How Valuable are Noninvasive Tests as Indicators of IBD Activity and Severity in the Primary Health Care

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Background and study aims: Inflammatory bowel disease (IBD) is a chronic inflammatory and destructive disease of the bowel wall. Chronic inflammation is associated with ulcerations, strictures, perforations and is a risk factor for dysplasia and cancer. To reduce these long-standing complications, IBD patients are continuously in a need for treatment and monitoring. Primary health care centers lack specific IBD management facilities although they receive a reasonable number of IBD patients. Markers, such as ESR, CRP, fecal calprotectin (FC) have been widely used as noninvasive parameters for IBD monitoring. The aim of the current study was to evaluate readily available non-invasive tests (FC, ESR, Platelets, serum total proteins, serum albumin and hemoglobin level) in predicting IBD activity and severity in the primary health care.

Patients and Methods: This prospective study included 96 newly diagnosed IBD patients. Patients from many primary health care centers covering the landscape of Kafr-Elshikh governorate in the North of Egyptian Nile delta after giving complete history, clinical examination, and laboratory investigation were referred to IBD clinic at Kafrelsheikh University Hospital for assessment and ileoclonoscopy with biopsies.

Results: Seventy-eight (81.2%) patients were ulcerative colitis and 18(18.8%) were Crohn's disease, with mean age was 34.40 and 30.94 years respectively (P 0.380).FC, serum total proteins, ESR and HB level showed statistically significant difference between baseline levels and levels at time of remission. However, platelet count and serum albumin were not statistically different. The mean FC level at the time of diagnosis was 823.61±545.457µg/mg and after remission was 165.18±202.255µg/mg (P 0.000).

Conclusion: Markers (FC, ESR, serum total proteins and HB level) can be used as non-invasive markers for monitoring IBD activity and severity in the primary health care centers. Fecal calprotectin correlate with endoscopic disease activity. Furthermore, the initial level of FC is a predictor of early dysplasia in ulcerative colitis patients .

INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic inflammatory and destructive disease of the gastrointestinal tract. Chronic inflammation is associated with ulcerations, strictures, perforations and there is a real risk for dysplasia and malignancy **[1]**.The optimal target in IBD management is to achieve mucosal healing. A treat-to-target approach and close monitoring of the disease status improve the outcomes for those patients **[2].**

The European Crohn's and Colitis Organization [ECCO] reported that, every patient should have a biochemical assessment at the time of diagnosis with full blood count, inflammatory markers (ESR, CRP, fecal calprotectin (FC)), electrolytes, liver enzymes, and a stool sample for microbiological analysis, including C. difficile. In monitoring patients with IBD an endoscopic (or cross-sectional

in CD) reassessment should be considered in cases of relapse, persistent disease activity, new unexplained symptoms, and prior to switch of therapy. However, endoscopy needs experience and special centers for many patients it is time consuming, expensive, invasive, and needs bowel cleansing i.e. it is uncomfortable and inconvenient to many patients. Serum markers, such as have been widely used as noninvasive parameters for assessing the severity and activity of IBD **[3-5]**.

The primary health care (PHC), which lacks endoscopic facilities, performs laboratory tests as a common practice and these if found valuable, may be used as simple markers to monitor IBD patients in the setting of primary health care.

Calprotectin is a calcium binding protein that is found mainly in neutrophils and to a lesser extent in monocytes and reactive macrophages. It belongs to a sub-group of proteins of the S100 family (calgranulin A, S100A8; calgranulin B, S100A9 and calgranulin C, S100A12) that is associated with acute/chronic inflammatory disorders of the bowel and increasingly became available **[6,7]**. Other tests including ESR, CRP, serum total protein, hemoglobin levels (HB) and serum albumin are readily available in primary health care centers.

The current study aimed at evaluating the readily available non-invasive tests (FC, ESR, CRP, serum total protein, HB levels and serum albumin) in predicting IBD activity and severity in primary health care.

PATIENTS AND METHODS

This was a cross-sectional observational study In this prospective study, ninety-six patients were included. Patients with probable diagnosis of IBD in many primary health care centers covering Kafrelsheikh governorate (in the North of the Egyptian Nile Delta) were invited to complete their endoscopic evaluation in the IBD clinic, Department of Hepatology, Gastroenterology and Infectious Diseases, Kafrelsheikh University Hospital, Egypt after doing non-invasive laboratory tests in the period from November 2016 to October 2019.

Inclusion criteria: predefined as follows:

1- Age above 18 years
2- Endoscopic and histopathologically proven cases of IBD
3- Patients accepted to participate and perform all relevant investigations

Exclusion Criteria:

1- Patients previously diagnosed to have IBD and already started treatment
2- Patients who refuse follow up and evaluation.
3- Failure to obtain the consent.
4- Pregnant females.

Baseline evaluation: All patients were subjected to detailed medical history and complete clinical examination. Blood samples were tested for complete blood count (CBC), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), FC, Serum total protein, serum albumin. All Patients performed an iliocolonscopic examination and biopsies were taken from the terminal and all parts of the colon with special care to suspected lesions. Biopsies were sent for confirmation by histopathology.

Fecal Calprotectin Measurement:

Calprotectin was measured, using quantitative enzyme-linked immunosorbent assay (ELISA) (Genova Diagnostics, Asheville, NC). Laboratory personnel, who were blinded from the current clinical and endoscopic disease activity of the patients, performed the analyses.

Endoscopic Examination:

Endoscopy was done under conscious sedation, in spontaneous breathing with oxygen mask support. Colonoscopy was done by single endoscopist at the same endoscopy unit using **Pentax EG3890 colonoscope** with complete examination was done up to the cecum with ileal intubation. Biopsies were taken from the ileum to exclude any histopathological disease. All parts of the colon were biopsies as well with special care to suspected lesions. Complementary upper endoscopy using **Pentax EG29-i10**endoscope was done for those patients with suspected Crohn's disease to calculate the Montreal classification **[8]** (Table 1). Endoscopic evaluation was performed blinded from the results of FC and other labs measurements.

Histology:

Biopsies site and numbers: For optimal diagnosis and classification of IBD there should be samples of the ileum, at least four colonic sites, and the rectum, with a minimum of two biopsies from each site. Biopsies should be

submitted in such a way that their site of origin can be determined using multiple specimen container

Two pathologists assessed all biopsies and report histology utilizing a standardized chick list that includes histologically normal, quiescent, mild, moderate or severe disease. Histologically normal is defined as completely normal mucosa with no features of chronicity present. Histologically quiescent is defined as having features of chronicity including crypt atrophy or branching but no active inflammation, such as erosions, crypt abscesses or focal neutrophil infiltration. Histologically active is defined as presence of any epithelial infiltration by neutrophils, crypt abscesses, erosions or ulceration and is further classified into mild, moderate or severe **[9]**.

Follow up:

Patients were assessed on a monthly basis until they entered into remission clinically and laboratory and endoscopically. During follow up visits the following markers were measured: CBC, ESR, CRP, FC, Platelet, Serum Total protein, HB level and Serum Albumin.

Statistical Analyses:

Data collected and entered in spread sheets of Microsoft Excel before being transferred to the Statistical package for social Sciences (SPSS) software (SPSS Inc., Chicago, IL, USA) version 16 for Windows 7 (Microsoft Corp., Redmond, WA) to be analyzed.

Wilcoxon's test and Paired Student's t-test will be used to compare paired data, whereas Chi-Square test, Fisher's exact test and Mann–Whitney U-test will be used to compare unpaired data.

RESULTS

During the period of the study, a total of 100patientsrecently diagnosed as IBD who fulfilled the inclusion criteria were enrolled in the current study. A total of 78 (81%) were ulcerative colitis and 18 (18.8%) were Crohn's disease. The dropout rate was 4%,`two patients did not attend the scheduled follow up, while two patients withdrawn from the study, so final analysis was done on 96 patients, 55 were females (57.3%) and 41 were male (42.7%).

The mean age was34.4yearsfor patients diagnosed as ulcerative colitis and 30.94 years for patients diagnosed Crohn's disease (Table 2) with no statistical significant difference between both groups of patients (P value 0.380).

The following laboratory investigations (Fecal calprotectin level, platelet count, serum total proteins, serum albumin, ESR and hemoglobin level) were used as anon invasive markers because it demonstrated difference with IBD activity on the follow up. The difference was statistically significant for fecal calprotectin, serum total proteins, ESR and HB level. However, for platelet count and serum albumin, the difference was not statistically significant (Table 3).

The mean fecal calprotectin level in correlation to endoscopic severity of IBD at baseline according to Montreal classification in both types is shown in Table 4, which showed that the highest mean fecal calprotectin level in ulcerative colitis (E3) was1006±513 µg/mg and the highest mean FC level inpatients with Crohn's disease was (1540±0 µg/mg). The highest mean level among patients with Crohn's disease comprised patients with classification A1L3B2 (i.e. with ileocolonic and strictring pattern).

An additional finding of the current study, was the relationship between FC and the development of dysplasia on mucosal biopsy. The initial higher fecal calprotectin level is associated with early occurrence of low grade dysplasia. The mean FC level for patients with early dysplasia was 978.76 µg/mg and the mean fecal calprotectin for patients who didn't develop dysplasia after three years of follow up was 742.35 µg/mg (Figure 1).

Table (1): Montreal classification (8) for Crohn's disease.

	Montreal
Age at diagnosis	A1 below 16 y
	A2 between 17 and 40 y
	A3 above 40 y
Location	L1 ileal
	L2 colonic
	L3 ileocolonic
	L4 isolated upper disease*
Behavior	B1 non-stricturing, non-penetrating
	B2 stricturing
	B3 penetrating
	p perianal disease modifier†

*L4 is a modifier that can be added to L1–L3 when concomitant upper gastrointestinal disease is present.
†"p" is added to B1–B3 when concomitant perianal disease is present.

Table (2): Age and gender of the studied patients.

	Ulcerative colitis n=78	Crohn's n=18	P-Value
Mean Age (years)	34.40± 5.7	30.94± 4.8	0.380
Gender M:F	39:37	3: 17	0.05

Table (3): Laboratory investigation correlate with IBD activity.

	At time of diagnosis	**After Remission**	**P. value**
Fecal calprotectin level (µg/mg)	823.61±545.457	165.18±202.255	0.000
PLT 10^3/microliter	350 ±120.54	242.7±73.02	0.019
Serum total Protein (gm/l)	69.64±4.67	57.13±4.77	0.001
Albumin (gm/dl)	3.54+.501	3.75+.481	0.010
ESR (First hour)	51.21+31.186	17.10±12.3	0.009
Hemoglobin (mg/dl)	11.52±2.12	12.65±1.91	0.001

Table 4: Fecal Calprotectin as Key indictor of Severity in IBD

Type of IBD	**Montreal Classification**	**Mean level of FC (µg/mg)**	**percent of total number**	**P Value**
Crohn's	A1L2B1	150+ 0	1.0%	0.005**
	A1L3B1	1039.5+819.158	12.5%	
	A1L3B2	1540+0	1.0%	
	A2L3B2	795+341.8	12.5%	
	A2L3B3	1000+0	1.0%	
Ulcerative Colitis	E1	439.55+248.5	22.9%	
	E2	910+541.4	28.1%	
	E3	1006+513	20.8%	

** Statistically significant difference ($p<0.05$)

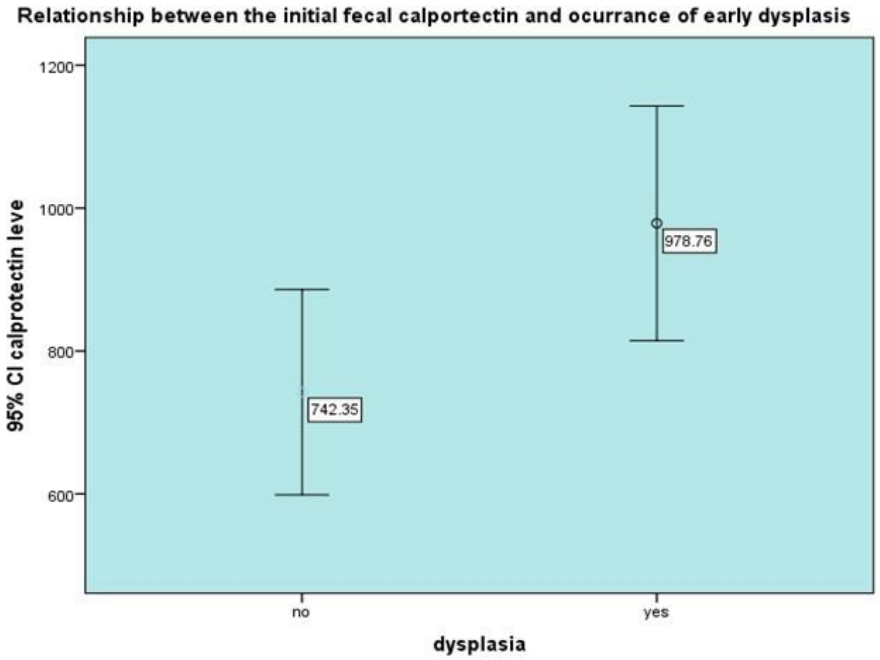

Figure (1): Relationship between initial FC level and early dysplasia.

DISCUSSION

An important question is, Is there a real need for PHC in management of IBD? And the answer can be driven from recognizing the growing burden of not only the frequency [**10]** but also the cost of management of IBD being a real example of chronic diseases **[11]**. Literature search found that 30-70% of IBD patients receive specialist's care **[12,13]** consequently large proportion of patients receives their care in PHC **[14-16]**.

Still estimation of severity and activity of the gastrointestinal inflammation in IBD is a problematic dilemma. Endoscopy with biopsy remains the most reliable method **[14]**. However, the fact that the endoscopic interventions are invasive and frequently disturbing and the symptoms are usually not conclusive, has led to the use of laboratory investigations in the assessment of disease activity among IBD patients **[17]**.

A number of studies have reported that FC can be used as diagnostic aid, and in assessment of disease remission and exacerbation. To the best of our knowledge, It's the first Egyptian report to correlate it with endoscopic severity and estimate the initial level as a predictor of early dysplasia and its validity for monitoring IBD patients in primary health care.

Vermeireetet. al. **[18]** showed that some acute phase reactants increase in active inflammatory conditions and CRP is among the most commonly used acute phase reactants. In the current study we noticed that there is significant difference between the levels of HB, ESR, serum albumin, serum total proteins and PLT count in patients at the time of diagnosis and after remission irrespective of being UC or Crohn's disease. Based on its availability on the PHC centers and its correlation with IBD activity, these parameters can be used as noninvasive markers for IBD remission monitoring.

In the current study the mean level of FC level at time of diagnosis was 823.61±545.457 µg/mg and it was 165.18±202.255µg/mg at remission. This result disagree with Costa and his colleagues **[19]**, who demonstrated a 14-fold greater risk of relapse in UC patients if the calprotectin was more than 150 g/g, but this was not the situation for CD patients. The difference in the mean levels may be due to poor sanitation and frequent intestinal infections in developing countries than developed country with presumably higher level in developing countries.

One meta-analysis focusing non-invasive tests in IBD management at pediatric age group found that in children whose pediatrician is considering an endoscopy, symptoms were not accurate enough to identify low-risk patients in whom an endoscopy can be avoided. FC, CRP, and albumin findings were potentially of clinical value, given their ability to select children at low risk (negative FC test result) or high risk

(positive CRP or albumin test result) for IBD **[20,21]**.

The correlation between FC and endoscopic features in IBD has been reported by Aomatsu et. al. **[22]**. Infact the current study reported similar findings; the highest FC level in Crohn's disease (A1L3B2) was 1540 µg/mg, they were young age patients with ileocolonic phenotype complicated with stricture but, in ulcerative colitis (Montreal E3) the level was 1006 µg/mg.

In fact, the finding that FC in the current study is associated with early dysplasia in IBD reinforce the findings of other authors. Kristinssonet et. al. **[23]** found that FC is a valuable marker for colorectal cancer development in patients with cancer colon. However, in IBD FC proved its predictive value as a noninvasive marker of intestinal inflammation and was found suitable for predicting clinical relapse in ulcerative colitis than in Crohn's disease **[24]**.

We noticed that higher initial level of fecal calprotectin associated with early dysplasia on follow up and this might warrant close follow up and trigger PHC physicians to refer their IBD patients for colonoscopy and screening early and at regular intervals without delay. One study found persistently high fecal calprotectin is correlated with lack of mucosal healing **[25]**.

This study although shade the light on an important sector of health care; the PHC in the management of IBD it has some limitations. It is a single center study recruiting a small number of patients with a short period of follow up. Future multicenter studies with large number of participating centers with a large number of patients would reach meaningful recommendations.

CONCLUSION

In conclusion serum markers (ESR, serum total proteins, HB level) besides FC can be used as non- invasive markers for IBD activity monitoring in primary health care centers. Fecal calprotectin correlates with endoscopic disease activity. Furthermore, the initial level of FC is a predictor of early dysplasia in ulcerative colitis patients.

Disclosure:

The authors have no conflicts of interest with respect to the contents of this article.

Abbreviations:

IBD, Inflammatory bowel disease; ESR, Erythrocyte sedimentation rate; CRP, C-Reactive Protein; FC,Fecal calprotectin, ,HB, Hemoglobin.

Ethical considerations: This study was performed in accordance with the Declaration of Helsinki, Good Clinical Practice and applicable regulatory requirements. A written informed consent were obtained from all patients after explanation of the research idea.

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Author contribution: All authors equally shared in formulating the idea, conception, data collection statistics, writing and drafting the manuscript.

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