ROLE OF FECAL CALPROTECTIN AS A BIOLOGICAL MARKER IN DIFFERENTIAL DIAGNOSIS BETWEEN FUNCTIONAL AND ORGANIC COLONIC DISORDERS

THESIS

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ABSTRACT

Background: There is growing evidence showing the importance of fecal calprotectin assay in differentiating organic from functional colonic disorders. It is a simple, non-invasive technique than colonoscopy.

Objectives: The aim of this study was to assess the value and sensitivity of fecal calprotectin (FCP) in patients with signs and symptoms suggestive of colonic disorders and to distinguish between organic and functional colonic pathology, to avoid unnecessary invasive techniques.

Methods: A single stool sample was collected from each patient presenting with signs and symptoms suggestive of colonic disorders (abdominal pain, chronic diarrhea, weight loss, and anemia).
Calprotectin concentration was measured by commercial ELIS System.

Results: ESR results showed significant difference between organic non IBD group and all other studied groups. While, non significant difference was detected in between control, IBS & IBD groups. Comparison of fecal calprotectin results between all studied groups demonstrated no significant difference between control and IBS group, while a significant difference was noticed between IBS and IBD group and IBS and organic non IBD group.

Conclusion: Fecal calprotectin has potential as a screening procedure to differentiate between patients with functional colonic disorders from other organic intestinal diseases as calprotectin concentration is rarely within the normal range in patients with IBD or colorectal cancer / adenomatous polyps.

Keywords: Fecal Calprotectin, Functional colonic disorders, inflammatory bowel disease.

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List of Abbreviations

CC	**Colorectal Cancer**
CD	**Crohn's Disease**
FAP	**Familial Adenomatous Polyposis**
FC	**Fecal Calprotectin**
g	**Gram**
HNPCC	**Hereditary Non-Polyposis**
IBD	**Inflammatory Bowel Disease**
IBS	**Irritable Bowel Syndrome**
JP	**Juvenile Polyposis**
P-JP	**Peutz-Jeghers Polyposis**
UC	**Ulcerative Colitis**
ug	**Microgram**

INTRODUCTION

Functional colonic disorders are a frequent cause of primary care visits and gastroenterology consultations that on some occasions even requires hospitalization. The main challenge is to differentiate it from an organic pathology, which may mean multiple diagnostic tests with the risks that these may entail from their side effects and with loss of work hours.

Many clinical symptoms present in functional colonic disorders are also common in other organic disorders, in particular inflammatory bowel disease (IBD). When there is suspicion of IBD, it is important to carry out a colonoscopy and biopsy in order to confirm the diagnosis and to establish the inflammation extent. This is an invasive technique with an inherent risk of complication, and general anesthesia may be needed especially in children.

The classic biochemical parameters of inflammation, ESR (erythrocyte sedimentation rate) and C-reactive protein, lack diagnostic specificity and sensitivity. In recent years calprotectin in feces is becoming a new useful marker for diagnosis of intestinal inflammatory pathology. Also various studies have shown that there is an association between calprotectin levels and severity of inflammation; as a consequence, this could be used to monitor treatment response and to predict risk of relapse.

Nowadays an improved assay is available to determine calprotectin using ELISA system. When compared to the original method it requires smaller samples, and increases the performance of the extraction solution. It is a simple, sensitive, accurate, reproducible and cheaper technique.

The objective of this study was to evaluate the use and sensitivity of fecal calprotectin in patients with signs and symptoms suggestive of IBD with the aim of avoiding unnecessary invasive techniques, and to be able to discriminate between organic and functional GI pathology.

LARGE INTESTINE

The large intestine is approximately 1.5 m in length and extends from the ileum to the anus. Its size decreases gradually from the caecum, where it is approximately 7 cm in diameter, to the sigmoid, where it is approximately 2.5 cm in diameter **(Keshav 2003)**. The large intestine has four segments: the caecum, colon, rectum and anal canal. The colon is divided into four sections: the ascending colon, transverse colon, descending colon and sigmoid colon.

Blood supply of the colon

The blood supply to the right colon as far as the distal transverse colon is through the superior mesenteric artery and then by the inferior mesenteric artery, which anastomoses with the pudendal vessels to a minor degree in anal canal. The venous drainage from the right colon is predominantly to the right hepatic lobe and from the rectum and left colon to the left hepatic lobe owing to the "streaming" of blood in the portal vein **(Ellis 2004)**. The lymphatic vessels pass along the respective arteries to the Para-aortic glands, with smaller glands located on the colonic wall and midway between this and the aorta **(Watson 2000)**.

Nerve supply of the colon

The nerve supply of the smooth muscle fibers, themselves in direct communication with each other by gap junctions, consists of an intramural plexus with connections from the extrinsic nerves. The sympathetic components pass from the aortic plexuses along the arteries. These nerves carry inhibitory impulses mediated through α, β, and non-α, and non-β purine receptors. There are possibly also some stimulatory fibers. The main motor or parasympathetic fibers come from the vagus nerve to the gut segment supplied by the superior mesenteric artery; more distally the gut receives parasympathetic nerves from the sacral outflow. These sandwich the inferior mesenteric vessels and run up the bowel wall to reach as far as the distal transverse colon **(Siegfried 2002)**.

Caecum

The small intestine terminates at the posteromedial aspect of the caecum. The caecum is fixed to the right side near the iliac crest. At the opening to the caecum there is a fold of mucous membrane known as the ileocaecal valve, which allows the passage of materials from the small intestine into the large intestine and prevents the reflux of

contents from the colon back into the ileum. The caecum is a dilated portion and has been described as a blind pouch approximately 6cm in width and 8cm in length. It is continuous with the ascending colon superiorly and has a blind end inferiorly. Attached to the caecum is a coiled tube closed at one end called the vermiform appendix and it is usually 8–13 cm in length although this can vary from 2.5 cm to 23 cm and has the same structure as the walls of the colon; however, it contains more lymphatic tissue **(Moore & Dalley 1999).**

Colon

Ascending colon

The ascending colon is approximately 15 cm long and joins the caecum at the ileocaecal junction. The ascending colon is covered with peritoneum anteriorly and on both sides, however, its posterior surface is devoid of peritoneum. It ascends on the right side of the abdomen to the level of the liver where it bends acutely to the left. At this point it forms the right colic or hepatic flexure and then continues as the transverse colon **(Thibodeau & Patton 2002).**

Transverse colon

This is a loop of colon approximately 45 cm long that continues from the hepatic flexure across to the left side of the abdomen to the left colic flexure. It passes in front of the stomach and duodenum and then curves beneath the lower part of the spleen on the left side as the left colic or splenic flexure and then passes acutely downward as the descending colon **(Watson 2000).**

Descending colon

This section of the colon passes downwards on the left side of the abdomen to the level of the iliac crest. It is approximately 25 cm in length. The descending colon is narrower and more dorsally situated than the ascending colon.

Sigmoid colon

The sigmoid colon begins near the iliac crest and is approximately 36 cm long. It ends at the centre of the mid-sacrum, where it becomes the rectum at about the level of the third sacral vertebra. It is mobile and is completely covered by peritoneum and attached to the pelvic walls in an inverted V shape **(Ross et al 2001).**

Rectum

The rectum is approximately 13 cm in length and begins where the colon loses its mesentery. It lies in the posterior aspect of the pelvis and ends 2–3 cm anteroinferiorly to the tip of the coccyx, where it bends downwards to form the anal canal **(Tortora & Grabowski 2002).**

Anal canal

This is the terminal segment of the large intestine and is approximately 4 cm in length opening to the exterior as the anus. The mucous membrane of the anal canal is arranged in longitudinal folds that contain a network of arteries and veins. The anus remains closed at rest. The anal canal corresponds anteriorly to the bulb of the penis in males and to the lower vagina in females and posteriorly it is related to the coccyx. The internal anal sphincter is composed of smooth muscle and is the upper of the two sphincters. It is about 2.5cm long and can be palpated during rectal examination. It controls the upper two thirds of the anal canal. The external sphincter is made up of skeletal muscle and is normally closed except during elimination of feaces. The nerve supply is from the perineal branch of the fourth sacral nerve and the inferior rectal nerves **(Martini 2004).**

Lining of the GI tract

The walls of the GI tract consist of four layers. These are from outwards inwards:

- Adventitia.
- Muscularis.
- Submucosa.
- Mucosa.

Adventitia

The adventitia or outer layer consists of a serous membrane composed of connective tissue and epithelium. In the abdomen it is called the visceral peritoneum. It forms a part of the peritoneum, which is the largest serous membrane of the body **(Thibodeau & Patton 2002).**

Muscularis

The muscularis mostly consists of two layers of smooth muscle, which contract in a wave like motion. The two smooth muscle layers consist of longitudinal fibers in the outer layer and circular fibers in the inner layer. Between the two muscle layers the blood vessels, lymph vessels and the major nerve supply to the GI tract can be found. The nerve supply is called the mesenteric or Auerbach's plexus, and it consists of both sympathetic and parasympathetic nerves. It is mostly responsible for GI motility, which is the ability of the GI tract to move spontaneously **(Tucker 2002; Martini 2004).**

Submucosa

The submucous layer is highly vascular as it houses plexuses of blood vessels, nerves and lymph vessels, and tissue. It consists of connective tissue and elastic fibers. It also contains the submucosal or Meissner's plexus, which is important in controlling the secretions in the GI tract **(Martini 2004).**

Mucosa

The mucosa is a layer of mucous membrane that forms the inner lining of the GI tract. It is made up of three layers:

• A lining layer of epithelium composed of absorptive (tall columnar), goblet and endocrine cells. Ratio of the number of tall columnar cells to goblet cells is 4:1. Paneth cells are usually present in the caecum & proximal colon (usually confined to the crypt bases).

• The lamina propria, which supports the epithelium by binding it to the muscularis mucosa and is made up of loose connective tissue that contains blood and lymph vessels;

• The muscularis mucosa layer, which contains smooth muscle fibers **(Siegfried 2002).**

Functional Bowel Disorders

Functional bowel disorders are functional gastrointestinal disorders with symptoms attributable to the middle or lower gastrointestinal tract. These include the irritable bowel syndrome (IBS), functional bloating, functional constipation, functional diarrhea, and unspecified functional bowel disorder. To separate these chronic conditions from transient gut symptoms, they must have occurred for the first time ≥6 months before the patient presents, and their presence on ≥3 days per month during the last 3 months indicates current activity **(Thompson et al 2000)**. Previous diagnostic criteria presumed the absence of a structural or biochemical disorder.

Irritable Bowel Syndrome (IBS)

Definition

IBS is a functional bowel disorder in which abdominal pain or discomfort is associated with defecation or a change in bowel habit, and with features of disordered defecation.

Epidemiology

Throughout the world, about 10%–20% of adults and adolescents have symptoms consistent with IBS, and most studies find a female predominance **(Saito et al 2002), (Longstreth 2005)**. IBS symptoms come and go over time, often overlap with other functional disorders **(Whitehead et al 2002)**, impair quality of life **(Wilson et al 2004)**, and result in high health care costs **(Longstreth et al 2003)**.

Pathophysiology

Numerous mechanisms have been supposed to explain the cause of disease **(Rothstein 2000).** The failure of laboratory studies to show any morphologic, histological, microbiologic, or biochemical abnormalities in patients with IBS previously supported the concept that IBS is primarily a disorder of gastrointestinal motility **(Olden & Schuster 1998).** More current theory suggests that the syndrome represents a multiple of aberrations. Dysmotility, visceral hypersensitivity, abnormal brain-gut responses, psychosocial factors, sensor motor dysfunction, and alterations in local reflex mechanisms or extrinsic and central neural connections have been postulated as the basis for disease **(Rothstein 2000).**

Altered gut motility

There have been many reports, often conflicting, of abnormal motility in IBS. In healthy persons, high-amplitude peristaltic contractions are seen six to eight times in 24 hours. These contractions tend to cluster around meals and bowel movements **(Gorard et al 1995).** The frequency of these contractions appears to be markedly diminished in constipation-predominant IBS. Some studies have demonstrated hypermotility and high-amplitude contractions over long segments of intestine in patients with IBS during episodes of crampy abdominal pain **(Kellow et al 1991).** Other studies have not found these differences and were unable to show correlation between symptoms, including diarrhea, and motor abnormalities **(Gorard et al 1994).**

Visceral hypersensitivity

In the majority of patients with IBS, sensory perception of pain is abnormal **(Maxwell et al 1997).** Patients with IBS had pain at lower volumes of visceral balloon distension **(Maxwell et al 1997).** Increasing evidence suggests that long term changes in the thresholds and gain of the visceral afferent pathways are present in patients with functional bowel disorders; this type of change is referred to as *visceral hyperalgesia* **(Mayer & Gebhart 1994).** This exaggerated sensory perception may explain the symptoms that are produced in IBS even with small volumes of gas or stool **(Almy 1951).**

Psychosocial factors

Psychological disorders are frequently seen concomitantly in patients with IBS **(Walker et al 1992).** In fact, several studies have demonstrated that approximately 50% of patients with IBS have a coexisting psychiatric disorder at the time of presentation **(Blanchard et al 1990).** A recent survey of 206 female patients with IBS reported a 44% incidence of physical or sexual abuse in childhood, a rate that was 3 to 11 times higher than in control patients **(Drossman et al 1990).** Although no specific personality profile appears to be associated with IBS **(Frigerio et al 1992),** certain psychiatric disorders are increasingly prevalent with IBS. Panic disorder, characterized by paroxysmal attacks of intense fear and autonomic arousal, has been associated with IBS. Other studies have shown that patients with IBS tend to be more preoccupied with illness than healthy subjects; they report more illness not related to IBS and more debilitation from other illness than a control group **(Sheikh & Wright 1999).** This association may suggest that

illness behavior persists because it has been supported by social reinforcement, which provides secondary gain **(Sheikh & Wright 1999).**

Brain-gut axis

The enteric nervous system (ENS) is a diffuse network of ganglia running from the smooth muscle of the esophagus to the rectum. It independently controls gut function, including the migrating motor complex and peristalsis. Neural transmission within the ENS is controlled by a plethora of neurotransmitters and neuromodulatory peptides. It is the integration of intestinal motor, sensory, autonomic, and central nervous system activity interacting through the brain-gut axis that is responsible for normal gastrointestinal function. The numerous neurotransmitters found in the brain and gut which link visceral afferent sensation and intestinal motor function with higher cortical centers include enkephalins, substance P, calcitonin gene-related polypeptide, nitric oxide, cholecystokinin, and 5-hydroxytryptamine (5-HT) **(Azia & Thompson 1998; Mayer et al 1998; Gershon 1999).** The gut contains more than 95% of the body's 5-HT, and concentrations of 5-HT in the bowel is on an order of magnitude higher than that in the brain. Depending on conditions, 5-HT can make the bowel contract or relax, secrete or not secrete **(Gershon 1999).** It appears that 5-HT stimulates both vagal and enteric afferent nerve fibers, initiating responses as diverse as nausea, vomiting, intestinal secretion, and the peristaltic reflex. Recent studies have shown that 5-HT3 receptors exist on gut afferent neurons and that their activation by locally released 5-HT leads to visceral nociceptive stimulation in addition to increased neuronally mediated motor and secretory activity **(Humphrey et al 1999; Lydiard et al 1994).**

Diagnostic Criteria for Irritable Bowel Syndrome

Recurrent abdominal pain or discomfort at least 3 days per month in the last 3 months associated with 2 or more of the following:

1. Improvement with defecation.
2. Onset associated with a change in frequency of stool.
3. Onset associated with a change in form (appearance) of stool.

Criteria fulfilled for the last 3 months with symptom onset at least 6 months prior to diagnosis.

Discomfort means an uncomfortable sensation not described as pain and in pathophysiological researches and clinical trials, a pain/discomfort frequency of at least 2 days a week during screening evaluation for subject eligibility **(Longstreth 2005).**

Classification:

The Rome II working team suggested 2 systems for classifying patients into diarrhea predominant and constipation predominant and subgroups based on the first 6 supportive symptoms **(Thompson et al 2000; Thompson et al 1999),** includes: Diarrhea predominant: 1 or more of b, d, f, and none of a, c, e, or 2 of b, d, f, and 1 of a or e (c, hard/lumpy stool excluded); and Constipation predominant:1 of a, c, e, and none of b, d, f, or 2 of a, c, e, and 1 of b, d, f **(Thompson et al 2000).**

Supportive symptoms:

Not a part of the diagnostic criteria and include abnormal stool frequency.

[a] ≤3 bowel movements per week.

[b] >3 bowel movements per day, abnormal stool form.

[c] lumpy/hard stool.

[d] loose/watery stool.

[e] Defecation straining.

[f] Urgency, or also a feeling of incomplete bowel movement, passing mucus, and bloating.

Clinical Evaluation

Diagnosis depends on careful interpretation of the temporal relationships of pain/discomfort, bowel habit, and stool characteristics. Pain/discomfort related to defecation is likely to be of bowel origin, whereas that associated with exercise, movement, urination, or menstruation usually has a different cause. Fever, gastrointestinal bleeding, weight loss, anemia, abdominal masses, and other “alarm” symptoms or signs are not due to IBS, but may accompany it. Heartburn, fibromyalgia, headache, backache, genitourinary symptoms, and others are often associated with IBS, but are not useful in diagnosing it. These symptoms increase as the severity of IBS increases and may be associated with psychological factors **(Whitehead et al 2002)**. Obviously, a common disorder such as IBS may coexist with organic gastrointestinal disease. There are no discriminating physical signs of IBS, but abdominal tenderness may be present. Tensing the abdominal wall increases local tenderness associated with