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Fecal Calprotectin – india Perspective Role in Diagnosis of Patients with Chronic Non-Bloody Diarrhea

KEYWORDS

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ABSTRACT • ***Aims:*** *Fecal calprotectin (FC) has been found to be a marker of intestinal inflammation particularly in the context of inflammatory bowel disease in the West. This study was thus done to evaluate the efficacy of Fecal Calprotectin as a screening test to discriminate organic from functional diarrhea.*

• ***Methods:*** *Eighty three adult patients with chronic diarrhea of unknown origin were consecutively enrolled in this study. Fecal Calprotectin was also measured by ELISA.*

• ***Results:*** *Patients with organic diarrhea included Inflammatory Bowel Disease (IBD), Parasites, Polyps. Fecal Calprotectin showed a sensitivity of 70 % (CI 56-82%) and specificity of 60% (CI 45-72%) for the diagnosis of organic diarrhea using a cut off at 250µg/g.*

• ***Conclusions:*** *Fecal Calprotectin was found to be a reliable biomarker of intestinal inflammation in patients with chronic diarrhea of unknown origin. The best estimate cut off of Fecal Calprotectin in our population appears to be >250µg/g to distinguish organic from functional disease and >600µg/g to diagnose inflammatory bowel disease.*

Introduction

Irritable bowel disease (IBS) is very common cause of chronic diarrhea in the western world and has been reported to affect upto 6–20%(1) of the population. The data from Asia had shown that affluent cities like Singapore and Tokyo, to have prevalence of 8.6% and 9.8%, respectively(2). India on the other hand has had the lowest prevalence of 4.2%(2). Although clinical criteria based on a simple medical history and examination should be used to make a positive diagnosis of IBS, many tests are often performed to rule out organic disease. The yield of colonoscopy is poor being negative in upto 60% in persons with bleeding, 70% in those with non-bloody diarrhea and 88% of the time with other symptoms(3).

Fecal Calprotectin (FC) is one of the most promising test and there has been a extensively studied. A meta-analysis by Van Rheenen et al(4) which concluded that it was a good screening test to discriminate IBS from other patients with organic diseases in patients presenting with chronic diarrhea. Subsequently in has also been shown to be useful in assessing disease activity and predicting rates of relapse and mucosal healing in IBD(5)

Unfortunately there is no data from India on the levels of calprotectin in IBS or IBD. Given the higher levels protozoan and helminthic infections(6) and the presence of tropical enteropathy and colopathy in India(6) . It is important to evaluate the usefulness of FC for the above indications in India. So this study was performed to assess the performance of Fecal Calprotectin to discriminate IBS from organic causes of diarrhea.

Objectives

The aim of this study was to assess performance of fecal Calprotectin among colonoscopy referral patients with chronic diarrhea of unknown origin.

PATIENTS & METHODS:

This study was conducted in the Gastroenterology department of a tertiary care University teaching hospital in South India between November 2010 and January 2012. We enrolled all consecutive patients referred for colonoscopy after presenting for chronic diarrhea in outpatient clinics of the Department of Gastroenterology.

Inclusion criteria:

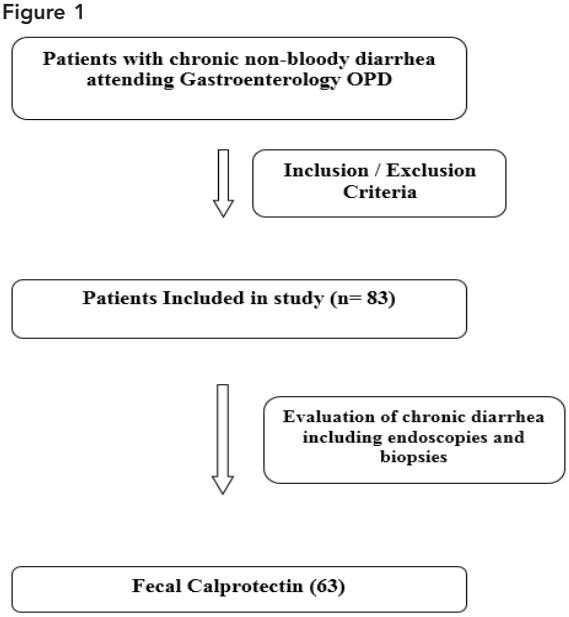
Adult patients (age >18 yrs) referred for Colonoscopy as part of evaluation ofchronic diarrhea (>4 wk duration) of unknown origin

Exclusion criteria:

- Overt GI bleeding
- GI endoscopy performed within the preceding 2 wks
- Menstruation
- Pregnancy
- Known colorectal neoplasia / familial adenomatous polyposis and hereditary non polyposis colorectal cancer syndrome.

Those who refuse informed consent

Figure 1



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20 patients did not give stool for faecal calprotectin and three did not undergo colonoscopy so could not be included in the analysis.Details of the study were explained to the patients and informed consent for participation in the study taken. Clinical and demographic data were collected in a prospective manner prior to subjecting them for Colonoscopy or stool tests and entered in a detailed proforma. The study was approved by the Institution review board.

Laboratory tests:
Patients were investigated as per the protocol used by our department for evaluation of chronic diarrhea. Decision on tests to be ordered were taken by the treating physician. Gastrointestinal (GI) endoscopy has a pivotal role in the diagnosis of suspected IBD(3). Here our patients will undergo endoscopies, as well as biopsies if needed, as part of the routine protocols. The lab will be performing the tests blinded to the patient details

Estimation of Calprotectin:
One stool sample for calprotectin estimation was collected and returned by each study participant within 1 week of the first visit prior to colonoscopy preparation. The stool samples were stored at −20 °C and assayed for Calprotectin within 4 months. After thawing the stool sample, Calprotectin concentrations were measured by a commercial ELISA system (BÜHLMANN Calprotectin assay) based on polyclonal antibodies against Calprotectin. The BÜHLMANN Calprotectin assay requires 50-100 mg of stools . Stools can be stored at 2-8°C for upto 6 days or -20% for upto 4 months. The cut off value is 50 µg/g Calprotectin in feces. This is a routine ELISA test which requires about 2 hours to perform.The test has a sensitivity of 84%, specificity of 95% and a negative predictive value of 93% . The laboratory performing the tests were blinded to the patient details. The gastroenterologist who made the final diagnosis was unaware of the Fecal Calprotectin results throughout the study.

Analysis
Data entry and Descriptive statistics were calculated using the Statistical Package for the Social Sciences {SPSS}.

Using colonoscopy with or without biopsy as the gold standard the sensitivity and specificity of the FC assay and its positive and negative predictive values, along with their 95% confidence intervals, were calculated by standard statistical methods. The Fishers two tailed tests of significance used to assess the significance. The Mann–Whitney test was used to compare the means of FC values recorded in various groups studied. The interpretation of results will in the light of clinical and other lab results

This is a new test in the Indian population. It is a quantitative ELISA, Receiver operating characteristics (sensitivity and specificity) were assessed by curve analysis as described by Henderson will be used to establish the best cut-off. Since the gold standard is endoscopy with or without biopsies, the results will be considered positive only when concordant

Using the available literature with a sensitivity of 83% given a prevalence of 26% there should the samples sizes for 15 % precision was 96

Results
Calprotectin ELISA
The majority of the patients were men 70% and women were 30%. The mean age of the patients in our study was 41 years . So most of the participants were middle aged and the range was from 20 to 71 as shown in. This study did not include children.

A total of 83 consecutive patients with chronic diarrhea were initially included in the study. All the patients were adults. 20 patients who had initially consented to take part in the study did not give fecal calprotectin, 3 other patients did not undergo colonscopy and so were excluded from the final analysis. All underwent clinical and laboratory evaluation decided by the treating physician.

In adult patients, individuals with an organic cause of diarrhea had significantly higher FC concentrations than individuals with IBS (P = 0.011, Mann–Whitney test). As this was significantly different it was decided to proceed with further evaluation. The number of patients with organic cause of diarrhea was 30 while those with IBS (controls) was 30 as well so the group was equally divided.

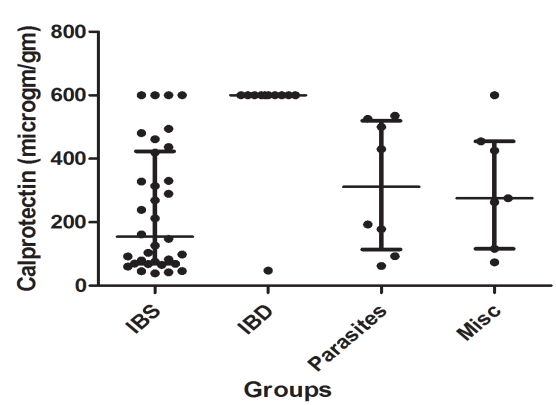
The Results of the ELISA had a range up to 600. As 600 was the maximum value the exact value of those with this value is not available. Hence 600 has been used as the value in all the analysis

Of the 10 cases of IBD the value of calprotectin is >600 in all except for 1 in which it is <50. In the below box plot in Figure 2 the IBD column / second group reflects the same as only value (<50). The rest are marked as 600 as the ELISAs upper limit was 600. In the IBD group there were 6 patients with Ulcerative colitis and 4 patients with Crohns disease(all ileo-colic). Those with Ulcerative colitis 3 had left sided colitis, 2 had pancolitis and 1 had a narrowing in lumen and complete colonoscopy could not be completed. The patient with Calprotectin which was normal was a patient with Left sided colitis

Calprotectin ELISA distribution based on diagnosis
Table 1

Group 1 (30)	IBS - 220.67 (39.08 – 600)
Group 2 (10)	IBD - 549.77 (47.44 – 600)
Group 3 (9)	Parasites -314.91(62.55 – 535.34)
Group 4 (11)	Miscellanous (Includes bacterial infection/polyp/HIV/collagenous colitis) -354.86 (263.07- 455.18)

Figure 2



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Here when the difference in means as shown in Figure 2 was assessed overall between the different means by Krushal-Whellis method it was significant P-0.001

The parasites isolated Isospora Belli, H.nana, Trichuris trichura, Giardiasis, amoebiasis and Ascariasis. Two patients with Isospora Belli had concurrent HIV infection. The bacterial infection isolated were Aeromonas and Salomonella (atypical)

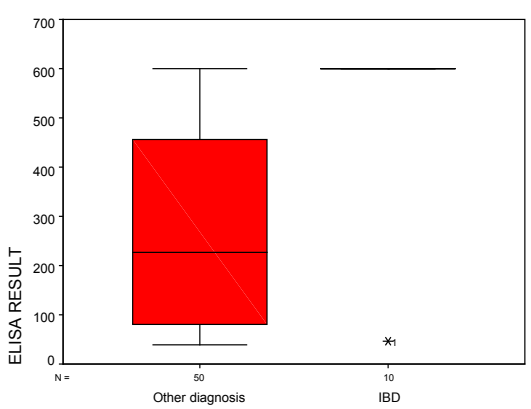
A cross-tabulation of the calprotectin results obtained in adult patients, according to the final diagnoses, is shown in below tables summarizes the sensitivity, specificity values of the FC assay in distinguishing between organic causes of diarrhea and IBS calculated in the study population according to three cutoff limits 50, 150 and 250 µg/g of stool. The higher cut off had poorer sensitivity but better specificity (250 µg/g). The ROC curve showed 250 to be the best cut off to differentiate between organic and functional disease with sensitivity, specificity and odds ratio of 70%, 60% and 3.5 respecitively.

Table 2

Calprotectin	Sensitivity	specificity	Odds ratio	P value	Calprotectin
>50	93 (86-99)	10 (3-15)	1.5	1	>50
>150	80(66-91)	53 (40-64)	4.5	0.01	>150
>250	70 (56-82)	60(45-72)	3.5	0.03	>250

All of the consecutive adult patients with chronic diarrhea suffering from Inflammatory bowel disease had FC concentrations (median, 550 µg/g; range, 47–600 µg/g). The ROC curve plotted from the data of the consecutive patients with final diagnoses of IBS or IBD showed that the calprotectin value with the highest diagnostic accuracy was 600 µg/g: it was 90% sensitive and 88% specific in the diagnosis of Inflammatory bowel disease. The negative predictive value of 600 was 97%

Figure 3



Others Vs IBD

Discussion

Fecal calprotectin is a simple non-invasive tests that may predict the presence to colorectal inflammation. It would appear to have a role as a pre-endoscopic triage tool for patients with chronic diarrhea.

In our study 50% of patients had an organic pathology and ~16% of the study population had Inflammatory bowel disease. The distribution in this series was similar to an earlier series with chronic diarrhea from Italy (10) (7) but different from Schroder et al(12) (9)where the prevalence of IBD was higher and Limberg et al (11) (9) where the level of organic disease was only 25%. The prevalence of parasites(13%) was high but rare in other studies except the study by Carracio et al(31) (9)study on children from Italy where giardiasis was an important cause of chronic diarrhoea.

The sensitivity and specificity of FC to discriminate IBS from organic disease in our study were 70% and 60% respectively. This is comparable to studies from the west which were similar in design to our study (Table 15) . The best results were in the study by Limburg et al with over 80% sensitivity and specificity.ROC curve in our study showed that the best cut off was achieved with 250 µg/g. This study suggests that the cutoff value in Indian patients should be higher than the west.

Table 3
Sensitivity and Specificity of Fecal Calprotectin for IBS and IBD

Studies (Cutoff value of FC which predicts Organic disease) / Study (Cutoff value of FC which predicts IBD)	Sensitivity	Specificity
Our study/ >250	70%	60%
Our study/ >600 n-10/60	90%	88%
Carroccio et al/ >50(10)	64%	80%
Carroccio et al/ >170(10) n- 9/70	100%	95%
Limburg et al/ >100(11)	83%	83%
Limburg et al/ >100(11) n-16/110	94%	90%

Subgroup analysis of patients with Inflammatory bowel disease showed that 9 out 10 patients had FC levels more than 600 µg/g . The cut off value of FC to differentiate between IBD Vs non-IBD was higher than for Organic Vs Functional disease. Similar trend noted with other studies as well

Limitation of the study is the small number of patients studied . Another limitation in our study was that evaluation of patients was decided by the treating physician . So all our patients did not have colonoscopy biopsy. It is therefore not possible to definitively exclude microscopic or collagenous colitis in patients who had normal colonoscopy but did not have biopsy.

Conclusion

In conclusion fecal Calprotectin was found to be a reliable biomarker of intestinal inflammation in patients with chronic diarrhea of unknown origin. Fecal Calprotectin had a sensitivity of 70% and specificity of 60% in discriminating between IBS and organic causes of diarhoea , and 90 & 88% respectively for discriminating between IBD and other pathologies. The best estimate cut off of fecal calprorectin in our population appears to be >250 µg/g to distinguish organic and functional disease and >600 µg/g suggests Inflammatory bowel disease

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Reference

1. Drossman DA, Whitehead WE, Camilleri M. Irritable bowel syndrome: a technical review for practice guideline development. Gastroenterology. 1997 Jun;112(6):2120–37.
2. Gwee K-A, Lu C-L, Ghoshal UC. Epidemiology of irritable bowel syndrome in Asia: something old, something new, something borrowed. J. Gastroenterol. Hepatol. 2009 Oct;24(10):1601–7.
3. Lasson A, Kilander A, Stotzer P-O. Diagnostic yield of colonoscopy based on symptoms. Scand. J. Gastroenterol. 2008 Mar;43(3):356–62.
4. van Rheenen PF, Van de Vijver E, Fidler V. Faecal calprotectin for screening of patients with suspected inflammatory bowel disease: diagnostic meta-analysis. BMJ. 2010;341:c3369.
5. Lewis JD. The utility of biomarkers in the diagnosis and therapy of inflammatory bowel disease. Gastroenterology. 2011 May;140(6):1817–1826.e2.
6. Ramakrishna BS, Venkataraman S, Mukhopadhya A. Tropical malabsorption. Postgrad Med J. 2006 Dec;82(974):779–87.
7. Tibble JA, Bjarnason I. Non-invasive investigation of inflammatory bowel disease. World J. Gastroenterol. 2001 Aug;7(4):460–5.
8. Fagerhol MK, Dale I, Anderson T. Release and Quantitation of a Leucocyte Derived Protein (L1). Scandinavian Journal of Haematology. 1980 Dec 1;24(5):393–8
9. Desai D, Faubion WA, Sandborn WJ. Review article: biological activity markers in inflammatory bowel disease. Alimentary Pharmacology & Therapeutics. 2007 Feb 1;25(3):247–55.
10. Carroccio A, Iacono G, Cottone M, Di Prima L, Cartabellotta F, Cavataio F, et al. Diagnostic accuracy of fecal calprotectin assay in distinguishing organic causes of chronic diarrhea from irritable bowel syndrome: a prospective study in adults and children. Clin. Chem. 2003 Jun;49(6 Pt 1):861–7.
11 Limburg PJ, Ahlquist DA, Sandborn WJ, Mahoney DW, Devens ME, Harrington JJ, et al. Fecal calprotectin levels predict colorectal inflammation among patients with chronic diarrhea referred for colonoscopy. Am. J. Gastroenterol. 2000 Oct;95(10):2831–7.
12. Schröder O, Naumann M, Shastri Y, Povse N, Stein J. Prospective evaluation of faecal neutrophil-derived proteins in identifying intestinal inflammation: combination of parameters does not improve diagnostic accuracy of calprotectin. Aliment. Pharmacol. Ther. 2007 Oct 1;26(7):1035–42.