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Fecal Lactoferrin as a New Marker of Disease Activity in Inflammatory Bowel Diseases

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ABSTRACT

Background: Inflammatory bowel diseases (IBD) including ulcerative colitis (UC) and Crohn's disease (CD) are organic inflammatory diseases, caused by chronic mucosal inflammation of the gasrtointetinal tract. As the presenting manifestations of IBD and other diseases are similar, obtaining a clinical diagnosis can be difficult, and further invasive diagnostic procedures may be required in order to obtain a confirmed diagnosis. The aim of this study is to evaluate the diagnostic utility of measuring fecal concentrations of lactoferrin as a simple and noninvasive indicator of disease activity in patients IBD and to be correlated with endoscopic findings and disease activity index and acute inflammatory response including leucocytic count, high sensitive CRP, ESR. **Methods:** This study was carried on 40 patients with IBD; 24 patients with active IBD (16 UC patients and8 CD patients) and 16 patients with inactive IBD (10 UC patients and 6 CD patients) versus 40 healthy controls. All patients underwent blood and stool sampling as well as an interview to assess the disease severity utilizing UC activity measured by the Truelove and Witts Severity Index and Crohn's Disease Activity Index. Measurement of FLA levels at different stages of inflammatory bowel disease activity to detect its role in assessment of disease severity. **Results:** This study showed that FLA levels were highest in patients with IBD in comparison with healthy group. FLA levels also correlated significantly with disease severity in patients with IBD where higher levels of FLA were found in patients with severe UC or Crohn`s disease. At cutoff value 9.68 ug/ml FLA showed 100% sensitivity and specificity in identification of patients with IBD from healthy subjects.

Conclusions: FLA is a sensitive and specific biochemical marker of inflammation for use in the diagnosis of suspected IBD cases, and its level correlates well with both clinical disease activity indices.

Keywords: Inflammatory Bowel Disease, Ulcerative Colitis, Crohn's Disease, Fecal lactoferrin.

INTRODUCTION

Inflammatory Bowel Disease (IBD) includes Crohn's Disease (CD) and Ulcerative Colitis (UC). These are chronic idiopathic conditions, marked by recurrent episodes of inflammation of the gastrointestinal tract, interspersed with periods of remission [1].

Determining disease activity in IBD is difficult, as patients might have a concurrent source of gastrointestinal symptoms, such as irritable bowel syndrome (IBS) or infection. Attributing certain clinical symptoms to IBD has traditionally been accomplished either by examining biopsy specimens or by using radiologic imaging. However, these methods are not without risks [2] and there is much interest in assessing disease severity in a noninvasive fashion. The gold standard for assessing intestinal damage is fecal excretion of 111indium-labeled leukocytes, but because this process involves patient exposure to radiation as well as prolonged collection of feces, it is rarely used in clinical practice [3]. Initial attempts to noninvasively gauge disease activity had employed serologic markers,
such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR).These markers have been more recently thought to be less sensitive and specific than fecal makers [4].

Biological markers are a noninvasive way of objectively measuring inflammation and can play an adjunctive or primary role in the assessment of disease activity. These markers can be classified into serological and fecal categories [5]. Among the various serological biological markers available, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), anti-neutrophil cytoplasmic antibodies (ANCA), and anti-Saccharomyces cerevisiae antibodies (ASCA) stand out. However, these systemic markers have low sensitivity and specificity for intestinal inflammation and correlate poorly with symptoms and disease

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activity indexes.Fecal markers, however, have the theoretical advantage of having higher specificity for the diagnosis of gastrointestinal diseases such as IBD because their levels are not raised in extra digestive processes [1]. These findings led to the idea that an increased translocation of granulocytes into the intestinal mucosa in conditions of inflammation might give increased levels of proteins from such cells in feces. Calprotectin and lactoferrin are the most used and use fulfecal markers of intestinal inflammation [6]. Fecal markers selected and studied as indicators of inflammation include neutrophil granule proteins, lactoferrin and calprotectin [7].

Some authors consider a colonoscopy with biopsy to be the best means for evaluating inflammation location, extent, and severity; aside from being an invasive method, this approach carries risks of complications [8].

Lactoferrin is an iron-containing glycoprotein secreted by the majority of mucosal membranes. It is the main component of secondary polymorphonuclear granules, which are the prime cells of an acute inflammatory response. Other hematopoietic cells, such as monocytes and lymphocytes, do not contain lactoferrin. In intestinal inflammation, leukocytes invade the mucosa, which results in an increase in the excretion of lactoferrin into the feces [5].

Fecal lactoferrin can be used as amarker for monitoring disease activity in IBD and to discriminate between IBD and Inflammatory Bowel syndrome [9].

SUBJECTS AND METHODS

This study was case control, analytical, observational study, and has been conducted on convenient non-probability sample of 40 patients presented with (IBD) inflammatory bowel diseases from Outpatients clinic and inpatients of internal medicine Department of El- Hussein university hospital, AL-AZHAR University in addition to 40 healthy persons as control in the period from October 2014 to May 2016.

They were divided as follows:

Group I: *40 patients with IBD, they were subdivided into two subgroups:*

- **Subgroup A:** 24 patients with active IBD.
- **Subgroup B:** 16 patients with inactive IBD (controlled by treatment).

Group II: *40 healthy persons as controls.*

The following patients were excluded:

- Patients with positive stool culture.
- Patients with past history of colorectal carcinoma.
- Patients with past history of major gastrointestinal surgical procedures.
- Patients with liver cell failure, chronic renal failure or congestive heart failure.
- Patients with bleeding tendency.
- Patients on non steroidal anti-inflammatory drugs.

All patients were subjected to the following:

1) Full history taking with special emphasis on abdominal pain, weight loss, rectal bleeding, diarrhea, constipation, malaise, lethargy, anorexia, nausea, tenesmus, abdominal distension, passage of mucous, vomiting and low-grade fever. Past history of appendectomy or other operations and positive family history of IBD.
2) Full clinical examination
3) Laboratory investigations: Including CBC, PT, PTT and INR, fasting and postprandial blood glucose, serum creatinine, blood urea nitrogen, serum Na, serum K, serum total protein, serum albumin, AST, ALT, serum bilirubin total and direct.
4) ESR and CRP titre.
5) Complete stool analysis and stool culture and sensitivity to exclude the presence of infection.
6) Colonoscopy.
7) **Patient concent:** All procedures will follow Al-Azhar university ethical committee regulation, and patient concent will be taken from all patients.
8) **Measurement of activity indices in IBD patients**:

- Crohn's Disease Activity Index (CDAI) scores between 150 and 220 are mild and scores between 221 and 400 are moderate; more than 400 points is considered severe disease, and remission is defined as CDAI score less than 150, while UC activity was measured by the Truelove and Witts Severity Index (mild, moderate and severe) [10].
- The Crohn's Disease Activity Index consists of eight factors, each summed after adjustment with a weighting factor. The components of the CDAI and weighting factors are the following:

1. Number of liquid/very soft stools in 7 days (weighting factor 2)
2. Sum of 7 days abdominal pain ratings (Subjective grading: 0 = none, 1 = mild, 2 = moderate, 3 = severe) (weighting factor 5).
3. Sum of 7 days general well-being ratings (Subjective grading: 0 = well, 1 = average, 2 =

poor, 3 = very poor, 4 = terrible) (weighting factor 7).

4. Extraintestinal features (1 per finding): perianal disease (fissure/fistula/abscess), external fistula, mucocutaneous or cutaneous lesions, iritis/uveitis, arthritis/arthralgia, febrile episode in the past week (>100 °F) (weighting factor 20).
5. Use of antidiarrheal drugs (Lomotil or opiates): yes = 1, no = 0 (weighting factor 30).
6. Presence of abdominal mass: none = 0, equivocal = 2, definite = 5 (weighting factor 10).
7. Hematocrit deviation from normal (Typical {average 47 in males and 42 in females} minus current hematocrit) (weighting factor 6).
8. Percentage deviation from standard weight: 100 x [(standard weight-actual body weight) / standard weight] (weighting factor 1).
9. Total score between 0 and 750, sum score based on a 7 day aggregate of each item scored daily and current hematocrit and weight measurement. Total CDAI = sum of each item score x its weighting factor =1x2 + 2x5 + 3x7 + 4x20 + 5x30 + 6x10 + 7x6 + 8x1 **[10].**

The Truelove and Witts Severity Index in measurement of UC activity:

Severe:

- Six or more bowel movements per day
- Mean evening body temperature greater than 37.5°C
- Mean pulse rate greater than 90 beats per minute
- Hemoglobin less than 10.5 g/dL
- Erythrocyte sedimentation rate (ESR) greater than 30 mm/h.

Mild:

- Less than four bowel movements per day; scant amounts blood
- No fever or tachycardia
- Mild or absent anaemia
- ESR less than 30 mm/h.

Moderate:

- Somewhere in between mild and severe **[10].**

9) **Fecallactoferrin test**

AssayMax™ (manufactured by Assaypro LLC 3400 Harry S Truman BlvdSt. Charles, MO63301) formeasurement of FLA (for all groups):

A single stool sample (about 5 gm weight) placed in a suitable disposable container is sent to the laboratory on the same day under temperature < 30ºC. About 100 mg of the faecal sample is added to 4.9 ml of diluted extraction solution in a screw cap tube which is then shaked vigorously for 30 seconds by means of a vortex mixer then homogenized 30 minutes on a shaker or roller. 1 ml of the homogenate is transferred to an Eppendorf tube and centrifuged at 10 000 rpm for 20 minutes. Then 0.5 ml of the clear extract supernatant is transferred to another Eppendorf tube and tested immediately by an ELISA technique named Assay Max™ which uses a polyclonal antibody against lactoferrin in an enzyme linked immune-sorbent assay system. Lactoferrin presented in the diluted sample is bound by the antibody adsorbed to the surface of the plastic well. The enzyme conjugated antibody binds to the captured antigen and subsequently the enzyme catalyses the conversion of the substrate to a coloured product. The intensity of the colour is proportional to the amount of conjugate bound, and thus to the amount of captured lactoferrin.

Concentration of lactoferrin in the samples is calculated using the provided samples.

10) **Abdominal ultrasound:** To exclude the presence of associated diseases or complications.
11) Statistical analysis of the results (Data management).

<u>Group II (n= 40)</u>

Forty (40) normal individuals (control) were included in this group with no confirmed abnormality in the upper or lower digestive tract.

All were subjected to

1) Complete clinical examination.
2) Laboratory tests.

- Complete blood count (CBC).
- Erythrocyte sedimentation rate, C-reactive protein levels correlate imperfectly with inflammation and disease activity.
- Liver enzyme and function testing—international normalized ratio (INR), bilirubin, albumin.
- Renal function tests (BUN, Cr)
- Stool analysis
- Fecal lactoferrin test

Statistical analysis

Data were analyzed using Statistical Program for Social Science (SPSS) version 20.0. Quantitative data were expressed as mean± standard deviation (SD). Qualitative data were expressed as frequency and percentage.

The following tests were done:

- Independent-samples t-test of significance was used when comparing between two means.
- A one-way analysis of variance (ANOVA) when comparing between more than two means.
- Chi-square (X^2) test of significance was used in order to compare proportions between two qualitative parameters.

- Pearson's correlation coefficient (r) test was used for correlating data.
- Diagnostic validity test
- Sensitivity = (True positive) / (True positive + False negative) x 100.
- Specificity = (True negative) / (True negative + False positive) x 100.
- PPV (Positive predictive value) = (True positive) / (True positive + False positive) x 100.
- NPV (Negative predictive value) = (True negative) / (True negative + False negative) x 100.
- Accuracy = (True positive + True negative) / (True positive + True negative + False positive + False negative) x 100.
- Receiver operating characteristic (ROC curve) analysis was used to find out the overall predictivity of parameter in and to find out the best cut-off value with detection of sensitivity and specificity at this cut-off value.
- Probability (P-value)
  - P-value ≤0.05 was considered significant.
  - P-value ≤0.001 was considered as highly significant.
  - P-value >0.05 was considered insignificant.

RESULTS

Table (1): Characteristics of the studied subjects as regard the age and sex.

Demographic Data		Patients	Control	t/x2*	p-value
Age (years)	Mean ±SD Range	38.65±15.52 20-76	40.20±14.59 19-61	0.106	0.747
Sex	Male Female	24 (60%) 16 (40%)	28 (70%) 12 (30%)	0.440*	0.507

Table (1) shows no significant difference between study groups regarding age and sex where 52 male subjects (65%) and 28 female subjects (35%) were involved in the study divided as shown above.

Table (2): Comparison of p-values (significance) between the 2 studied groups regarding laboratory data.

Laboratory data		Patients	Control	t-test	p-value
HB(gm/dl)	**Mean±SD** **Range**	10.10±3.14 5-15	12.90±2.22 10-17	10.580	**0.002**
WBCx10^3/UL	**Mean±SD** **Range**	9.41±2.97 4.5-14	7.520 ± 2.762 3.7-12	7.455	**<0.001**
PLTx10^3/UL	**Mean±SD** **Range**	417.05±172.57 155-674	294.40±67.91 150-400	8.748	**0.005**
ESR(mm)	**Mean±SD** **Range**	68.50±16.44 10-109	10.10±2.97 7-17	22.849	**<0.001**
CRP	**Mean±SD** **Range**	16.4±3.94 6-24	7.2±2.53 6-12	11.028	**<0.001**
Na(mEq/L)	**Mean±SD** **Range**	139.70±3.01 135-144	139.25±3.01 134-144	0.224	0.639
K(mEq/L)	**Mean±SD** **Range**	4.21±0.48 3.6-5	4.12±0.45 3.6-4.9	0.334	0.567
ALT(IU/L)	**Mean±SD** **Range**	17.85±6.85 8-31	20.90±7.38 11-32	1.835	0.184
AST(IU/L)	**Mean±SD** **Range**	21.45±5.15 6-35	20.35±5.89 8-32	0.819	0.415
ALBUMIN(g/dL)	**Mean±SD** **Range**	3.34±0.87 1.4-4.6	4.30±0.44 3.6-5	19.269	**<0.001**
T.BILIRUBIN	**Mean±SD** **Range**	0.52±0.12 0.2-0.7	0.57±0.15 0.3-0.8	1.646	0.535
INR	**Mean±SD** **Range**	1.34±0.25 0.9-1.7	1.05±0.13 0.9-1.2	21.964	**<0.001**
CREAT(mg/dL)	**Mean±SD** **Range**	0.78±0.26 0.2-1.3	1.12±0.30 0.6-1.9	14.419	**<0.001**
BUN	**Mean±SD** **Range**	13.6±4.79 6-23	11.80±3.15 7-18	0.145	0.534

Table (2) shows statistically significant difference between groups regarding Hb, PLT, WBC ESR, CRP, Albumin, INR and Creat. in comparison of those with IBD and control group.

Table (3): The percentages of types of IBD in our groups.

Type of IBD	Patients	Control	Chi-square	p-value
Chrons	14 (35%)	0 (0%)	40.000	**<0.001**
UC	26 (65%)	0 (0%)		
No	0 (0%)	40 (100%)		
Total	40 (100%)	40 (100%)		

Table (3) shows highly statistically significant difference between groups according type of IBD.

Table (4): Descriptive analysis of patients with inflammatory bowel disease (group 1) regarding disease activity (acc. to clinical activity index).

Clinical activity index		Patients	
		No.	%
CDAI (CD)	*Remission*	6	42.9%
	Mild	2	14.3%
	Moderate	2	14.3%
	Severe	4	28.6%
Truelove and witts severity index (UC)	*Remission (Mild)*	10	38.5%
	Moderate	8	30.8%
	Severe	8	30.8%

Table (5): Remission or activity distribution of the patients group.

Remission or activity	Patients	
	No.	%
Active	24	60.0%
Remission	16	40.0%
Total	40	100.0%

Table (6): Relation between remission and activity regardingclinical activity index in patients group.

Clinical activity index	Rendition or activity				Chi-square test	
	Active		Remission		x^2	p-value
	No.	%	No.	%		
CDAI (CD)						
Remission	0	0.0%	6	100.0%	7.000	0.072
Mild	2	25.0%	0	0.0%		
Moderate	2	25.0%	0	0.0%		
Severe	4	50.0%	0	0.0%		
Truelove and wits severity index (UC)						
Remission	0	0.0%	10	100.0%	13.000	**0.005**
Moderate	8	50.0%	0	0.0%		
Severe	8	50.0%	0	0.0%		

Table (7): Comparison between different study groups regarding fecal Lactoferrin levels.

Fecal lactoferrin (μg/gm)	Patients	Control	t-test	p-value
Mean±SD	759.15±182.2	1.61±0.39	17.446	**<0.001**
Range	50-2446	1.70-9.68		

Table (7) shows a significant elevation of fecal lactoferrin levels in IBD patients in comparison with control group.

Table (8): Relation between remission and activity regarding laboratory data in patients group.

Laboratory data	Remission or in activity				t-test	
	Active		Remission			
	Mean	±SD	Mean	±SD	t	p-value
Age (years)	40.08	10.55	36.50	8.68	0.496	0.626
HB(gm/dl)	9.17	2.79	11.50	3.30	-1.706	0.105
PLTx10^3/UL	485.33	159.17	314.63	75.51	2.431	**0.026**
WBCx10^3/UL	11.39	1.95	7.55	1.81	3.737	**0.002**
ESR(mm)	54.08	12.97	12.5	3.51	1.494	0.161
CRP	19.00	4.56	6.00	0.01	2.879	**0.013**
Na(mEq/L)	139.42	2.81	140.13	3.44	-0.505	0.619
K(mEq/L)	4.14	0.49	4.30	0.47	-0.719	0.481
ALT(IU/L)	16.08	3.86	20.50	4.92	-1.452	0.164
AST(IU/L)	20.08	4.8	23.50	6.65	-0.918	0.371
ALBUMIN(g/dL)	3.01	0.82	3.83	0.75	-2.258	**0.037**
BILIRUBIN	0.43	0.16	0.55	0.21	-1.423	0.172
INR	1.28	0.24	1.43	0.24	-1.369	0.188
CREAT(mg/dL)	0.77	0.18	0.80	0.19	-0.275	0.786
UREA	21.50	4.56	21.75	5.44	-0.111	0.913

p-value<0.05 significant

Table (9): Relation between fecal lactoferrin and type of IBD in patients group.

Fecal lactoferrin (µg/gm	Type of IBD		t-test	
	CD	UC	T	-value
Mean±SD Range	495.7±118.97 190-1050	901±216.24 50-2446	703	0.208

p-value>0.05 non significant

Table (10): Relation between fecal lactoferrin and clinical activity index in patients group.

Clinical activity index	Fecal lactoferrin (µg/gm)		ANOVA test	
	Mean	±SD	F	p-value
CDAI (CD)				
Remission	233.3	40.4	388	**0.050**
Active	692.5	253.56		**(S)**
Mild	460	0.0		
Moderate	590	0.0		
Severe	860	268.7		
Truelove and witts severity index (UC)				
Remission (Mild)	69.4	12.3	100	**0.001 (HS)**
Active	1420.75	496.07		
Moderate	1192.5	125.5		
Severe	1649	647.7		

Table (10) shows a positive correlation and significant between clinical activity index with fecal lactoferrin (µg/gm).

Table (11): Correlation between Fecal lactoferrin (µg/gm and other parameters, using Pearson Correlation Coefficient in patients group.

Patients	Fecal lactoferrin (µg/gm)	
	R	**p-value**
Age (years)	0.095	0.691
HB(gm/dl)	-0.096	0.688
PLTx10^3/UL	0.533	**0.015**
WBCx10^3/UL	0.401	**0.040**
ESR(mm)	.604**	**0.005**
CRP	.570**	**0.009**
Na(mEq/L)	-0.375	0.103
K(mEq/L)	-0.117	0.624
ALT(IU/L)	-0.184	0.438
AST(IU/L)	-0.234	0.322
ALBUMIN(g/dL)	-.514*	**0.021**
BILIRUBIN	0.420	**0.35**
INR	-0.372	0.106
CREAT(mg/dL)	-0.353	0.126
UREA	-0.001	0.997

r- Pearson Correlation Coefficient

Table (11) shows that there is significant Positive correlation between Fecal lactoferrin (µg/gm) and PLT, WBC, ESR and CRP, while albumin showed significant negative correlation.

Table (12): Diagnostic value of FLA levels in discriminating IBD patients from healthy subjects.

Cut-off.	Sen.	Spe.	PPV	NPV	Accuracy
≥9.68	100%	100%	100%	100%	100%

Receiver operating characteristics (ROC) curve was used to define the best cut off value of Fecal lactoferrin (µg/gm) was ≥9.68, with sensitivity of 100% specificity of 100% positive predictive value of 100%, negative predictive value of 100% with diagnostic accuracy of 100%.

Table (13): Diagnostic Performance of Fecal lactoferrin (µg/gm) in discrimination of CD into active and remission cases.

Cut-off.	Sen.	Spe.	PPV	NPV	Accuracy
≥260	100%	83.33%	88.9%	100%	96.9%

Receiver operating characteristics (ROC) curve was used to define the best cut off value of Fecal lactoferrin (µg/gm) was ≥260, with sensitivity of 100% specificity of 83.33% positive predictive value of 88.9%, negative predictive value of 100% with diagnostic accuracy of 96.9%.

Table (14): Diagnostic Performance of Fecal lactoferrin (µg/gm) in discrimination of UC into active and remission cases.

Cut-off.	Sen.	Spe.	PPV	NPV	Accuracy
≥80	100%	90%	94.1%	100%	99.1%

Receiver operating characteristics (ROC) curve was used to define the best cut off value of Fecal lactoferrin (µg/gm) was ≥80, with sensitivity of 100% specificity of 90% positive predictive value of 94.1%, negative predictive value of 100% with diagnostic accuracy of 99.1%.

DISCUSSION

Gastroenterologists are sometimes faced with the diagnostic difficulty of those with organic intestinal pathology, in particular inflammatory bowel disease (IBD). They feel compelled to exclude all other organic and non organic diseases (eg IBS) using invasive diagnostic investigations as objective evidence for there being no other significant pathology [11].

Endoscopic examination and histological analysis of biopsy specimens remain the "gold standard" methods for detecting and quantifying bowel inflammation; however, these techniques are costly, invasive and repeated examinations are unpopular with patients. Disease activity questionnaires and laboratory inflammatory markers, although widely used, show an unreliable correlation with endoscopy and histology. New markers are needed for detecting and quantifying bowel inflammation [3].

The serologic panel for IBD is rapidly expanding. So far, ASCA and atypical P-ANCA are the most widely studied markers and remain the best characterized markers in IBD.

The ASCA+ve/atypical P-ANCA–ve phenotype is characteristic of CD, while the ASCA –ve/atypical PANCA+ve phenotype is seen primarily in UC [12].

As serum markers of inflammation can be elevated in a variety of conditions, it seems likely that faecal markers of inflammation, in the absence of enteric infection, would be more specific for IBD [13].

Fecal markers comprise a heterogeneous group of substances that either leak from, or are generated by the inflamed intestinal mucosa. The main use of these markers is likely to be in diagnosing and assessingdisease activity in difficult cases. They may also have a role in assessing treatment effect and prediction of relapse [14].

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 [15].

Lactoferrin is an iron binding glycoprotein with a molecular mass of about 80 kDa that is present in various secretory fluids, such as milk, saliva, tears, and nasal secretions [16]. LF is a component of the innate immune system, with antimicrobial activity as a bactericide and fungicide, as well as being a major constituent of neutrophil granules that is released during apoptosis [17].

Elevated LF has been used as a marker of active IBD and for monitoring patients for response to treatment [18].

The aim of this study is to assess the fecal lactoferrin levels in patients with IBD and to compare them with normal subjects to detect its sensitivity and specificity as a non invasive biomarker in identification of such patients. Our study also included measurement of FLA levels at different stages of inflammatory bowel disease activity to detect its role in assessment of disease severity.

This study was conducted on 40 patients with IBD; 24 patients with active IBD (16 UC patients and 8 CD patients) and 16 patients with inactive IBD (10 UC patients and 6 CD patients) versus 40 healthy persons as control.

IBD patients were 24 males (60%) and 16 females (40%), their mean age was 38.65±15.52, while controls were28 males (70%) and 12 females (30%), their mean age was 40.20±14.59. The mean age and sex difference was statistically non significant (P>0.05). However in epidemiological studies*;* ***Clark and Silk*** [19] found that Crohn`s disease was slightly commoner in females (M: F=1: 1.2) than ulcerative colitis (M: F=1.2:1) and they might affect people of any age.

This study showed significant elevation of Fecal lactoferrin levels in patients with IBD with range (50-2446ug/ml) more than normal control group with range (0.70-9.68ug/ml), (p-value <0.001) as shown in table (7). These findings agreed with ***Walker et al.*** who reported higher levels of FLA levels in patients with IBD with mean 1880 ±565ug/mL for patients with UC and 1701±382 for patients with crohn`s disease, while upper limit for control group was 7.2 ug/ml. This can be attributed to presence of active inflammatory cells in patients with IBD with production of lactoferrin at higher levels in patient's stools than healthy groups [20]. These results also agree with ***Sidhu et al.*** who found that levels of FLA varied significantly in

patients with IBD when compared to those with IBS or healthy subjects (p-value <0.001) [21].

This study did not detect a significant difference in FLA levels between patients with UC and those with CD (p-value 0.208) as shown in table (9), in agreement with ***Walker et al.*** who found no significant difference in FLA levels between patients with UC and those with CD (p-value 0.603) [20].

On analysis of biochemical profile of patients with IBD at different stages of disease activity in correlation to healthy controls, a significant difference in serum albumin and serum creatinine level was found between patients with IBD in correlation to healthy control levels asshown in table (2). This could be attributed to protein loss due to prolonged diarrhea in IBD patients. On the other hand no significant difference was found regarding serum electrolytes between patients and control group. This was supported by ***Cucino and Sonnenberg*** [22] as they found that severe cases of UC and CD were associated with protein/calorie malnutrition, hypoalbuminemia, hypoproteinemia and electrolyte disturbances.

Regarding the serological markers of disease activity (e.g: ESR, CRP) and blood picture, our study showed that their values varies significantly between IBD patients and control group (p-value <0.001) as shown in table (2), being higher in patients with IBD than control group. Also, ESR and CRP levels varied significantly with disease activity table (8). It was found that CRP was helpful in differentiating active IBD from inactive so it might be used as a marker of disease activity with p-value 0.013, but p-value of ESR in differentiating active IBD from inactive is 0.161 as in table (8).

But**,** in ***Tibble et al.*** [23] found that the median ESR and CRP values in patients with active CD were significantly higher compared to patients with inactive CD. Also**,** ***Xiang et al.*** [24] found that the patients with active UC had higher levels of CRP and ESR than the patients with inactive UC and the controls. This may be due to involvement of larger surface area in UC.

On correlating fecal lactoferrin levels to other biochemical parameters in patients with IBD, FLA levels were found to correlate negatively with serum albumin (p-values 0.021) table (11), while FLA levels were not found to correlate significantly with serum electrolytes levels (Na, K) at different stages of disease activity with p-values 0.103, 0.624 respectively. Also, FLA levels were found to correlate significantly with ESR and CRP levels (p values 0.005, 0.009 respectively as shown in table (11), where higher degrees of inflammation are usually associated with presence of higher levels of lactoferrin in stool.

This study also showed that fecal lactoferrin levels correlated significantly with other hematological parameters as WBCs, platelet counts in IBD patients at different stages of activity ((p-values 0.040, 0.015, respectively as shown in table (11)). This comes in agreement with ***Walker et al.*** whose study showed FLA levels correlated significantly with serum albumin, platelets, ESR with p-value <0.05.

On the other hand, no significant correlation was found between FLA level and Hb level in this study (p-value 0.688) as shown in table (11), which wasn`t coinciding with ***Walker et al.*** whose study showed a significant correlation of FLA levels with Hb level [20].

Receiver operating characteristic curves comparison demonstrated that FLA levels displayed high sensitivity and specificity in identifying patients with IBD from healthy controls and also in differentiating IBD in to active and remission cases.

Comparing FLA levels in patients with IBD and healthy controls, FLA was found to be highly sensitive (sensitivity 100 %) and highly specific (specificity 100 %) in differentiating patients with IBD from healthy subjects with cut with cutoff value >9.86 ug/ml. This agrees with ***Sidhu et al.*** who found high sensitivity and specificity for FLA in differentiation of patients with IBD from healthy controls (sensitivity 71 %, specificity 100 %) [21]. Higher values of sensitivity at our study is much more related to lower number of study group in comparison with other studies and meta analysis which involved hundreds of patients.

The most important in this study FLA levels were significantly increased in patients who were in flares more than the patients in remission, after evaluation of FLA measurements in patients with inactive disease and others during flares at the time of specimen collection and FLA has Diagnostic Performance in discrimination of CD into active and remission cases as it had a sensitivity,

specificity, PPV and NPV of 100%, 83.33%, 88.9%, and 100% respectively with cut off value was ≥260 with diagnostic accuracy of 96.9% and for UC it had a sensitivity, specificity, PPV and NPV of 100%, 90%, 94.1%, and 100% respectively with cut off value was ≥80 with diagnostic accuracy of 99.1%. For discrimnation of UC into active and remission cases. This agrees with ***Sidhu et al.*** as before.

Conclusion

- Fecal lactoferrin assay levels were significantly elevated in patients with inflammatory bowel disease in comparison with patients with healthy subjects, so it can be used as a useful non invasive diagnostic tool for diagnosis of inflammatory bowel disease.
- Levels of fecal lactoferrin varied significantly with disease severity in patients with inflammatory bowel disease, so FLA can be used in monitoring disease activity in such patients without need for recurrent endoscopic interventions.
- Fecal lactoferrin levels correlated significantly with other serological markers used for assessment of IBD activity.

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