The level of fecal calprotectin with reference to disease activity in children with Crohn’s disease – preliminary data

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Abstract

Current knowledge suggests that calprotectin (CLP), a calcium-binding protein which is found in abundance in neutrophils, may act as a marker of intestinal inflammation. The aim of our study was to estimate the levels of fecal CLP and to relate them to disease activity in children with Crohn’s disease (CD). The study included 16 patients either with active inflammation (n=12) or in remission (n=4). The control group consisted of 5 children with negative family history for inflammatory bowel disease. The stool samples of all patients were collected and the levels of CLP were measured using immunoenzymatic method. Increased CLP concentrations were found in all CD patients with active inflammation, while in those in remission and controls, normal ranges of CLP were detected. The levels of calprotectin corresponded with clinical conditions of the patients as well as with endoscopic and histological findings. It also correlated with the extent of inflammation expressed by C-reactive protein but not the amount of leukocytes. Our preliminary data present that fecal CLP is a simple, non-invasive and sensitive marker of disease activity in those who already have a firm diagnosis of CD.

Key words: Crohn's disease, calprotectin, inflammatory bowel disease

Introduction

Calprotectin (CLP) is a calcium-binding protein with in vitro bacteriostatic and fungistatic properties. It is found in abundance in neutrophils, where it accounts for 60% of the protein in the cytosol. Lower CLP concentrations are found in monocytes and reactive macrophages [6, 8]. CLP is probably involved in the regulation of inflammatory reactions. Study evidence has shown that fecal CLP is significantly increased in Crohn’s disease (CD), ulcerative colitis (UC) and neoplasms, whereas normal values are found in patients with irritable bowel syndrome and in healthy subjects [3, 4]. Clinically active disease CD and UC showed higher CLP levels than those observed in patients with quiescent disease, therefore it proved to be an even stronger predictor of clinical relapse in inflammatory bowel disease, which makes the test a promising non-invasive, cheap, and simple tool for monitoring and optimizing therapy [3, 4, 9]. Other studies revealed that in unselected outpatients referred for colonoscopy, a single measurement of fecal CLP is not sufficiently accurate to identify those with significant disease. However, a normal result can help in ruling out organic disease among patients with diarrhoea and those with abdominal pain and/or constipation in children [2, 9]. It seems that CLP is a sensitive, but not disease specific, marker to easily detect inflammation throughout the whole gastrointestinal tract. It may
help in identifying an organic disease characterized by intestinal mucosa inflammation and in the differential diagnosis of functional bowel disorders.

The aim of this study was to estimate the levels of fecal CLP with reference to disease activity in children with CD, and compare it with laboratory indices of inflammation.

Materials and methods

Patients

We enrolled 16 children with different CD activity and 5 control children to this study. Each patient underwent endoscopy with biopsies in order to estimate histological grading of disease activity. Laboratory tests including blood inflammatory markers such as C-reactive protein (CRP) and leukocytes were also performed. The studied group included patients with active conditions (n=12) and those in remission (n=4). Control group included children randomly chosen from the cohort of patients with negative IBD family history from the Department of Gastroenterology, Hepatology and Immunology in the Children’s Memorial Health Institute.

Determination of fecal calprotectin

CLP was detected in fecal samples of all patients. Stools were collected any time of day in a plain universal containers and frozen at –20°C for long term storage. The levels of fecal CLP were determined using commercial ELISA kit (PhiCal Calprotectin ELISAKit, Immunodagnostik AG) according to manufacture instructions. Each specimen was manually weighted within the range of 80–120 mg and 5 ml of extraction buffer was added not depending on the sample amount. Then, after centrifugation the supernatants of the extractions were taken and diluted 1:50 with wash buffer. Standards, controls and diluted samples were added to wells of microplate coated with a monoclonal anti-human CLP antibody, and then after incubation and washing a peroxidase labeled conjugate was added to each wells. To visualize the reaction, TMB was used as a substrate for peroxidase. Finally, an acidic stop solution was added to terminate the reaction. The CLP concentrations were read from standard curve using ELISA microplate reader (Scientific Point). Results <10 mg/l were recognized as normal ranges, those >15 mg/l as increased values. Results 10-15 mg/l were classified as so called “grey area”.

Statistical analysis

Statistical analysis was performed by non-parametric Mann-Whitney test using Graph Instat program.

Results

CLP was detected in minute amounts in control group whereas CD patients presented different CLP concentrations. In children with remission average concentration of CLP was within normal ranges (10,3 mg/l), however in one children CLP was a slightly higher (17,2mg/l). Histological analysis of tissue specimens of this patient showed minimal inflammatory changes. CLP concentration in patients presenting active conditions was significantly (p<0,004) higher than in those with remission. In all patients except one CLP was much above average (mean concentration 356,1±918,3 3 mg/ml). Histological analysis of tissue specimens of the patients with lowest CLP showed local inflammation limited to ileum, the rest of the intestine was not pathologically changed. We also observed that patients conditions correlated with the extent of blood inflammatory marker such as CRP. The average level of CRP in control group was 0,1±0,18, in children with remission – 0,06±0,06, and in those with active CD – 3,21±2,6, respectively. Leukocytes serum concentrations, however, corresponded neither with clinical nor with endoscopic or histological disease activity. Table 1 presents the exact values of fecal CLP concentrations and blood inflammation markers found in studied groups.

Discussion

Fecal CLP has been proposed as an ideal marker of disease activity in CD. The test is cheap, and simple to perform, with a marker that is stable at room temperature for up to seven days (thereby permitting postage of samples). The usefulness of measuring this neutrophil-derived protein, in the non-invasive

Table 1

Fecal calprotectin (CLP) concentrations and blood inflammation markers of studied groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of children</th>
<th>Leukocytes in blood serum (K/µl)</th>
<th>CRP in blood serum (mg/dl)</th>
<th>Fecal CLP (mg/l)</th>
<th>The minimum and maximum level of CLP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control children</td>
<td>5</td>
<td>6,8±1,4</td>
<td>0,1±0,18</td>
<td>0,01±0,01</td>
<td>0,0 – 0,1</td>
</tr>
<tr>
<td>CD children in remission</td>
<td>4</td>
<td>6,7±1,7</td>
<td>0,06±0,06</td>
<td>10,3±7,6</td>
<td>0,0 – 17,5</td>
</tr>
<tr>
<td>CD children with active inflam</td>
<td>12</td>
<td>6,8±1,8</td>
<td>3,21±2,6</td>
<td>356,1±918,3</td>
<td>11,0-3237,0</td>
</tr>
</tbody>
</table>

The results are expressed as arithmetical means ± standard deviations.
diagnosis and management of IBD and other gut disorders has been investigated for some years both in adults and children as it is often difficult to distinguish between non-inflammatory and chronic inflammatory bowel disease [5]. This leads in many cases to extensive and unnecessary colonscopic examinations, when the CLP test may allow clear differentiation between the two patient groups [1, 7, 10].

Fecal calprotectin correlates well with histological inflammation as detected by colonoscopy with biopsies and has been shown successfully to predict relapses and detect pouchitis in patients with IBD. However, in Tibble’s study there was a significant, albeit weak correlation between fecal CLP and clinical conditions expressed by CDAI (Cron’s Disease Activity Index) [13]. The absence of a stronger correlation was probably caused by the fact that symptoms in CD may derive both from non-inflammatory processes such as fibrotic strictures and bile-salt-induced diarrhoea after previous ileal resection, and inflammatory processes. In our patients both CLP and CRP corresponded properly with their clinical conditions, which means that in our groups disease symptoms were caused mainly by inflammatory process. Additionally, Schmidt et al showed that CLP measures can reveal treatment failure in IBD, thus avoiding prolonged ineffective steroid courses [11]. It may be able to predict relapse in inflammatory bowel disease before this becomes apparent clinically [12]. It is specially helpful in pediatric patients as lasting steroid therapy results in many serious side effects including growth failure.

In our preliminary study we analyzed the levels of fecal CLP with reference to disease activity in children with CD and relate them to blood inflammatory markers such as CRP or amounts of leukocytes. As fecal CLP is significantly increased in IBD patients we supposed to detect higher levels of CLP in CD children comparison with the control group. Indeed, we found differences in fecal CLP between studied groups. Our results proved, that children with negative IBD family history had normal ranges of CLP (<10 mg/l). Significantly increased levels of CLP (>15mg/l) were found in all children with severe inflammation. Patients in remission had CLP slightly higher than normal (average level 10,3±7,6). Additionally, the amounts of CLP corresponded well with endoscopic and histological findings, which is in accordance with other reports. Also CRP correlated properly with our patients clinical conditions. According to those observations, it seems that fecal CLP, although not specific enough to replace invasive tests for the diagnosis of IBD, has real potential to evolve as a simple, cheap, non-invasive, and sensitive marker of disease activity and/or its response to treatment in those who already have a firm diagnosis. Concluding, our preliminary studies showed that fecal CLP may be a marker of intestinal inflammation in patients with already diagnosed CD. It may play a role in clarifying the presence of pathology, reducing the need for endoscopy in some patients, and provide good prognostic information. However, further studies on bigger group are needed.

Acknowledgements

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References