Internal Medicine

Study of Fecal Calprotectin in Adults with non Bloody Chronic Diarrhea

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ABSTRACT

Background: Fecal Calprotectin is a surrogate marker of neutrophil influx into the bowel lumen because it is resistant to bacterial proteolytic destruction during transit through the gastrointestinal tract, has long-term stability in faeces (>7 days) at different temperatures, and is easy to assess As a result, FC has been suggested.

Aim of the work: Study the accuracy of fecal calprotactin as a non-invasive diagnostic modality in determining organic causes in cases of chronic non bloody diarrhea in adult patients.

Patients and Methods: This was a case control study included 90 persons divided into 2 groups: 60 patients with chronic diarrhea as a study group and 30 persons without diarrhea as a control group, period from January 2021 to January 2022. Fecal calprotectin was performed for all persons in both groups. Colonoscopy was performed for patients with chronic diarrhea. As results of colonscopy, patients then were divided according to presence of inflammatory bowel disease.

Result: Fecal calprotectin was significantly higher among chronic diarrhea group. Also, number of patients with fecal calprotectin above 50; 100 were significantly higher among the study group. Fecal calprotectin was significantly higher among inflammatory bowel disease patients. Also, number of patients with fecal calprotectin above 50; 100 were significantly higher among the study group. At cut-off value equal 50 μg/ mg, fecal calprotectin had sensitivity equal to 92% and specificity equal to 88% to diagnose inflammatory bowel disease.

Conclusion: Fecal calprotectin has the potential to be a simple, noninvasive, and reliable screening technique for IBD.

Keywords: *Fecal calprotectin; Nonbloody chronic diarrhea; inflammatory bowel disease.*

INTRODUCTION

Chronic diarrhea is defined as three or more loose stool per day that lasts for at least 4 weeks. Chronic diarrhoea can have a significant influence on a patient's quality of life, ranging from little inconvenience to incapacity and death in its most severe form.[1]

A complete blood count, CRP, anti-tissue transglutaminase immunoglobulin A (IgA), total IgA, and a basic metabolic panel are all recommended. Profile should all be conducted to rule out celiac disease or inflammatory bowel disease.[2]

Irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), infections, endocrine diseases such as diabetes mellitus and hyperthyroidism, food allergies, and drugs such as NSAIDs, antacids, and antibiotics are among the various causes of chronic diarrhoea.[3]

Irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), celiac disease, and microscopic colitis are all common causes. Chronic diarrhoea can be caused by a number of things, but a comprehensive medical history and physical examination can help narrow down the options.[4]

Eosinophil cationic protein, leukocyte elastase, leukocyte esterase, myeloperoxidase, lysozyme, lactoferrin, and calprotectin are just a few of the leukocyte-derived proteins that have showed early promise as inflammatory biomarkers.[5]

Calprotectin may have a performance advantage over these other possibilities due to its biological properties. This 36.5-kD nonglycosylated polypeptide trimer, in particular, is responsible for up to 60% of the cytosolic proteins identified in neutrophils and macrophages.[6]

Early research has found that faecal calprotectin levels are higher in patients with inflammatory bowel disease, and that they appear to correspond with disease activity as well.[7]

The aim of our study was to study the accuracy of fecal calprotectin as a non-invasive diagnostic modality in determining organic causes in cases of chronic non bloody diarrhea in adult patients.

PATIENTS AND METHODS

This was a case control study included 90 persons divided into 2 groups: Group A: 60 patients with age (20 - 60) with chronic diarrhea was chosen from Al-Hussein University Hospital, and Group B: 30 healthy individuals corresponding to group A in age and sex, as a control group, period from January 2021 to January 2022.

Inclusion criteria: A history of chronic diarrhea of unknown origin lasting for more than 4 weeks, with or without abdominal pain.

Exclusion criteria: previous evaluation of chronic diarrhea, overt gastrointestinal bleeding, anorectal diseases, familial adenomatous polyposis, and colorectal cancer.

All the participants was subjected to the following:

- Full history taking.
- Physical examination.
- Laboratory investigations which include (CBC, ESR, CRP, TSH, HBA1C, liver functions and renal functions tests).
- Stool analysis.
- Fecal calprotactin (was done by ELISA assay technique).
- Pelviabdominal Ultrasonography.(patients)
- Colonoscopy (with biopsy if needed) for patients who have high leval fecal calprotactin.

Statistical analysis: The data was collected, edited, coded, and entered into the IBM SPSS version 20 statistical package for social science. When the distribution of qualitative data was determined to be parametric, it was provided as a number and a percentage, whereas quantitative data was presented as a mean, standard deviations, and ranges. When the predicted count in any cell was less than 5, the Chi-square test and/or Fisher exact test were employed instead of the Chi-square test to compare two groups with qualitative data. To compare two independent groups with quantitative data and parametric distribution, the Independent t-test was utilised. The acceptable margin of error was set at 5%, while the confidence interval was set at 95%. A P value of less than 0.05 was considered significant.

RESULTS

	Study group (60 patients)	Control group (30 subjects)	Test of significance	P value
Age (years) mean±SD	56.3±9.3	57.69±8.7	T= 0.84	**0.4**
Sex No. (%) Male Female	24 (60%) 36 (40%)	14 (46.67%) 16 (53.33%)	X^2= 0.54	**0.63**
Body mass index (kg/M^2) mean±SD	24.68±2.9	24.1±2.8	T= 1.11	**0.27**
Smokers No. (%)	18 (30%)	8 (26.67%)	X^2= 0.16	**0.69**
Diarrhea characters Duration (months) Frequency (time/day)	7.3±1.09 6.4±1.8	- -	-	**-**
Fecal calprotectin (µg/mg) mean±Sd	57.2±25.3	39.1±5.02	T= -5.4	**0.0001**
Fecal calprotectin > 50 µg/mg No. (%)	26 (43.33%)	3 (10%)	X^2= 24.05	**0.0001**
Fecal calprotectin > 100 µg/mg No. (%)	12 (20%)	0	X^2= 10.43	**0.0012**

T: student t test, X^2: Chi square, level of significance < 0.05

Table 1: Demographics and baseline, diarrhea characters and fecal calprotectin levels among both groups.

There was no statistically significant difference between both groups regarding demographics and baseline characteristics. Duration of diarrhea in the study group ranged from 5 months to 9 months with mean 7.3±1.09. Fecal calprotectin was significantly higher among chronic diarrhea group. Also, number of patients with fecal calprotectin above 50; 100 were significantly higher among the study group (Table 1).

	Organic bowel disease (25 patients)	Control group+ IBS (65 patients)	Test of significance	P value
Fecal calprotectin (µg/mg) mean±Sd	92.85±16.1	39.8±7.2	T= -23.29	**0.0001**
Fecal calprotectin > 50 µg/mg No. (%)	23 (92%)	6 (9.2%)	X^2= 23.14	**0.0002**
Fecal calprotectin > 100 µg/mg No. (%)	12 (48%)	0	X^2= 10.43	**0.0012**

T: student t test, X^2: Chi square, level of significance < 0.05

Table 2: Fecal calprotectin levels between organic bowel disease and both normal endoscopy patients and control group:

Fecal calprotectin was significantly higher among inflammatory bowel disease patients. Also, number of patients with fecal calprotectin above 50; 100 were significantly higher among the study group (Table 2).

	Organic bowel disease (25 patients)	Irritable bowel syndrome (35 patients)	Test of significance	P value
Hemoglobin (g/dL) mean±Sd	10.3±1.3	11.5±2.1	T= 2.5	**0.015**
White blood cells (/mm^3) mean±Sd	9.5±2.1	6.4±1.8	T= -6.1	**0.0001**
Platelets (/mm^3) mean±Sd	283.9±20.3	260.3±30.7	T= -3.35	**0.0014**
Serum creatinine (mg/dL) mean±Sd	0.9±0.3	0.83±0.25	T= -0.98	**0.33**
Serum albumin (g/dL) mean±Sd	3.1±0.2	3.2±0.5	T= 0.95	**0.35**
C-reactive protein (mg/L) mean±Sd	9.6±2.4	3.3±0.6	T= -14.93	**0.0001**
ESR 1st hour(mm/hour) mean±Sd	48.36±13.8	17.9±7.8	T= -10.87	**0.0001**
Fecal calprotectin (μg/mg) mean±Sd	90.6±20.2	44.7±15.3	T= -10.019	**0.0001**
Fecal calprotectin > 50 μg/mg No. (%)	23 (92%)	3 (8.6%)	X^2= 41.34	**0.0001**
Fecal calprotectin > 100 μg/mg No. (%)	12 (48%)	0	X^2= 17.51	**0.00002**

T: student t test, X^2: Chi square, level of significance < 0.05

Table 3: Laboratory investigations between functional and organic bowel disease:

Hemoglobin was lower among organic bowel disease while white blood cell and platelet counts were higher with statistically significant difference. CRP and ESR were higher was statistically significant difference. Fecal calprotectin was significantly higher among inflammatory bowel disease patients. Also, number of patients with fecal calprotectin above 50; 100 were significantly higher among organic bowel disease (Table 3).

	Cut of point	AUC	Sensitivity	Specificity	+PV	-PV	P valve
Fecal calprotectin	50 μg/mg	0.87	92%	88%	98	70	0.001

Table 4: Receiver Operator Curve analysis of Fecal calprotectin to diagnose inflammatory bowel disease:

At cut-off value equal 50 μg/ mg, fecal calprotectin had sensitivity equal to 92% and specificity equal to 88% to diagnose irritable bowel disease (Table 4).

	Below 50 μg/mg (61 patients)	Above 50 μg/mg 29 patients)	Test of significance	P value
Age (years) mean±SD	57.58±11.6	56.89±9.3	T= -0.292	0.77
Sex (male) No. (%)	24 (39.34%)	14 (48.27%)	X^2= 0.26	0.7
Body mass index (kg/M^2) mean±SD	24.9±2.3	24.8±2.4	T= -0.202	0.84
Smokers No. (%)	16 (26.2%)	10 (34.3%)	X^2= 4.1	0.09
Hypertension No. (%)	18 (29.5 %)	14 (48.27%)	X^2= 0.045	0.9
Diabetes No. (%)	10 (16.3%)	8 (27.6%)	X^2= 0.14	0.6
Chronic liver disease No. (%)	6 (9.8%)	3 (10.3%)	X^2= 1.79	0.18
Autoimmune disease No. (%)	1 (1.6%)	4 (13.79%)	X^2= 7.3	0.009
Stone kidney disease No. (%)	0	5 (17.24%)	X^2= 10.3	0.001
Irritable bowel syndrome No. (%)	32 (35.16%)29	3 (10.34%)	X^2= 6.55	0.016
Organic bowel disease No. (%)	2 (2.19%)	23 (79.3%)	X^2= 79.29	0.00001

T: student t test, X^2: Chi square, level of significance < 0.05

Table 5: Risk factors for increased fecal calprotectin above 50 μg/ mg (univariate analysis):

At this cut-off value, patients were divided into 2 groups. Autoimmune disease and stone kidney disease incidence were higher among patients with higher fecal calprotectin. Irritable bowel disease was associated with lower fecal calprotectin values. On the other hand, organic bowel disease was associated with higher fecal calprotectin values (Table 5).

	B estimate	95% confidence interval		P value
		Lower	Upper	
Age (years) mean±SD	0.00897	-0.13659	0.36987	0.52
Sex (male) No. (%)	0.073728	-0.26603	0.413487	0.66
Body mass index (kg/M^2) mean±SD	-0.13052	-0.455532	0.194483	0.42
Smokers No. (%)	0.00396	-0.26438	0.272301	0.97
Hypertension No. (%)	-0.00699	-0.25432	0.240338	0.95
Diabetes No. (%)	0.068654	-0.17491	0.312215	0.57
Chronic liver disease No. (%)	0.0981	-0.14677	1.342974	0.49
Autoimmune disease No. (%)	0.157093	-0.07597	0.390155	0.18
Stone kidney disease No. (%)	0.238082	-0.15454	1.242311	0.18
Irritable bowel syndrome No. (%)	0.199642	-0.36908	1.768362	0.01
Organic bowel disease No. (%)	-0.00018	-0.01109	11.010722	0.00001

Linear regression for continuous data, binary logistic regression for categorical data, level of significance < 0.05

Table 6: Risk factors for increased fecal calprotectin above 50 μg/ mg (multivariate analysis):

Mutlivariate analysis showed that irritable bowel disease and organic bowel disease incidence affected fecal calprotectin values (p value: 0.01; 0.00001) (R: 0.23, adjusted R: 0.047, Significance F: 0.37, p value<0.05 of significance) (Table 6).

	r2	P value
ESR	0.5	0.01
CRP	0.3	0.3

Table 7: Correlation between fecal calprotectin and disease severity among inflammatory bowel disease patients (ESR and CRP):

Disease severity represented by ESR and CRP correlated positively with fecal calprotectin. However, the correlation was significant with ESR but the correlation with CRP was not of statistical significance (Table 7).

DISCUSSION

In the current study, control group was matched to chronic diarrhea group in age and sex. Mean age in diarrhea group was 56.3±9.3 years which is considered relatively older than what was reported in a study reporting chronic diarrhea in Japanese population and revealed predominance at younger age.[8]

Callan et al.[9] In women going through the menopause transition, researchers discovered that the severity of diarrhoea decreased with age. According to Singh et al.10, the prevalence of diarrhoea skyrocketed after the age of 60.

Chronic diarrhea group had female predominance in this study. This could be explained by the increased incidence of irritable bowel syndrome among females. The explanation for this could be because female steroid hormones oestrogen and progesterone are said to reduce gastrointestinal motility by reducing colonic smooth-muscle contractility and increasing intestinal permeability.[11,12] In spite that BMI was higher among chronic diarrhea patients, there was no statistically significant difference when compared to control group. This is against Zhao et al.[13] who reported higher incidence of diarrhea among high BMI patients but most of his patients had functional not organic diarrhea. Another study in

united states reported higher incidence of chronic diarrhea among obese patients.[14,15]

There was no statistically significant difference between both groups regarding smoking. Zhao et al.[13] also did not find association between smoking and chronic diarrhea. Scallan et al.[16] found increase incidence with smoking and alcohol consumption.

In the current study, chronic diarrhea was diagnosed mainly depending on stool frequency (6.4±1.8 / day). Some studies reported a relation between stool characters and stool frequency as Matsumoto et al.[8]. However, Singh et al.[10] did not find a correlation between stool characters and frequency of diarrhea.

Anemia is common among diarrhea patients especially iron deficieny anemia. However, other nutrients loss is a common cause of anemia as viramin B12, folic acid and albumin and megaloblastic anemia may occur.[17] In the current study, there was statistically significant difference between both groups regarding hemoglobin levels. Also, anemia was more severe in patients with irritable bowel disease. This comes in hand with Akpınar et al.[18].

In our study, there was leucocytosis and thrombocytosis as inflammatory markers in patients with chronic diarrhea and more severe in

inflammatory bowel disease patient. This comes in agreement with Gazelakis et al.[19] and Nabih[20]. However, Nabih[20] and Khalil et al.[21] did not report leucocytosis with IBD patients. He found only thrombocytosis.

C-reactive protein as an inflammatory marker was high with statistically significant difference among chronic diarrhea group and also among IBD patients. It come in hand with Smalley et al. [22], Burgers et al.[2]. Same finding were reported regarding ESR.[21]

In the current study, there was significant difference between the levels of fecal calprotectin in patients with inflammatory bowel disease and both no diarrhea group and irritable bowel syndrome patients. It was very high among patients with IBD.

Khalil et al.[21] In his research, he found the same association.

Many more investigations have verified the use of faecal calprotectin as a diagnostic for separating IBD from IBS. 23,24

In the current investigation, we discovered that faecal calprotectin had a sensitivity of 92 percent and a specificity of 88 percent for diagnosing irritable bowel disorder at a cut-off value of 50 g/ mg.

According to a literature-based meta-analysis, FC has a high overall sensitivity of 0.97 (95 percent CI 0.92–0.99) and a modest specificity of 0.71 (0.59–0.80) for detecting IBD.25

According to Khalil et al.21, faecal calprotectin had an 86.8% specificity and a 66.7 percent sensitivity in distinguishing IBD from non-IBD cases.Licata et al.26 found that the FC assay had The FC assay had 75.4 percent sensitivity and 88.3 percent specificity for histologic inflammation, with 81.7 percent positive and 83.7 percent negative predictive values, indicating that it could be a viable and noninvasive screening tool for inflammatory causes of chronic, nonbloody diarrhoea. Vernia et al.27 validated that the best available data supports the use of FC as a diagnostic and monitoring tool for reliably assessing the degree of intestinal inflammation in a meta-analysis. As a result, FC should always be addressed in the treatment of IBD patients.

Disease severity represented by ESR and CRP correlated positively with fecal calprotectin in this study. This comes in hand with D'Haens et al.[7] and Khalil et al.[21]. Another studies found the same correlation with CRP only.[28,29]

These findings highlight the importance of faecal calprotectin in determining the severity of IBD and possibly other non-IBD colitides. The link between faecal calprotectin and and diagnosis of IBD was confirmed in our study by multivariate analysis for risk factors for increased FC and it was significant only for IBD positively and IBS negatively.

CONCLUSION

Fecal calprotectin has the potential to be IBD screening that is simple, noninvasive, and accurate. Based on the assessment of faecal calprotectin levels,

our findings suggest that resource utilisation, particularly of expensive and invasive procedures like colonoscopy, should be more logically and cost efficiently directed.

Conflict of interest : none

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