Faecal lactoferrin as a predictor of positive faecal culture in south Indian children with acute diarrhoea

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Summary  Faecal lactoferrin, an iron-based glycoprotein found concentrated in secondary granules of neutrophils, may serve as a surrogate marker of inflammation in the intestine. We evaluated the usefulness of faecal lactoferrin as a predictor of infection with invasive enteropathogens in 262 children with diarrhoea. Lactoferrin at a dilution of 1:50 had the highest sensitivity for detection not only of conventionally cultured invasive enteropathogens but also of all other enteropathogens. Neither individual clinical symptoms nor the identification of faecal leucocytes by microscopy significantly predicted isolation of invasive enteropathogens from the faeces of children with diarrhoea. Faecal lactoferrin is a simple test which showed promise in predicting which children with diarrhoea are likely to be infected with invasive pathogens and can be incorporated as a screening test before faecal cultures are undertaken in this population.

Introduction

Diarrhoecal diseases are a major cause of morbidity and mortality in children in developing countries. Diarrhoea can range from a self-limiting, benign condition to severe, life-threatening illness, the complications of which are often related to infection with pathogens that invade the mucosa to cause inflammation. The management of diarrhoea with non-invasive organisms requires only rehydration and electrolyte replacement, but infection with several invasive microorganisms might require specific antimicrobial therapy. Algorithms to manage diarrhoea emphasise the need for faecal culture in patients with bloody diarrhoea because of the potential complications related to antibiotic use in patients with enterohaemorrhagic E. coli infection, but quite often diarrhoea caused by invasive enteropathogens is not associated with the passage of blood in faeces. Faecal culture in all patients presenting with acute diarrhoea is impractical, expensive and time-consuming, and results in a very low yield of positive cultures, as demonstrated by two studies in which only 1.5% and 2.4% of all diarrhoeal stools tested were culture-positive. It was estimated from these studies that the costs per positive faecal culture were $1200 and $952, respectively. In resource-poor developing countries it would be particularly inappropriate to order faecal culture indiscriminately in patients with diarrhoea.

In order to improve the cost-benefit ratio, patients with diarrhoea have been screened...
by various tests to determine the need for faecal culture. Microscopic examination of stools for the presence of leucocytes can indicate the presence of colonic inflammation. Studies using faecal leucocyte detection in freshly obtained specimens to select those for culture increased the yield of positive results with a reduction in cost per positive culture to $30. In developing countries, many factors limit the use of faecal microscopy for leucocyte detection, including the need for fresh specimens for examination, decreased sensitivity with swab/nappy specimens, and variations in the skill and experience of the microscopist.

A latex agglutination test for detecting faecal lactoferrin has been proposed as an alternative, since it provides a semiquantitative marker for the presence of polymorphonuclear leucocytes (PMNL) in faecal specimens. Lactoferrin is an iron-binding glycoprotein found concentrated in the secondary granules in PMNL and is not found in lymphocytes or monocytes. The latex agglutination test for faecal lactoferrin has been evaluated and found to be useful in the screening of invasive enteropathogens in travellers’ diarrhoea. Faecal lactoferrin is positive in infection with enteropathogenic Escherichia coli, entero-aggregative Escherichia coli, Clostridium difficile, Shigella and Campylobacter. It has been used as a screening test for inflammatory diarrhoea. Of the two studies that have assessed the value of faecal lactoferrin in diagnosing inflammatory diarrhoea in children, the first reported that faecal lactoferrin showed a greater overall sensitivity while the second reported that faecal leucocytes of more than five per high power field (hpf) had the best sensitivity and specificity. A meta-analysis of faecal screening tests found that faecal lactoferrin was the most accurate index whereas faecal leucocytes were poor indicators of inflammatory diarrhoea.

The present study was undertaken to assess the usefulness of the faecal lactoferrin assay as a screening test in an unselected population of children presenting with acute diarrhoea in a tertiary care hospital in a developing country and thereby provide guidance on its use in actual clinical practice.

**Subjects and Methods**

Faecal specimens were obtained from 262 children aged 0–12 years with acute diarrhoea (≥3 stools per day for ≤2 weeks) treated by a paediatric unit in a tertiary care hospital in south India. All specimens were collected in clean, screw-top plastic containers. Fresh stool specimens were examined for parasites and a faecal leucocyte count was performed after methylene blue staining. The stool was cultured for bacterial pathogens. Colonies of Escherichia coli were saved for adherence testing which was done in batches by detecting adherence patterns on HEp-2 cell monolayers, as described previously. The stool was also checked for Cryptosporidium parvum using a direct fluorescent antibody test. The presence or absence of gross blood and/or mucus in stool, abdominal pain and fever was noted in each child.

Faecal lactoferrin was assayed using a kit from Tech Labs Inc. (Blacksburg, VA, USA). Faecal specimens were diluted 1:50 and 1:200 using the diluent (buffered protein solution containing 1% sodium azide) provided with the kit. A slide agglutination reaction was then carried out using the reagent provided. The active reagent consisted of latex beads coated with antibodies against lactoferrin. The presence of lactoferrin was detected by a positive agglutination reaction. Appropriate controls were used.

**Statistical analysis**

Statistical analyses were carried out using the Epi Info version 6.0 statistical package. Conventional invasive enteropathogens were defined as Shigella, Campylobacter and Salmonella species. The association of faecal lactoferrin, faecal leucocytes, blood and mucus in stool, fever and tenesmus with isolation of any pathogen or conventional
invasive enteropathogens was tested. The \( \chi^2 \) test (Fischer’s exact test) was used to determine statistically significant differences. Sensitivity, specificity and negative and positive predictive values were calculated for the variables of interest.

Results

There were 262 patients (174 boys and 88 girls), of whom 162 were < 1 year of age and 97 between 1 and 12 years of age.

Detection of enteropathogens

At least one putative enteropathogen was identified in 162 (61.8%) children (Table 1). However, the majority of the pathogens isolated were entero-adherent *Escherichia coli* (EAEC). Shigella, Campylobacter and Salmonella species, which are conventional invasive enteropathogens, accounted for only 42 isolates. Cryptosporidium was also detected in the stool in a significant number of cases.

Faecal lactoferrin

As can be seen from Table 2, the presence of blood in stool or faecal leucocytes > 3/hpf were significantly associated with the isolation of conventional invasive enteropathogens from the faeces, as were fever and tenesmus. However, these features were not very sensitive and only faecal lactoferrin at a 1:50 dilution was sufficiently sensitive in predicting the isolation of these pathogens. When all isolated pathogens were taken into consideration (Table 3), faecal lactoferrin at both 1:50 and 1:200 dilutions showed significant associations, while faecal leucocytes showed a trend towards significant association. When the performance of each of these markers as a screening test for isolating pathogens was evaluated, faecal lactoferrin positivity at a 1:50 dilution showed the best overall characteristics with a high sensitivity and negative predictive value, especially for conventional, culture-positive invasive enteropathogens (89.95%).

Discussion

The presence of faecal lactoferrin at a 1:50 dilution proved to be the most sensitive

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>None isolated</td>
<td>100</td>
</tr>
<tr>
<td>Shigella</td>
<td>27</td>
</tr>
<tr>
<td>Campylobacter</td>
<td>14</td>
</tr>
<tr>
<td>Salmonella</td>
<td>1</td>
</tr>
<tr>
<td>Vibrio</td>
<td>1</td>
</tr>
<tr>
<td>Cryptosporidium</td>
<td>24</td>
</tr>
<tr>
<td>Locally adherent <em>E. coli</em> (LA)</td>
<td>20</td>
</tr>
<tr>
<td>Diffusely adherent <em>E. coli</em> (DA)</td>
<td>61</td>
</tr>
<tr>
<td>Mixed pattern <em>E. coli</em> (LA &amp; DA)</td>
<td>22</td>
</tr>
<tr>
<td>Aggregative <em>E. coli</em></td>
<td>30</td>
</tr>
</tbody>
</table>

Numbers add up to more than 262 because more than one pathogen was detected in 30 children.

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>NPV</th>
<th>PPV</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactoferrin at 1:50 dilution</td>
<td>83.3</td>
<td>28.2</td>
<td>90.0</td>
<td>18.1</td>
<td>0.17</td>
</tr>
<tr>
<td>Lactoferrin at 1:200 dilution</td>
<td>66.7</td>
<td>45.9</td>
<td>87.8</td>
<td>19.0</td>
<td>0.18</td>
</tr>
<tr>
<td>PMNs</td>
<td>45.2</td>
<td>78.6</td>
<td>88.3</td>
<td>48.8</td>
<td>0.002</td>
</tr>
<tr>
<td>Gross blood in stool</td>
<td>35.7</td>
<td>91.4</td>
<td>88.2</td>
<td>44.1</td>
<td>0.000</td>
</tr>
<tr>
<td>Gross mucus in stool</td>
<td>54.3</td>
<td>50.0</td>
<td>88.0</td>
<td>19.7</td>
<td>0.12</td>
</tr>
<tr>
<td>Fever</td>
<td>64.2</td>
<td>60.0</td>
<td>89.8</td>
<td>23.5</td>
<td>0.006</td>
</tr>
<tr>
<td>Tenesmus</td>
<td>45.2</td>
<td>74.1</td>
<td>87.6</td>
<td>25.0</td>
<td>0.019</td>
</tr>
</tbody>
</table>

NPV, negative predictive value; PPV, positive predictive value; PMNs, neutrophils > 3/high power field.
TABLE 3. Performance parameters of different screening variables in predicting the isolation of any pathogen from faeces of children with diarrhoea.

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>NPV</th>
<th>PPV</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactoferrin at 1:50 dilution</td>
<td>78.4</td>
<td>34</td>
<td>49.3</td>
<td>68.4</td>
<td>0.038</td>
</tr>
<tr>
<td>Lactoferrin at 1:200 dilution</td>
<td>63.6</td>
<td>56</td>
<td>48.7</td>
<td>70.0</td>
<td>0.002</td>
</tr>
<tr>
<td>PMNs</td>
<td>29.0</td>
<td>81</td>
<td>41.3</td>
<td>71.1</td>
<td>0.09</td>
</tr>
<tr>
<td>Gross blood in stool</td>
<td>15.4</td>
<td>91</td>
<td>39.9</td>
<td>75.5</td>
<td>0.188</td>
</tr>
<tr>
<td>Gross mucus in stool</td>
<td>51.9</td>
<td>47</td>
<td>61.3</td>
<td>37.6</td>
<td>0.95</td>
</tr>
<tr>
<td>Fever</td>
<td>45.1</td>
<td>58</td>
<td>63.5</td>
<td>39.3</td>
<td>0.72</td>
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<tr>
<td>Tenesmus</td>
<td>32.2</td>
<td>76</td>
<td>40.9</td>
<td>68.4</td>
<td>0.2</td>
</tr>
</tbody>
</table>

NPV, negative predictive value; PPV, positive predictive value; PMNs, neutrophils > 3/high power field.

The presence of lactoferrin in human breast-milk is considered to result in false positives. The presence of faecal polymorphonuclear leucocytes of > 3/hpf and clinical criteria such as gross blood in the stool, fever and tenesmus showed significant association only with the isolation of conventional invasive enteropathogens (Shigella, Salmonella and Campylobacter). Their association with the isolation of other pathogens such as EAEC and Cryptosporidium was not significant. On the other hand, faecal lactoferrin was associated significantly with the isolation of all pathogens. Although Shigella, Salmonella and Campylobacter are traditionally viewed as invasive enteropathogens, both EAEC and Cryptosporidium may be considered invasive since they adhere to or invade epithelial cells and cause mucosal inflammation. The ability of the lactoferrin test to sensitively detect mucosal inflammation might explain why it is more significantly associated with all isolated enteropathogens than faecal polymorphonuclear leucocytes. Since the small intestine rather than the large intestine is the primary site of infection with EAEC and Cryptosporidium, it is possible that inflammation could have produced enough lactoferrin to be detected in stool, while faecal leucocytes might not have been detected because of degeneration during passage through the intestine. Faecal lactoferrin has been demonstrated to be positive even when leucocytes are morphologically lost in swab specimens or by storage and transportation.

The presence of lactoferrin in human breast-milk is considered to result in false positives. Breast-milk specimens tested elsewhere did not give lactoferrin latex agglutination titres of > 1:8 and a coarse agglutination of control normal immunoglobulin G-coated beads have been noted when treated with human milk specimens. Of our patients, 61.8% were < 1 year of age and might still have been on breast-milk. Previous antibiotic use, recorded in as many as 67 (22.6%) of our patients, could have led to a decrease in the test’s specificity by reducing the number of culture-positive specimens. Since we wanted to assess the use of a faecal lactoferrin assay in actual clinical practice, we decided not to exclude these patients.

Owing to its low specificity, the potential of the faecal lactoferrin test lies in its high negative predictive value. The absence of faecal lactoferrin makes the presence of an invasive pathogen unlikely. The faecal lactoferrin test could appropriately be used as a screening test for deciding on stool culture in the paediatric outpatient setting. Based on these results, it is possible to calculate that if negative faecal lactoferrin at a 1:50 dilution were used as a screening test for withholding conventional stool cultures, then 23.4% of stool samples would not have been processed. In this situation, a false negative rate of 16.6% would mean that two-to-three culture-positive samples would have been missed per 100 patients screened. On the
other hand, if faecal PMNs < 3 per/hpf were the cut-off, then as many as 74.8% of stool cultures could have been avoided, but at the cost of a high false-negative rate of 54.8%.

The lactoferrin assay is a useful, quick and easily performed laboratory test that is likely to be useful in the cost-effective diagnosis and management of acute diarrhoeas in the paediatric outpatient setting. Future studies need to focus on the role of breast-milk lactoferrin and develop assays that differentiate leucocyte and breast-milk lactoferrin, and study the clinical implications in actual practice of not doing stool cultures on lactoferrin-negative specimens from children with acute diarrhoea.

References
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