Scholars LITERATURE

La Prensa Medica Argentina

Research Article

DOI: https://doi.org/10.47275/0032-745X-234
Volume 106 Issue 5

Role of Fecal Calprotectin in Diagnosis of Colorectal Carcinoma and Inflammatory Bowel Disease

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Abstract

Background: Testing for fecal calprotectin has become an accepted technique of non-invasive screening for colorectal neoplasia and IBD activities. The ability of fecal calprotectin testing to distinguish between patients with inflammatory bowel disease and non-inflammatory bowel disease with FDA-approved indication for the fecal calprotectin test. Generally, studies have shown that the fecal calprotectin test is reasonably accurate for this purpose when used with clinical suspicion of colorectal neoplasia and inflammatory bowel disease based on examination and history.

Aim of study: The study aims to assess and compare the sensitivity and specificity of fecal calprotectin and in depending on Colonoscopy for IBD activity and colorectal neoplasia among Iraqi patients.

Patients and Methods: Fecal calprotectin was assessed in 80 tested patients in IBD, colorectal neoplasia with patients referred for Colonoscopy and histopathological assessment. The fecal calprotectin was tested by calprotectin ELISA assay kit as positive or negative results.

Results: The calprotectin cut-off level representing a positive value was equal or greater than 50 µg/g as stated by the manufacturer ,showing in this study, sensitivity 84% and specificity 60% with positive predictive value 77.8%, and negative predictive value 69.2% with accuracy 75% and p value <0.004 which is significant in predicting IBD activities, while sensitivity 89.3% and specificity 58.3% with positive predictive value 83.3%, and negative predictive value 70% with accuracy 80% and p value <0.001 which is significant in predicting colorectal carcinoma.

Conclusion: Fecal calprotectin is helpful as an adjunctive tool in overall evaluation of patients with colorectal neoplasia and IBD to monitor disease activity. It is less invasive than use of Colonoscopy and can help guide management.

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Citation: Al-Kufi YY, Ghadhban JM (2020) Role of Fecal Calprotectin in Diagnosis of Colorectal Carcinoma and Inflammatory Bowel Disease. Prensa Med Argent, Volume 106:5. 234. DOI: https://doi.org/10.47275/0032-745X-234.

Received: March 11, 2020; **Accepted:** April 02, 2020; **Published:** April 06, 2020

Introduction

Differentiating patients with colorectal neoplasia and inflammatory bowel disease from those with functional disorders may be difficult in patients with nonspecific symptoms, such as diarrhea, and abdominal pain. Most often, invasive procedures, such as endoscopy, with biopsies are required. A marker or a set of markers that can accurately detect inflammation and monitor disease activity would be useful clinically. Fecal calprotectin because of their direct contact with the intestinal mucosa, may be more accurate in determining gastrointestinal inflammation [1]. The mucosa of actively inflamed colon contains a large number of neutrophils. Fecal proteins derived from neutrophils have the potential to be ideal markers of intestinal inflammation and lesion. One of these fecal proteins, calprotectin has been studied extensively [2].

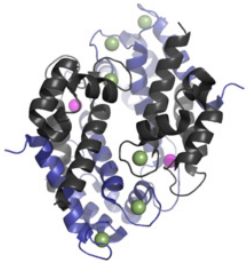
Fecal Calprotectin

Fecal calprotectin is a calcium- and zinc-binding neutrophilic cytosolic protein that is found in proportion to the degree of inflammation present. Fecal calprotectin is resistant to colonic bacterial degradation, is evenly distributed and stable in the stool for up to 1 week at room temperature, and can be measured by a commercially available enzyme-linked immunosorbent assay (ELISA) with less than 5 g of stool [2]. Calprotectin plays a regulatory role in the inflammatory process and is a very sensitive marker for detection of inflammation in the gastrointestinal tract. Calprotectin has developed a rapid test (FDA approved previously) that can be done within minutes and correlates well with its conventional ELISA equivalent. Fecal markers are more accurate than serum calprotectin markers. However, fecal markers are not specific for IBD, colorectal neoplasia and may be elevated other gastrointestinal conditions such as food intolerance, NSAID enteropathy, microscopic colitis, and gastroenteritis [3].

Fecal lactoferrin is also a fecal biomarker and a sensitive and specific marker in identifying intestinal inflammation but not involved in this study [4]. Fecal calprotectin can still differentiate inflammatory disease or colorectal mass from functional bowel disorders. Fecal calprotectin correlated well to mucosal healing and histologic improvement.

Calprotectin is a complex of the mammalian proteins S100A8 and S100A9 [5,6] In the presence of calcium, calprotectin is capable of sequestering the essential nutrients manganese and zinc [6,7]. This metal sequestration affords the complex antimicrobial properties [6,7].

Calprotectin is the only known antimicrobial manganese sequestration protein complex [8]. Calprotectin comprises as much as 60% of the soluble protein content of neutrophil cytosol [6,9,10] and is secreted by an unknown mechanism during inflammation [11]. Fecal calprotectin has been used to detect intestinal inflammation and can serve as a marker for inflammatory bowel diseases [9,12].



Structure

Crystal structure of Mn^{2+} and Ca^{2+} loaded calprotectin, showing two S100A8-S100A9 dimers. The grey and blue chains represent S100A8 and S100A9, respectively. Purple spheres represent Mn^{2+} and green spheres represent Ca^{2+}. Only one manganese ion can bind per calprotectin dimer. The human homologue of calprotectin is a 24 kDa dimer [8] and is formed by the protein monomers S100A8 (10,835 Da) and S100A9 (13,242 Da) [11]. The primary structure of calprotectin can vary between species.

Metal binding

Calprotectin has a high affinity for calcium, zinc and manganese [9,10,13] Each of S100A8 and S100A9 contain two EF-hand type Ca^{2+} binding sites [8,11] and calprotectin is able to bind a total of four calcium ions per dimer or eight calcium ions per tetramer [14]. Calcium binding induces a conformational change in the complex that improves its affinity for transition metals, and promotes tetramer formation [6,8] A maximum of two transition metal ions may bind to each calprotectin S100A8-S100A9 dimer [8].

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A calprotectin dimer can bind only one manganese ion with high affinity, and it can do this only in the presence of calcium [8,15]. Zinc can bind at two sites within the calprotectin dimer, and this can occur in the absence of calcium [6]. Calcium, however, improves calprotectin's affinity for zinc [8]. While calprotectin metal-binding occurs at the interface of S100A9 and S100A8 monomers, the independent monomers have some capacity for zinc binding and may contribute to zinc homeostasis within mammals [6,8].

His6 coordination of Mn^{2+} in calprotectin. S100A8 histidine residues are colored grey, S100A9 histidine residues are colored purple.

The first of the two calprotectin metal-binding sites consists of a His3Asp motif, with S100A8 contributing two histidine ligands (His83 and His87), and S100A9 contributing a histidine and an aspartic acid ligand (His20 and Asp30) [8]. The second site can coordinate metals through a tetra-histidine (His4) or a hexahistidine (His6) binding motif. In the case of His4 binding, S100A8 coordinates through both His17 and His27 while S100A9 coordinates through His91 and His95.8 In hexahistidine binding two further histidine residues, His103 and His105, are recruited from the C-terminal end of S100A9 to enable octahedral coordination of the transition metal. Manganese is bound by the calprotectin dimer at this His6 site [8]. Zinc can be bound to either of the sites that form at the interface between S100A8 and S100A9 monomers [8,15].

Antimicrobial properties

Transition metals are essential to the survival of all organisms [16]. Mammals strictly limit metal availability as a part of the innate immune system, and this helps prevent infection by microbes and fungi [16]. Calprotectin was first described in the 1980s as a mammalian antimicrobial protein that acts through the sequestration of zinc. [5,6,8]. It is now known that calprotectin also has antibacterial and antifungal properties that arise from its ability to sequester manganese [7,8]. Calprotectin is the only known antimicrobial agent that acts through manganese sequestration [8]. Calprotectin constitutes up to 60% of soluble protein content in the cytosol of neutrophil granulocytes [5,9,10] and it can be found at a lower concentration in monocytes, macrophages, and squamous epithelial cells [6,9,10]. Calprotectin enters into pus and abscess fluid during neutrophil cell death, along with other antimicrobial proteins [6].

Mammalian cells secrete calprotectin during the inflammatory response. For instance, calprotectin is secreted in the mouth during inflammation of the gingiva and oral candidiasis infection [17,18]. People who have mutations in the calprotectin gene appear susceptible to serious gum infections [17]. Manganese sequestration by calprotectin is likely important during lung inflammation [7]. The exact mechanism by which S100A8 and S100A9 is secreted by mammalian cells during inflammation remains unknown [11].

Calprotectin becomes available in the intestinal lumen via leukocyte shedding [5] active secretion [6,10] cell disturbance, and cell death [5,6]. This results in elevated fecal calprotectin levels, which can be detected in the stool [5,10]. Elevated fecal calprotectin levels therefore indicate migration of neutrophils into the intestinal mucosa, which occurs during intestinal inflammation and colorectal neoplasia [5]. As people with bowel neoplasia and active inflammatory bowel diseases (IBD) such as ulcerative colitis or Crohns disease have as much as a 10-fold increase in fecal calprotectin levels, [9] the measurement of fecal calprotectin can serve as a biochemical test for these diseases.

Although,a fecal calprotectin is regularly used as diagnostic marker for IBD [12]. Fecal calprotectin tests can also use in distinguishing patients with irritable bowel syndrome from those with IBD [5,10]. Calprotectin is useful as a marker, as it is resistant to enzymatic degradation, and can be easily measured in faeces [19]. Although fecal calprotectin correlates significantly with disease activity in IBD [20]. Importantly, intake of proton pump inhibitor is associated with significantly elevated calprotectin values [21]. Furthermore; positive fecal calprotectin does not help in localizing site of tumor or

distinguishing ulcerated colitis from Crohns disease [5]. Calprotectin levels vary depending on age, comorbidity, and may vary day-to-day within individuals. Fecal calprotectin could be used as a preliminary screen in otherwise functional patients suspected of having IBD, or as a means of following mucosal healing [5].

IBD and fecal calprotectin

Multiple studies have shown that fecal calprotectin is predictive of activities in IBD; values above 50 μg/g are often predictive of activities, whereas levels below 50 μg/g are often predictive of inactivities [24]. Fecal calprotectin has been shown to discriminate IBS from Crohn's disease with excellent sensitivity and specificity, although it is important to know that false-positive testing occurs with NSAID use [26,27].

Colorectal neoplasia and fecal calprotectin

Fecal calprotectin is elevated in patients with colorectal cancer. The majority of patients with colorectal cancer have increased fecal concentration of calprotectin. One single fecal spot seems to be sufficient for determination of the calprotectin level. Measurement of fecal calprotectin may become of value as a marker for colorectal cancer, although calprotectin, similar to fecal occult blood (FOB) tests, is a non-specific test for colorectal neoplasia. Further investigation of its specificity is therefore needed. It is unlikely that calprotectin can predict specific colonic disorders [28].

NSAID induced enteropathy and fecal calprotectin

Fecal excretion of calprotectin can be used to assess intestinal inflammation that caused by NSAID enteropathy. Fecal calprotectin levels correlate with fecal excretion of indium-111.22 After 2 weeks of diclofenac, 75% of patients had increased fecal calprotectin levels and 68% showed injury on CE [23].

Patient and methods

This is a prospective cross sectional study including 80 patients who attended at Gastroenterology and Hepatology hospital in Medical City in Baghdad during period from July 2015-June 2016.

Patients who taken selectively in two groups, first, 40 colorectal neoplasia (10 males, 18 females were CRN, total 28) and (4 males, 8 females were control, total 12), second, 40 IBD (19 males, 6 females were IBD, total 25) and (10 males, 5 females were control, total 12) with age above and below 50 years but starting age was 40 years up to 70 years).

Detailed history was taken including the age, sex, history of melena, weight loss, anemia, BPR, abdominal discomfort, colorectal neoplasia, IBD, gastroenteritis, NSAID and PPI use. All patients underwent routine and invasive investigations like CBC, ESR, CRP, Colonoscopy Pentax and Olympus with biopsies for histopathological assessments. Single samples of stool obtained from patients sent them for fecal Calprotectin and provided before any administered bowel preparation.

Patients who diagnosed as IBD and colorectal neoplasia according to diagnostic criteria like histopathology were also involved to provide samples for fecal Calprotectin [1,3].

Patients who taking NSAID were excluded from this study as these have been shown to cause an enteropathy and causing false positive fecal calprotectin [21,22]. All patients attended our hospital explained to them nature of the study.

Fecal Calprotectin measurement

Calprotectin was measured in a single stool sample in all patients and returned it on the same day. Patients were instructed to collect the sample must be 24 hours before bowel preparation for endoscopy. The method was done by automated Calprotectin ELISA system Biotek (washer and reader) that measures quantitative calprotectin, optical density (405 nm: measured on a Micro Tracer plate reader. The calprotectin cut-off level representing a positive value was equal or greater than 50 μg/g as stated by the manufacturer [29].

Aliquots of 100 mg feces were homogenized in 5 mL extraction buffer and homogenized for 45 seconds. Microtitre plates were coated by adding of anticalprotectin [33]. Calprotectin standards 3.75-60 mg/l [30-32].

Picture A: Reader

Picture B:washer

Boitek spectrophotometry

Colonoscopy and histopathological assessment

The Colonoscopy, Pentax and Olympus system was done for patients with colorectal neoplasia and IBD by well-trained doctor under supervision of expert and the biopsies examined microscopically by the expert pathologist in our hospital.

Results

A total of 80 patients who had been included in the study. There were two groups, the first group was colorectal neoplasia 28 patients with 12 control and the second group was IBD 25 patients and 15 control.

Colorectal neoplasia

There were 7 (25%) CRN and 2 (16.7%) control below or equal 50 years but starting age was 40 years and 21 (75%) CRN and 10 (83.3%) control more than 50 years age up to 70 years, with gender distribution males 10 (35.7%) CRN and 4 (33.3%) control, with females 18 (64.3%) CRN and 8 (66.7%) control and as shown in (Table 1, Figures 1 and 2).

IBD

There were 20 (80%) IBD and 9 (60%) control below or equal 50 years but starting age was 40 years and 5 (20%) IBD and 6 (40%) control more than 50 years age up to 70 years, with gender distribution

Table 1: Demographic characteristics of patients with colorectal neoplasia and control group for age and gender.

		Colorectal neoplasia (n=28)	Control (n=12)
Gender	Male	10(35.7%)	4(33.3%)
	Female	18(64.3%)	8(66.7%)
Age/years	≥50	21(75%)	10(83.3%)
	<50	7(25%)	2(16.7%)

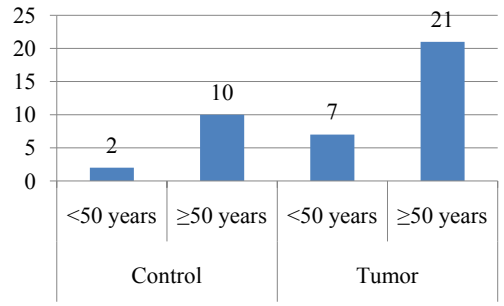

Figure 1: Age distribution of colorectal neoplasia and control group.

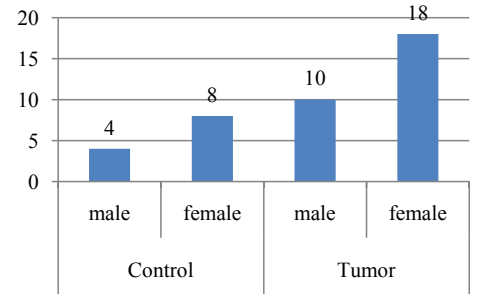

Figure 2: Gender distribution of colorectal neoplasia patients and their control group.

Table 2: Demographic characteristics of patients with IBD and control group.

		IBD (n=25)	Control (n=15)
Gender	Male	19(76%)	10(66.7%)
	Female	6(24%)	5(33.3%)
Age/years	≥50	5(20%)	6(40%)
	<50	20(80%)	9(60%)

for males 19 (76%) IBD and 10 (66.7%) control, with females 6 (24%) IBD and 5 (33.3%) control and as shown in (Table 2, Figures 3 and 4).

Discussion

Calprotectin, although present in blood, enters the bowel lumen as part of an inflammatory process rather than bleeding from the tumour 1. Calprotectin is a cytosolic protein in neutrophilic granulocytes and macrophages [33,35,36]. We have made a direct comparison of fecal calprotectin and Colonoscopy in patient who had have colorectal neoplasia and IBD. In this study, use of fecal calprotectin for patients referred for Colonoscopy with finding consistent with colorectal neoplasia, 83.3% had strongly positive test with negative predictive value 70.% (p value significant <0.001). This study consistent with Tibble J, et al. (2001) where 78.2% positive test than patients without findings 21.8% [37]. The sensitivity for colorectal neoplsia using calprotectin was 89.3% in comparison with 100% sensitivity of Colonoscopy. These accords well with the findings of Kristinsson J (1998) who demonstrated a sensitivity of 94% for colorectal neoplasia when using fecal calprotectin [34].

In IBD Tibble J, et al. (2001) found that an abnormal calprotectin

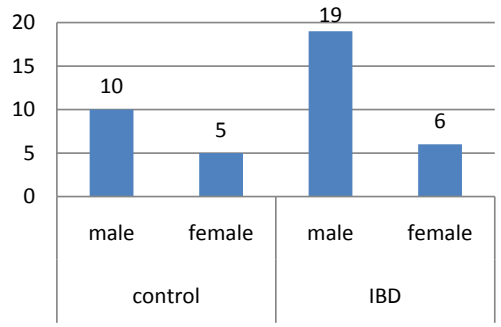

Figure 3: Gender distribution of IBD patients and their control group.

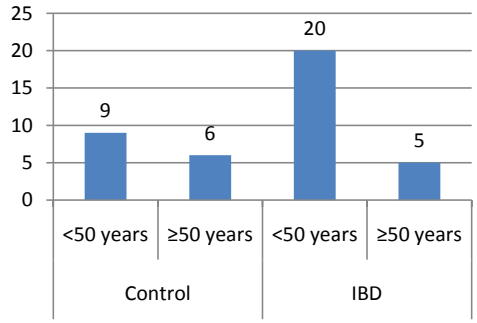

Figure 4: Age distribution of IBD and their control group.

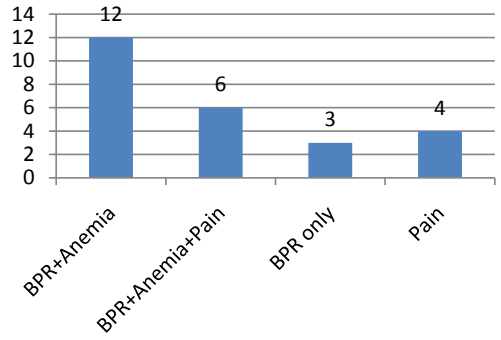

Figure 5: Clinical presentation of IBD,(n=25).

Table 3: Validity of fecal calprotectin compared to colonoscopy in diagnosis of colorectal neoplasia.

		Neoplasia	Non Neoplasia	Total	P value
Fecal calprotectin	Positive	25	5	30	0.001
	Negative	3	7	10	
Total		28	12	40	

Sensitivity=89.3%, Specificity=58.3%, Positive predictive value=83.3%, Negative predictive value=70%, Accuracy=80%.

Table 4: Validity of fecal calprotectin compared to colonoscopy in diagnosis of IBD activities.

		IBD		Total	P value
		Active	Inactive		
Fecal calprotectin	Positive	21	6	27	0.004
	Negative	4	9	13	
Total		25	15	40	

Sensitivity=84%, Specificity=60%, Positive predictive value=77.8%, Negative predictive value=69.2%, Accuracy=75%.

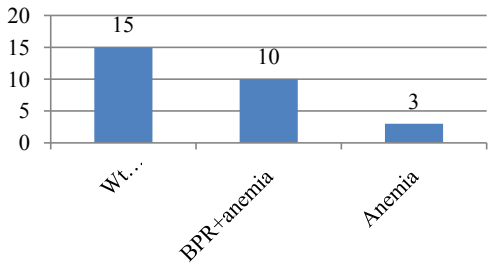

Figure 6: Clinical presentation of colorectal neoplasia (n=28) .

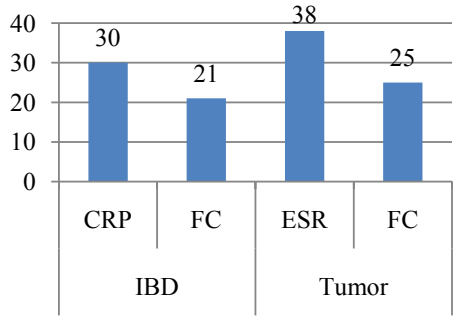

Figure 7: CRP, ESR and Fecal calprotectin in IBD and Colorectal neoplasia cases.

test to be 95% sensitive and 91% specific for IBD activities [26,37] had an odds ratio (OR) for inflammatory bowel disease confirmed by colonoscopic examination of 27.8 (significant P".0001).

In this study we found that abnormal fecal calprotectin to be sensitive 84% and specific 60% for differentiates IBD and its activities, p value (P<0.004) and statistically significant. This result was consistent with Tibble J, et al. (2001) (p value was significant p<0.0001).

Limitations

The limitation of this study we did not systematically assess the presence of mucosal lesions in the small-bowel, We acknowledge that this is a limitation of our study as increased fecal calprotectin has been shown in small-bowel enteropathy,41 this might have influenced fecal measurement of calprotectin.

Conclusion

- The study showed that fecal calprotectin values are elevated significantly in patients with colorectal neoplasia but does not help in localizing site of colorectal neoplasia.
- Role of fecal calprotectin in diagnosis of colorectal neoplasia in comparison with Colonoscopy is valuable as the test is simple, cheap and less invasiveness.
- We confirmed excellent ability of fecal calprotectin to identify mucosal lesion in IBD and can be used for monitoring IBD activities, yet, positive fecal calprotectin does not distinguishing ulcerative colitis from Crohns disease

Recommendations

- Clinicians should be aware to judge the cost effectiveness, inappropriate use endoscopy especially in follow up and use simple tests like fecal calprotectin as simple and cheap test.
- Further prospective studies directly comparing recommended guidelines of appropriateness for Colonoscopy with fecal calprotectin measurements are warranted to establish the value of a biomarker.

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