Original Article

Diarrhea in Crohn’s Disease: Investigating the Role of the Ileal Hormone Fibroblast Growth Factor 19

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Abstract

Background: Bile acids [BA] are usually reabsorbed by the terminal ileum, but this process is frequently abnormal in Crohn’s disease [CD]. BA malabsorption occurs, and excess colonic BA cause secretory diarrhea. Furthermore, the hormone fibroblast growth factor 19 [FGF19] is synthesized in the ileum in response to BA absorption and regulates BA synthesis. We hypothesized that reduced serum FGF19 levels will be associated with diarrheal symptoms and disease activity in both ileal-resected [IR-CD] and non-resected CD [NR-CD] patients.

Methods: Fasting serum FGF19 levels were measured in 58 patients [23 IR-CD patients and 35 NR-CD patients]. Disease activity was assessed using the Harvey Bradshaw Index and C-reactive protein [CRP]. Stool frequency, Bristol Stool Form Scale and length of previous ileal resection were recorded. FGF19 levels were also compared with healthy and diarrhea control patients.

Results: FGF19 levels were inversely correlated with ileal resection length in IR-CD patients \( r = -0.54, p = 0.02 \). In NR-CD patients, median FGF19 levels were significantly lower in patients with active disease compared with inactive disease [103 vs 158 pg/ml, \( p = 0.04 \)] and in those with symptoms of diarrhea compared with those without [85 vs 145 pg/ml, \( p = 0.035 \)]. FGF19 levels were inversely correlated with stool frequency, Bristol stool form and CRP in NR-CD patients with ileal disease.

Conclusions: Reduced FGF19 levels are associated with ileal resection, diarrhea and disease activity. FGF19 may have utility as a biomarker for functioning ileum in CD. This study supports a potential role of FGF19 in guiding treatments for diarrhea in Crohn’s disease.

Keywords: Crohn’s disease; bile acid diarrhea; bile acid malabsorption; FGF19; ileitis

1. Introduction

Diarrhea is a major feature of active disease in Crohn’s disease [CD] and a major influence on the ill health experienced by patients. Clinical disease activity scores are heavily influenced by the frequency of liquid stools. Stool frequency and form are among the least subjective components of the symptomatic scores but do not necessarily reflect active inflammatory disease.

The pathophysiology of diarrhea in CD is multifactorial but there are two key factors. Colonic water and electrolyte absorption can be impaired directly by colonic inflammation or indirectly by increased concentrations of bile acids [BA] having secretory effects, referred to as BA-induced diarrhea [BAD]. In healthy subjects, approximately 95% of luminal BAs within the small intestine are reabsorbed at the terminal ileum, with only small amounts entering the colon. In CD...
patients, increased colonic BAs result from impaired reabsorption of BAs at the terminal ileum. This can be either due to ileal inflammation or resection, and may also relate to reduced expression of the ileal apical sodium-dependent bile acid transporter [ASBT]. This bile acid malabsorption [BAM] can be regarded of as part of the usual phenotype of CD.

Options for making the diagnosis of bile acid malabsorption have recently been reviewed. The gold standard test for diagnosing BAM is the measurement of increased concentrations of fecal BA. This is not commonly done as it is unpopular with patients and laboratory staff. Other more indirect markers for BAM are measures of BA retention (Se-homococholic acid taurine [SeHCAT] scan). This involves the oral administration of a radiolabeled BA [SeHCAT] and measurement of its retention within the enterohepatic circulation. Seven-day retention is usually reported, and reduced retention levels reflect increased losses of BA into the feces. Reduced SeHCAT retention levels are associated with chronic diarrhea in quiescent CD in the vast majority [90%] of patients with previous ileal resection and in as many as 50% of those patients without previous ileal resections. The majority of these patients gained symptomatic benefit from the BA sequestrants, such as cholestyramine. More recent studies using a newer BA sequestant, colesevelam, has also demonstrated positive effects on symptoms of diarrhea in CD.

Another indirect marker of BAM is the BA precursor 7α-hydroxy-4-cholesten-3-one [C4]. Serum levels of C4 are a measure of new bile acid synthesis and increase in response to BA losses from the enterohepatic circulation. Measuring C4 involves high performance liquid chromatography [HPLC] or tandem mass spectrometry which requires specialized techniques. Increased levels of C4 have been shown to be associated with chronic diarrhea in pediatric CD.

FGF19 is a hormone produced in the enterocytes of the ileum in response to absorbed BAs. BAs bind to the farnesoid X receptor [FXR] in enterocytes and increase transcription of FGF19. Serum FGF19 levels are thus a direct marker of BA absorption and we have shown that they are reduced in BA malabsorption. Reduced levels of FGF19 have been demonstrated in patients with both ileal resected and non-resected CD. FGF19 is easily measured using commercially available enzyme-linked immunosorbent assay [ELISA]. FGF19 levels and C4 are inversely correlated in several groups including CD. Receiver operating characteristic [ROC] curve analysis has revealed that the optimal level of FGF19 for predicting severe BAM [defined by C4 >50 ng/mL] was >60 pg/mL with 80% sensitivity and 68% specificity in CD. We have previously shown that an optimal level of FGF19 at <145 pg/mL has a sensitivity of 58% and a specificity of 79% to detect C4 levels of >28 ng/mL in a cohort of BAD patients including CD.

We investigated the relationship in patients with CD between serum FGF19 levels and CD disease activity scores, and in particular symptoms of diarrhea [the main symptomatic consequence of BAM]. We also wanted to investigate the contribution of the terminal ileum to the production of serum FGF19 by studying the relationship between FGF19 levels and length of previous ileal resection.

2. Materials and Methods

A total of 58 patients with CD were prospectively recruited and gave informed consent. Blood samples were taken after an overnight fast and serum was separated and stored at -80°C until analysis. Bile acid sequestrants were stopped 48 h prior to sampling in all patients. The diagnosis of Crohn’s had been confirmed in all patients by histology. Of the 58, 23 patients had undergone previous ileal resection [IR-CD] and 35 had no previous intestinal resections [non-resected CD, ‘NR-CD’]. In 18 of the 23 IR-CD patients, the precise length of ileum resected was available according to macroscopic surgical specimen records. For comparison, additional results were included from 68 previously studied patients with idiopathic chronic diarrhea [without evidence of inflammatory bowel disease, previous resections or bile acid diarrhea and SeHCAT >15%] as an ‘idiopathic diarrhea control’ group; 19 previously studied healthy control subjects were also included.

Stool frequency and Bristol Stool Form Scale [BSFS] were recorded for all patients. Disease activity was assessed using the Harvey Bradshaw Index [HBI] and C-reactive protein [CRP] in those with CD. For comparisons between patient groups, diarrhea was arbitrarily defined as a stool frequency of ≥3/day, with stool form of 6 or 7 as in previous studies. A Stool Index [SI] was calculated as [daily stool frequency x BSFS] + loperamide use [mg*3]. None of the NR-CD patients were taking loperamide during this study. Distribution of CD was assessed using standard endoscopic and radiologic techniques. Of the NR-CD patients, 21 had evidence of ileal involvement [10 ileal only, 11 ileo-colonic CD] and 14 had disease affecting the colon only [colonic CD]. Details of the patients are shown in Table 1.

Eleven patients [six with ileal involvement and five colonic] with active disease [HBI≥4] requiring treatment [seven corticosteroids and four biologics] to induce remission were part of a longitudinal study. Repeat fasting samples were taken following resolution of diarrhea during a period of medically induced remission [HBI ≤4]. Where possible, samples were taken before the start of treatments used for inducing remission in patients with active disease. This was possible in all patients starting biologics and in three of the six starting corticosteroids. The three patients who had already commenced corticosteroids during active disease had been on these for no longer than 48 h.

Three patients with CD and ileal resection or inflammation [one NR-CD with ileal involvement, and two IR-CD] underwent serial blood sampling for FGF19 pre and post standardized breakfast and lunch. Four ‘diarrhea control’ patients with chronic diarrhea, who had no evidence of inflammatory bowel disease and SeHCAT retention levels >10% to exclude significant BA malabsorption [range 11–53%] were also studied for comparison.

FGF19 levels were measured using the Quantikine ELISA [R&D Systems, Minneapolis, MO] according to the manufacturer’s protocol. Non-parametric statistics were used and results are expressed as medians with interquartile ranges [IQR]. Mann—Whitney U tests were used for comparisons of medians and Wilcoxon matched signed rank tests were used for paired samples. Spearman rank coefficients were used to express correlations.

2.1 Ethical considerations

The study was approved by the local institutional review board, the Hammersmith, Queen Charlotte’s and Chelsea Hospitals’ Research Ethics Committee.

3. Results

3.1 FGF19 levels in ileal resected [IR]-CD patients

FGF19 levels were lower in IR-CD patients [median 68 pg/mL] than in the healthy controls [231 pg/mL; p = 0.0082], confirming previous findings. We found a significant inverse correlation between the recorded length of ileum resected and the fasting serum FGF19 in IR-CD patients [Spearman r = -0.55, p = 0.02, Figure 1]. Over half of the IR-CD patients, 14/23 [60%], were suffering from symptoms of diarrhea. Five of these patients were taking regular BA sequestrants.
FGF19 and diarrhea in Crohn's disease

3.2 FGF19 levels in non-resected CD patients

The median FGF19 levels in NR-CD [112 pg/ml] were 52% lower than in the healthy controls [p <0.0001]. Comparisons with diarrhea controls were also highly significantly different. Median FGF19 levels were similar in healthy control patients and diarrhea control patients [231 and 237 pg/ml, respectively]. Within the cohort of NR-CD patients, those with diarrhea had median FGF19 levels 41% lower than those without diarrhea [86 vs 145 pg/ml, p = 0.035, Figure 2]. Median FGF19 levels were 35% lower in patients with NR-CD and active disease [as shown by the HBI] compared with those with inactive disease [103 vs 158 pg/ml, p = 0.04 Figure 3].

Of the 19 NR-CD patients with active disease at the time of sampling, 11 of these patients were suffering from diarrhea. Symptoms of diarrhea were present in both colonic CD [5/6 patients] and in those with ileal involvement [6/13 patients]. In the patients with ileal involvement, median FGF19 levels were 71% lower in patients with diarrhea than those without [40 vs 177 pg/ml, respectively]. The similar trend in the patients with colonic CD was non-significant [Figure 4]. Patients with ileal involvement and diarrhea had significantly lower median FGF19 levels than those with colonic CD and diarrhea [p = 0.004]. Significant inverse correlations between FGF19 levels and stool frequency, BSFS, CRP and stool index were found;

Table 1. Details of the patients studied.

<table>
<thead>
<tr>
<th>Details</th>
<th>NR-CD</th>
<th>IC-CD</th>
<th>Idiopathic diarrhea</th>
<th>Healthy controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ileal</td>
<td>colonic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>21</td>
<td>14</td>
<td>23</td>
<td>68</td>
</tr>
<tr>
<td>Gender</td>
<td>Female</td>
<td>10 [48%]</td>
<td>10 [71%]</td>
<td>12 [52%]</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>11 [52%]</td>
<td>4 [29%]</td>
<td>9 [48%]</td>
</tr>
<tr>
<td>Clinical indices</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HBI</td>
<td>6 [3–7]</td>
<td>4 [0–6]</td>
<td>4 [2–6]</td>
<td>N/A</td>
</tr>
<tr>
<td>Resection length median [cm]</td>
<td>40</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resection length range [cm]</td>
<td></td>
<td>10–96</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current medications</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-ASAs</td>
<td>4 [20%]</td>
<td>3 [20%]</td>
<td>3 [13%]</td>
<td>-</td>
</tr>
<tr>
<td>Immunomodulators</td>
<td>7 [33%]</td>
<td>4 [29%]</td>
<td>8 [34%]</td>
<td>-</td>
</tr>
<tr>
<td>Biologics</td>
<td>4 [20%]</td>
<td>2 [14%]</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>5 [24%]</td>
<td>2 [14%]</td>
<td>1 [0.5%]</td>
<td>-</td>
</tr>
<tr>
<td>No medication</td>
<td>7 [33%]</td>
<td>5 [36%]</td>
<td>11 [48%]</td>
<td>-</td>
</tr>
</tbody>
</table>

NR-CD, non-resected Crohn’s Disease; IC-CD, ileal-resected Crohn’s Disease; CRP, C-reactive protein; BSFS, Bristol Stool Form Scale; 5-ASAs, aminosalicylates; HBI, Harvey Bradshaw Index.

Medians and interquartile ranges are shown, or percentages where indicated.

Figure 1. Significant inverse correlation between fasting FGF19 vs previous ileal resection length obtained from surgical records. Spearman’s rank correlation coefficient is shown. NR-CD, non resected Crohn’s disease.

Figure 2. Fasting levels of FGF19 in groups of patients with and without diarrhea [stool frequency ≥3, BSFS >5]. NR-CD, non-resected Crohn’s disease; IC-CD, ileal-resected Crohn’s disease. Median and inter quartile ranges are shown; Mann—Whitney U test was used for comparisons.
these inverse correlations were strongest in those patients with ileal disease [Figure 5, Table 2].

Using a cut-off value of 145 pg/ml [previously shown to have an 80% sensitivity and 60% specificity as a marker of excess BA losses assessed by SeHCAT retention levels in PBAD], levels below 145 pg/ml were significantly commoner in those with symptoms of diarrhea in all 35 NR-CD patients studied [p = 0.028, Fisher’s exact test]. Using a lower cut-off value of 60 pg/ml [previously shown to have an 80% sensitivity and 68% specificity as a marker of more severe BA losses assessed by C4 levels in IBD] revealed that levels below 60 pg/ml were significantly associated with symptoms of diarrhea in the 21 NR-CD patients with ileal disease [p = 0.046, Fisher’s exact test].

3.3 Serial FGF19 levels during the day
Repeated FGF19 sampling pre and post meals were compared between patients with CD and diarrhea control patients. Serial
FGF19 levels remained low with no evidence of diurnal variation pre and post meals in the three CD patients compared with the four diarrhea control patients [Figure 6].

3.4 Serial FGF19 in patients on treatment
In the longitudinal studies, initial serum FGF19 levels were measured in patients in clinical remission on different days, to look for intra-individual variability. There was no significant difference between the paired levels \( n = 6 \), Wilcoxon \( p = 0.68 \). Median FGF19 levels were significantly lower in the patients with ileal involvement while their disease was active [median 40 pg/ml, range 3–92] compared with paired levels taken after medically induced clinical remission and resolution of diarrhea [median 140 pg/ml, range 106–184, \( p = 0.03 \), paired Wilcoxon]. Median FGF19 levels were not statistically different in the five patients with colonic CD during active disease [median 113 pg/ml, range 50–120] compared with paired levels taken during medically induced clinical remission and resolution of diarrhea [median 70 pg/ml, range 40–120, \( p = 0.03 \), Wilcoxon, Figure 7]. The median time interval between samples taken during disease activity and medically induced remission was not significantly different between these groups [median interval: colonic 98 days, range 28–168 vs ileal involvement 83 days range 40–159, \( p = 0.66 \) Mann–Whitney U test].

4. Discussion:
Overall, significantly reduced median FGF19 levels were associated with active disease and more specifically with symptoms of diarrhea in NR-CD. Median FGF19 levels were found to be significantly lower in those patients with ileal disease and diarrhea than in those with disease restricted to the colon and diarrhea. In addition to this, FGF19 levels increased in NR-CD patients who received anti-inflammatory treatments leading to clinical remission and resolution of diarrhea in those patients with ileitis but not colonic CD. As excess fecal BAs are more likely to contribute to the diarrhea of patients with ileal rather than those with pure colonic disease, these

Table 2. FGF19 correlations with clinical indices.

<table>
<thead>
<tr>
<th>Indices</th>
<th>Stool freq/ day</th>
<th>BSFS</th>
<th>SI</th>
<th>HBI</th>
<th>CRP [mg/dl]</th>
</tr>
</thead>
<tbody>
<tr>
<td>NR-CD all ( [n = 35] )</td>
<td>Spearman ( r )</td>
<td>-0.34</td>
<td>-0.36</td>
<td>-0.35</td>
<td>-0.24 -0.37</td>
</tr>
<tr>
<td>( p )-Value</td>
<td>0.04</td>
<td>0.03</td>
<td>0.04</td>
<td>0.15</td>
<td>0.03</td>
</tr>
<tr>
<td>NR-CD ileitis ( [n=21] )</td>
<td>Spearman ( r )</td>
<td>-0.49</td>
<td>-0.45</td>
<td>-0.52</td>
<td>-0.39 -0.58</td>
</tr>
<tr>
<td>( p )-Value</td>
<td>0.02</td>
<td>0.04</td>
<td>0.02</td>
<td>0.08</td>
<td>0.006</td>
</tr>
<tr>
<td>Diarrhea controls ( [n = 68] )</td>
<td>Spearman ( r )</td>
<td>0.07</td>
<td>0.04</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td>( p )-Value</td>
<td>0.56</td>
<td>0.80</td>
<td>0.07</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

BSFS, Bristol Form Stool Index; SI, Stool Index; HBI, Harvey Bradshaw Index; CRP, C-reactive protein.

Figure 6. Diurnal variation in FGF19 levels in CD patients vs non-inflammatory diarrhea control patients. Closed shapes and solid lines = three patients with CD and diarrhea [one with ileal disease and the other two with previous ileal resections]. Open shapes and dotted lines = 4 idiopathic diarrhea control patients [without evidence of primary BA diarrhea, SeHCAT retention >10%]. Arrows indicate standardized meals [9 am and 12 midday].

Figure 7. FGF19 levels during a period of disease activity followed by a repeat sample during clinical remission after anti-inflammatory treatments used to induce remission [biologics or corticosteroids]. Two out of five patients with colonic CD were treated with corticosteroids and five out of six patients with ileitis were treated with corticosteroids. The remaining patients were treated with biologics [infliximab or adalimumab] only. The open circles represent patients who were still receiving corticosteroids when the repeat FGF19 sample was taken [paired values were compared using the Wilcoxon signed rank test].
differences support the role of FGF19 as a marker of both BAD and ileal involvement in NR-CD.

The association between ileal inflammation and markers of BA malabsorption have previously been demonstrated in NR-CD patients using older markers of BA loss techniques.\textsuperscript{6} Similar to our findings with FGF19, inverse relationships between symptoms of disease activity and SeHCAT retention values have also been demonstrated previously in NR-CD.\textsuperscript{12,13} A significant inverse correlation was found between fasting FGF19 and length of terminal ileal resection despite the possible confounding variables that may affect this [such as total small bowel length, type of anastomosis, and gut microbial metabolism of bile acids]. We also observed a complete loss of normal post-prandial diurnal variation in FGF19 levels throughout the day in the patients with ileal resection or inflammation. The pattern is similar to those observed in healthy controls who remain fasted throughout the day.\textsuperscript{14} These findings suggest that the terminal ileum is critical to the basal production of serum FGF19.

The higher FGF19 levels post treatment in patients with ileitis may also have been influenced by the use of corticosteroids. Budesonide treatment has been shown to increase the expression of ASBT protein in the terminal ileum of healthy volunteers.\textsuperscript{15} In vitro reporter assays have revealed a glucocorticoid receptor [GR] response element within the promoter region of ASBT, and various animal models have demonstrated an increase in ASBT expression and function after corticosteroid treatments.\textsuperscript{16,17} Hence, the higher post treatment FGF19 levels seen in some patients in this study may have been influenced by corticosteroid-induced increases in ASBT expression and BA absorption.

It is important to note that FGF19 synthesis occurs after absorbed BAs bind and activate the intracellular BA receptor farnesoid X receptor [FXR].\textsuperscript{18} The most FXR-responsive BA regulatory gene in human ileum is FGF19.\textsuperscript{19} However, FXR activation may also enhance anti-inflammatory genes and down-regulate pro-inflammatory genes.\textsuperscript{20,21} Oxbeticholic acid, a potent semisynthetic FXR agonist, has demonstrated anti-inflammatory and antifibrotic properties within the liver,\textsuperscript{22,23} and possibly within the intestine.\textsuperscript{31,32} Perhaps the most relevant application in human IBD will be in ileal CD, as this phenotype is most likely to be associated with a deficit in endogenous intestinal FXR activation.\textsuperscript{33} Hence reduced fasting circulating FGF19 levels may also serve as a biomarker of impaired FXR activation.

FXR agonists may therefore have dual functional effects in ileal CD. Stimulating FGF19 may have beneficial symptomatic effects by the inhibitory actions on BA synthesis reducing excess colonic BAs. The FXR agonist obeticholic acid has shown positive effects on diarrhea symptoms in an open-label phase II study of primary and secondary BAD patients.\textsuperscript{24} Additional positive effects on ileal FXR-mediated barrier function may also result and require further study.

It has already been established that the majority of patients with chronic diarrhea and previous ileal resection are likely to be suffering from BAD and will have symptomatic benefit following treatment BA sequester therapy.\textsuperscript{9} However, using reduced FGF19 as a marker of BAD in this study has revealed that the majority of NR-CD patients with ileal involvement, disease activity and symptoms of ileal disease are also likely to gain symptomatic benefit from treatments for BAD. FGF19 levels may have utility in the work-up of an NR-CD patient suffering from an acute flare of disease activity and diarrhea. FGF19 associated with higher levels of FGF19 is more likely to be associated with colonic inflammation and less likely to respond to such treatments. Levels less than 60 pg/ml are likely to be associated with severe BAM, ileal inflammation and BAD. These patients are likely to benefit from BA sequestrants or potential future treatments to restore FGF19 levels and reduce excess colonic BA, such as FXR agonists.

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**Conflict of Interest**

JW has been a consultant for Intercept Pharmaceuticals. The other authors declare no conflicts.

**References**