Interleukin-6 as a reliable biomarker for inflammatory bowel diseases

Islam Abd El-Hamid El-Zayyadi[a], Aya M. El-Noby[b], Yasmin N. Kamel[c], Sahar T. Mohamed[c]

Departments of [a]Hepatology and Gastroenterology, [b]Department of Microbiology, New General Mansoura Hospital, Mansoura, Egypt, [c]Medical Microbiology and Immunology, Faculty of Medicine, Mansoura University

Correspondence to Islam Abd El-Hamid El-Zayyadi, MD, Department of Hepatology and Gastroenterology, Specialized Medicine Hospital, Elgomhoria Street, Mansoura City, Egypt Tel: +20 100 751 8149/20 114 448 3991; e-mail: islamelzayyadi@gmail.com

Received: 28 January 2021
Revised: 29 June 2021
Accepted: 8 July 2021
Published: 27 May 2022

Kasr Al Ainy Medical Journal 2021, 27:14–21

Background
Inflammatory bowel diseases (IBD) include ulcerative colitis and Crohn's disease, both disorders have genetic predisposition. The study aims to compare the levels of serum interleukin-6 (IL-6) in correlation with fecal calprotectin (FC) in IBD patients and healthy controls to assess its diagnostic and prognostic role in IBD.
Patients, methods, and results
This study enrolled 90 participants with mean age of 36.09 and 34.72 years for cases and controls, respectively. Both FC and IL-6 had significantly higher values in cases compared with controls ($P<0.001$). Also, C-reactive protein levels were significantly higher in the same group (13.9 vs. 3.02 mg/l; $P<0.001$). On the other hand, cases had significantly lower hemoglobin levels compared with controls (9.22 vs. 13.5; $P<0.001$). Also, in active IBD cases, FC and IL-6 had significantly higher values compared with inactive ones ($P<0.001$).
Conclusion
Based on our findings, serum IL-6 compared with a valid IBD parameter, such as FC, was a sensitive and reliable marker in IBD diagnosis and prediction of disease activity with a significant correlation with FC.

Keywords:
Crohn's, disease, fecal calprotectin, inflammatory bowel disease, interleukin, ulcerative colitis

Kasr Al Ainy Med J 27:14–21

1687-4625

Introduction

Inflammatory bowel disease (IBD) encompasses two types of idiopathic intestinal disease that are differentiated by their location and depth of involvement in the bowel wall, ulcerative colitis (UC), and Crohn's disease (CD). Both disorders have a genetic predisposition and they both carry vast morbidity [1].

UC is limited to the colon with major involvement of the mucosa [2], while CD can affect any segment of the gastrointestinal tract from the mouth to the anus with 'skip lesions' as a characteristic feature and inflammation is transmural [3]. A genetic predisposition for IBD was documented and patients are more liable to malignancy [4].

Both UC and CD share similar clinical symptoms, including chronic diarrhea, abdominal pain, weight loss, and growth failure [5]. The clinical course of both is characterized by repeated episodes of relapse and remission, in spite of treatment [6].

Although the exact cause of IBD is still unknown, there is evidence to support an essential role of the mucosal immune system in the initiation of inflammation [7].

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 [8].

A significantly increased interleukin-6 (IL-6) production was reported in stimulated monocytes from patients with active IBD in comparison with samples from inactive disease phases or healthy control individuals [9].

IL-6 reflects inflammatory activity in patients with CD and UC, and overall appears to be a good predictor of IBD activity. Given a prominent role of IL-6 signaling in both CD and UC pathogenesis, IL-6 can be considered as an important target for cytokine-specific therapies [10].

There is good evidence that IL-6 levels, leading to activation of the pro-inflammatory signal transducer, are strongly elevated in the inflamed mucosa in IBD [11]. IL-6 is the main inducer of C-reactive protein (CRP), which has been shown to be positively associated with CRP levels in IBD [12].

Fecal calprotectin (FC), a calcium-binding and zinc-binding protein, represents 60% of the cytosolic protein in the granulocytes [13]. The amount of calprotectin in feces is proportional to the amount of neutrophil migration during the disease activity [14]. Additionally, the FC concentration is stable for up to 7 days at room temperature and resistant to degradation [15]. FC is a favorable marker for assessing intestinal activity with endoscopy as a reference standard, compared with conventional serum markers [16].

FC test is a reliable marker for assessing IBD disease activity and may have greater ability to evaluate disease activity in UC than CD [17].

In this study, we compared the levels of serum IL-6 in correlation with FC in IBD patients and healthy controls to assess IL-6 role in IBD diagnosis and prediction of the disease activity.

Patients and methods

This case–control study was conducted on 90 participants (73 patients with IBD and 17 healthy controls) over a period of 12 months from April 2019 to March 2020. Patients were selected from the outpatient clinic of IBD. The 73 patients were divided into 56 UC patients (33 active and 23 inactive) according to Truelove and Witts severity index, depending on the frequency of bowel motion, presence of blood in stool, temperature, pulse, and CRP or erythrocyte sedimentation rate and hemoglobin, and 17 CD patients (seven active and 10 inactive) according to CD activity index, depending on abdominal pain, diarrhea, weight, hematocrit, general well-being, complications, and use of opiate for diarrhea. Patients with past history of any malignant condition, major gastrointestinal surgical procedures, liver cell failure and/or chronic renal failure, congestive heart failure, and/or bleeding tendency and even patients on NSAIDs were excluded from the study. Written consents from patients who participated in the study or from their families were obtained and approved by Mansoura Medical Ethics Committee (MMEC) of Faculty of Medicine (Code: R.19.02.487).

All patients were subjected to history and full clinical examination with insisting on symptoms of IBD such as diarrhea (nocturnal, postprandial), rectal bleeding, tenesmus, crampy abdominal pain, anorexia, nausea, vomiting, fever, and weight loss.

Laboratory assessment

All of the enrolled patients were subjected to complete blood count, CRP, and determination of the levels of both serum IL-6 and FC. Blood samples of 5 ml were obtained to be immediately inoculated into sterile tubes, they were transported to the Medical Microbiology and Immunology Department to be processed to obtain serum, and kept frozen at −20°C for further analysis of IL-6. We used ELISA Kit (Sun Red Bio 201-12-0091, Shanghai, China). A single fresh fecal sample (weight 50–100 mg) was collected from each study participant using a calibrated inoculation loop that was put in a clean container to be inoculated in Epitope Diagnostics Fecal Sample Collection Tube supplied by ELISA kits. They were sent to the laboratory at ambient temperature, on the same day to be stored at −20°C until further analysis. EDI Quantitative Fecal Calprotectin ELISA KT-849 (Epitope Diagnostics Inc., USA) was used.

Statistical analysis

Data were entered and statistically analyzed using the Statistical Package for Social Sciences (SPSS), version 22. Data were entered and analyzed using IBM-SPSS software (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.). Data analysis was done using the χ^2 test and Monte Carlo test for comparison of two or more groups of categorical variables as appropriate. Quantitative data were described as median and range after testing normality by Kolmogorov–Smirnov test. Kruskal–Wallis test and Mann–Whitney tests were used for comparison between groups. Receiver-operating characteristics were used to calculate validity (sensitivity and specificity) of continuous variables with calculation of the best cut-off point. Spearman correlation coefficient (r) was used for nonparametric correlation between continuous variables. Significant predictors in the bivariate analysis were entered into the regression model using Enter method. Adjusted odds ratios and their 95% confidence interval were calculated. P values less than or equal to 0.05 were considered to be statistically significant.

Results

Patient sociodemographic characteristics

This study included 90 participants that were divided into 73 cases and 17 controls. Their mean age was

36.09 and 34.72 years for cases and controls, respectively. No significant difference was detected between cases and controls regarding that parameter (P=0.220). On the other hand, there was a significant difference between the two groups regarding sex (P=0.015). Males represented 35.6 and 58.8% of patients in both groups, respectively.

Significance of fecal calprotectin and interleukin-6 in inflammatory bowel diseases diagnosis

Both FC and IL-6 had significantly higher values in cases compared with controls (P<0.001). Also, CRP levels were significantly higher in the same group (13.9 vs. 3.02 mg/l; P<0.001). On the other hand, cases had significantly lower hemoglobin levels compared with controls (9.22 vs. 13.5; P<0.001). These data are illustrated in (Tables 1 and 2).

Significance of fecal calprotectin and interleukin-6 in inflammatory bowel diseases activity

In UC cases, FC was significantly higher in active cases compared with inactive ones (542.17 vs.184.48 μg/g; P<0.001). Likewise, IL-6 showed the same difference (395.43 vs. 133.17 pg/ml; P<0.001). Additionally, significant elevated CRP levels were detected in active cases compared with chronic ones (20.2 vs. 5.1 mg/l; P=0.001). Conversely, hemoglobin levels were significantly lower in active cases compared with inactive ones (8.81 vs. 12.9 gm/dl; P<0.001).

As regards CD, compared with inactive ones, active cases showed significantly elevated levels of FC (1314.05 vs. 244.48 μg/g; P<0.001), IL-6 (447.43 vs. 154.37; P<0.001), and CRP (23.45 vs. 6.34; P=0.001). However, active cases had significantly lower hemoglobin levels compared with inactive ones (8.73 vs. 12.61 g/dl; P=0.009).

Correlation between fecal calprotectin and interleukin-6

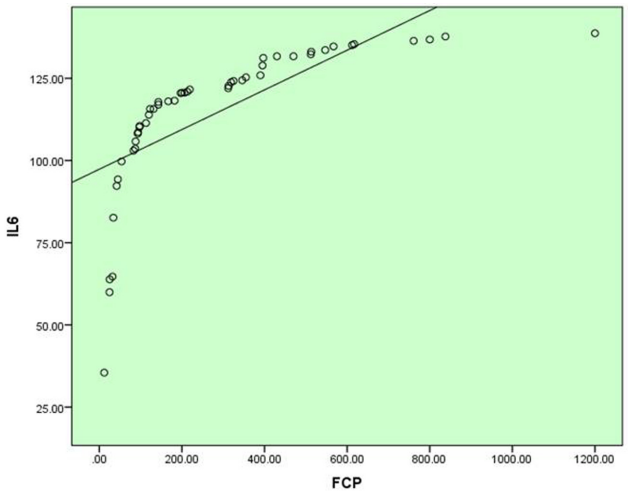
There was a significant positive correlation between FC and IL-6 levels (r=0.637; P=0.001). Nevertheless, no significant correlation was detected between CRP and either of the previously mentioned markers (P>0.05).

Sensitivity and specificity of fecal calprotectin and interleukin-6

Using a cut-off value of 53.95 μg/g, FC had sensitivity and specificity of 97.8 and 95.7%, respectively, to identify cases with IBD, with a diagnostic accuracy of 95.2%.

Using a cut-off value of 30.98 pg/ml, IL-6 had sensitivity and specificity of 94 and 92.5%, respectively, to identify cases with IBD, with a diagnostic accuracy of 93.8% (Figs 1–3 and Tables 3–7).

Figure 1



Correlation between IL-6 and FC. FC, fecal calprotectin; IL-6, interleukin-6.

Table 1 Sociodemographic characteristics of the groups included in the study

	Groups [*n* (%)]		
	IBD cases (*N*=73)	Control (*N*=17)	*P* value
Age	36.09±10.63	34.72±8.57	*t*=–1.286 *P*=0.22
Men	26 (35.6)	10 (58.8)	χ^2=3.104
Women	47 (64.4)	7 (41.2)	*P*=0.015*

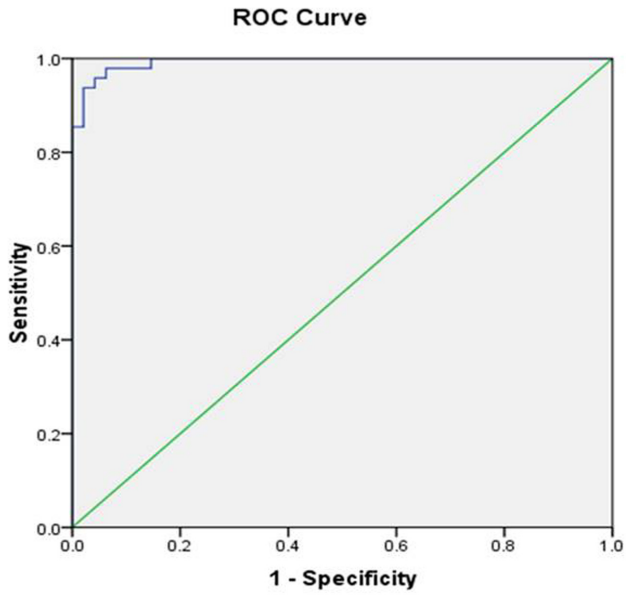
χ^2, χ^2 test; IBD, inflammatory bowel disease; *t*, independent-sample *t* test. **P* value <0.05 was considered statistically significant.

Table 2 Significance of interleukin-6 and fecal calprotectin in diagnosing inflammatory bowel disease

	Groups		
	IBD cases (*N*=73)	Control (*N*=17)	Test of significance
FC (μg/g)	566.48±127.19	24.38±6.56	*t*=22.871 *P*<0.001*
IL-6 (pg/ml)	276.87±18.74	9.32±2.13	*t*=23.509 *P*<0.001*
Hemoglobin (g/dl)	9.22±1.76	13.5±2.06	*t*=–3.447 *P*=0.009*
CRP (mg/l)	13.9±3.7	3.02±0.87	*t*=5.625 *P*=0.001*

CRP, C-reactive protein; FC, fecal calprotectin; IBD, inflammatory bowel disease; IL-6, interleukin-6. **P* value <0.05 was considered statistically significant.

Figure 2



Representing the ROC curve for FC to predict the presence of IBD. FC, fecal calprotectin; IBD, inflammatory bowel diseases; ROC, receiver-operating characteristic.

Discussion

IBDs are chronic diseases with activation and remission periods. It is required to find out noninvasive, easy, inexpensive, and accurate methods to evaluate the activity of the disease, decide therapy, and distinguish the IBD flairs, which have been noted in 20–30% of the patients suffering from IBD [18].

The utility of FC has been confirmed as a screening tool to identify patients with gut inflammation [19]. IL-6 is an important cytokine of the inflammatory process. It is reported that IL-6 receptor signaling is involved in the development of IBD [20].

Multiple studies have found that the increased serum levels of IL-6 reveal the increased severity degrees of certain diseases in addition to the blood levels of acute-phase reactants (erythrocyte sedimentation rate and CRP), and subsequently the serum levels of IL-6 may be utilized together with other biological tests to follow up the activity of the disease [21].

The present study included a total of 90 participants who were divided into two groups: cases (including 73 IBD cases) and controls (17 healthy controls). The IBD group was subdivided into four subgroups: active CD, inactive CD, active UC, and inactive UC.

In the current study, the mean age of the included patients was 36.09 and 34.72 years for cases and controls, respectively. No significant difference was detected between cases and controls regarding that parameter (P=0.220).

Nancey *et al.* [22] have also reported the mean age of 34 and 32 years in cases and control groups, respectively. There was no significant difference between the two groups similar to our results (P>0.05).

In another study handling the same perspective, there was no significant difference between cases and controls regarding the age of participants. It had median values of 51.7, 52.7, and 50.4 years in control, CD, and UC groups, respectively. Although the median age was much older compared with our patients, there was no significant difference between cases and controls regarding that parameter, and that comes in line with our findings [23].

In our study, there was a significant difference between the two groups regarding sex (P=0.015). Males represented 35.6 and 58.8% of patients in both groups, respectively.

Another study also reported a significant difference between cases and controls regarding sex like our study (P<0.05). Females represented 78.57, 49.01, and 75% of cases in the CD, UC, and control groups, respectively [24].

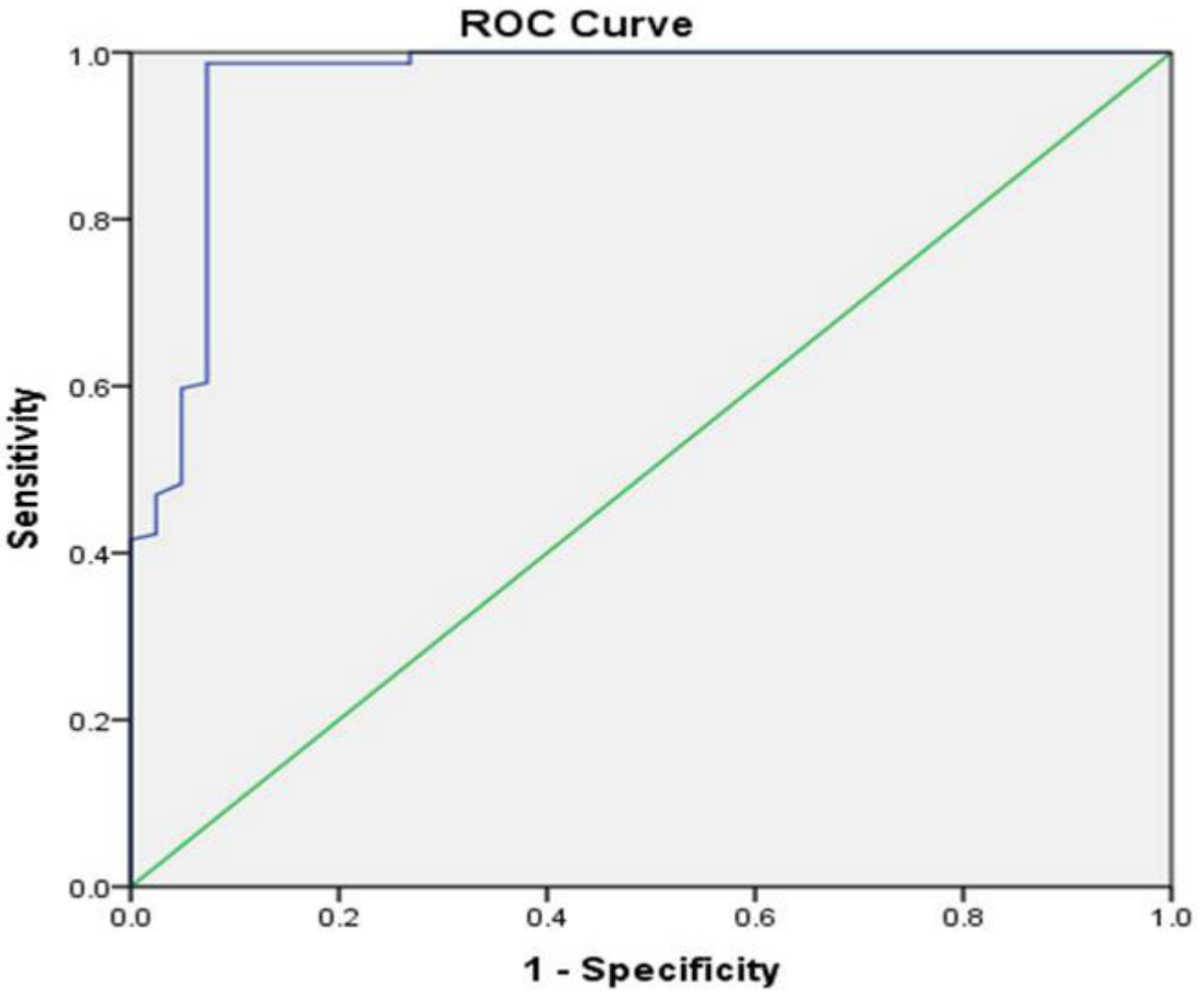
In the current study, cases had significantly lower hemoglobin levels compared with controls (9.22 vs. 13.5 g/dl; P<0.001). This could be attributed to the presence of bloody diarrhea in UC cases, along with malnutrition in CD cases, and both of them could lead to a significant drop in hemoglobin levels.

In a previous study, there was a significant difference between cases and controls regarding hemoglobin levels (P=0.001). Hemoglobin levels were significantly higher in controls (mean=14.89 g/dl) compared with UC (mean=12.8 g/dl) and CD (mean=11.61 g/dl) [24]. This coincides with our results.

In our study, CRP levels were significantly higher in cases compared with controls (13.9 vs. 3.02 mg/l; P<0.001). In accordance with our findings, Erbayrak *et al.* [24] reported that CRP levels were significantly elevated in IBD cases compared with controls (P<0.05). CRP had mean values of 17.53, 33.83, and 4.28 mg/dl in UC, CD, and controls, respectively.

Lochhead *et al.* [23] have also confirmed our findings regarding CRP levels. Nevertheless, that study has

Figure 3



Representing the ROC curve for IL-6 to predict the presence of IBD. IBD, inflammatory bowel diseases; IL-6, interleukin-6; ROC, receiver-operating characteristic.

Table 3 Significance of interleukin-6 and fecal calprotectin in detecting activity of ulcerative colitis

	Groups		
	Active UC (*N*=33)	Inactive UC (*N*=23)	Test of significance
FC (μg/g)	524.17±48	184.48±13.3	t=12.414 P<0.001*
IL-6 (pg/ml)	395.43±34.17	133.17±20.57	t=9.326 P<0.001*
Hemoglobin (g/dl)	8.81±1.2	12.9±0.96	t=−2.668 P=0.008*
CRP (mg/l)	20.2±2.70	5.1±1.62	t=4.192 P=0.001*

CRP, C-reactive protein; FC, fecal calprotectin; IL-6, interleukin-6; UC, ulcerative colitis. *P value <0.05 was considered statistically significant.

Table 4 Significance of interleukin-6 and fecal calprotectin in detecting activity of Crohn's disease

	Groups		
	Active CD (*N*=7)	Inactive CD (*N*=10)	Test of significance
FC (μg/g)	1314.05±224.31	244.48±51.98	t=31.217 P<0.001*
IL-6 (pg/ml)	447.43±47.5	154.37±31.47	t=11.326 P<0.001*
Hemoglobin (g/dl)	8.73±1.46	12.61±2.18	t=−2.539 P=0.009*
CRP (mg/l)	23.45±3.86	6.34±1.07	t=4.739 P=0.001*

CD, Crohn's disease; CRP, C-reactive protein; FC, fecal calprotectin; IL-6, interleukin-6. *P value <0.05 was considered statistically significant.

Table 5 Matrix correlation between different parameters in the study

	FC		IL-6	
	r	*P*	*r*	*P*
FC			0.637	0.001*
IL-6	0.637	0.001*		
CRP	0.237	0.126	0.303	0.063

CRP, C-reactive protein; FC, fecal calprotectin; IL-6, interleukin-6; *r*, Spearman's correlation. *Statistically significant ($P<0.005$).

Table 6 Analysis of the diagnostic ability of fecal calprotectin to predict the presence of inflammatory bowel disease

Diagnostic parameters	FC
AUC	0.937
Cut-off point	>53.98
Sensitivity	97.8%
Specificity	95.7%
Positive predictive value	96.4%
Negative predictive value	94.3%
Accurateness	95.2%
Probability	<0.001*

AUC, area under the curve; FC, fecal calprotectin. *P value <0.05 was considered statistically significant.

Table 7 Analysis of the diagnostic ability of interleukin-6 to predict the presence of inflammatory bowel disease

Diagnostic parameters	IL-6
AUC	0.874
Cut-off point	> 30.98
Sensitivity	94%
Specificity	92.5%
Positive predictive value	95.2%
Negative predictive value	92.6%
Accuracy	93.8%
Probability	<0.001*

AUC, area under the curve; IL-6, interleukin-6. *P value <0.05 was considered statistically significant.

assessed high-sensitive CRP levels. It was significantly elevated in CD (median, 2.3 mg/l) and UC (median, 2.2 mg/l), compared with controls (median, 1.5 mg/l) ($P<0.05$).

In the current study, CRP levels were significantly elevated in active cases compared with chronic ones. In UC cases, it had mean values of 20.2 and 5.1 mg/l, respectively ($P=0.001$). Additionally, it had mean values of 23.45 and 6.34 mg/l in active and chronic CD cases, respectively ($P=0.001$).

Our study demonstrated that FC levels were significantly elevated in cases versus controls (566.48 vs. 24.38 μg/g; $P<0.001$). Using a cut-off value of 53.95 μg/g, FC had sensitivity and specificity of 97.8 and 95.7%, respectively, to identify cases with IBD, with a diagnostic accuracy of 95.2%.

Fukunaga *et al.* [18] reported that the levels of FC were majorly greater among the cases suffering from UC in comparison with the levels of FC among the patients in the control group. This comes in line with our findings.

Erbayrak *et al.* [24] have also reported that FC had mean values of 164.73, 261.45, and 16.72 mg/kg in UC, CD, and control groups, respectively. FC levels were significantly reduced in controls compared with cases ($P<0.05$).

Higher levels of FC were reported in IBD cases compared with controls (mean values 674, 92, and 34 μg/g in active, inactive, and controls, respectively). The cut-off value of FC of 50 μg/g showed a specificity and sensitivity of 78 and 88%, respectively, with a negative predictive value of 87% and a positive predictive value of 79%. The area under the receiver-operating characteristic curve was 0.84. On the other hand, utilizing a cut-off value of 100 μg/g, the sensitivity was elevated to 97% with a small decrease in the specificity to 76% but with a negative predictive value of 97% and a positive predictive value of 75%. The area under the receiver-operating characteristic curve was 0.88 [25].

In the current study, in UC cases, FC was significantly higher in active cases compared with inactive ones (542.17 vs. 184.48 μg/g; $P<0.001$). Moreover, the same findings were noticed in CD. Active cases showed significantly elevated levels of FC (1314.05 vs. 244.48 μg/g; $P<0.001$).

It was shown that FC concentrations correlate with endoscopic findings [26]. Thus, the ability to quantify FC with different severity levels of inflammation enables monitoring using FC to determine treatment response or failure in patients with active disease who are initiated on new therapy and thus, decreasing the need for repeated endoscopic assessment [27].

In our study, IL-6 was significantly higher in cases compared with controls ($P<0.001$). It had mean values of 276.87 and 9.32 pg/ml in cases and controls, respectively. Using a cut-off value of 30.98 pg/ml, IL-6 had sensitivity and specificity of 94 and 92.5%, respectively, to identify cases with IBD, with a diagnostic accuracy of 93.8%.

Nikolaus *et al.* [28] reported that serum IL-6 concentrations were significantly higher in IBD

cases compared with controls, and that agrees with our findings. The median value of IL-6 levels in controls was less than 3 pg/ml (range, <3–6), while it ranged between less than 3 and 32.761 pg/ml in CD cases and between less than 3 and 195 pg/ml in UC cases.

Furthermore, Lochhead *et al.* [23] reported that the median values of IL-6 were 1.0, 1.7, and 1.2 pg/ml in controls, CD, and UC cases, respectively. IL-6 concentrations were elevated in IBD cases compared with controls.

When it comes to IL-6 levels and disease activity in the present study, it had significantly higher values in active UC cases compared with inactive ones (395.43 vs. 133.17 pg/ml; $P<0.001$). In addition, it showed the same significance in CD cases (447.43 vs. 154.37 pg/ml; $P<0.001$).

Previous publications demonstrating an overall increase in IL-6 levels in patients with IBD, particularly in those with active disease [22,29], were confirmed by our findings.

Nikolaus *et al.* [28] also confirmed our findings regarding IL-6 levels and its relation with disease activity. IL-6 levels were significantly elevated in active cases compared with inactive ones ($P<0.05$). In CD cases, IL-6 levels ranged between less than 3 and 32.671 pg/ml in active cases, while it ranged between less than 3 and 6.872 pg/ml in inactive cases. As regards UC cases, it ranged between less than 3 and 195 pg/ml in active cases, whereas it ranged between less than 3 and 27 pg/ml in inactive cases.

Additionally, another study reported that active CD was associated with significantly higher plasma IL-6 concentrations than inactive CD (80±9 and 50±4 pg/ml, respectively; $P<0.001$) [22].

Our results showed that there was a significant positive correlation between FC and serum IL-6 levels ($P=0.001$). This is a reasonable result as both of these markers showed a significant increase in cases against controls, and in active against inactive cases.

Conclusion

Based on our findings, serum IL-6 compared with FC as a valid IBD parameter was a sensitive and reliable marker in the diagnosis of IBD and prediction of disease activity with a significant correlation with FC.

Acknowledgements

The authors would like to thank all patients who participated in the study.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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