БИОХИМИЯ, ГЕНЕТИКА И МОЛЕКУЛЯРНАЯ БИОЛОГИЯ

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Original article

STUDY OF ALBUMIN, C-REACTIVE PROTEIN AND CALPROTECTIN AS NON-INVASIVE INDICATORS FOR THE DIAGNOSIS AND DIFFERENTIATION OF INFLAMMATORY BOWEL DISEASE

Aamer M. Ali, Hayder H. Abed, Eman Mobder Nayif, Hamzah Abdulrahman Salman

Background. *Inflammatory bowel disease (IBD) is defined as an "inflammatory conditions of the colon and small intestine". The main forms of IBD are ulcerative colitis and Crohn's disease. The study aims to evaluate fecal calprotectin with albumin and C-reactive protein as non-invasive indicators of (IBD), mainly in patients with digestive symptoms, who are offered for endoscopic-assessment.*

Methods. *The study includes 104 participants, who are divided into the control, Crohn's disease, and ulcerative colitis groups. Gender, age, BMI, albumin, C-reactive protein (CRP), as well as fecal calprotectin are measured.*

Results. *The results show a significant increase in C-reactive protein and calprotectin (26.77 ± 1.485 mg/l and 419.8 ± 16.99 µg/mg, respectively) in patients with ulcerative colitis, compared to the control group, which shows a mean value of 6.567 ± 1.802 mg/l and 92.98 ± 10.94 µg/mg for C-reactive protein and fecal calprotectin, respectively. The highest value of the CRP result in patients with Crohn's disease is 40.82 ± 2.191 mg/l and the highest significant difference in calprotectin value is 296.2 ± 17.87 µg/mg, related to the control.*

Conclusion. *The results show high and significant differences in serum C-reactive protein and fecal calprotectin in the patients with ulcerative colitis and Crohn's*

disease compared to the healthy people in the control group. In addition, the results show a significant difference in serum C-reactive protein and fecal calprotectin between the ulcerative colitis and Crohn's disease groups. The results of the study suggest the use of these tests as non-invasive indicators to assess the diagnosis and its differentiation.

Keywords: *calprotectin; Crohn's disease; ulcerative colitis; inflammatory bowel disease*

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Научная статья

ИССЛЕДОВАНИЕ АЛЬБУМИНА, С-РЕАКТИВНОГО БЕЛКА И КАЛЬПРОТЕКТИНА КАК НЕИНВАЗИВНЫХ ИНДИКАТОРОВ ДЛЯ ДИАГНОСТИКИ И ДИФФЕРЕНЦИРОВКИ ВОСПАЛИТЕЛЬНЫХ ЗАБОЛЕВАНИЙ КИШЕЧНИКА

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Обоснование. *Воспалительное заболевание кишечника (ВЗК) определяется как «воспалительное заболевание толстой и тонкой кишки». Основными формами ВЗК являются язвенный колит и болезнь Крона. Целью исследования является оценка фекального кальпротектина с альбумином и С-реактивным белком в качестве неинвазивных индикаторов ВЗК, главным образом у пациентов с симптомами пищеварения, которым предписано эндоскопическое исследование.*

Методы. *В исследовании приняли участие 104 участника, которые были разделены на контрольную группу, группу с болезнью Крона и группу больных с язвенным колитом. Измерялись пол, возраст, ИМТ, альбумин, С-реактивный белок (СРБ) и фекальный кальпротектин.*

Результаты. *Результаты показывают достоверное увеличение С-реактивного белка и кальпротектина (26,77 ± 1,485 мг/л и 419,8 ± 16,99 мкг/мг соответственно) у пациентов с язвенным колитом по сравнению с кон-*

трольной группой (6,567±1,802 мг/л и 92,98±10,94 мкг/мг для С-реактивного белка и фекального кальпротектина соответственно). Наибольшее значение результата СРБ у больных болезнью Крона составляет 40,82 ± 2,191 мг/л, а наибольшее значимое различие кальпротектина составляет 296,2 ± 17,87 мкг/мг по отношению к контрольной группе.

Заключение. *Результаты показывают высокие и достоверные различия уровня сывороточного С-реактивного белка и фекального кальпротектина у больных язвенным колитом и болезнью Крона по сравнению со здоровыми людьми контрольной группы. Кроме того, результаты показывают значительные различия уровня сывороточного С-реактивного белка и фекального кальпротектина между группами больных язвенным колитом и болезнью Крона, что позволило использовать эти тесты в качестве неинвазивных индикаторов для оценки правильности постановки и дифференциации диагноза.*

Ключевые слова: *кальпротектин; болезнь Крона; язвенный колит; воспалительные заболевания кишечника*

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Introduction

Inflammatory bowel disease (IBD) is known as a disorder of the small intestine and colon. The main forms of IBD are ulcerative colitis and Crohn's disease [1]. IBD are reflected to be autoimmune diseases, wherein, the body defense mechanisms target elements of the intestinal tract [2]. IBD is a term involving longstanding (chronic) inflammation of tissues in the digestive tract, generally used to define two conditions, i.e., ulcerative colitis and Crohn's disease [3].

Ulcerative colitis disease is an inflammation that causes sores (ulcer) in the digestive tract. Ulcerative colitis affects the large intestine lining, rectum and the colon [4]. In most people, ulcerative colitis begins in the rectum and may extent to the colon. Its signs may be stable or may come and go. They include weight loss, diarrhea, anemia, abdominal-cramping, and pus or blood in the intestines. There is no treatment or drug for ulcerative colitis disease [5]. Drugs can help decrease symptoms of the inflammation [6]. Surgery is a first and last option for more difficult cases. Crohn's disease is known as inflammatory bowel disease (IBD). It leads to inflammation of the tissues (swelling) in the digestive system, which can cause severe diarrhea, abdominal pain, weight loss, malnutrition, and fatigue

[7]. There are no defined causes of Crohn's disease. Different reasons may be rising threat of developing of symptoms and disease, including smoking cigarettes and autoimmune genes. There is neither drug nor treatment for Crohn's disease, and medication can be usually used to control or decrease the symptoms [8]. The IBD diagnosis using standard test is endoscopy (colonoscopy) [9], which is considered as invasive and expensive. Besides, blood indicators of inflammation are believed to be inadequate markers to diagnose and discriminate between the types of IBD [10]. Calprotectin is a protein biomarker that is present in the feces when intestinal inflammation occurs [11]. Calprotectin acts as a competitive inhibitor (zinc-dependent enzymes) in biostatic action against microorganism, by zinc chelation, inflammation regulation, and apoptosis initiation in cancerous cells (malignant cells) [12]. Fecal calprotectin test is a biochemical test of the calprotectin protein amount in the patients' stool. Increasing fecal calprotectin directs the passage of neutrophils cells to the intestinal mucosa, which happens during inflammation of intestine, as well as inflammation caused by IBD [13]. In a particular clinical situation, the test may replace the requirement for radiolabeled white cell scanning or an invasive colonoscopy [14]. This study aims to evaluate fecal calprotectin with albumin and C-reactive protein as non-invasive indicators of IBD, particularly in patients with digestive disorder symptoms, who are prescribed an endoscopic diagnostic test.

Material and Methods

This cross-sectional study was carried out in the teaching Al-Hussein Hospital, Al-Muthanna, Iraq, from June 2021 to April 2022. This study included 104 participants (48 females and 56 males), who were classified into three groups. The first group consisted of 21 volunteers (10 males and 11 females) as the control group with no inflammatory bowel disease, whereas, the second group comprised 58 patients (29 males and 29 females) with ulcerative colitis disease. The third group comprised 25 patients (9 males and 16 females) with Crohn's disease. All patients were diagnosed with inflammatory signs diseases (ulcerative colitis or Crohn's disease), who had their diagnoses made by using the endoscopic technique. Data including age, BMI, gender, and family history were collected. All the volunteers signed consent forms.

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study. Ethical approved reference No. (UAM /EC/5/2021) and all procedures performed in studies involving participants were in accordance with the ethical

standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Specimen Collection

About five milliliters of venous blood samples were collected from patients attending the Al-Hussein Teaching Hospital in Samawah, Iraq. Venous blood samples of up to 5 ml were taken from the vein by using a 5 ml syringe. The samples were kept for 30 minutes at room temperature and then the tubes were transported to centrifugation at 1800 rpm for twenty minutes at room temperature [15]. Serum was kept in separate tubes under frozen conditions until the samples were used for albumin and C-reactive protein tests. Feces (50 mg) was collected in a clean plastic container, using the Quantitative Fecal Calprotectin ELISA Kit.

Albumin Assay Kit

Serum albumin was assayed by using the ALB2 assay kit / Roche and Cobas c311 analyzers (Hitachi, Japan). In the colorimetric test, at 4.1 pH value, albumin showed an adequately cationic-character, which is able to bind with bromocresol green (BCG), an anionic dye, formation of a green blue complex. photometrically concentration for the blue green was directly relative to the sample albumin amount and was measured photometrically [16].

C-reactive protein test

Determination of the C-reactive protein **(**CRP) quantitatively was done using the C-reactive protein (Gen.3 assay kit / Roche and Cobas C311 analyzers) (Hitachi, Japan). Human-CRP (agglutinates with latex particles) were covered with monoclonal anti-CRP antibodies. The aggregates particles were measured using turbidimetric-testing [17; 18].

Calprotectin Elisa Test

Quantitative determination of fecal calprotectin was done with an assay using the Calprotectin Elisa kit (Epitope Diagnostics, Inc., USA). This Calprotectin ELISA Assay Kit was created, developed, and manufactured to test human calprotectin in stool samples quantitatively. The ELISA Assay Kit of Calprotectin proteins uses the two site sandwich technique with two selected antibodies that bind changed epitopes of the human calprotectin [19].

Statistical Analysis

All the figures, tables, and results were obtained using the GraphPad prism software program (Version 6) and the Excel 22016 software program. The results were analyzed using one-way ANOVA test and the unpaired T-test.

Results

The samples of the control group were obtained from 10 males and 11 females, whereas samples of the ulcerative colitis group comprised were obtained from 29 females and 29 males, and from 9 males and 16 females of the last group of patients with Crohn's disease comprised, as shown in Figure 1.

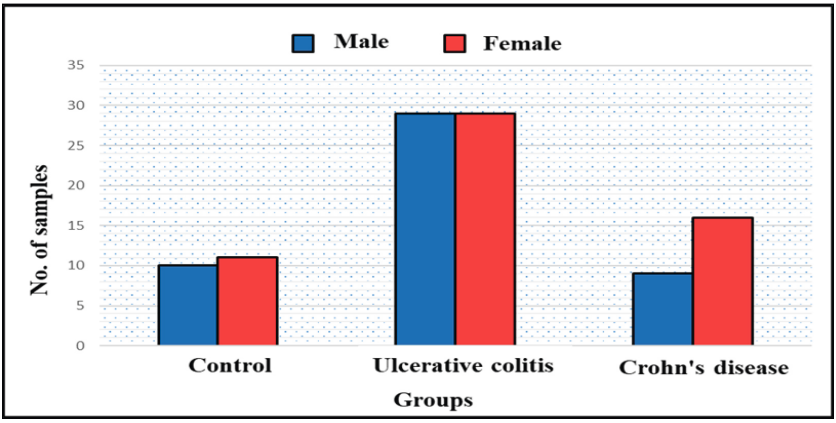

Fig. 1. Gender distribution of the groups members

The mean ages of the members of the three groups in years were 33.43 ± 1.705, 29.83 ± 1.180, and 37.04 ± 1.581 for the control, Crohn's disease, and ulcerative colitis respectively, and there were no significant differences between them and a ***p***-value of ≤ (0.05) as shown in Figure 2 and Table 1.

Table 1.

Age mean value of the groups members

Age in years	N	Mean ± SEM	*p*-value	Range	95% confidence interval	R square
Control	21	33.43 ± 1.705	-	19- 43	-	-
Ulcerative colitis	58	29.83 ± 1.180	0.1080 NS	16- 44	-8.010 to 0.8084	0.03320
Crohn's disease	25	37.04 ± 1.581	0.1279 NS	19- 54	-1.079 to 8.302	0.05188

NS – non-significant, S – significant

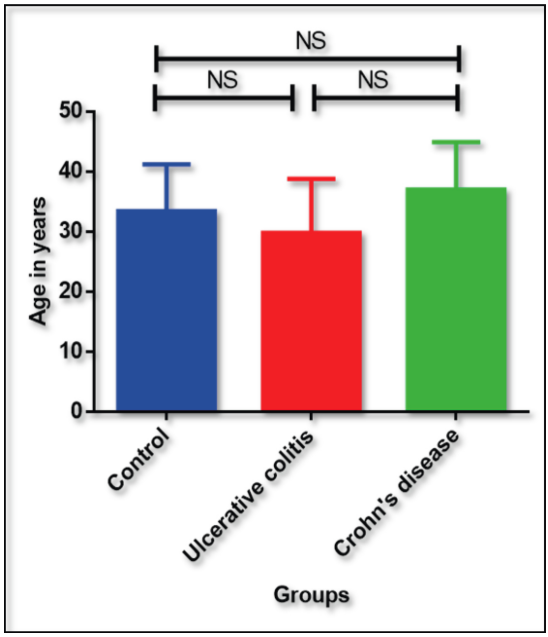

Fig. 2. Age distribution of the groups members, *p*-value ≤ 0.05

The mean BMIs of the three the groups members were 26.29 ± 0.7240, 25.18 ± 0.5390, and 24.89 ± 0.7809 of the control, Crohn's disease and ulcerative colitis groups, respectively, there were no significant differences between them and a ***p***-value of (≤ 0.05), as shown in Figure 3 and Table 2.

Table 2.

BMI value of the groups members

BMI	N	Mean ± SEM	*p*-value	Range	95% confidence interval	R square
Control	21	26.29 ± 0.7240	-	20.2- 31.3	-	-
Ulcerative colitis	58	25.18 ± 0.5390	0.2721 NS	17.6- 33	-3.089 to 0.8826	0.01564
Crohn's disease	25	24.89 ± 0.7809	0.2037 NS	17.2- 34.3	-3.571 to 0.7833	0.03645

NS – non-significant, S – significant

The mean values of albumin in g/l were 44.74 ± 1.210, 42.63 ± 1.261, and 41.36 ± 2.278 in the Control, the Crohn's disease and the ulcerative colitis

groups, respectively. There were no significant differences between them and a ***p***-value ≤ of 0.05, as shown in (Figure 4 and Table 3).

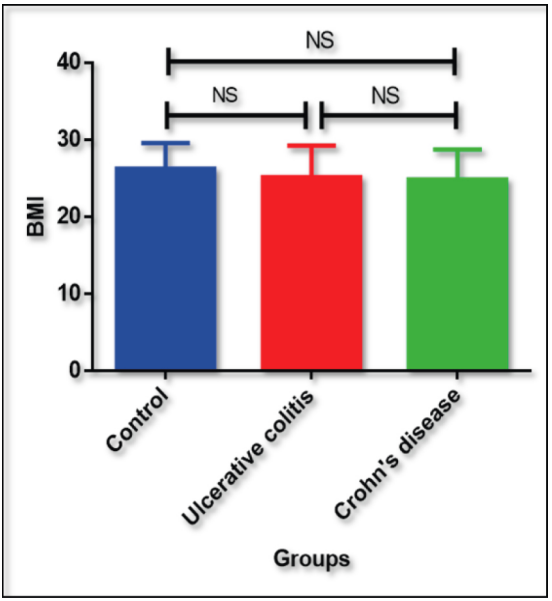

Fig. 3. BMI distribution in the groups members, *p*-value ≤ 0.05

Table 3.

Albumin value in the groups members

Albumin	N	Mean ± SEM	*p*-value	Range	95% confidence interval	R square
Control	21	44.74 ±1.210	-	22.4- 64.3	-	-
Ulcerative colitis	58	42.63 ± 1.261	0.6044 NS	21.2- 73.2	-3.574 to 6.105	0.003330
Crohn's disease	25	41.36 ± 2.278	0.2215 NS	33.1- 54.3	-2.112 to 8.870	0.03377

NS – non-significant, S – significant

C-reactive protein values were 6.567 ± 1.802, 26.77 ± 1.485, and 40.82 ± 2.191 for the Control, the Crohn's disease and Ulcerative-colitis groups, respectively. The result of ulcerative colitis and Crohn's disease groups showed a significant difference in both groups (***p***-value < 0.0001) compared to the control as shown in Table 4 and Figure 5.

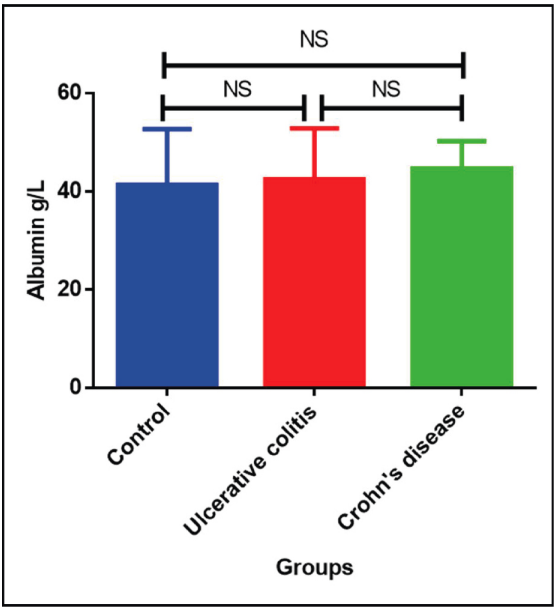

Fig. 4. Albumin value in the groups, *p*-value of ≤ 0.05

Table 4.

C-reactive protein value in mg/l of group samples

Groups	n	Mean ± SEM	*p*-value	Range	95% confidence interval	R square
Control	21	6.567 ± 1.802	-	0-33	-	-
Ulcerative colitis	58	26.77 ± 1.485	< 0.0001 S****	10.20- 61.30	14.82 to 25.58	0.4208
Crohn's disease	25	40.82 ± 2.191	< 0.0001 S****	22.30-65.30	28.40 to 40.12	0.7593

NS – non-significant, S – significant, S**** – High significant ***p***-value < 0.0001

The mean values of fecal calprotectin µg/mg were 92.98 ± 10.94, 419.8 ± 16.99, and 296.2 ± 17.87, of the control, Crohn's disease and ulcerative colitis groups, respectively.

The result of the ulcerative colitis and Crohn's disease groups showed high significant difference in both groups (***p***-value < 0.0001) compared to the control as in Table 5 and Figure 6.

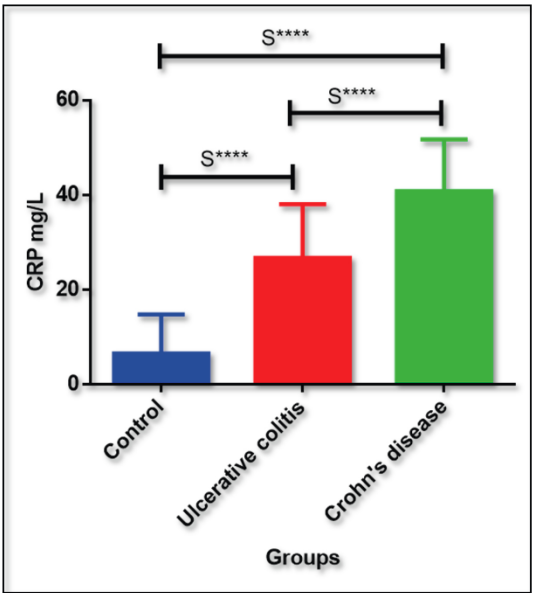

Fig. 5. CRP value in mg/l of group samples with *p*-value < 0.0001

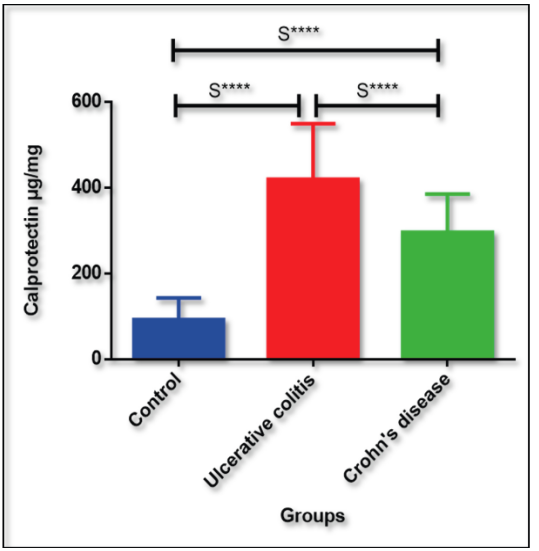

Fig. 6. Fecal calprotectin in µg/mg for group samples with *p*-value of < 0.0001

Table 5.

Fecal Calprotectin in µg/mg of group samples

Calprotectin µg/mg	n	Mean ± SEM	*p*-value	Range	95% confidence interval	R square
Control	21	92.98 ± 10.94	-	24.12- 168.3	-	-
Ulcerative colitis	58	419.8 ± 16.99	< 0.0001 S****	141.6- 745.3	268.9 to 384.8	0.6212
Crohn's disease	25	296.2 ± 17.87	< 0.0001 S****	142.5- 432.4	158.9 to 247.4	0.6608

NS – non-significant, S – significant, S**** – High significant p value < 0.0001

Discussion

Calprotectin is a protein produced from activated leukocytes and usually can be utilized as an indicator for leukocyte activation [20]. Feces calprotectin can be utilized to identify active leukocyte in various body fluids in addition to using as indicator for inflammation of bowel disease [21]. In addition, according to another research, there are no significant differences according to gender (males and females), to commend a reference interval, based on males and females (0.3 to 2.6 mg/L) [22]. Besides, there are no significant differences in this study between groups according to gender and BMI. CRP (the acute phase reactant) rises with the increase in age and plasma concentrations as biological indicators of frailty, crucial increasing vulnerability for mortality and diseases, as well as with aging [23]. Serum albumin levels decrease with age in both men and women [24], although there are no significant differences between groups according to age or albumin value of samples, as shown in Figure 4 and Table 3.

C-reactive protein is the most common biomarker used as indicator of inflammation in IBD. Elevated value of CRP service to distinguish mucosal active disease from "quiescent of IBD" [25]. Many situations are connected with a CRP response including inflammatory diseases, infectious stimuli (fungal, bacterial, or viral), stress, tissue-necrosis, neoplasia, and childbirth [26]. There is a high correlation between inflammation and some disorder disease and strong C-reactive protein response including Crohn's disease and rheumatoid arthritis [27; 28]. Results in Table 4 show high significant differences in the CRP values of patients with Crohn's disease and ulcerative- colitis, and the highest value in CD patients. A recent study has concluded that in CD, level of CRP high relate with activity of diseases: CRP value is higher in severe CD compared to moderate CD, which is on its turn higher than mild Crohn's disease [29]. For UC, the similar tendency can be detected, even though CRP is generally much

less than in Crohn' disease [30] and agrees with the results of this study as in Figure 5.

Calprotectin protein is produced in neutrophils and the presence of fecal calprotectin is a biomarker for gastrointestinal inflammation, higher levels of fecal calprotectin representing severe inflammation [31]. According to the results of this study, there is an increase in the fecal calprotectin level in Crohn's disease patients and an upper level in the ulcerative colitis group, with high significant differences, as compared to the control group, as shown in Table 5 and Figure 6.

To achieve these objectives, i.e., a cheap screening and non-invasive tests, calprotectin will be used to provide a preliminary diagnosis of inflammatory bowel disease. Calprotectin is a main protein present in the cell cytosol in the inflammatory diseases [32]. The protein test is constant in fecal samples for up to 7 days at normal conditions, i.e., room temperature and usually less than 5 g per sample is enough for a reliable test [33]. Due to these characteristics, stool samples may be collected at home and transported to the lab room with possible delays. Fecal calprotectin is considered a good indicator for IBD-diagnosis, specifically when screening patients with digestive disorder symptoms, who are offered an endoscopic test. Anyway, only a few studies, depending on a quite small number of patients, have assessed fecal calprotectin level in patients with other forms of bowel inflammations less public than CD and UC, such as, nonspecific colitis, eosinophilic, and lymphocytic, any way calprotectin levels have been established in symptomatic patients to accurately differentiate between IBD and non organic disease and, when elevated, urge early endoscopic evaluation to rule out IBD and other organic disorders [34]. Calprotectin (a neutrophil protein) is present in both blood and stool serum. Its concentration significantly elevates through inflammatory in (IBD) and infections conditions. Increasing level of fecal calprotectin (FC) appears to be a screening test to determine patients needing further invasive diagnostics [35].

Conclusion

The conclusion of this study shows high significant differences in serum C-reactive protein and fecal-calprotectin in patients with Crohn's disease or ulcerative colitis linked to healthy people in the control group. In addition, the results show a significant difference in serum C-reactive protein and fecal calprotectin between Crohn's disease and ulcerative colitis, which allows the use of these tests as non-invasive indicators for the diagnosis and differentiation between them.

Conflict of Interest. The authors of this study declare that they have no conflict of interest.

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