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FECAL CALPROTECTIN AND LACTOFERRIN IN COMPARISON WITH COLONOSCOPY IN DIFFERENTIATING OF INFLAMMATORY BOWEL DISEASE FROM IRRITABLE BOWEL SYNDROME

[1,] *Eman G. Behiry, [2]Rami A. Metwalli and [2]Ahmed M. Hussein

[1]Department Clinical Patholog, Faculty of Medicine Benha University, Egypt
[2]Department Internal Medicine, Faculty of Medicine Benha University, Egypt

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ABSTRACT

Inflamatory Bowel Disease (IBD) is an idiopathic ailment, most likely including an invulnerable response of the body to its own particular intestinal tract. Separation amongst chrons disease (CD) and ulcerative colitis (UC) is still troublesome. Faecal neutrophil-derived proteins (mainly calprotectin and lactoferrin) assessment is receiving increasing attention as promising tools to differentiate organic bowel diseases and functional bowel diseases. The aim of this study is to evaluate and compare fecal calprotectin and lactoferrin as non invasive rapid test in comparison with colonoscopy invasive test in diagnosis and differentiating inflammatory bowel disease (Ulcerative Colitis / Crhon'sdisease) from Intestinal Bowel Syndrome. Subjects and Methods: This study has been conducted on 30 patients divided as follows: Group I: 10 patients with IBD (4 males and 6 females), Group II: 10 patients with IBS(5 males and 5 females) (Diagnosis was based on Rome III criteria),Group III: 10 healthy volunteers (4 males and 6 females) as control group , they were recruited from the outpatient clinic of internal medicine in Banha University Hospital. Results: Cer Test Calprotectin + Lactoferrin combo card tests had a sensitivity, specificity, PPV and NPV of 90%, 100%, 100% and 90.91% respectively by CerTest Calprotectin + Lactoferrin combo card tests with AUC of 0.95. Conclusion: calprotectin-lactoferrin appear to be a clinically useful marker in differentiating IBD from IBS, moreover it can be used as an activity marker in IBD

INTRODUCTION

Inflamatory Bowel Disease (IBD) is an idiopathic ailment, most likely including an invulnerable response of the body to its own particular intestinal tract (Rowe, 2007). It incorporates ulcerative colitis (UC) and Crohn`s illness (CD) (Shapiro, 2006). Separation amongst CD and UC is still now and then troublesome. In roughly 10% of instances of colitis, no separation can be made. Ailment in these patients is delegated uncertain colitis (IC). IC was viewed as an interim grouping until a last determination was set up amid postliminary (Joossens *et al.,* 2002).As serum markers of inflammation can be elevated in a variety of conditions, it seems likely that faecal markers of inflammation, in absence of enteric infection, would be more specific for IBD. The fecal markers lactoferrin (Lf), calprotectin (Cal) is able to differentiate IBD from IBS (irritable bowel syndrome) (Langhorst, 2008).

Corresponding author: Eman G. Behiry,
Department Clinical Pathology, Faculty of Medicine Benha University, Egypt.

Although the classic IBS symptoms of lower abdominal pain, bloating, and alteration of bowel habits are easily recognizable to most physicians, diagnosing IBS remains a challenge. This is in part caused by the absence of autonomic and physiologic markers (Olden, 2002). Several longitudinal follow-up studies, suggest that a diagnosis based on positive-symptom criteria, requires no additional investigation because the likelihood of organic disease is quite low. Only if alarm symptoms "red flags" are present, which include: Onset after 50 years of age, Weight loss, Refractory diarrhea, Nocturnal symptoms, Blood in stools, History of antibiotic use, Family history of colon cancer (Malagelada and Malagelada, 2006). Faecal neutrophil-derived proteins (mainly calprotectin and lactoferrin) assessment is receiving increasing attention as promising tools to differentiate organic bowel diseases and functional bowel diseases, they could be the putative ideal test for non-invasive assessment of intestinal inflammation (Costa *et al.,* 2007). The combined use of presence/absence of alarm features, Rome criteria and calprotectin test proved to be a non-invasive, effective mean of screening patients for organic intestinal disease (Costa *et al.,* 2007).

Endoscopic examination and histological analysis of biopsy specimens remain the "gold standard" methods for detecting and quantifying bowel inflammation; however, these techniques are costly, invasive and repeated examinations are unpopular with patients (Bossuyt, 2006). Calprotectin, a 36 KDa calcium and zinc binding protein, is probably the most easy to measure, resistant to proteolysis and stable in stool for 7 days, and thus has been proposed as a simple non invasive investigative tool (Fagerberg *et al.,* 2005).

Lactoferrin is a glycoprotein that is created by neutrophils, mononuclear phagocytes and epithelial cells and is contained in the secretory liquids, for example, salivation and bosom milk. Its capacity is to square bacterial development by constraining the accessibility of iron. Lactoferrin may serve as a marker of aggravation in the digestive tract. The significant reason for fecal neutrophils in patients with constant loose bowels is incessant provocative digestive tract ailment of the colon (i.e., Crohn's Disease and Ulcerative Colitis). Lactoferrin has been likewise concentrated on as an indicator of contamination with intrusive enteropathogens in kids with loose bowels. Bacterial provocative the runs might be brought on by Shigella, Salmonella, Campylobacter and Clostridium difficile (Amemoto *et al.,* 1996)

SUBJECTS AND METHODS

Subjects: This prospective study has been conducted on 30 patients presented with manifestations suggesting (IBD) inflammatory bowel diseases versus (IBS) irritable bowel syndrome; they were recruited from the outpatient clinic of gastroenterology in a Benha University Hospital in the period from April 2015 to July 2015. Ethical approval was taken from Benha University.

They were divided as follows

Group I: 10 patients with IBD.
Group II: 10 patients with IBS.(Diagnosis was based on Rome III criteria).Group III: 10 healthy persons as control.

Exclusion criteria

- Patients with positive stool culture.
- Patients with past history of any malignant.
- Patients with past history of major gastrointestinal surgical procedures
- Patients with liver cell failure, chronic renal failure or congestive heart failure.
- Patients with bleeding tendency.
- Patients on non steroidal anti-inflammatory drugs.

Written consents were obtained from all participants in the study.

Group III: 10 healthy persons as control.

Methods: All patients were subjected to;

a- Full history taking with special emphasis on abdominal pain, weight loss, rectal bleeding, diarrhea, constipation, malaise, lethargy, anorexia, nausea, tenesmus, abdominal distension, passage of mucus, vomiting and low-grade fever. Past history of appendectomy or other operations and positive family history of IBD.

b- Full clinical examination

c-Laboratory investigations

- CBC, AST, ALT,
- ESR and CRP titre.
- Complete stool analysis to exclude the presence of infection.

Fecal Calprotectin and Lactoferrin

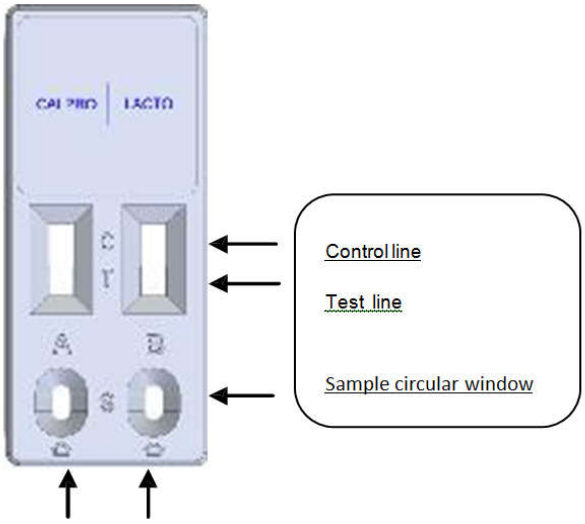

Fig. 1. CerTest Calprotectin+Lactoferrin combo card

I) **(**http://www.certest.es/products/combos/calprolacto1.html#combo**)**.

Fecal Calprotectin and Lactoferrin

Materials provided: CerTest Calprotectin+Lactoferrin combo card tests and Stool collection tubes with diluents (Fig. 1.)

Specimen collection and preparation: Stool samples were collected in clean containers, stored in the refrigerator (2-8ºC) prior to testing, the sample was thawed and to room temperature and homogenized before testing.

Specimen reparation

- The stick was used to pick up sufficient sample quantity. Then, the stick was introduced once into 4 different parts of the stool sample, fecal sample was added to the stool collection tube.
- The tube was closed with the diluent and stool sample, then the tube was shaked in order to assure good sample dispersion.

Assay procedure

- Four drops were dispensed in the circular window in CerTest Calprotectin+ Lactoferrin card, marked with the letter A and 4 drops, using the same tube, in the circular window marked with the letter B.

- The result was read at 10 minutes. The intensity of the red colored bands in the test lines (T) in the results windows will vary depending on the concentration of human calprotectin and human lactoferrin present in the specimen. Internal procedural controls are included in the test. The green lines appearing in the control lines (C) in the results windows are internal controls, which confirm sufficient specimen volume and correct procedural technique.

Measurement of activity indices in IBD patients: Crohn's Disease Activity Index (CDAI) scores between 150 and 220 are mild and scores between 221 and 400 are moderate; more than 400 points is considered severe disease, and remission is defined as CDAI score less than 150, while UC activity was measured by the Truelove and Witts Severity Index (mild, moderate and severe) (Tamboli, 2007). The Crohn's Disease Activity Index consists of eight factors, each summit after adjustment with a weighting factor. The components of the CDAI and weighting factors are the following (1) Number of liquid/very soft stools in 7 days (weighting factor 2), (2) Sum of 7 days abdominal pain ratings (Subjective grading: 0 = none, 1 = mild, 2 = moderate, 3 = severe) (weighting factor 5) (3) The sum of 7 days general well-being ratings (Subjective grading: 0 = well, 1 = average, 2 = poor, 3 = very poor, 4 = terrible) (weighting factor 7) (4) Extraintestinal features (1 per finding): perianal disease (fissure/fistula/abscess), external fistula, mucocutaneous or cutaneous lesions, iritis/uveitis, arthritis/arthralgia, febrile episode in the past week (> 100 °F) (weighting factor 20) (5) Use of antidiarrheal drugs (Lomotil or opiates): yes = 1, no = 0 (weighting factor 30) (6) Presence of abdominal mass: none = 0, equivocal = 2, definite = 5 (weighting factor 10) (7) Hematocrit deviation from normal (Typical {average 47 in males and 42 in females} minus current hematocrit) (weighting factor 6) (8) Percentage deviation from standard weight: 100 x [(standard weight-actual body weight) / standard weight] (weighting factor 1)

Total score between 0 and 750, sum score based on a 7 day aggregate of each item scored daily and current hematocrit and weight measurement. Total CDAI = sum of each item score x its weighting factor =1x2 + 2x5 + x7+ 4x20 + 5x30 + 6x10 + 7x6 + 8x1 (Tamboli, 2007). The Truelove and Witts Severity Index in measurement of UC activity: (1) Mild: Less than four bowel movements per day; scant amounts blood , No fever or tachycardia , Mild or absent anemia , ESR less than 30 mm/h (2) Moderate: Somewhere in between mild and severe (3) Severe: Six or more bowel movements per day, Mean evening body temperature greater than 37.5°C, mean pulse rate greater than 90 beats per minute, Hemoglobin less than 10.5 g/dL , Erythrocyte sedimentation rate (ESR) greater than 30 mm/h (Tamboli, 2007).

Statistical analysis: Data were analyzed using Statistical Program for Social Science (SPSS) version 18.0. Quantitative data were expressed as mean± standard deviation (SD). Qualitative data were expressed as frequency and percentage.

- Independent-samples t-test of significance was used when comparing between two means.
- Chi-square (X^2) test of significance was used in order to compare proportions between two qualitative parameters.
- Receiver operating characteristic (ROC curve) analysis was used to find out the overall predictivity of parameter in and to find out the best cut-off value with detection of sensitivity and specificity at this cut-off value.
- Sensitivity: Probability that a test result will be positive when the disease is present and Specificity: Probability that a test result will be negative when the disease is not present
- PPV (positive predictive value): probability that the disease is present when the test is positive and NPV (negative predictive value): probability that the disease is not present when the test is negative
- Probability (P-value)

RESULTS

This study was conducted on 10 patients with IBD; 7 patients with active IBD (2 UC patients and 5 CD patients) and 3 patients with inactive IBD (2 UC patients and 1 CD patients) versus 10 patients with IBS (10 IBS-D patients) in addition to 10 healthy persons as control (Table 1).

IBD patients were 4 males (40%) and 6 females (60%), their mean age was 27.70±9.06, while IBS patients were 5 males (50%) and 5 females (50%), their mean age was 38.9±12.72, and controls were 4 males (40%) and 6 females (60%), their mean age was 39.90±12.40. The mean age and sex difference was statistically non significant (P>0.05)

Table 1. Distribution of patients according to pathology

Pathology Result	IBD	
	No.	%
Active CD	5	50
Active UC	2	20
Inactive CD	1	10
Inactive UC	2	20

Results of this study a highly statistically significant difference between IBD and control groups as regard CALP with sensitivity: 90%, specificity: 100%, PPV: 100%, NPP: 90.91%. There is a statistically significant difference between IBD and control as regard LACOF, using Chi-square test (Table 2).

Table 2. Comparison between IBD and control as regard CALP and LACOF

	IBD (CALP)	Control	IBD (LACOF)
	No.(%)	No.(%)	No.(%)
Positive	9 (90%)	0 (0%)	7 (70%)
Negative	1 (10%)	10 (100%)	3 (30)
x2	18.000		7.571
p-value	<0.001		0.011

Also there is no statistically significant difference between IBS and control as regard CALP, or LACOF, using Chi-square test P=0.305 (Table 3).

Table 3. Comparison between IBS and control as regard CALP and LACOF

	IBS (CALP)	Control	IBS (LACOF)
	No.(%)	No.(%)	No.(%)
Colitis (positive)	1(10%)	0(0 %)	1(10%)
Negative	9 (90%)	10(100%)	
x2	1.053	1.053	
p-value	0.305	0.305	

There is a highly statistically significant difference between IBD and IBS as regard CALP (P=<0.001), and a statistically significant difference as regard LACOF (P= 0.006), using Chi-square test (Table 4 and 5). The table 4 shows statistically significant difference between pathology results and LACOF, using Chi-square test. In IBS group, there is a statistically significant difference between pathology results and CALP, LACOF using Chi-square test, with p-value <0.05 (Table 6 and 7)

Table 4. Comparison between CALP regarding pathology results in IBD group

Pathology Result	CALP			
	Positive		Negative	
	No.	%	No.	%
Active CD	5	55.6	0	0
Active UC	2	22.2	0	0
Inactive CD	1	11.1	0	0
Inactive UC	1	11.1	1	100

Table 5.Comparison between LACOF regarding pathology results in IBD group

Pathology Results	LACOF			
	Positive		Negative	
	No.	%	No.	%
Active CD	5	71.43	0	0.00
Active UC	2	28.57	0	0.00
Inactive CD	0	0.00	1	33.33
Inactive UC	0	0.00	2	66.67

Table 6. Comparison between CALP regarding pathology results in IBS group

Pathology Result	CALP			
	Positive		Negative	
	No.	%	No.	%
Colitis	0	0.0	1	11.1
Non specific colitis	1	100.0	0	0.0
Normal	0	0.0	8	88.9
x2		10.000		
p-value		0.007		

Table 7. Comparison between LACOF regarding pathology results in IBS group

Pathology Result	LACOF			
	Positive		Negative	
	No.	%	No.	%
Colitis	0	0	1	11.1
Non specific colitis	1	100	0	0.0
Normal	0	0	8	88.9
x2		10.000		
p-value		0.007		

DISCUSSION

Inflamatory Bowel Disease (IBD) is an idiopathic ailment, most likely including an invulnerable response of the body to its own particular intestinal tract. Separation amongst chrons disease (CD) and ulcerative colitis (UC) is still troublesome. Faecal neutrophil-derived proteins (mainly calprotectin and lactoferrin) assessment is receiving increasing attention as promising tools to differentiate organic bowel diseases and functional bowel diseases. The aim of this study is to evaluate and compare fecal calprotectin and lactoferrin as non invasive rapid test in comparison with colonoscopy invasive test in diagnosis and differentiating inflammatory bowel disease (Ulcerative Colitis / Crhon'sdisease) from Intestinal Bowel Syndrome

In the present study, active IBD patients had a statistically highly significant higher TLC, PLT count than IBS patients, inactive IBD patients and controls, however there was no statistically significant difference between inactive IBD patients, IBS patients and control. This was in agreement with Tibble *et al.,* (2000) who found higher TLC and PLT count in active CD patients in comparison to patients with quiescent disease, IBS patients and control, while no significant difference was found on comparing the results of patients with quiescent disease, IBS patients and control. This could be clarified by the way that these parameters are expanded in incendiary conditions as intense stage reactants. As respect fecal calprotectin, it gave off an impression of being clinically valuable in separating IBD from IBS. So also, Tibble *et al.,* (2000) found that all patients with CD had increased faecal calprotectin concentrations which differed significantly from patients with IBS and normal controls. Carroccio *et al.,* (2003) concluded that their data fully confirmed that the faecal calprotectin assay in adults could distinguish between IBD and IBS being higher in patients with IBD. Also, Schoepfer *et al.,* (2007) found that faecal calprotectin was significantly elevated in IBD patients compared to IBS patients.

In addition, faecal calprotectin was helpful in differentiating active from inactive IBD patients. Several studies reported the same results as in the study done by Sipponen *et al.,* (2007) who found that faecal calprotectin level was significantly lower in CD patients with inactive than with active disease. Xiang *et al.,* (2008) found that the faecal calprotectin concentrations were significantly higher in the active UC than in the inactive UC patients. Also, Langhorst (2008) found that the UC or CD patients with active inflammation demonstrated significantly higher levels of faecal calprotectin when compared to patients with inactive inflammation as well as patients with IBS. Moreover, faecal calprotectin values were higher in inactive IBD patients compared to IBS patients and control. Likewise, Tibble *et al.,* (2000) found that inactive IBD patients had higher faecal calprotecin compared to IBS patients and control. Also, Xiang *et al.,* (2008) results showed that faecal calprotectin concentrations were higher in the patients with inactive UC than in the controls. Furthermore, in the present study faecal calprotectin correlated significantly with the TLC, PLT count, ESR, CRP and UC activity index.

This was in agreement with Tibble *et al.,* (2000) who found a good correlation between faecal calprotectin, TLC, PLT count, ESR and CRP in CD patients. Also, this was supported by Xiang *et al.,* (2008) who found a good correlation between the concentrations of faecal calprotectin, ESR, CRP and UC activity index in UC patients. However, this study found an insignificant, weak correlation between faecal calprotectin and CD activity index. Conflicting resuts were found in other studies concerning this correlation, as Tibble *et al.,* (2000) found a weak correlation between CD activity index and faecal calprotectin. While, Vermiere *et al.,* 2004 found a good correlation, but Gaya *et al.,* (2005) found no significant correlation.

This might be explained by the small sample size of CD patients. Most importantly in this study, faecal CerTest Calprotectin+Lactoferrin combo card tests had a sensitivity, specificity, PPV and NPV of 90%, 100%, 100% and 90.91% respectively by CerTest Calprotectin+Lactoferrin combo card tests with AUC of 0.95. However in other studies; Tibble *et al.,* (2000) found that at a cut off point of 30 mg/l using the Calprest® test, faecal calprotectin had 100% sensitivity and 97% specificity in discriminating between active Crohn's disease and the irritable bowel syndrome. The high cut off value in this study could be explained by the presence of active CD patients' sample. While, Tibble *et al.,* 2002 found that faecal calprotectin at cut off value of 10 mg/L using the Calprest® test had maximal sensitivity and specificity of 89% and 79% respectively with a PPV of 76% and a NPV of 89% in separating patients with natural and non natural intestinal infections. Carroccio *et al.,* 2003 found that the calprotectin esteem with the most astounding indicative exactness was 170 µg/g stool, utilizing the Calprest® test: it was 100% delicate and 95% particular in separating CD from IBS grown-up patients.

The explanation for this high cutoff value was that the CD patients included in this study were only 9 patients who were suffering from active disease, also the Calprest® value in µg/gm is 2.5 times higher than when measured in mg/L. Schoepfer *et al.,* (2007) used another method named the PhiCal test in the measurement of faecal CerTest Calprotectin+Lactoferrin combo card tests and found that it had specificity, sensitivity, PPV and NPV of 83%, 100%, 100% and 74% at a cutoff value of 50 µg/ml faeces (the cut off value provided by the manufacturer) in differentiating IBD from IBS patients. In this study, CRP had lower diagnostic value than faecal CerTest Calprotectin+Lactoferrin combo card tests in differentiating IBD from IBS, as at its best cut off value of 2.4 mg/L, CRP had a NPV of 76% to exclude IBS patients with a sensitivity of 70% and a PPV to confirm IBD of 93.33% with a specificity of 95% and AUC of 0.863. Similar results were found in Tibble *et al.,* (2002) study who found that the diagnostic values of CRP in differentiating organic from non organic intestinal diseases were lower than that of faecal calprotectin as at a cut off value of 5 mg/L, it had sensitivity of 50%, specificity of 81% with a PPV of 56% and a NPV of 89%. Also, Schoepfer *et al.,* (2007) found that CRP at a cut off value of 5 mg/L had 64% sensitivity and 92% specificity with a PPV of 94% and a NPV of 55% in differentiating IBD from IBS patients and these values were also lower than the faecal calprotectin values.

Finally, both faecal Cer Test Calprotectin+Lactoferrin combo card tests and CRP showed a 100% diagnostic accuracy in discriminating active from inactive IBD at values of 25.5 mg/L and 5.5 mg/L respectively. However, Gaya *et al.,* (2005) study on CD patients showed that the best cut off value of faecal calprotectin was of >100 µg/g using Calprest® test which had a sensitivity of 80%, specificity of 67%, PPV of 87%, NPV of 64% and an accuracy of 87% in identifying those with and without any inflammation. Also, Xiang *et al.,* (2008) found that the faecal calprotectin at a cut off value of 50 µg/g (the cut off value provided by the manufacturer using the faecal test purchased from "Nycomed, Norway") and CRP at a cut off value of 5 mg/L had a specificity of 79.4%, 69% and a sensitivity of 91.9%, 62.2% respectively in differentiating active from inactive UC patients. This could be explained by the small sample size of the IBD patients.

Conclusion and Recommendation

The diagnostic validity of calprotectin-lactoferrin faecal CerTest calprotectin +Lactoferrin combo card tests appear to be a clinically useful marker in differentiating IBD from IBS as well as active from inactive IBD.. calprotectin-lactoferrin can be recommended in the initial work up of patients presented with symptoms suggestive of IBS vs IBD. calprotectin-lactoferrin can replace endoscopy in the surveillance for IBD activity.

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