age z-score was similar in both groups: –0.76 (SD 1.54) in acute diarrhoea and –0.77 (SD 1.28) in persistent diarrhoea patients.

In the age group < 4 months, 20 out of 36 (55.6%) were exclusively breastfed and in the age group < 6 months 26 out of 73 (35.6%) were exclusively breastfed. The proportion of exclusively breastfed infants was significantly higher in the persistent diarrhoea than the acute diarrhoea group, analysing only those aged < 2 years (14.5% versus 4.4%, P = 0.001). There was no difference between the acute and persistent diarrhoea groups in the frequency of breastfed and non-breastfed children (age < 2 years only).

Two deaths occurred during this study and both were related to the underlying disease of the patient and not to the diarrhoeal disease.

**Enteropathogens in stool specimens**

Laboratory testing of stool specimens for enteropathogens gave a positive result in only 42% of acute and 36% of persistent diarrhoea cases (Figure 2). EAEC was the most frequently isolated enteropathogen in both groups. No clear difference in the profile of infection was found between cases of acute and persistent diarrhoea (Table 2). *Salmonella* spp. was the only pathogen with a significantly higher prevalence in persistent diarrhoea (2/83 cases) than acute diarrhoea cases (0/341 cases) (P = 0.038), although the number of cases was very small.

**Discussion**

Due to the fact that this was a hospital-based study performed in a referral centre, a high frequency of persistent diarrhoea was expected. The prevalence of persistent diarrhoea (19.6% overall and 21.8% in children aged < 5 years) was higher than that reported in a study from west of Islamic Republic of Iran (7.7%). This may be because of the difference in admission status of the cases (inpatient versus outpatients). The rate of persistent diarrhoea is lower than that from a hospital-based study in Brazil (56.9%) [8] but higher than in Nigeria (11.7%) [9]. Age is a major determinant in the development of persistent diarrhoea. Our results showed that diarrhoea was more likely to be persistent in younger children and this is consistent with the findings of previous studies in Kenya, Peru, Egypt and Guatemala [2,10–13].

Comparing some related symptoms and characteristics of the diarrhoeal disease, we found that the illness was similar in severity in persistent diarrhoea and acute diarrhoea cases. Studies from Nigeria and Egypt have reported similar findings [9,12]. A major dietary change (such as introducing complementary food or animal milk to the child) 2 weeks prior to admission was more common in persistent diarrhoea than the acute diarrhoea cases. This may underscore the role of non-infectious causes, especially allergies, in the development of persistent diarrhoea.
diarrhoea. The frequency of exclusive breastfeeding was not very high among the children, so most of them were already exposed to foodborne infections. A diet change such as introducing animal milk or complementary food to an infant who is not exclusive breastfed or changing formula brand may indicate other causes of diarrhoea. Supported by the results of previous studies, we noticed that antibiotic and anticholinergic drug use during the initial days of a diarrhoeal episode was associated with longer duration of the disease [14–17].

Enteropathogens were identified in only 42% of acute and 36% of persistent diarrhoea cases. There are several reasons for this relatively low rate. First, we did not exclude patients treated with antibiotics prior to stool examination, which might result in negative bacterial growth. Secondly, we did not test the specimens for some bacterial enteropathogens—e.g. *Clostridium difficile*, *Aeromonas* spp. and *Campylobacter* spp.—or for any viral enteropathogens. Thirdly, a proportion of diarrhoeal episodes may be due to non-infectious causes such as food allergies or chronic non-specific diarrhoea of childhood, as noted by Vernacchio et al. [18].

Several studies from Bangladesh, India and Brazil have recognized EAEC as an important pathogen in developing persistent diarrhoea [19–22]. We also found it as the most prevalent pathogen in cases of both acute and persistent diarrhoea albeit with no significant difference between the 2 groups. Surprisingly, protozoa, especially *Cryptosporidium* spp. which is presumed to be one of the main etiological agents of persistent diarrhoea, was not associated with prolonged diarrhoea in our study [2,17,23]. *Salmonella* spp. was the only pathogen with a significantly higher prevalence in persistent diarrhoea than acute diarrhoea cases in this study, although the numbers were very small.

When assessing malnutrition, a demonstrated risk factors for persistent diarrhoea [9,24,25], we found no difference between the 2 groups.

Breastfeeding is a protective factor for persistent diarrhoea, as indicated in several studies [5,7,23], but in our study

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### Table 2 Frequency of enteropathogens in stool specimens of children hospitalized with acute and persistent diarrhoea

<table>
<thead>
<tr>
<th>Enteropathogen</th>
<th>Acute diarrhoea (n = 341)</th>
<th>Persistent diarrhoea (n = 83)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Giardia lamblia</em> trophozoite</td>
<td>1</td>
<td>1</td>
<td>0.35</td>
</tr>
<tr>
<td><em>Cryptosporidium</em> spp.</td>
<td>6</td>
<td>1</td>
<td>0.00</td>
</tr>
<tr>
<td><em>Blastocystis hominis</em></td>
<td>13</td>
<td>1</td>
<td>0.32</td>
</tr>
<tr>
<td><em>Salmonella</em> spp.</td>
<td>0</td>
<td>2</td>
<td>0.04</td>
</tr>
<tr>
<td><em>Shigella</em> spp.</td>
<td>7</td>
<td>2</td>
<td>0.69</td>
</tr>
<tr>
<td>Enteroaggregative <em>Escherichia coli</em> (EAEC)</td>
<td>60</td>
<td>15</td>
<td>0.92</td>
</tr>
<tr>
<td>Enteropathogenic <em>E. coli</em> (EPEC)</td>
<td>18</td>
<td>5</td>
<td>0.79</td>
</tr>
<tr>
<td>Shiga-toxin-producing <em>E. coli</em> (STEC)</td>
<td>25</td>
<td>6</td>
<td>0.97</td>
</tr>
<tr>
<td>Enterotoxigenic <em>E. coli</em> (ETEC)</td>
<td>21</td>
<td>5</td>
<td>0.96</td>
</tr>
<tr>
<td>Enteroinvasive <em>E. coli</em> (EIEC)</td>
<td>31</td>
<td>5</td>
<td>0.37</td>
</tr>
</tbody>
</table>
the frequency of breastfeeding in children < 2 years of age was similar in the acute and persistent diarrhea groups. However, it should be mentioned that the proportion of exclusively breastfed infants was significantly higher in the persistent diarrhea than the acute diarrhea group (14.5% versus 4.4%). We also observed that only 55.6% of infants under 4 months and 35.6% under 6 months of age were exclusively breastfed. This, along with the fact that mothers tend to discontinue breastfeeding during a diarrheal episode, implies the need for an extended public health education programme for diarrheal disease in the Islamic Republic of Iran.

Follow-up of the children showed no deaths associated with acute or persistent diarrhea. This may be a result of good hospital care especially in the case of rehydration and nutritional care.

This study presents some data about persistent diarrhea in a hospital setting and although the findings are not representative of the community, they clarify some aspects of persistent diarrhea in Iranian children. Persistent diarrhea seems to be a benign disease in the Iranian paediatric population. Young age, antibiotic and anticholinergic drug use, a history of a significant diet change 2 weeks before the onset of diarrhea and presence of Salmonella spp. are some factors that may be involved in persistent diarrhea.

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