European Journal of Molecular & Clinical Medicine
ISSN 2515-8260 Volume 7, Issue 2, 2020

FECAL CALPROTECTIN AS INDICATOR OF INFLAMMATORY BOWEL DISEASE AND INTESTINAL MALIGNANCY IN PATIENTS OF CHRONIC DIARRHEA

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Abstract - Background: Calprotectin is consisting of a protein binding zinc and calcium mainly found in Neutrophils. It is generally associated with inflammation. Elevated fecal Calprotectin can be detected when there is gastric or colorectal malignancy, or when there is an inflammation such as in inflammatory bowel disease (Crohn's disease and ulcerative colitis). Objectives: To assess the role of fecal Calprotectin as an indicator for inflammatory bowel disease and malignancy in chronic diarrhea. Methodology: Cross-sectional study conducted in Al-Sadr Teaching Hospital from January till the end of March 2020, and included (100) patients with chronic diarrhea who underwent fecal Calprotectin test and colonoscopy. Results: Mean age of participants was (36.61 ± 13.09) years, with equal gender distribution. Comparison of fecal Calprotectin level among colonoscopy findings was performed using ANOVA test. There was significant association between the two variables (FCP and colonoscopy finding), P-value < 0.001. In Crohn's disease, fecal Calprotectin level was significantly higher than that of normal group, with a mean difference of (44.53) µg/g, P-value < 0.001. Similarly, ulcerative colitis had significantly higher fecal Calprotectin level than normal group with mean difference of (54.29) µg/g, P-value < 0.001. Conclusions: This study concludes that the level of fecal Calprotectin increases significantly in patients with inflammatory bowel disease and colon malignancy, and can be used to predict the abnormal findings in colonoscopy. However, this indicator cannot discriminate between ulcerative colitis and Crohn's disease.

Keywords: Fecal Calprotectin, colon malignancy, inflammatory bowel disease, ulcerative colitis, Crohn's disease, chronic diarrhea, colonoscopy, biological therapy

INTRODUCTION

Calprotectin related to S100 protein family (A8/A9) of cytoskeleton proteins. It is a calcium and zinc binding protein that is mainly found in Neutrophils, accounting for up to 60% of their cytoplasmic matrix protein content. It is also present in epithelial cells and monocytes [1]. The release of Calprotectin is generally associated with cellular disruption or death [2]. Fecal Calprotectin is highly resistant to enzymatic degradation within the gastrointestinal tract (GIT), and can be easily detected using the standard enzyme-linked immunosorbent assays (ELISA) [3]. Increased Calprotectin level is generally associated with inflammation, and considered to be an acute phase protein [4]. It is usually observed in cases of ongoing inflammatory process and malignancy, and can be detected in blood plasma, synovial fluid, cerebrospinal fluid (CSF), urine, as well as in feces. Elevated fecal Calprotectin (FCP) can be detected when there is gastric or colorectal malignancy, or when there is an inflammation such as in

inflammatory bowel disease (IBD) [5]. Inflammatory bowel disease is defined as "chronic inflammation of the bowel which is caused by interactions between host and microbes in an individual who is susceptible genetically". It consists of a number of autoimmune diseases characterized by inflammation of the large and small intestine. Crohn's disease and ulcerative colitis are the two main types of IBD [6]. Crohn's disease (CD) is the condition in which a trans-mural granulomatous inflammatory process that can affect the entire gastrointestinal tract, but occurs mostly in the ileum or colon [7]. Its prevalence is continually increasing worldwide, posing a significant impact on the quality of life of affected patients, limiting their capability of work. Its prevalence in Europe is estimated to be the highest worldwide, reaching up to 294 persons per 100000 people [8]. It has also been linked to higher mortality [9]. The disease is characterized by skip lesions that affect the gastrointestinal tract tissues, and three main phenotypes of Crohn's disease exist, these are [10]:

- Inflammatory
- Structuring
- Penetrating

Clinical features of the disease vary widely among patients, but generally include diarrhea, loss of weight, abdominal pain, nausea and vomiting, as well as fever in some patients [10]. The main principles of management of Crohn's disease include the induction of remission by using biological therapy (infliximab, adalimumab, certolizumab) and immune-modulation (corticosteroids, thiopurines, Methotrexate) while optimizing the nutritional status of the patient in order to maintain remission [11-12] by instruct the patient to eat low residue diet like eggs, rice, bananas, oatmeal, lean poultry, fish and avoid all foods which worsen the disease like fatty, fried foods, spicy foods, meats, high fiber foods and alcohol. Ulcerative colitis (UC) is another inflammatory bowel disease that causes continuous inflammation of the mucosa of the colon. It usually extends from the rectum to the more proximal parts of the colon. It is considered to be more common than Crohn'sdisease [13]. It is classically presented as bloody diarrhea, which can be associated with mucus. It is often associated with tenasmus and abdominal pain. Constipation is also reported in some patients. The symptoms of the disease are characterized by occurring in occasional or discontinuous order (intermittent attacks), reflecting the relapsing/remitting course of the disease. Management of ulcerative colitis requires early identification and intervention of the intermittent attacks and acute relapses, and the choice of therapy is made on the basis of severity and extent of the disease. Therapeutic options include systemic immune-suppressive medications (oral budesonide, thiopurines) local application of medical therapy (5-ASA enema or suppositories), and surgical intervention [14].

OBJECTIVE OF THE STUDY

The objective of the study is to assess the role of fecal Calprotectin as an indicator for IBD and intestinal malignancy in chronic diarrhea.

PATIENTS AND METHODS

Study design, site, and time

This is a cross-sectional study conducted in Al-Sadr Teaching Hospital, Al-Najaf city from January 2020 till the end of March 2020.

Study population

The study included patients with chronic diarrhea who have no contraindications for colonoscopy.

Exclusion criteria

Patients with the following criteria were excluded from the study:

- Clinically unstable patients.
- Severe toxic mega colon and Fulminant colitis.
- Patients who refused to participate in the study.

Data collection tools

Data was collected using a special form that includes demographic characteristics of the patients. Patients underwent fecal Calprotectin investigation followed by colonoscopy to detect the type of diarrhea. Calprotectin detected using the standard enzyme linked immunosorbent assay (ELISA). The colonoscopy was done by intubation of the terminal ileum by pentax video scope system model EPK-P100.

Statistical analysis

SPSS® Software (version 23.0 for Linux®) was used to perform statistical analysis. Qualitative data are presented as numbers and percentages, while continuous numerical data are presented as mean ± standard deviation. P-value of < 0.05 was considered statistically significant.

Ethical and administrative arrangements

1. Permissions were obtained from the Arab board for medical specializations and Al-Sadr Teaching Hospital before conducting the study.

2. Informed verbal consent was obtained from the participants before the examination, after explaining the aim of this study and describing the data needed for acquisition for the present study.

3. Data was treated with confidentiality during collection, processing, and analysis.

Results

This study included (100) patients who complained of chronic diarrhea, and underwent laboratory investigation to obtain the fecal Calprotectin level, followed by colonoscopy. Age of participants ranged from (20-60) years, with a mean age of (36.61 ± 13.09) years with a median of (35) years. No significant difference in age was observed between males (36.86 ± 13.33) and females (36.36 ± 12.98), Student t-test = 0.19, P-value = 0.850. Males and females were equally distributed, with a female-to-male ratio of 1:1. Fecal Calprotectin level was variable highly variable among study participants, with a mean of (45.96 ± 33.84) µg/g. It was observed that (30%) of patients had normal fecal Calprotectin level (up to 10 µg/g), while the remaining (70%) had abnormally elevated level of fecal Calprotectin (>10 µg/g). No significant difference was observed between males and females regarding elevation of fecal Calprotectin, P-value = 0.663, table (1).

Table (1): Elevation of fecal Calprotectin level by gender

Gender	Fecal Calprotectin Level (µg/g)			P-value
	Normal (≤ 10 µg/g)	Elevated (> 10 µg/g)	Total	
Male	14 (28.0%)	36 (72.0%)	50 (100%)	0.663
Female	16 (32.0%)	34 (68.0%)	50 (100%)	
Total	30 (30.0%)	70 (70.0%)	100 (100%)	

Regarding colonoscopy findings, the majority of patients (40.0%) were found to have ulcerative colitis, followed by Crohn's disease in (28.0%) of them, as illustrated in table (2). Highest level of fecal Calprotectin was found among patient with ulcerative colitis, followed by patients with CA colon.

Table (2): Fecal Calprotectin level by colonoscopy finding

Colonoscopy	Fecal Calprotectin Level (µg/g)		
	Mean	SD	Range
Normal	8.68	11.09	0.3 – 36.0
Crohn's disease	53.21	32.22	1.0 – 100.0
Ulcerative colitis	62.98	26.05	3.0 – 100.0
CA Colon	62.80	36.19	7.0 – 93.0
Microscopic colitis	47.00	-	-

Comparison of fecal Calprotectin level among colonoscopy findings was performed using ANOVA test. There was significant association between the two variables, ANOVA F = 19.14, P-value < 0.001. To

further elaborate this finding, post-hoc test was performed to identify significant differences between each pair of colonoscopy findings by grouping patients according to their colonoscopy findings. In Crohn's disease group, fecal Calprotectin level was significantly higher than that of normal group, with a mean difference of (44.53) µg/g, P-value < 0.001. Similarly, ulcerative colitis group had significantly higher fecal Calprotectin level than normal group with mean difference of (54.29) µg/g. CA colon patients had also significantly higher fecal Calprotectin level compared to normal group, with mean difference of (54.12) µg/g. However, no significant difference in fecal Calprotectin level was observed between microscopic colitis group and normal group, P-value = 0.147, table (3) summarizes the findings, no significant differences in fecal Calprotectin level was observed between any two of the abnormal colonoscopy findings, P-value > 0.05.

Table (3): Correlation between colonoscopy groups with normal group

Colonoscopy Group	Correlation to normal group	
	Mean difference	P-value
Crohn's disease	44.53	< 0.001*
Ulcerative colitis	54.29	< 0.001*
CA Colon	54.12	< 0.001*
Microscopic colitis	38.32	0.147

* Significant at P < 0.05

Receiver operating characteristics (ROC) curve was utilized in order to assess the reliability of the cut-off value (sensitivity & specificity). Area under the curve (AUC) for Calprotectin as indicator for abnormal colonoscopy finding was (0.93) with P-value of < 0.001 (95% C.I.: 0.89 – 0.97). A cut-off value of (11 µg/g) had a sensitivity of 87.8% and specificity of 80.8% in discriminating between normal and abnormal colonoscopy findings, table (3) provides the ROC curve for the analysis. Comparison between abnormal colonoscopy findings and abnormal elevation of fecal Calprotectin level was performed. Proportion of patients with abnormal colonoscopy who had elevated fecal Calprotectin level was significantly higher than the proportion of those who had normal Calprotectin level, P-value < 0.001 (Table 3-4).

Table (4): Elevation of fecal Calprotectin level by colonoscopy findings

Colonoscopy	Fecal Calprotectin Level (µg/g)			P-value
	Normal(≤ 10 µg/g)	Elevated(> 10 µg/g)	Total	
Normal	21(80.8%)	5(19.2%)	26(100%)	<0.001*
Abnormal	9(12.2%)	65(87.8%)	74(100%)	
Total	30(30.0%)	70(70.0%)	100(100%)	

* Significant at P < 0.05

The odds ratio for patients with elevated fecal Calprotectin level, above 10 µg/g to have abnormal colonoscopy findings was (30.33), with (95% C.I: 9.15 – 100.59), this means that patients with fecal Calprotectin higher than (10 µg/g) are 30 times more likely to have abnormal findings on colonoscopy.

DISCUSSION

The present study had demonstrated that more than two-thirds of patients with chronic diarrhea (68.0%) were found to have inflammatory bowel disease (IBD). This proportion was markedly higher than the proportion of (23.7%) reported by Licata et al. in their study conducted in Italy, which included a total of 382 patients with chronic diarrhea [15]. On the other hand, approximately one-quarter (26.0%) of the patients enrolled in the present study had normal colonoscopy, in contrast to the finding by Licata et al. who reported a proportion exceeding two-thirds (69.9%). This higher proportion of patients with IBD in the present study may reflect the effect of certain differences in diet and lifestyle between the Iraqi population and Italian population regarding the development of the disease. ROC analysis have shown that sensitivity and specificity for fecal Calprotectin in the detection of abnormal colonoscopy findings were 87.8% and 80.8%, respectively, with area under the curve (AUC) of 0.93 (95% C.I: 0.89 – 0.97), P-value

< 0.001. Al-Kufi and Ghadhban in their study conducted in Baghdad in 2015-2016 had reported that fecal Calprotectin had a sensitivity of 84.0% and specificity of 60.0% in detecting IBD [16]. Slightly lower findings were reported by D'inca et al. who found that fecal Calprotectin provided a sensitivity of 78.0% and a specificity of 70.0%, with an AUC of 0.75 (95% C.I. : 0.60 – 0.91), P-value = 0.015 [17]. The present study had demonstrated that Calprotectin level is significantly higher than normal in cases of IBD (Crohn's disease and ulcerative colitis), CA colon, and microscopic colitis, which are detected by colonoscopy. Patients with fecal Calprotectin level above 10 µg/g were found to be 30 times more likely to have abnormal findings on colonoscopy. Patients with colon malignancy in the present study had also significantly higher levels of fecal Calprotectin, with P-value of < 0.001. Al-Kufi and Ghadhban had also concluded that fecal Calprotectin test is an effective tool for the diagnosis of colorectal malignancy in comparison to colonoscopy, due to its lower cost and less invasiveness [17]. Elevated fecal Calprotectin level reflects the migration of Neutrophils to the lumen of the intestine during inflammatory process, as it is released following the activation of Neutrophils [18]. The use of Calprotectin as an indicator for inflammatory bowel disease and GIT malignancy is considered more acceptable by the patients, as the use of colonoscopy is considered invasive, expensive, and often requires sedation of the patient [19]. Due to these advantages, fecal Calprotectin has been recommended by the clinical guidelines to be considered as part of the regular diagnostic work-up for patients with Crohn's disease and ulcerative colitis [20-21].

CONCLUSIONS

This study concludes that the level of fecal Calprotectin increase significantly in patients with inflammatory bowel disease and colon malignancy, and can be used to predict the abnormal findings in colonoscopy. However, this indicator cannot discriminate between Crohn's disease and ulcerative colitis.

Ethical clearance- Taken from The Institution's Ethical Committee approval

Source of funding- Self

Conflict of Interest -Nil

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