Effect of Colonoscopy on Fecal Calprotectin Testing

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Abstract

Background:

In IBD patients, colonoscopy is essential for diagnosis, determining severity, tracking therapeutic response, screening for malignancy, and making critical therapeutic decisions. A non-invasive diagnostic test is necessary to enhance the filtering of patients who need a colonoscopy. Faecal calprotectin (FC) provides a non-invasive method for measuring disease activity in patients with inflammatory bowel disease (IBD).

Aim:

Investigating the effect of colonoscopy on testing faecal calprotectin results after the procedure.

Patients and Methods:

In a quasi-experimental study (a before-and-after design), 34 patients underwent colonoscopy after being tested for fecal calprotectin within one week before the procedure. All patients received the same bowel cleansing preparation. FC was measured at 3 points: (a) specimen 1 (FC1): within one week before bowel cleansing by a laxative, (b) specimen 2 (FC2): within the first 48 hours after colonoscopy, (c) specimen 3 (FC3): one to two weeks after colonoscopy.

Results:

The FC level significantly increased ($p = 0.000$) in FC2 in 100% of the study group. The median of the difference in FC levels between FC1 and FC2 was 78 (10-836). FC level increased in FC2 by more than 100 µg/g in 22/34 (64.7%). The greater difference between FC2 and FC1 levels was significantly associated with the colonoscopy's longer duration ($p = 0.006$). The FC level decreased significantly ($p = 0.000$) in FC3 to a level lower than FC2 in 100% of the study group. FC3 decreased to a level lower than or equal to FC1 in 20 out of 34 (58.8%).

Conclusion:

Testing for FC can be inaccurate and unreliable if performed too soon after a colonoscopy.

Keywords: *colonoscopy, fecal calprotectin, inflammatory bowel disease, Stool tests, endoscopy*

What is already known about this topic? Before this study, this point was not of genuine interest, and no clear information was available. To the best of our knowledge, in real-life practice, physicians order FC testing either before or after a colonoscopy without any reference guidelines regarding this issue.

What this study adds – This study shows that testing FC after colonoscopy is neither accurate nor reliable for diagnosis or follow-up.

How this study might affect research, practice, or policy – Physicians would not test for FC early after colonoscopy, either for diagnosis or follow-up, revising the data of all patients who received treatment based on post-colonoscopy FC testing. Furthermore, additional studies should be conducted to investigate the optimal time for FC testing following colonoscopy. FC testing, performed early after colonoscopy, may contribute to the causes of false-positive results.

Abbreviations:

IBD: Inflammatory Bowel Disease

FC: Fecal Calprotectin

Introduction:

More than 50% of patients with inflammatory bowel disease (IBD) need surgical intervention within ten years of being diagnosed because of the severe and progressive nature of the

condition [1-3]. Timely referral is essential, which necessitates filtering patients with a high likelihood of IBD from a larger group of patients who primarily have irritable bowel syndrome (IBS) [4].

In IBD patients, colonoscopy is a crucial procedure for diagnosis, determining disease severity, monitoring therapeutic response, screening for malignancy, and informing critical therapeutic decisions [5-7].

In the absence of a non-invasive diagnostic test, referral rates by general practitioners range from 10% to 20% of patients complaining of abdominal symptoms [8,9]. Among referred patients, 75% do not have an organic disorder, and only one-third of those with organic disease have IBD [10,11].

Clinically useful biomarkers of intestinal inflammation, including lactoferrin or fecal calprotectin (FC), have emerged, offering a way to measure disease activity in patients with inflammatory bowel disease (IBD) [12–18]. The number of inflammatory cells infiltrating the intestinal mucosa is reflected in the quantity of these neutrophil-derived indicators in feces [19]. The daily fluctuations in fecal surrogate marker levels in inflammation are not well-reported. Moum et al. [20] demonstrated a significant degree of intraindividual variability in FC levels, primarily in the higher values rather than those indicating a positive or negative status for the patient. The solid endoscope that comes in close contact with the colonic mucosa, theoretically, could be injurious and may affect post-colonoscopy FC testing. This study aims to investigate the accuracy and reliability of testing for FC after colonoscopy.

Methods:

Ethical considerations and patient involvement:

The study was conducted in accordance with the ethical guidelines of the 1975 Helsinki Declaration and was approved by the local ethics committee of the Faculty of Medicine, New Valley University (Registration No.: 20240230004). All participants provided written informed consent to be enrolled in the study. Enrolment of patients started on March 1, 2024, and ended on March 20, 2024. There was enough discussion with each patient about the study details and its benefits to them and others; they were all aware of the study. Not only that, but we were also interested in sharing our preliminary results with them, and they were all inspired by these results, agreeing to disseminate and publish our findings.

In a quasi-experimental study (before-and-after study) [21], 34 patients were enrolled in the study. They were presented with different abdominal manifestations and were referred for colonoscopy. The study group underwent colonoscopy after testing for fecal calprotectin within one week before the procedure. FC was measured at 3 points: (a) specimen 1 (FC1): within one week before bowel cleansing by a laxative [22], (b) specimen 2 (FC2): within the first 48 hours after colonoscopy, (c) specimen 3 (FC3): one to two weeks after colonoscopy. The study was conducted at New Valley University, Aswan University, and Assiut University Hospitals.

All patients received the same bowel cleansing preparation, which consisted of a fixed-dose oral laxative containing macrogol 3350, sodium sulfate, sodium chloride, potassium chloride, ascorbic acid, and sodium ascorbate. Colonoscopy required fasting for 8 hours before the procedure, and they were all anaesthetized using propofol. All eligible patients who accepted to participate were enrolled in the study.

Inclusion criteria: 1. Presentations that are indicated for colonoscopy, 2. Patients who agree to be involved in the study, 3. No contraindication to colonoscopy.

Exclusion criteria: Any indication for carrying out upper endoscopy in the same setting with colonoscopy.

Quantitative measurement of Calprotectin in stool:

FC was released from the stool with an extraction medium in the Stool Extraction Tube (ORG 282). Then, the extracted FC level was measured using an Indirect ELISA assay (ORGENTEC Diagnostika GmbH, Cat No. ORG 580, Carl-Zeiss-Straße 49, 51512 Mainz, Germany) according to the manufacturer's instructions. The cut-off value was 50 µg/g.

Interpretation of results was as follows: normal range: < 50 µg/g, slightly elevated values: 50 - 200 µg/g, and significantly elevated values: > 200 µg/g [23-37].

Sample size:

The sample size was calculated using OpenEpi, version 3, an open-source calculator with the expected frequency of increased FC in 75% of 8 patients who underwent colonoscopy in Summerton et al. 2002, versus 22% of the other nine patients [37] at a 95% confidence level and an effect size of 1. The resulting sample size was 34 patients.

Statistical analysis:

The data was collected and entered into the statistical package for Social Science (SPSS) version 25; the qualitative data was presented as numbers and percentages, while quantitative data was presented as mean, standard deviations, range, and median with inter-quartile range, and according to their distribution, the suitable test was used; the Wilcoxon. Regression analysis was conducted to identify potential predictors of the outcome. The confidence interval was set at 95%, and the accepted error margin was 5%. Therefore, the p-value was considered significant if it was less than 0.05. There was no missing data.

Results:

The study group consisted of 18 males (52.9%) and 16 females (47.1%). The mean age was 38±13.3 (8-60) years old. Among them, 11 (32.4%) were smokers and 6 (17.6%) were taking nonsteroidal drugs.

Indications of colonoscopy were bleeding per rectum in 21 patients, chronic abdominal pain in 8 patients, chronic diarrhea in 6 patients, and chronic undiagnosed constipation in 8 patients.

Macroscopic normal colonic mucosa was observed in 14 patients, including eight patients with hemorrhoids. Eight patients had polyps, either with or without dysplasia. Two patients had ulcerative colitis, one patient had abundant eosinophils, four patients had chronic nonspecific colitis, and one patient had oxyuris proctosigmoiditis.

Table 1. Data of colonoscopy and fecal calprotectin:

	Study group n=34 (%)	p value
Biopsy	13 (38.2)	
Colonoscopy duration (min.) (mean±SD, min-max)	23.4±8.5 (12 - 45)	
FC1 (µg/g) (median, IQR)	50, 86	
FC2 (µg/g) (median, IQR)	167, 342	
FC3 (µg/g) (median, IQR)	50, 80	
FC2 > FC1 (no. of patients)	34 (100)	
FC2 was elevated more than 100 µg/g (no. of patients)	22 (64.7)	
Difference between FC2 and FC1 (µg/g) (median, IQR)	78, 208	p = 0.000*
Difference between FC2 and FC1 (log) (mean±SD, min-max)	2±0.5, (1–2.9)	
FC3 < FC2 (no. of patients)	34 (100)	
FC3 ≤ FC1 (no. of patients)	20 (58.8)	

FC: Fecal calprotectin, FC1: Specimen 1 within one week before receiving preparation for colonoscopy, FC2: Specimen 2 within 48 hours after colonoscopy, FC3: Specimen 3 one to two weeks after colonoscopy

* p-value ≤ 0.05 is significant, ns: Non-significant

As shown in Table 1, the duration of colonoscopy was 23.4±8.5 (12 - 45) minutes. The median FC1, FC2, and FC3 levels were 50 (8-2215), 167 (40-2912), and 50 (20-2319), respectively.

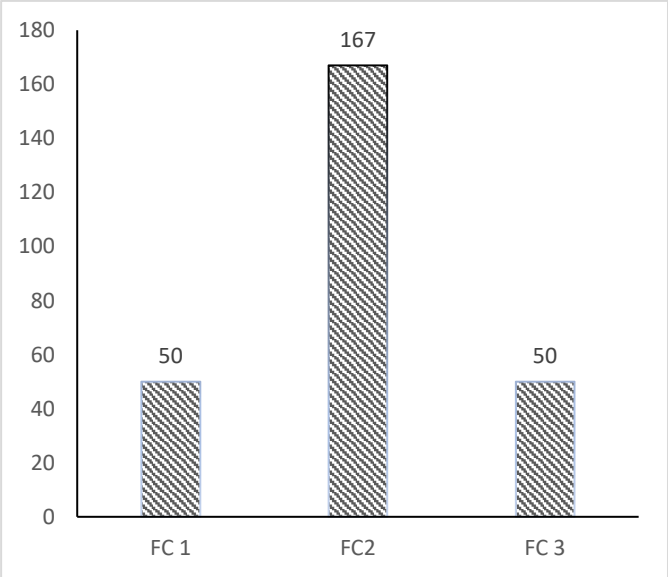

Fig 1.shows the median of FC1, FC2, and FC3 measured in µg/g. FC1: specimen 1 Fecal calprotectin, FC2: specimen 2 Fecal calprotectin, FC3: specimen 3 Fecal calprotectin.

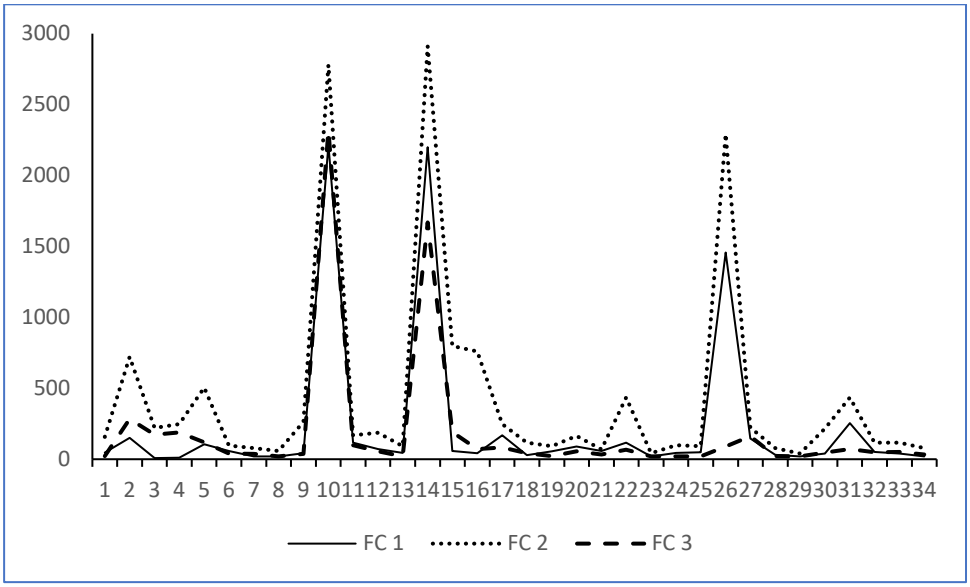

Figure 2. Shows fecal calprotectin (FC) level measured in µg/g in the study group patients. FC1: specimen 1 Fecal calprotectin, FC2: specimen 2 Fecal calprotectin, FC3: specimen 3 Fecal calprotectin.

FC level increased significantly (p=0.000) in FC2 in 100% of the study group (Fig. 2). The median difference between FC1 and FC2 levels was 78 (10-836). FC2 level increased by more than 100 µg/g in 22/34 patients (64.7%).

This level of increase was more pronounced in males than in females, with 14/18 (77.8%) and 8/16 (50%), respectively (p = 0.09). In addition, it was found that this increase is related to patients who are not receiving nonsteroidal anti-inflammatory drugs (NSAIDs) rather than those who are taking NSAIDs; 20/28 (71.4%) and 2/6 (33.3%), respectively (p = 0.076) (Table 2). The longer duration of colonoscopy was significantly related to the increased difference between FC2 and FC1 levels (p = 0.006) (Table 3).

Table 2. Relationship between Gender and NSAIDs and increased fecal calprotectin levels post-colonoscopy in FC2:

	Study group n=34 (%)	p value
Gender		
Male	14/18 (77.8%)	0.09
Female	8/16 (50%)	
NSAIDs		
Yes	2/6 (33.3%)	0.076
No	20/28 (71.4%)	

Table 3. Relationship between Duration of colonoscopy and increased fecal calprotectin levels post-colonoscopy in FC2:

	Beta	t	p value
Duration of colonoscopy	0.460	2.931	0.006*

* p-value ≤ 0.05 is significant, ns: Non-significant

FC level decreased significantly (p=0.000) in FC3 to a level lower than FC2 in 100% of the

study group (Fig. 2). FC3 decreased to a level lower than or equal to FC1 in 20/34 (58.8%). This decrease was more pronounced in females than in males, with 12/16 (75%) and 8/18 (44.4%) cases, respectively (p = 0.07). Additionally, this decrease was more pronounced among nonsmokers than smokers, with 16/23 (69.6%) and 4/11 (36.4%) cases, respectively (p = 0.066). In addition, it was found that this decrease was related to not taking a biopsy more than taking a biopsy; 15/21 (71.4%) and 5/13 (38.5%), respectively (p = 0.058) (Table 4).
There was no significant difference between FC1 and FC3 (p=0.357).

Table 4. Relationship of Gender, smoking, and taking biopsy to decreased fecal calprotectin levels in FC3:

	Study group n=34 (%)	p value
Gender		
Male	8/18 (44.4%)	0.07
Female	12/16 (75%)	
Smoking		
Yes	4/11 (36.4%)	0.066
No	16/23 (69.6%)	
Biopsy		
Yes	5/13 (38.5%)	0.058
No	15/21 (71.4%)	

Discussion

The present study found that FC increased within 48 hours after colonoscopy in 100% of the study group and then decreased in 100% of the study group after one to two weeks following the procedure. The longer duration of colonoscopy was significantly related to the increased difference in FC level within 48 hours after colonoscopy. Female gender and patients receiving NSAIDs showed a lower degree of increased FC level early after colonoscopy.
Male patients, smokers, and those who underwent a biopsy showed a lower degree of return to their pre-colonoscopy FC level one to two weeks after colonoscopy.
A study carried out by Summerton et al. (2002 found that there was an insignificant increase in FC level one week after colonoscopy or sigmoidoscopy in 7/9 (77.8%) [37]. These results align with our findings, which describe the rise in FC level one to two weeks after colonoscopy. In the present study, the FC level showed an insignificant increase in FC3 compared to FC1 in 14 out of 34 (41.2%) cases. However, Summerton et al. (2002 carried out their study on a small sample size, did not describe FC level 24 to 48 hours after colonoscopy, and did not investigate the possible predictors for FC level rise. Summerton et al. (2002) stated that there were no definite differences between pre- and post-endoscopy FC, and they found that it is better to rely on pre-endoscopy FC for the rest of the patients in the same study [12]. We think that our results are more conclusive and more precise in describing the effect of colonoscopy on FC testing.
Kolho et al. 2012 studied 10 pediatric patients with IBD [22]. They found that FC levels decreased significantly on the day of bowel cleansing in 100% of the study group and increased dramatically after colonoscopy in 6 out of 7 (85.7%) participants. The post-colonoscopy results are in concordance with our results. Their results about FC level during bowel cleansing showed that the only factor causing post-colonoscopy FC rise is colonoscopy itself [22].
The results of the present study suggest that colonoscopy itself may induce some injury and inflammatory process in the colonic mucosa, resulting in FC rising early post-colonoscopy.

This is also supported by the results of the effect of colonoscopy duration, NSAIDs, and taking colonic mucosal biopsies on changes in FC2 and FC3. We suggest that longer duration and taking biopsy means more mucosal injury, and receiving NSAIDs, which are anti-inflammatory drugs, is associated with a lower degree of FC level rise.

The main limitation of the study was the small sample size, which was attributed to the high cost of FC testing. Additionally, we were unable to determine the exact time for reliable FC testing after colonoscopy. Further randomized controlled studies are recommended, considering these limitations, to generalize these results.

Conclusion:

Testing for FC can be inaccurate and unreliable if performed too soon after a colonoscopy. It could be more accurate for diagnosis if it is done before preparation for colonoscopy or after colonoscopy, with enough time, more than two weeks. Still, the exact time should be investigated accurately in further studies.

Footnotes.

Ahmed Agrodey (Professor of internal medicine, gastroenterology, and hepatology unit), Mohamed Emara (Professor of gastroenterology, hepatology, and infectious diseases department), and Amany Mohamed (Professor of family medicine and biostatistician) were peer reviewers.

E- Editor: Salem Youssef Mohamed, Osama Ahmed Khalil, Amany Mohammed.

Ethics approval

The study was approved by the local ethics committee of the Faculty of Medicine, New Valley University (Registration No.: 20240230004). The study was conducted in accordance with relevant guidelines and regulations. All participants provided informed consent to be enrolled in the study.

Data and materials availability: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests.

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This work was conducted following the STROBE guidelines.

Authors' contributions:

Mohamed Farouk, Helal Hetta, and Amel Ahmed Moustafa revised the results and shared them during the manuscript writing and editing process. Essam Eldeen M.O. Mahran, Mohamed Abdelghani, Manal M. Darwish, Ebtisam Shawky Ahmed Hassanin, and Mohamed Abdel Fatah Mohamed Sayed conceived the study and analyzed the data. Amel Ahmed Moustafa, Essam Eldeen M.O. Mahran, Mohamed Abdelghani, Manal M Darwish, and Ebtisam Shawky Ahmed Hassanin conceived the idea, contributed to interpreting the results, and revised the manuscript. Mohamed Farouk designed the study and conducted the data analysis. Mohamed Abdel Fatah Mohamed Sayed conducted clinical studies, collected data, and wrote the manuscript in collaboration with others. Mohamed Farouk and Helal Hetta collected data, analyzed results, and prepared the manuscript. All authors read, revised, and approved the final manuscript.

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