Evaluation of Faecal Lactoferrin as a Diagnostic Marker in Egyptian Patients with Irritable Bowel Syndrome, Ulcerative Colitis and Colorectal Carcinoma

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Abstract: Background: Elevated LF has been used as a marker of active IBD and for monitoring patients for response to treatment. Some studies report a high sensitivity of LF for active IBD in comparison with IBS. **Aims:** To investigate the utility of faecal lactoferrin as a marker of inflammation in patients with UC, IBS and CRC. **Methods:** A cross sectional study was conducted on 60 persons who fulfilling the designed inclusion criteria and classified into five groups, Group I include 10 healthy persons, Group II: 10 patients known to have IBS, Group III: 15 patients known to have UC in remission, Group IV: 15 patients known to have UC in active state, Group V:10 patients known to have colorectal carcinoma. Fecal Lactoferrin level was measured in all patients using a highly sensitive enzyme – linked immunosorbent assay (ELISA). **Results**: The mean ± lactoferrin concentration (µg//ml) was 0.5 ±23 for IBS patients, 23 ± 4800 for UC patients, 2.5 ± 62 for CRC patients and 0,5 ±7.1 for healthy controls. Lactoferrin levels were significantly higher in UC patients compared with IBS/healthy controls ($P < 0.001$). The mean lactoferrin concentrations were significantly higher in active UC patients compared with inactive patients ($P = 0.02$). The mean lactoferrin concentrations were significantly higher in CRC group compared to control group. The sensitivity, specificity, positive and negative predictive values of lactoferrin in distinguishing active UC from IBS/healthy controls were 96.7% and 100%, 100% and 90.9% respectively. The sensitivity, specificity, positive and negative predictive values of lactoferrin in distinguishing CRC from control group 93.3%, and 100%, 100% and 90,9% respectively. **Conclusions:** Fecal Lactoferrin is useful to differentiate between UC and IBS and can be used as an adjuvant to blood parameters to determine patients who have ongoing colorectal carcinoma.
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1. Introduction

Inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS) are common conditions that may present with a similar symptom complex of abdominal pain and altered bowel habits. However, the two conditions differ markedly in their pathophysiology, prognosis and therapeutic approaches **(Bernstein et al., 2010).** Colorectal cancer is the third most commonly diagnosed cancer in the world, but it is more common in developed countries. Colorectal cancer represents about 12% of all cancers (National cancer statistics 2005). It is the third most common cancer in women after breast and lung cancer whereas in men it also ranks third after prostate and lung cancer, in Egypt, the incidence of colorectal cancer ranges between 2 and 6 percent of the total number of cancer cases reported annually **(Abd al salam, 2010).**

The prevalence of colorectal cancer in patients with ulcerative colitis is approximately 3.7%. The risk for colorectal cancer increased with duration of disease; there was a 2% incidence of cancer after 10 years, a 9% incidence after 20 years and a 19% incidence after 30 years of disease. The development of cancer accounts for one third of deaths related to ulcerative colitis **(Fernando et al., 2010).** Several neutrophil-granular proteins released by activated neutrophils may constitute fecal markers of intestinal inflammation, including lactoferrin (LF), calprotectin (Cal), polymorphonuclear neutrophil-elastase (PMN-e), and lysozyme (Lys), with Cal and LF appearing to be the most promising surrogate biomarkers**(Poullis et al., 2002).**

One such potential marker is fecal lactoferrin, determination of this marker may serve as a prescreening test in qualification for endoscopy**, (Pfefferkorn et al., 2010; Walker et al., 2007; Kane et al., 2003; Fine et al., 1998; Sugi et al., 1996 and Sudo et al., 1993)** were the first to show that patients with IBD had a significantly higher fecal lactoferrin levels than the individuals with irritable bowel syndrome. The aim of this study was to investigate the clinical utility of LF as a marker of GI inflammation in

patients with active and inactive IBD compared with patients with IBS, CRC and healthy controls.

2. Patients and methods:

Sixty consecutive patients with were recruited from the out-patient clinic, as well as from the inpatient unit of the Tropical medicine department - Al-Azhar University. Inclusion criteria included was Egyptian patients above 18 years old, confirmed diagnosis of UC was ascertained using conventional clinical, endoscopic, radiological and histopathological criteria, and confirmed diagnosis of IBS after full work up. Patients with irritable bowel syndrome, inflammatory bowel disease were questioned about their general well being, the frequency of bowel habit, the presence/absence of abdominal pain or blood in the stool, and clinical examination were completed to all the studied patients. All patients were investigated and treated according to the guidelines. Patients with positive stool culture, medical history of major gastrointestinal surgical procedures especially resection anastomosis operation, liver cell failure, chronic renal failure, congestive heart failure and non-steroidal anti-inflammatory drugs or Proton pump inhibitors were excluded from the study. All patients were subjected to full medical history, through clinical examination, laboratory investigations (CBC, ESR, CRP, Stool analysis, stool culture and sensitivity, Liver function tests, kidney function tests, and Fecal lactoferrin). Abdominal ultrasonography, Endoscopic examination and biopsy, and histopathological examination of tissue samples were done for selected patients. Fecal lactoferrin assay: Fecal lactoferrin levels were determined for all samples using quantitative enzyme-linked immunosorbent assay (ELISA) using Lactoferrin human ELISA kit by assaypro company, 3400 Harry S Truman Blvd St. Charles, MO 63301-4046, USA .

Statistical analysis:

The data was analysed using Microsoft Excel 2010 and statistical package for social science (SPSS version 24.0) for windows (SPSS IBM., Chicago, IL). Results was expressed as mean ± SE with 95% confidence interval using medians for quantitative variables, and using the frequencies and percentage for qualitative ones; a p value < 0.05 will be considered statistically significant.

3. Results:

Sixty individuals will be involved in this study and classified into five groups: Group I: Including 10 healthy persons as control group, Group II: Including10 patients known to have IBS with normal colonoscopy and histopathological examination, Group III: Including 15 patients known to have UC, in remission state, Group IV: Including 15 patients known to have UC, in active state and Group V: Including 10 patients known to have colorectal carcinoma.

Kruskal–Wallis ANOVA in **Table (1)** shows a highly significant elevation of fecal lactoferrin levels in UC patients (group III & IV) in comparison with IBS patients (group II), CRC patients (group V) and control group (group I). Also a significant elevation of fecal lactoferrin levels in CRC patients (group V) in comparison with control group (group I). But no significance showed between IBS patients (group II) and control group (group I).

Table (2): Shows a significant elevation of fecal lactoferrin levels in UC patients with severe activity index in comparison with lower stages of disease activity.

Table (1) Comparison between different study groups regarding fecal Lactoferrin levels.

	lactoferrin			Kruskal-Wallis Test	
	Range	Median	InterquartileRange	Mean rank	X^2	P-value
Group I	0.5 - 7.1	2.15	2.95	10.15	39.397	0.000
Group II	0.5 - 23	5.5	5.475	16.65		
Group III	23 - 305	150	112	45		
Group IV	1.6 - 4800	105	909	43.1		
Group V	2.5 - 62	24.5	42.75	24.05		

Mann-Whitney Test									
I&II	**I&III**	**I&IV**	**I&V**	**II&III**	**II&IV**	**II&V**	**III&IV**	**III&V**	**IV&V**
0.069	0.000	0.00	0.007	0.000	0.00	0.096	0.020	0.000	0.004

Table (2): Comparison of FL levels in UC patients at different stages of activity:

				ANOVA	
	Range	Mean ± SD		F	P-value
Inactive	23 - 305	153.667 ± 77.064		18.346	0.000
Mild	105 - 950	363.000 ± 336.927			
Moderate	1.6 - 105	50.800 ± 35.000			
Severe	1200 - 4800	2626.667 ± 1912.625			

Inactive & Mild	Inactive & Moderate	Inactive & Severe	Mild & Moderate	Mild & Severe	Moderate & Severe
0.881	0.976	0.000	0.768	0.000	0.000

Table (3): Shows a significant elevation of fecal lactoferrin levels in UC patients with high activity in colonoscopy in comparison with lower stages of disease activity.

Table (4): shows that FL levels have 93.3% sensitivity and 100% specificity in identification of UC patients from those with IBS with cutoff value 23 ug/ml.

Table (5): Shows that FLA levels have 93.3% sensitivity and 95% specificity in identification of CRC patients from those with IBS with cutoff value 23 ug/ml.

Table (6): Shows that FLA levels have 90% sensitivity and 50% specificity in identification of IBS patients from healthy subjects with cutoff value 1.8ug/ml.

Table (7): Shows that FLA levels have 82 sensitivity and 100% specificity in discriminating patients with IBS (functinonal) from patients with UC and CRC (organic).

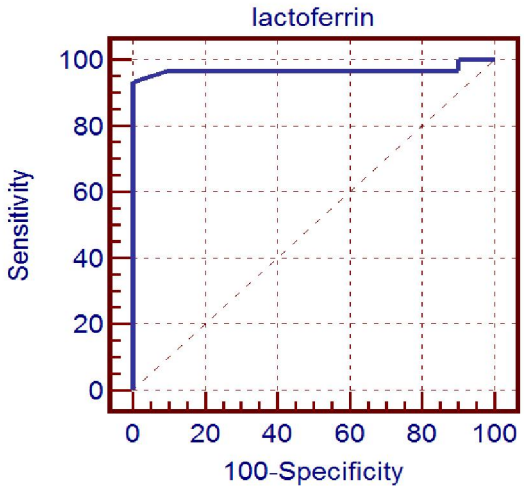

Figure (1): Receiver Operator Curve Analysis was done to estimate the diagnostic value of fecal lactoferrin levels in discriminating patients with UC (Group III+IV) from IBS (Group II) patients.

Table (3): Comparison of FL levels in UC patients as regard colonoscopic finding.

				ANOVA	
	Range	Mean ± SD		F	P-value
Normal	0.5 - 180	49.213 ± 65.295		6.118	0.001*
Inactive	23 - 305	163.200 ± 91.757			
Mild	1.6 - 85	38.520 ± 30.028			
Moderate	58 - 230	141.600 ± 74.433			
High	320 - 4800	1830.000 ± 1751.771			

Normal& Inactive	Normal& Mild	Normal& Moderate	Normal& High	Inactive& Mild
1.000	0.999	1.000	0.007	0.997
Inactive& Moderate	**Inactive& High**	**Mild& Moderate**	**Mild& High**	**Moderate& High**
1.000	0.002	0.999	0.004	0.007

Table (4): Diagnostic value of FL levels in discriminating patients UC from IBS patients.

ROC curve					
Cutoff	**Sens**	**Spec**	**PPV**	**NPV**	**Accuracy**
> 23	93.3	100	100	83.3	0.968

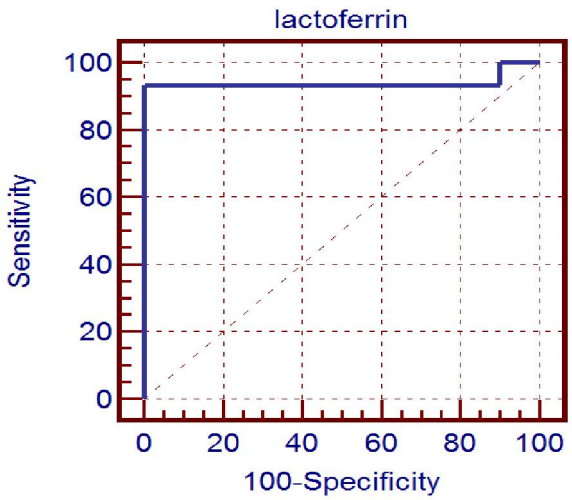

Figure (2): Receiver Operator Curve Analysis was done to estimate the diagnostic value of FL levels in discriminating patients with CRC from IBS patients.

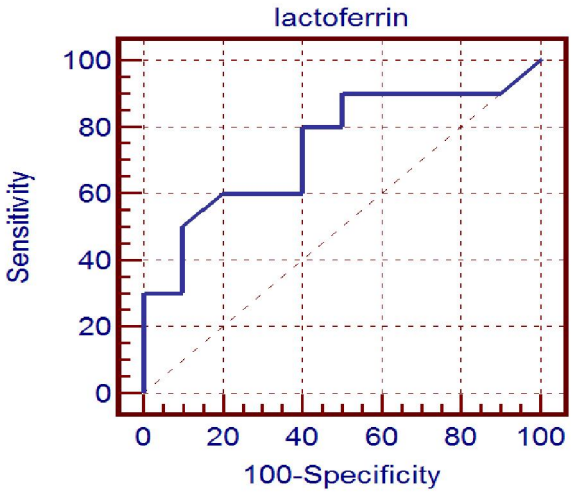

Figure (3): Receiver Operator Curve Analysis was done to estimate the diagnostic value of fecal lactoferrin levels in discriminating patients with IBS from healthy subjects.

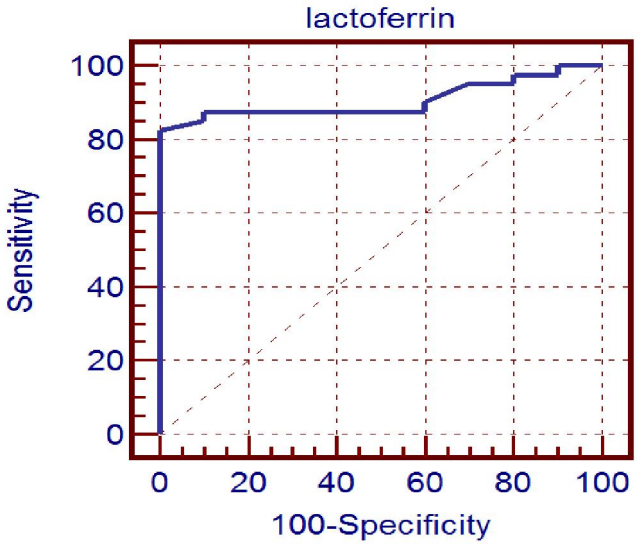

Figure (4): Receiver Operator Curve Analysis was done to estimate the diagnostic value of FL levels between functinonal (Group II) and organic (Group III, Group IV, Group V) groups.

Table (5): Diagnostic value of fecal lactoferrin levels in discriminating patients with CRC from IBS patients.

ROC curve					
Cutoff	**Sens**	**Spec**	**PPV**	**NPV**	**Accuracy**
>23	93.3	95	95	90.9	94

Table (6) Diagnostic value of fecal lactoferrin levels in discriminating IBS patients from healthy subjects.

ROC curve					
Cutoff	**Sens**	**Spec**	**PPV**	**NPV**	**Accuracy**
>1.8	90	50	64.3	83.3	0.74

Table (7): Comparison of FL levels between functinonal (Group II) and organic (Group III, Group IV, Group V) groups.

ROC curve					
Cutoff	**Sens**	**Spec**	**PPV**	**NPV**	**Accuracy**
> 23	82.5	100.0	100.0	58.8	90.6

4. Discussion

The gold standard investigation for the early detection of colorectal cancer is colonoscopy. However, the acceptance of this costly and invasive method is low. Only 1.7% of people entitled to colonoscopy under the national colorectal cancer screening program actually undergo the procedure **(Parkin et al., 2005).** If the ideal marker exists for IBD, it would greatly facilitate the work of the gastroenterologist or surgeon treating these patients and to increase the participation in colorectal cancer screening programs, an easy, fast and economical initial screening method, with good patient compliance, is absolutely necessary. This allows identification of those patients most likely to have colorectal cancer, who require further investigation by colonoscopy. One such potential marker is fecal lactoferrin, determination of this marker may serve as a prescreening test in qualification for endoscopy **Pfefferkorn et al., (2010).** Our aim in the current cross sectional study was to investigate the faecallactoferrin levels in patients with ulcerative colitis during exacerbation and remission and comparing with those obtained from patients with irritable bowel syndrome, colorectal carcinoma and control personsand to detect its sensitivity and specificity as a non invasive biomarker in identification of such patients. As the aim of our study is to evaluate the role of lactoferrine as a fecal biomarker. Lactoferrinin the current study showed that there was significant high level of fecal lactoferrinin UC groups (remission and exacerbation) compared to CRC, IBS and control groups. This can be attributed to presence of active inflammatory cells in patients with UC with production of lactoferrin at higher levels in patient's stools than healthy groups. Even with inactive disease, patients with UC still had significantly higher lactoferrin concentrations than healthy controls or patients with IBS (p value < 0.001). Also there is no significant difference between the level of fecal Lactoferrin in IBS and healthy control groups (p-value> 0.05). Also in the present study fecal lactoferrin levels varied significantly in patients with UC according to disease activity, higher levels were found in patients with severe UC than patients with inactive UC (p-value =0.000). On the other hand no significant difference in FLA levels was found between patients with inactive UC and those with mild or moderate activity (p-value> 0.05). Our results are in agreement with those conducted by **Sidhu et al (2010)** who reported that the median fecal Lactoferrin levels were significantly higher in patients with UC compared with patients with IBS ($P < 0.001$) and healthy controls ($P < 0.001$). As for stratification based on severity of disease activity, the median fecal Lactoferrin levels were significantly higher in patients with active disease compared with patients with inactive disease for UC patients ($P < 0.001$), also there is no significant difference between the level of fecal Lactoferrin in IBS and healthy control groups, Also **Anna et al., (2015)** reported that there is significant relationships between the fecal concentration of lactoferrin and the activity of IBD determined on the basis of clinical symptoms and various scoring systems.

In the present study fecal lactoferrin levels varied significantly in patients according to Endoscopic Activity Index of UC. Higher levels were found in patients with high grades of UC than patients with normal, inactive, mild or moderate colonoscopic finding (p value =0.001). Our findings was in agreement with **Langhorst et al., (2006)** who stated that lactoferrin levels were significantly increased in moderate to severe IBD and showed higher levels with increasing endoscopy scores. Also **Jones et al., (2008)** reported that lactoferrin has been shown to correlate well with clinical, endoscopic and histological grading of IBD disease activity.

Receiver operating characteristic curves comparison demonstrated that FLA levels displayed high sensitivity and specificity in identifying patients with UC from those with IBS, CRC and healthy controls.

In the present study Comparing FLA levels in patients with UC and IBS patients, fecal Lactoferrin levels was found to be highly sensitive (sensitivity 93.3%) and highly specific (specificity 100%) with positive predictive value (100%) and negative predictive value (83%) in differentiating patients with IBD from those with IBS with cutoff value >23ug/ml and 96% accuracy. also fecal lactoferrin levels between functinonal (IBS) and organic (UC patients in remission, exacerbation and CRC) patients, FLA levels showed a high sensitivity (82.5%) with high specificity (100%) with positive predictive value (100%) and negative predictive value (85.8%) in identification patients with IBS from other patients with cutoff value 23 ug/ml and 90% accuracy.

In the present study Comparing FLA levels in patients with UC and healthy controls, FLA was found to be highly sensitive (sensitivity 96.7%) and highly specific (specificity 100%) with positive predictive value (100%) and negative predictive value (90.9%) in differentiating patients with UC from healthy subjects with cutoff value >7.1ug/ml and 98% accuracy.

In the present study Comparing FLA levels in patients with UC patients in remission and exacerbation, FLA was found to be very low sensitivity (sensitivity 33.3%) and highly specific (specificity 100%) with positive predictive value (100%) and negative predictive value (60%) in differentiating patients with UC in remission and exacerbation, with cutoff value >305ug/ml and 50% accuracy.

On the other hand, comparing FLA levels in patients with IBS and healthy controls, FLA levels showed a high sensitivity (90%) but with very low specificity (50%) with positive predictive value (64.3%) and negative predictive value (83.3%) in identification patients with IBS from healthy controls with cutoff value 1,8ug/ml and 74% accuracy. The results are in agreement with **Dai et al., (2007)** Comparing FLA levels in patients with UC and IBS patients, fecal Lactoferrin levels was found to be highly sensitive (sensitivity 100%) and highly specific (specificity 100%) with cutoff value >24ug/ml, with no significant difference between IBS and control groups.

Sidhu et al., (2010) found that with cutoff value > 7.25µg/g fecal Lactoferrin levels was found to be sensitive (sensitivity 76%) and highly specific (specificity 96%) positive predictive value (87%) and negative predictive value (87%) in differentiating patients with IBD from IBS patients and healthy subjects, with no significant difference between IBS and control groups. **Otten et al., (2008)** found that with cutoff value >7.25µg/g fecal Lactoferrin levels was found to be sensitive (sensitivity 78%) and highly specific (specificity 90%) positive predictive value (67%) and negative predictive value (94%) in differentiating patients with IBD from IBS patients and healthy subjects, with no significant difference between IBS and control groups. **Zhou et al., (2014)** FL level had a pooled sensitivity of 0.78% and a pooled specificityof 0.94% in distinguishing IBD (active and inactive) from IBS. **Mahmoud et al., (2015)** reported that the pooled sensitivity and specificity estimates for fecal Lactoferrin were (95%) and (95%), respectively. The pooled positive and negative likelihood ratios were (95%) and (95%), respectively. The AUC was (95%). Also **Anna et al., (2015)** reported that the cut-off value of fecal lactoferrin concentration optimally distinguishing between the children with IBD and the controls was identified as 13 µg/g. The sensitivity and specificity of this cut-off value equaled 80.7% and 92.7%, respectively, and its PPV and NPV were 96.8% and 63.3%, respectively.

Wang et al., (2015) reported that the pooled FL sensitivity and pooled specificity were 82% and 95%, respectively. The positive and negative likelihood ratios were 16.63 and 0.18, respectively. The area under the receiver-operating characteristic curve was 95%.

In the present study Comparing FLA levels in patients with CRC and IBS patients, FLA was found to be highly sensitive (sensitivity 93%) and highly specific (specificity 95%) with positive predictive value (95%) and negative predictive value (90.9%) in differentiating patients with CRC from those with IBS with cutoff value >23ug/ml and 94% accuracy.

Also in comparing FLA levels in patients with CRC and healthy controls, FLA was found to be highly sensitive (sensitivity 93.3%) and highly specific (specificity 100%) with positive predictive value (100%) and negative predictive value (90.9%) in differentiating patients with CRC from healthy subjects with cutoff value >7.1ug/ml and 96% accuracy.

On the other hand, comparing FLA levels in patients with CRC and IBD, FLA levels showed a high sensitivity (100%) but with high specificity (80%) with positive predictive value (62.5%) and negative predictive value (100%) in identification patients with CRC from IBD patients with cutoff value 62ug/ml and 90% accuracy.

Our results in agreement with **Sidhu et al., (2010)** they found in a prospective study of patients undergoing routine colonoscopy, patients with an elevated lactoferrin (>7.25µg/g), colorectal pathology was likely to be identified at endoscopy with a high sensitivity of 78% in detecting cancer or IBD in patients above 50 years old. In patients below the age of 50, faecallactoferrin had a specificity of 96% in excluding colorectal disease. This demonstrates a potential role for faecallactoferrin in this group of patients as a screening tool to determine which individuals necessitate further lower gastrointestinal investigations. Also **Langhorst and Boone (2012)** reported that lactoferrin is nonspecific for IBD, and levels can be elevated by other infectious processes, such as those caused by Salmonella and Clostridium difficile, other forms of colitis, colon cancer or polyposis syndromes. **Hirata et al., (2007)** was shown that the detection of Lf concentrations in faecal matter was an effective prognostic for confirming colorectal cancer, Crohn's diseases, colon polyps or ulcerative colitis diseases. The expression of faecal Lf and haemoglobin shown that patients with colorectal cancer and Crohn's disease showed a significant

increase with more than 60% positive for Lf and haemoglobin expression. **D'Inca et al., (2006)** found that fecal LF concentration in patients with colon cancer showed a higher value than that in controls with with positive rate of lactoferrin in feces 87% (7/8 CRC patients). Also **Parsi et al., (2004)** found that the normal range for lactoferrin was<7.25 µg/g and have been demonstrated as raised in inflammatory, infective and neoplastic enteropathies. **Uchida et al., (1994)** stated that fecal LF concentration in patients with colon cancer showed a higher value than that in controls with positive rate of lactoferrin in feces 95.8% (23/24 CRC patients). In cancers, neutrophil leucocytes are known to be stimulated and accumulated within tumorousregions by cytokines such as tumor necrosis factor and interferon (IFN--y), and react as an antitumor agent. The mechanism of appearance of this reaction was also considered to be one of the factors increasing fecal LF concentration in colon cancer. LF concentration is not greatly affected by physiological bleeding and bleeding due to hemorrhoids. Therefore, this method is considered to be useful as a screening method for colon cancer.

FL is an inexpensive, simple, stable and useful screening marker with high specificity and modest sensitivity for differentiating between IBD and functional disorders, appearing to have greater ability to evaluate UC rather than CD. The fecal lactoferrin methods are the first line of techniques that allow non-invasive assessment of IBD **(Wang et al., 2015).** Different cut-off values are suggested for different patient categories, i.e, higher for patients with known inflammatory conditions while lower for screening purposes.

Conclusion

Fecal lactoferrin levels can be used in differentiating patients with IBD from those with IBS and in monitoring disease activity in patients with UC and can be used as an adjunct to blood parameters to determine patients who have ongoing colorectal carcinoma.

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