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Assesement of Leucine Rich Glycoprotein as a biomarker in Crohn's disease

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Abstract

Background: The amount of Serum Leucine Rich Glycoprotein (LRG) has a strong correlation with the clinical disease activity observed in patients with Crohn's disease (CD).

Aim: to determine the Leucine rich glycoprotein serum level in patients with CD.

Patients and Methods: This prospective cohort study was carried out at Internal Medicine and Clinical Pathology Departments, Zagazig University Hospitals on 16 patients complaining of CD. The following were done for all patients: detailed history, complete general examination, local abdominal examination, examination of other systems and upper endoscopy findings with biopsy. Colonoscopy was done if there was no contraindication. Colonoscopy was done by two senior experts and by OLYMPUS colonoscope. Fecal calprotectin was measured and serum LRG was quantified using ELISA kit at the baseline and after 6 months as the patients had their biological treatment and subjected to follow up for six months.

Results: Activity of CD had a significant relation with Harvey-Bradshaw score ($p=0.005$). On pairwise comparison, difference was significant between remission and each of newly diagnosed and relapse ($p=0.034$, and 0.002 respectively). Severity of CD had a significant relation with Harvey-Bradshaw score ($p<0.001$). On pairwise comparison, difference was significant between remission and moderate CD ($p<0.001$). The best cutoff of baseline LRG in diagnosis of Crohn's disease in remission is ≤61.4 µg/ml with area under curve 0.855 with sensitivity 80%, specificity 81.8%, positive predictive value 66.7%, negative predictive value 90% and overall accuracy 81.3% ($p=0.027$). There is statistically significant positive correlation between baseline LRG and Harvey-Bradshaw score, CRP and serum creatinine. There is significant decrease in LRG in remission and mild cases after treatment.

Conclusion: Leucine-rich glycoprotein could be a reliable serum biomarker for the assessment of clinical disease activity in patients with IBD. It can be an alternative to CRP and fecal calprotectin for the assessment of Crohn's disease.

Keywords: Leucine Rich Glycoprotein, Crohn's disease

Introduction

Crohn's disease [CD] represent primary clinical form of chronic inflammatory bowel disease [IBD]. CD is believed to arise from the interplay of genetic predisposition, environmental influences, and intestinal microbiota, leading to abnormalities in the immune response of the mucosal lining and changes in the function of the protective epithelial barrier**(1)**.

Crohn's disease (CD) is linked to substantial morbidity and has a pronounced effect on the patient's quality of life. The most prevalent symptoms of CD include stomach discomfort, diarrhea, rectal bleeding, weight loss, fever, and exhaustion. Moreover, inflammation outside the intestines often appears in the eyes, liver, skin, and joints, indicating the widespread nature of this devastating condition. In addition, a significant number of patients experience penetrating or structural difficulties, which result in the need for many surgeries and incapacity (2).

Endoscopic inspection and histological analysis of biopsy materials are now considered the most reliable approaches for identifying and measuring intestinal inflammation. However, these techniques are expensive, require intrusive procedures, and are not well-received by patients who are reluctant to undergo repeated examinations (3).

There is a need for a dependable surrogate marker that may accurately reflect intestinal inflammation and be used as an alternative to endoscopy. Blood-based biomarkers generally offer a non-intrusive assessment of the level of inflammation in IBD (4). Nevertheless, only a small number of blood-based indicators have undergone thorough validation in IBD, and even fewer are currently employed in routine clinical practice (5).

C-reactive protein (CRP) is one potential indication. The clinical level of the condition is determined by the intensity of the pathogenic activity, which triggers the formation of CRP (6). Nevertheless, certain patients fail to exhibit elevated levels of CRP, even in the presence of an active illness. Thus, there is a requirement for biomarkers with enhanced sensitivity (4).

Further research has indicated that levels of LRG are elevated in various inflammatory conditions, including Still's disease, Kawasaki illness, juvenile idiopathic arthritis, psoriasis, appendicitis, malignant diseases such gastric cancer and colorectal cancer, heart failure, diabetes, and obesity. The production of this substance is mostly obtained from neutrophils, macrophages, intestinal epithelial cells, and hepatocytes in response to tumor necrosis factor-α (TNF α), interleukin1β (IL -1β), IL-6, and IL-22 (7,8). Nevertheless, it is an independent biomarker that is not reliant on IL-6. LRG is also produced by cytokine-stimulated neutrophils and epithelium in the intestinal epithelial cells of people with IBD and is subsequently released into the bloodstream. LRG is a better indicator of intestinal inflammation compared to CRP (9).

The objective of this study was to ascertain the serum level of Leucine rich glycoprotein in patients with CD.

Patients and Methods

This prospective cohort study was conducted on 16 patients diagnosed with Crohn's disease who were either admitted to the hospital or receiving treatment at the outpatient clinic for inflammatory bowel disease (IBD OPD) in the Internal Medicine Department at Zagazig University Hospitals in Zagazig, Egypt. The patients underwent the administration of the biological treatment from March 2023 to February 2024. Prior to their involvement in this study, all participants were informed that blood samples would be taken from them for research purposes, and they all provided their consent after being fully informed. The authorization of the institutional review board – Faculty of human medicine, Zagazig university IRB was obtained (ZU-IRB#10650/2-4-2023).

Inclusion criteria:

Patients of both sexes were be enrolled in the study after their informed consent. Age of patients included in the study was more than 18 (adults patients only). Patients suffering from any lower GIT symptoms attending for colonoscopy, the patient's condition was determined to be inflammatory bowel disorders by a comprehensive assessment that included clinical, biochemical, stool, endoscopic, and histological studies. In cases when there is suspicion of CD, it may be required to employ radiological techniques to see the small intestine. It is important to rule out infectious colitis, namely Clostridium difficile infection **(10)**

Pregnant females, patients with acute infection, patients with chronic disorders such as chronic renal failure, congestive heart failure, thyroid disorders, malignancy and patients with another autoimmune disease, patients with other inflammatory diseases and patients under steroid therapy or none steroidal anti-inflammatory drugs were excluded from participation in this study.

The following were done for all patients:

1. ***Detailed history and Complete general examination:*** Personal history including name, age, sex, occupation, residence and Complete general and systemic examination.
2. ***Laboratory investigations*:**

Blood samples were obtained by venipuncture for CBC, ESR, CRP, urea, creatinine, total bilirubin, ALT, AST for each patient. two ml of blood was collected in BD vacutainer ® ESR tube, three ml of blood in ® plastic serum tube, BD vacutainer and two ml of blood in ® EDTA tube (Becton, Dickinson and company, NJ). Part of serum separated and stored frozen at – 80 C for LRG level assessment by ELISA later. Stool samples were collected for faecal calprotectin.

All patients underwent the following laboratory analysis: Complete blood count by fully automated cell counter (XN 1000 Sysmex, Germany). The automatic erythrocyte sedimentation rate (ESR) analyzer Vision B (Shenzhen. YHLO Biotech Co., Ltd., Shenzhen, China) was used to determine the ESR. C-Reactive Protein (CRP) was done by Cobas 6000, c501 module (Roche diagnostics, Mannheim, Germany) by immunoturbidimetry assay. Serum urea, creatinine, total bilirubin, ALT, AST by Cobas 8000, c501 module (Roche diagnostics, Mannheim, Germany). Fecal calprotectin performed on ichroma™ Boditech Med Inc, Korea, Catalog No. CFPC-83. Its assay range 10–1000 mg/kg, the reference value was

less than or up to 50 mg/kg feces, assay time 10 min, performance CV < 10%, and sample volume 10 mg (Feces).

Special investigation:

Serum LRG levels were measured by using double antibody sandwich enzyme-linked immunosorbent assay (ELISA) **(DL-develop Co, China) CAT: DLR-LRG1-HU**, according to the manufacturer's instructions. The LRG levels were calculated using a standard curve. The results were interpreted as µg/ml.

Colonoscopy was done by two senior experts and by OLYMPUS colonoscope, model /CV-190, serial NO 7336784 and histopathological assessment was done according to **(11,12).**

Scoring Disease Activity

The Harvey-Bradshaw index is a modified version of the Crohn's disease activity index (CDAI) used specifically for the collecting of data. The score varied from 0 and 16 or higher, with the maximum score being determined by the frequency of liquid stools per day. With higher scores indicating worse severity as remission cases had score less than 5, Mild Disease: 5-7, Moderate Disease: 8-16 and sever cases had score more than 16 **(13).**

Statistical analysis

The data analysis was conducted using SPSS (Statistical Package for the Social Sciences) software, specifically version 27. The categorical variables were analyzed by calculating their absolute frequencies in order to compare ordinal data across two groups. The chi-square test for trend was employed for this purpose. The Shapiro-Wilk test was employed to validate the assumptions necessary for the application of parametric tests. The quantitative variables were characterized by either their means and standard deviations or their medians and interquartile ranges, depending on the type of data. The Kruskal-Wallis test was employed to compare quantitative data among more than two groups, specifically for data that is not normally distributed. Conversely, the one-way ANOVA test was utilized for normally distributed data. When there was a notable difference, pairwise comparison and Bonferroni tests were employed to identify differences between each pair of individual groups. The Receiver Operating Characteristic (ROC) curve was employed to identify the optimal threshold for a certain quantitative parameter in the diagnosis of a particular health condition. The Pearson correlation coefficient was employed to evaluate the direction and magnitude of correlation between each pair of continuous variables. The paired sample t-test was employed to evaluate the change in a certain parameter between two time points. The threshold for statistical significance was established at a P-value of less than 0.05. A statistically significant difference was observed if the p-value was less than or equal to 0.001.

Results

This study comprised 16 patients with Crohn's disease with age range from 19 to 45 years with mean age 29.6 ± 8.6, Female represented 56.2% and more than 50% came from rural areas **(Table 1)**

Table (1) Demographic data of all patients:

	Crohn's Disease

	N=16	%
Gender:		
Male	7	43.8%
Female	9	56.2%
Age (year)		
[Mean ± SD]	29.6 ± 8.6	
Range	19 – 45	
Geographic:		
Rural	9	56.2%
Urban	7	43.8%

According to disease severity, 31.3% had moderate form and 37.5% were on relapse. Endoscopic examination revealed that 38.9% had left side colitis and 43.8% had chronic colitis. Histopathology revealed that 37.5% had active CD. Harvey-Bradshaw score ranged from 1 to 11 with median 5.5. According to clinical activity CD cases classified into newly diagnosed (25%), cases in remission (37.5%) and relapse (37.5%) (table 2).

Table (2) Clinical assessment of patients with Crohn's disease:

	N=16	%
Severity:		
Remission	5	31.3%
Mild	6	37.5%
Moderate	5	31.3%
Activity:		
Newly diagnosed	4	25%
Remission	6	37.5%
Relapse	6	37.5%
Endoscopic distribution:		
Proctitis with terminal ilium	2	12.5%
Terminal ilium	1	6.3%
Pancolitis	8	50%
Proctocolitis	2	12.5%
Descending colon	1	6.3%
Unremarkable	2	12.5%
Histopathology:		
Active CD	6	37.5%
Chronic colitis	7	43.8%
Chronic duodenitis, colitis	1	6.3%
Moderate chronic active ileitis	2	12.5%
Harvey-Bradshaw score	5.5(3 – 9.75)	1 – 11

Activity of CD showed a significant difference with Harvey-Bradshaw score (p=0.005) between the three studied groups. On pairwise comparison, difference was significant between remission and each of newly diagnosed and relapse (p=0.034, and 0.002 respectively). Severity of CD had a significant relation with Harvey-Bradshaw score

($p<0.001$). On pairwise comparison, difference was significant between remission and moderate CD ($p<0.001$) (Table 3).

Table (3) Clinical and laboratory data of Crohn' disease patients according to disease activity and severity:

	Newly diagnosed	**Remission**	**Relapse**	χ^2	**p**
	N=4(%)	**N=6(%)**	**N=6(%)**		
Gender **Female** **Male**	 3 (75%) 1 (25%)	 3 (50%) 3 (50%)	 1 (16.7%) 5 (83.3%)	0.16	0.89
Residence **Rural** **Urban**	 1 (50%) 3 (50%)	 3 (50%) 3 (50%)	 5 (83.3%) 1 (16.7%)	0.16	0.89
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	F	p
Age (year)	30.5 ± 7.0	32.8 ± 10.9	25.8 ± 6.7	1.016	0.389
Hemoglobin (g/dl)	11.2 ± 1.8	11.7 ± 1.7	12.0 ± 1.4	0.324	0.729
T. bilirubin (mg/dl)	0.8 ± 0.4	0.4 ± 0.09	0.5 ± 0.2	1.683	0.431
ALT (U/L)	20.3 ± 3.9	14.7 ± 4.7	13.7 ± 3.8	2.578	0.276
AST (U/L)	25.3 ± 2.4	19.3 ± 5.3	18.6 ± 5.0	1.427	0.49
LRG (µg/ml)	66.1 ± 11.3	58.1 ± 9.7	69.1 ± 7.9	1.903	0.165
	Median (IQR)	**Median (IQR)**	**Median (IQR)**	**KW**	**p**
WBCs (x10^3/uL)	11.5 (8.1 – 15.9)	5.9(5.1 – 10.3)	6.7(3.9 – 9.8)	4.263	0.119
Neutrophil (x10^3/uL)	8.5(4.9 – 13.5)	3.8(2.1 – 7.4)	3.2(2.1 – 8)	3.779	0.151
Lymphocytes (x10^3/uL)	2.5(2.0 – 3)	2(1.7 – 2.3)	1.5(1.4 – 2.4)	24.047	0.132
BUN (mg/dl)	19.2(14.8 – 21.9)	5.9(11.4 – 27)	14.8(11.2 – 24.5)	0.428	0.807
Creatinine (mg/dl)	0.8(0.5 – 1.0)	0.6(0.4 – 0.9)	1 (0.5 – 1.2)	1.301	0.552
Platelet (x10^3/uL)	231(154 –347)	291(240-333)	309(233 – 340)	1.226	0.542
CRP (mg/L)	21.9(14.8–79.7)	11.5(2.4–31.6)	16.1(2.8 – 33.4)	1.074	0.585
ESR (mm.)	30(15.5 – 43)	25(13 – 40)	15(8.8 – 24.5)	3.141	0.208
Calprotectin (mg/kg)	261.5(206.3–313.8)	220(195.8-449.3)	348(200.5–493.8)	0.755	0.686
Harvey-Bradshaw score	6(5 – 10)	2.5(2 – 3)	9.5(7 – 10)	10.543	**0.005***
Pairwise	**P_1 0.034***	**P_2 0.002***	P_3 0.486		
	Remission	Mild	Moderate	χ^2	p
	N=5 (%)	**N=6 (%)**	**N=5(%)**		
Gender Female Male	 3 (60%) 2 (40%)	 3 (50%) 3 (50%)	 1 (20%) 4 (80%)	1.524	0.217
Residence Rural	 3 (60%)	 2 (33.3%)	 4 (80%)	0.381	0.537

Urban	2 (40%)	4 (66.7%)	1 (20%)		
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	F	p
Age (year)	31.6 ± 11.7	32.0 ± 7.4	24.8 ± 5.6	1.164	0.343
Hemoglobin (g/dl)	11.4 ± 1.7	12.3 ± 1.1	11.3 ± 1.9	0.719	0.506
Platelet (x10^3/uL)	290 ± 74	290 ± 72	264 ± 78	0.203	0.819
LRG (µg/ml)	55.3 ± 7.8	65.6 ± 7.7	71.9 ± 9.3	2.1	0.2
	Median (IQR)	**Median (IQR)**	**Median (IQR)**	**KW**	**P**
WBCs (x10^3/uL)	5.6(4.8 – 8.2)	8.8(5.2 – 11.0)	8.3(5.8 – 15.8)	1.876	0.391
Neutrophil (x10^3/uL)	3.7(2.0 – 5.5)	5.6(2.5 – 8.8)	6.6(2.9 – 13.4)	1.871	0.392
Lymphocytes (x10^3/uL)	2.2(1.6 – 2.4)	2.1(1.7 – 2.4)	1.4(1.4 – 2.9)	0.427	0.808
BUN (mg/dl)	14.3(11.2 – 22.3)	19.2(12.3-30.3)	15.2(13.5 – 20.7)	0.685	0.71
Creatinine (mg/dl)	0.5(0.4 – 0.8)	0.9(0.6 – 1.1)	0.9(0.4 – 1.3)	2.021	0.364
ALT (U/L)	14(10 – 17)	20(12.3 – 22.5)	13(12.5 – 18)	1.899	0.387
AST (U/L)	17.3(13.9 – 23.8)	23.7(15 – 26.6)	22.6(17.5 – 24.2)	0.935	0.627
T bilirubin (mg/dl)	0.4(0.3 – 0.4)	0.7(0.4 – 0.8)	0.4(0.4 – 0.9)	2.614	0.271
CRP (mg/L)	4(2.1 – 25.2)	21.9(10.3 – 25.9)	26.6(5.1 – 76.2)	2.134	0.344
ESR (mm.)	25(10 – 45)	18(8.8 – 28)	22(15 – 38.5)	0.861	0.65
Calprotectin (mg/kg)	220(190.5 – 270)	293.5(215-334)	298(197 – 574.5)	4.16	0.125
Harvey-Bradshaw score	1.5(1 – 2)	5(5 – 5.5)	10(9.5 – 10.5)	13.565	**<0.001 ****
Pairwise	P_1 0.054	P_2 0.054	**P_3 <0.001****		

IQR interquartile range F One way ANOVA test KW Kruskal Wallis test χ^2 Chi square for trend test significant p1 difference between new cases and remission cases p2 difference between remission cases and relapse p3 difference between new cases and relapse cases of CD, IQR interquartile range F One way ANOVA test KW Kruskal Wallis test $\chi 2$ Chi square for trend test p1 difference between remission and mild CD p2 difference between mild and moderate CD p3 difference between remission and moderate CD **p0.001 is statistically highly significant

The best cutoff of CRP in diagnosis of moderate cases of CD in comparison to remission and mild cases was ≥19.5 mg/ml with area under curve 0.818 with sensitivity 63.6%, specificity 80 %, positive predictive value 87.5%, negative predictive value 50% and overall accuracy 68.75% (p=0.047). The best cutoff of fecal calprotectin in diagnosis of moderate cases of CD in comparison to remission and mild cases was ≥247.5 mg/ml with area under curve 0.818 with sensitivity 72.7%, specificity 60 %, positive predictive value 80%, negative predictive value 50% and overall accuracy 68.75% (p=0.047) (Table 4 and Figure 1, 2).

Table (4) Performance of baseline CRP in detection of CD severity:

	Cutoff	**AUC**	**Sensitivity**	**Specifi**	**PPV**	**NPV**	**Accuracy**	**p**

				city				
CRP (mg/L)	≥19.5	0.818	63.6%	80 %	87.5%	50 %	68.75%	0.047*
Fecal Calprotectin (mg/kg)	≥247.5	0.818	72.7%	60 %	80%	50 %	68.75%	0.047*

AUC area under curve **p≤0.001 is statistically highly significant *p<0.05 is statistically significant PPV positive predictive value NPV Negative predictive value

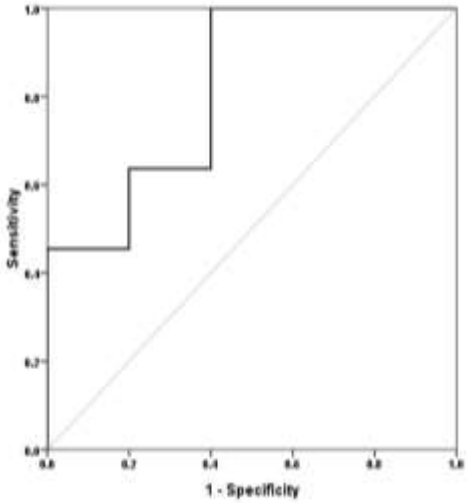

Figure (1) ROC curve showing performance of baseline CRP in detection of CD severity

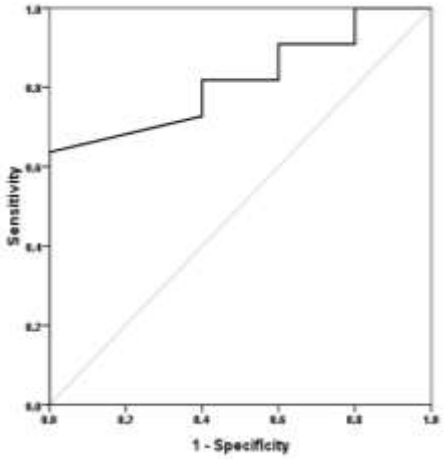

Figure (2) ROC curve showing performance of baseline calprotectin in detection of CD severity

The best cutoff of baseline LRG in diagnosis of Crohn's disease in remission is ≤61.4 µg/ml with area under curve 0.855 with sensitivity 80%, specificity 81.8%, positive predictive value 66.7%, negative predictive value 90% and overall accuracy 81.3% (p=0.027) (Table 5 and Figure 3).

Table (5) Performance of baseline LRG in detection of CD severity:

	Cutoff	AUC	Sensitivity	Specificity	PPV	NPV	Accuracy	P
LRG (µg/ml)	**≤61.4**	**0.855**	**80%**	**81.8%**	**66.7%**	**90%**	**81.3%**	**0.027***

AUC area under curve **p≤0.001 is statistically highly significant *p<0.05 is statistically significant PPV positive predictive value NPV Negative predictive value

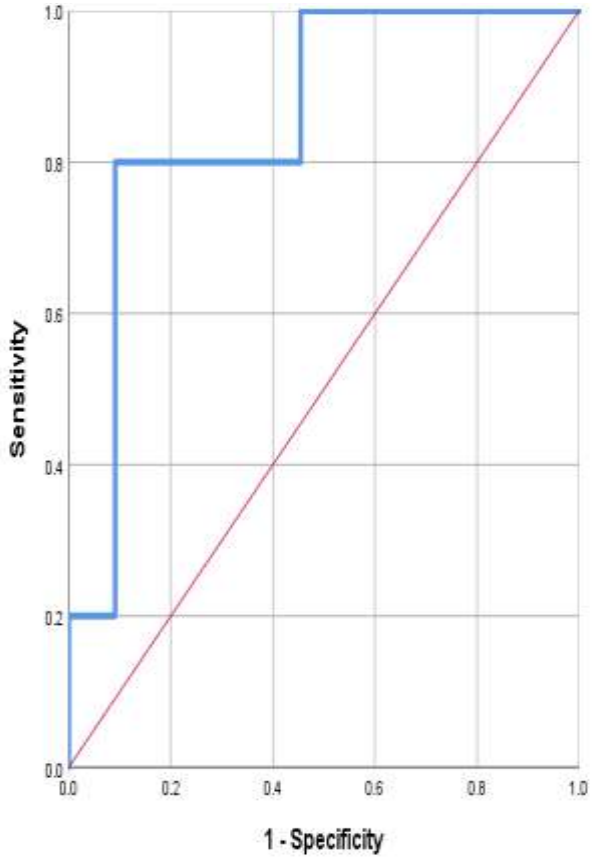

Figure (3) ROC curve showing performance of baseline LRG in detection of CD severity.

There is statistically significant positive correlation between baseline LRG and Harvey-Bradshaw score, CRP and serum creatinine. There is non-significant correlation between baseline LRG and either age or other laboratory data (Table 6).

Table (6) Correlation between baseline LRG and the studied parameters among Crohn's disease group:

	R	P
Age (year)	0.161	0.551
WBCs	0.424	0.102
Neutrophil	0.465	0.069
Lymphocyte	-0.065	0.81
Hemoglobin	-0.156	0.565
Platelet	0.076	0.779
CRP	**0.562**	**0.023***
ESR	-0.115	0.67
BUN	0.067	0.804
Creatinine	**0.511**	**0.043***
Total bilirubin	0.153	0.572
ALT	-0.095	0.726
AST	-0.025	0.75
Calprotectin	-0.086	0.75
Harvey-Bradshaw score	0.679	0.004
LRG after 6 months	0.456	0.076

r Pearson correlation coefficient *$p<0.05$ is statistically significant

There is statistically non-significant difference between the three groups according to activity of CD and baseline LRG, LRG after 6 months of treatment or percent change in LRG. Within each group with paired t test, there is significant change in LRG in new cases, cases in remission and cases in relapse (Table 7).

Table (7) Level of LRG before and after 6 months of treatment according to activity of Crohn's disease:

	Newly diagnosed (n=4)	Remission (n=6)	Relapse (n=6)	F	p
	Mean ± SD	Mean ± SD	Mean ± SD		
LRG (µg/ml)	66.1 ± 11.3	58.1 ± 9.7	69.1 ± 7.9	1.903	0.165
LRG after 6 months (µg/ml)	28.33 ± 10.7	35.6 ± 14.5	46.3 ± 19.1	0.771	0.471
p¥	0.015*	0.007*	0.015*		
	Median (IQR)	**Median (IQR)**	**Median (IQR)**	**KW**	**p**
% Change	54.5(41.1–73.3)	43.4(14.8–58.97)	36.8(13.4 – 55.4)	2.647	0.266

IQR interquartile range F One way ANOVA test KW Kruskal Wallis test ¥Paired sample t test *p<0.05 is statistically significant

There is statistically non-significant difference between severity of CD and baseline LRG, LRG after 6 months of treatment or percent change in LRG. There is significant decrease in LRG with paired t test in remission and mild cases while there is non-significant decrease in moderate CD (Table 8).

Table (8) Level of LRG before and after 6 months of treatment according to severity of Crohn's disease:

	Remission (n=5)	Mild (n=6)	Moderate (n=5)	F	p
	Mean ± SD	Mean ± SD	Mean ± SD		
LRG (µg/ml)	55.3 ± 7.8	65.6 ± 7.7	71.9 ± 9.3	2.1	0.2
LRG after 6 months (µg/ml)	30.2 ± 6.8	33.9 ± 16.2	50.1 ± 18.7	1.7	0.2
p¥	0.01*	0.004*	0.052		
	Median(IQR)	**Median(IQR)**	**Median(IQR)**	**KW**	**p**
% Change	47.7(27.3 –59.0)	52.8(31.7–62.0)	36.7(8.7 – 47.6)	2.3	0.3

IQR interquartile range F One way ANOVA test KW Kruskal Wallis test ¥Paired sample t test3

Discussion

Inflammatory bowel disease (IBD) is a term used to describe two long-lasting disorders that affect the digestive tract: ulcerative colitis (UC) and Crohn's disease (CD). These conditions involve ongoing inflammation mostly in the stomach, often accompanied by additional symptoms outside of the intestines. Although some patients may have temporary remission, the histological activity and digestive symptoms may persist, leading to ongoing systemic inflammation that could potentially cause additional issues outside of the intestines **(14)**

The implementation of the treat-to-target approach necessitates regular monitoring of the level of disease activity. The primary therapy goals for people with IBD are clinical

remission, endoscopic healing, improved quality of life, and absence of disability. These objectives are considered crucial for long-term management of the condition. Colonoscopy is the most reliable method currently used to evaluate the repair of the mucosal lining in individuals with inflammatory bowel disease (IBD). Nevertheless, the regularity of endoscopy poses challenges due to its time-consuming nature, invasive procedure, and labor-intensive requirements (15).

Serum C-reactive protein (CRP) is commonly employed as a serum biomarker to forecast the clinical activity of inflammatory illnesses, such as IBD. Nevertheless, increased CRP levels are not consistently observed in active IBD patients. Presently, fecal calprotectin has gained widespread usage as a precise biomarker for the restoration of the mucosal lining in cases of ulcerative colitis (UC). Nevertheless, the association between fecal indicators and clinical symptoms is moderate to low in ulcerative colitis (UC), and the effectiveness of fecal indicators in Crohn's disease (CD) is uncertain due to inconsistent accuracy **(7).**

The use of LRG as a non-invasive monitoring biomarker in patients with CD has been examined. A noteworthy association has been observed between LRG and endoscopic disease activity in individuals with Crohn's disease, which is pertinent to CRP and fecal calprotectin (16). In this study, LRG and fecal calprotectin were found to be the most effective biomarkers for evaluating the repair of the mucosa in small bowel Crohn's disease. Moreover, the simultaneous presence of LRG and fecal calprotectin, as well as LRG and fecal haemoglobin, might strengthen the association with endoscopic disease severity and forecast the outcome of CD, including parameters like hospitalization, surgery, and relapse (21).

Our study included a total of 16 participants diagnosed with Crohn's disease. We discovered that there is a higher occurrence of UC and CD in women, with no statistically significant difference seen between the groups in terms of gender. While the incidence rates for male and female patients with inflammatory bowel disease (IBD) are well-known (about 1:1.5 in Crohn's disease), there is less data available regarding the particular differences between sexes in terms of disease features in IBD. ***(22).***

Sex-based disparities are evident in the pathophysiology, epidemiology, clinical course, and consequences of IBD. There is a range of evidence indicating that the intricate interplay of well-defined determinants of disease development, such as genetic susceptibility, immunological dysfunction, environmental influences, and imbalances in gut bacteria, could be influenced by characteristics that differ between males and females ***(23).***

There is a statistically significant correlation between the activity of CD (Crohn's disease) and the Harvey-Bradshaw score. There is a substantial difference in pairwise comparison between remission and both newly diagnosed and relapse. There is no statistically significant relationship between the activity of CD and baseline or laboratory data.

Our study found that a CRP cutoff of ≥19.5 mg/l is the most effective in diagnosing moderate cases of CD compared to remission and mild cases. This cutoff has a sensitivity of 63.6%, specificity of 80%, positive predictive value of 87.5%, negative predictive value of 50%, and an overall accuracy of 68.75%.

These results were compatible with ***Yoshimura et al. (7)*** The individual who provided the data stated that in order to determine clinical remission, the threshold value for CRP (8 mg/L) had a sensitivity of 71.1% and a specificity of 73.6%. For individuals with CD, a CRP cutoff

value of 4.5 mg/L demonstrated a sensitivity of 81.8% and a specificity of 78.1%. ***Yoshida et al. (17)*** It was indicated that in patients with CD, the threshold level for CRP was 2.1 mg/L. CRP values showed a high area under the curve (AUC) in patients with UC. The CRP cut-off levels were set at 1.8 mg/L.

The optimal threshold for fecal calprotectin in diagnosing moderate cases of CD, as compared to remission and mild cases, was found to be ≥247.5 µg/g. This threshold yielded an area under the curve of 0.818, with a sensitivity of 72.7%, specificity of 60%, positive predictive value of 80%, negative predictive value of 50%, and an overall accuracy of 68.75% (p=0.047).

In agreement with **Penna et al (24)** After investigating CD, they determined that an FC cut-off value of 155µg/g had a high sensitivity of 96% and accuracy of 78% in diagnosing endoscopic activity. The study showed that a CRP reading of 6.7mg/L had a sensitivity of 75% and a specificity of 67%.

Our results matched with ***Romero et al. (18)*** The individual who stated that a Fecal calprotectin cut-off of 50 µg/g exhibited a high sensitivity (83%) but a low specificity (53%) in identifying small intestinal lesions in CD. However, a greater specificity was detected when the fecal calprotectin level was over 200 µg/g. The study indicated that patients with fecal calprotectin levels below 50 µg/g have a minimal likelihood of having lesions in the small bowel.

The study was revised by incorporating 14 studies (including 6 retrospectives) that demonstrated consistent findings. The use of an FC cut-off of 100 µg/g yielded a sensitivity and specificity of 73%. ***(19).***

Performing further evaluations of C-reactive protein (CRP) and fecal Calprotectin, in addition to considering symptoms, might help inform decisions about modifying treatment and ultimately increase the likelihood of achieving endoscopic remission. Therefore, CRP and fecal Calprotectin have been evaluated as reliable indicators of disease activity for IBD in the clinical management of the condition. Nevertheless, it is possible for patients with active mucosal inflammation to have normal serum CRP levels. While there is a correlation between fecal Calprotectin and mucosal inflammatory activity in ulcerative colitis (UC), there is limited research on whether it is also connected with disease activity during inflammation of the small intestine in Crohn's disease (CD). Hence, there is a need for blood biomarkers that can more precisely indicate the endoscopic activity of inflammatory bowel disease (IBD). ***(20).***

The baseline levels of LRG in CD were 55.3 ± 7.8, 65.6 ± 7.7, and 71.9 ± 9.3 µg/ml in instances of remission, mild severity, and moderate severity, respectively. Following a 6-month treatment period, the levels of LRG were lowered to 30.2 ± 6.8, 33.9 ± 16.2, and 50.1 ± 18.7 in cases with remission, mild, and moderate severity, respectively. LRG experiences a substantial decrease in remission and mild instances, whereas moderate CD shows a negligible decrease.

The current study found that a baseline LRG level of ≤61.4 µg/ml is the most effective cutoff for diagnosing CD in remission. The area under the curve was 0.855, indicating a high level of accuracy. The sensitivity of the test was 80%, meaning it correctly identified 80% of individuals with CD in remission. The specificity was 81.8%, indicating that it correctly ruled

out CD in remission in 81.8% of cases. The positive predictive value was 66.7%, meaning that 66.7% of individuals who tested positive actually had CD in remission. The negative predictive value was 90%, indicating that 90% of individuals who tested negative were truly free of CD in remission. Overall, the accuracy of the test was 81.3%.

This was in accordance with ***Yoshimura et al. (7)*** The investigation of individuals with CD revealed that the cutoff value for LRG (61.3 µg/mL) was reported to have a sensitivity of 77.3% and a specificity of 60.8%. ***Yoshida et al. (17)*** Reported that the area under the curve (AUC) for LRG in patients with CD was 0.894. The area under the curve (AUC) of LRG was substantially greater than that of CRP. The threshold concentration of LRG was 12.6 µg/ml.

The results of our study indicate that there was no statistically significant relationship between the severity of CD and the baseline LRG, LRG after 6 months of treatment, or the percent change in LRG. LRG experienced a notable decline in remission and mild illness, but moderate CD showed a considerable drop.

Yoshida et al. (17) Conducted ROC curve research to examine the discriminatory capacity of LRG and CRP in detecting mucosal healing. The area under the curve (AUC) of LRG was superior to that of CRP (0.894 vs. 0.689) in patients with CD. LRG serves as a valuable biomarker that correlates with the level of disease activity observed during endoscopic examination in individuals with inflammatory bowel disease (IBD). The cutoff limits for LRG and CRP in CD were 12.6 µg/ml and 21 µg/ml, respectively. The amount of LRG in patients with CD was below the suggested threshold of 16 µg/ml. This result suggests that a lower LRG cut-off level is more useful for the detection of mucosal healing in patients with CD.

Yasutomi et al. (16) examined the performance of LRG in CD patients, and the correlation with the endoscopic activity and predictability of MH of LRG were quite similar to those of CRP and comparable to those of Fecal calprotectin. The AUCs of both serum markers were higher in CD than in UC. Tus, LRG as well as CRP may be useful for evaluating the disease activity and MH in CD patients, even without fecal tests. In addition, the performance of LRG appears to be superior to that of CRP in CD with colonic involvement. Therefore, LRG might be particularly useful in CD patients with colonic involvement. ***Shimoyama et al. (15)*** demonstrated that LRG showed a significant relationship with clinical and endoscopic severity in CD.

There was a statistically significant positive correlation between baseline LRG and Harvey Bradshaw score, CRP and serum creatinine among CD group. ***Yoshida et al. (17)*** stated that in patients with CD, correlations were observed between serum LRG or CRP levels, and Harvey Bradshaw Index.

While studying multiple parameters in CD ***Kawamoto et al. (21)*** mentioned that LRG showed a sensitivity of 78% and specificity of 80% at a cutoff value of 13 µg/ml, whereas fecal calprotectin showed a sensitivity of 91% and specificity of 67% at a cutoff value of 151 µg/g. Dual positivity for LRG and fecal calprotectin, as well as LRG and fecal hemoglobin, both predicted ulcers with an improved specificity of 92% and 100%. A positive LRG or fecal calprotectin/hemoglobin showed an improved sensitivity of 96% and 91%. Positivity for LRG and either of the fecal biomarkers was associated with increased risk of hospitalization, surgery, and relapse.

In agreement with our findings, ***Yoshida et al. (17)*** stated that the examined application of serum LRG and CRP levels as validated, independent serum biomarkers for disease activity in IBD. In patients with CD, the correlation between disease activity and both serum LRG and CRP levels was statistically significant; however, LRG tended to be better than CRP. CRP has been reported to be a useful biomarker that correlates well with mucosal inflammation and recurrence in CD; however, the correlation between serum CRP levels and mucosal inflammation in the small intestine has been reported to be weak.

Yoshimura et al. (7) reported that there were statistically insignificant differences were found in LRG levels between patients with UC and those with CD. Patients with active CD had higher levels of LRG than did patients with inactive CD and healthy controls.

Conclusion

Leucine-rich glycoprotein could be a reliable serum biomarker for the assessment of clinical disease activity in patients with CD. It can be an alternative to CRP and fecal calprotectin for the assessment of Crohn's disease.

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