Fecal Calprotectin as a Marker of Severity of COVID-19 Disease: A Hospital-based Study

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Abstract:

Background: Although fecal calprotectin has prognostic value in assessing inflammatory bowel disease, the relationship of fecal calprotectin level with the severity of COVID-19 disease has not been determined yet. This study aimed to determine if fecal calprotectin may be used as a marker for the severity of COVID-19 disease.

Patients and Methods: A total of 50 patients with COVID-19 disease who presented with gastrointestinal manifestations, with or without respiratory manifestations and other symptoms, were categorized into four groups: 14 patients with mild symptoms, 12 patients with moderate manifestations, 12 patients with severe manifestations, and 12 critically ill patients in the intensive care unit (ICU). Fecal calprotectin, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), D-dimer, ferritin, and complete blood count (CBC) were measured at baseline and after 21 days, along with clinical follow-up.

Results: A substantial decrease in fecal calprotectin levels from baseline to 21 days across all cases and subgroups. Notably, critical patients exhibited significantly higher fecal calprotectin levels compared to severe, moderate, and mild cases at baseline and after 21 days (p-value <0.001). Additionally, critical patients had significantly elevated ESR, CRP, ferritin, and D-dimer levels compared to other groups. The duration of hospitalization was significantly longer for vital and severe patients compared to moderate and mild cases. Furthermore, the mortality rate within 21 days was significantly higher in critical and severe cases compared to other groups.

Conclusion: Our findings indicate a substantial correlation between fecal calprotectin levels and the severity of COVID-19. Notably, fecal calprotectin levels were considerably higher in the critical group compared to other groups, both at baseline and after 21 days. So, fecal calprotectin may be used as a marker for the severity of COVID-19 disease.

Keywords: *COVID-19 Disease, Gastrointestinal manifestations, Fecal calprotectin, Inflammatory markers.*

Abbreviations:

COVID-19**:** Coronavirus disease 2019

CRP: C-Reactive Protein

ESR: Erythrocyte sedimentation rate

GI: Gastrointestinal

IL-6: Interleukin-6

SARS-CoV-2: severe acute respiratory syndrome coronavirus 2

IBD: Inflammatory Bowel Disease

FC: Fecal calprotectin.

1. Introduction

The COVID-19 pandemic, caused by the SARS-CoV-2 virus, poses a significant global challenge. COVID-19 patients manifested mainly with respiratory symptoms, frequent gastrointestinal symptoms, and others. According to World Health Organization (WHO) information, the incidence and prevalence of this virus infection have been rising worldwide over the last few years as a pandemic **[1]**.

Fecal calprotectin has been suggested as a noninvasive indicator of intestinal inflammation in IBD. However, its widespread use is limited because elevated levels are associated with colorectal neoplasia and gastrointestinal infections. Additionally, relatives of IBD patients may exhibit increased fecal calprotectin levels with uncertain degrees of inflammation **[2]**.

A clinical study in Austria has provided evidence suggesting that SARS-CoV-2 can induce intestinal inflammation. Notably, patients who experienced current or resolved diarrhea exhibited higher fecal calprotectin concentrations than those without diarrhea. Furthermore, a significant correlation was observed between fecal calprotectin levels and serum interleukin-6 **[3]**.

Fecal calprotectin has diagnostic and prognostic values in gastrointestinal diseases, mainly inflammatory bowel diseases, which may play a role in assessing the progression of COVID-19.

A high fecal calprotectin level is commonly associated with GI manifestations of COVID-19 and has even been correlated with disease severity **[4]**.

The presence of live virus in the stool of COVID-19 patients, as detected by electron microscopy, raises the potential for fecal-oral transmission. However, this transmission mode remains unconfirmed. A study utilizing epidemiological and environmental data suggests that fecal aerosols generated during flushing may have caused a cluster of cases in a high-rise building in China, indicating the possibility of fecal-aerosol transmission of SARS-CoV-2 **[6]**.

In the context of COVID-19 infections, respiratory, constitutional, and hematological manifestations are the most frequently observed. Gastrointestinal symptoms such as diarrhea, nausea, and vomiting are commonly reported. Notably, diarrhea is the second most prevalent symptom, with a pooled prevalence of 12.5%. A comprehensive analysis of clinical studies examining diarrhea in COVID-19 patients revealed a prevalence of 10.4%, ranging from 2% to 50% **[7]**. Nevertheless, recent data from the United States revealed elevated percentages ranging from 23.7% to 33.7% **[8]**.

While some research has indicated the presence of mild diarrhea **[9,10]**, other studies have documented instances of severe diarrhea and acute hemorrhagic colitis in association with severe COVID-19 illness **[11,12]**.

The study aims to evaluate the relationship between fecal calprotectin and the clinical and biochemical parameters of patients with COVID-19 disease after 21 days from the baseline to determine if fecal calprotectin may be used as a marker of the severity of COVID-19 disease, especially in patients with GI symptoms with or without other manifestations. The outcome was the proportion of patients with SARS-CoV-2 RNA PCR negativity and the percentage of COVID-19 mortality within 21 days from the start of this study.

2. Patients and Methods

2.1. Study design:

A hospital-based observational cross-sectional study was carried out on fifty patients with COVID-19 who were admitted to the isolation unit of the Internal Medicine Department, Zagazig University Hospitals, from September 2021 to March 2022. Samples for laboratory tests were collected by medical staff using personal protective equipment and performed at the Clinical Pathology Department, Zagazig University Hospitals. All included fifty COVID-19 patients with GI manifestations with or without respiratory manifestations and others were classified according to disease severity classification system for COVID-19 disease based on the patient's pulse, systolic blood pressure, respiratory rate, oxygen saturation, and oxygen flow rate into four arms; the first arm included (Mild group), the second arm (Moderate group), the third arm (Severe group), the fourth arm (Critically ill group) **[13]**. Patients were treated according to the management protocol for COVID-19 patients version 1.4/November 2020 by the Egyptian Ministry of Health and Population during the study period. The Zagazig University institutional review board approved the study (ZU-IRB#9084-8-8-2021). All individual participants involved in the study provided written informed consent.

2.2. Patient Selection and Data Collection:

To qualify for this study, the patient had to meet the following criteria:

- Admitted to the isolation unit of internal medicine
- Over 18 years of age
- Symptomatic COVID-19 disease with gastrointestinal symptoms (with or without respiratory or other symptoms)
- Confirmed positive for SARS-CoV-2 RNA via real-time reverse-transcriptase polymerase chain reaction of nasopharyngeal swab

We excluded patients with:

- End-organ failure (e.g., decompensated liver disease, heart failure, renal failure)
- Inflammatory bowel disease (IBD)

- Gastrointestinal malignancies
- Pregnancy

2.3. Clinical and laboratory assessments

The following baseline data was collected for each eligible patient in this study: age, gender, body mass index (BMI), residency, smoking status, body temperature, heart rate, respiratory rate, blood pressure, and oxygen saturation. Baseline laboratory tests included fasting blood glucose, HbA1C, liver function, renal function, and coagulation tests. Additionally, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), D-dimer, serum ferritin, and fecal calprotectin were measured for all four groups involved in the study.

The fecal calprotectin cut-off was classified into: if the cut-off is equal to 50 mcg/g, specificity 0.60 (0.52-0.67) and sensitivity 0.92 (0.90-0.94), if the cut-off is equal to 100 mcg/g, specificity 0.66 (0.59-0.73) and sensitivity 0.84 (0.80-0.88), and if the cut-off is equal to 250 mcg/g, specificity 0.82 (0.77-0.86) and sensitivity 0.80 (0.76-0.84) **[14]**.

2.3.1. Samples:

Blood and stool samples were obtained in BD Vacutainer *(Becton, Dickinson and Company, Franklin Lakes, NJ)*. Six tubes were collected at baseline, including one citrate, one ESR, two plain, and two EDTA tubes from each patient. One EDTA tube was used to take the complete blood picture. The second EDTA tube was utilized to assess HbA1C. The plain vacutainer was allowed to clot for 30 minutes after collection. After this period, the sample was centrifuged at 1200 x g for 10 minutes to separate serum. The citrate sample was centrifuged at 2000 x g for 15 minutes to assess coagulation tests. After 7 and 21 days, tubes were collected from each patient, including citrate, ESR, and plain.

2.3.2 Methods:

The complete blood count was conducted using the XS500i Hematology analyzer (Sysmex, Kobe, Japan). Differential cell counts were obtained through blood film analysis. The ESR was measured using the Westergren method. Coagulation tests were performed with the Sysmex

CS2100i (Siemens, Munich, Germany). All biochemical tests were quantified using the Cobas 8000 Modular Analyzer (Roche Diagnostics, Mannheim, Germany), except for HbA1C and D-dimer, which were analyzed on the Cobas 6000 Modular Analyzer (Roche Diagnostics, Mannheim, Germany).

Fecal calprotectin (FC) concentration was determined via the Calprest ELISA (Eurospital, Trieste, Italy), adhering to the manufacturer's specifications.

2.4. Statistical analysis

Quantitative parameters were presented as the mean and standard deviation (SD), while categorical parameters were presented as absolutes and percentages. The student t-test and paired t-test were employed for unrelated and related quantitative variables, respectively. The chi-squared test was utilized to compare categorical variables. All statistical analyses were conducted using the statistical software program SPSS for Windows version 25.0 (SPSS; Chicago, IL, USA). A p-value less than 0.05 was considered statistically significant.

3. Results

All included fifty patients with COVID-19 disease who manifested with GI symptoms with or without respiratory symptoms, and others were classified into four arms: the first of whom 14 patients with mild symptoms, 12 patients with moderate manifestations, 12 patients with severe manifestations, lastly 12 critically ill patients in ICU.

Age, gender, residence, smoking, diabetes mellitus, diabetes and IHD, diabetes and HTN, gout GIT symptoms, respiratory symptoms, other diseases, BMI, fasting Glucose, HbA1c, white blood cells, hemoglobin, platelet count, total bilirubin, direct bilirubin, AST, ALT, albumin, ALP, INR, and BUN were insignificantly different among the four groups. Total protein was insignificantly different between mild and (moderate and severe), between moderate and (severe and critical) and between severe and critical. Total protein was significantly lower in the crucial group than in the mild group (P value =0.003). Creatinine was considerably lower in critical than (mild, moderate, and severe) (P value =0.040)., as presented in **Table 1.**

Table 1. Demographic and baseline characteristics of the four study groups (N=50).

Parameters	Mild Group (N=14)	Moderate Group (N=12)	Severe Group (N=12)	Critical Group (N=12)	Test	P-value
Age (year)	51.3 ± 4.56	49.8 ± 3.24	49.6 ± 3.34	51.7 ± 3.55	F 0.94	0.430
Gender (Male/Female)	9/5	8/4	7/5	8/4	X^2 0.241	0.971
Residence (Rural/Urban)	10/4	9/3	8/4	7/5	X^2 0.871	0.832
Smoking: (Non/Smoker)	9/5	7/5	8/4	8/4	X^2 0.241	0.970
Comorbidity:						
No	7 (50%)	6 (42.86%)	4 (28.57%)	3 (21.43%)		
Diabetes Mellitus	3 (21.43%)	3 (21.43%)	4 (28.57%)	4 (28.57%)		
Diabetes and IHD	1 (7.14%)	0 (0%)	2 (14.29%)	1 (7.14%)	X^2 9.158	0.689
Diabetes and HTN	2 (14.29%)	3 (21.43%)	0 (0%)	3 (21.43%)		
Gout	1 (7.14%)	0 (0%)	0 (0%)	1 (7.14%)		
Clinical manifestations:						
GIT symptoms	14 (100%)	12 (85.71%)	12 (85.71%)	11 (78.57%)		
Respiratory symptoms	7 (50%)	6 (42.86%)	7 (50%)	9 (64.29%)	X^2 2.693	0.615
others	0 (0%)	1 (7.14%)	2 (14.29%)	2 (14.29%)		
BMI (kg/m^2)	25.3 ± 2.05	24.7 ± 2.1	25 ± 1.35	24.3 ± 2.48	F 0.57	0.637
Fasting Glucose (mg/dL)	143.6 ± 55.34	121.8 ± 33.3	143 ± 41.74	140.7 ± 37.34	F 0.71	0.553
HbA1c (%)	8.2 ± 0.99	8.1 ± 1.07	7.8 ± 0.56	7.4 ± 0.59	F 1.62	0.212
White Blood Cells(10^9/L)	9.5 ± 1.57	8.8 ± 2.12	9.7 ± 1.92	8.5 ± 1.92	F 1.15	0.338
Hemoglobin (g/dL)	12.5 ± 0.83	12.3 ± 0.76	12.1 ± 0.74	12.4 ± 0.8	F 0.59	0.622
Platelet count (10^9/L)	311.1 ± 29.13	307.8 ± 30.63	276.8 ± 58.63	275.5 ± 52.36	F 2.41	0.079

Total bilirubin (mg/dL)	**0.8 ± 0.2**	**0.8 ± 0.19**	**0.8 ± 0.21**	**0.7 ± 0.13**	**F 0.29**	**0.832**
Direct bilirubin: (mg/dL)	**0.2 ± 0.05**	**0.2 ± 0.06**	**0.2 ± 0.16**	**0.2 ± 0.03**	**F 1.11**	**0.356**
AST (U/L)	**42 (36.75 – 45.5)**	**38.5 (36.25 – 45)**	**38 (37 – 48.25)**	**44.5 (38 - 48.75)**	**H 2.21**	**0.530**
ALT (U/L)	**35 (29.75 – 38.5)**	**27 (24.75 – 37.5)**	**26 (25 – 38.25)**	**35.5 (27.5 - 42)**	**H 2.69**	**0.442**
Albumin (g/dL)	**4 ± 0.29**	**4.1 ± 0.37**	**4.1 ± 0.35**	**4.2 ± 0.36**	**F 0.78**	**0.512**
Total protein (g/dL)	**7.7 ± 0.3**	**7.5 ± 0.37**	**7.4 ± 0.35**	**7.2 ± 0.45**		
P1		**0.213**	**0.096**	**0.003***	**F 4.89**	**0.005***
P2			**0.980**	**0.324**		
P3				**0.546**		
ALP (U/L)	**108.5 ± 22.38**	**100.3 ± 14.97**	**112.8 ± 28.07**	**100.2 ± 15.03**	**F 1.1**	**0.359**
INR	**1 ± 0.11**	**1 ± 0.07**	**1 ± 0.11**	**1 ± 0.08**	**F 0.72**	**0.544**
Creatinine (mg/dL)	**0.86 ± 0.09**	**0.91 ± 0.13**	**0.79 ± 0.12**	**0.79 ± 0.13**		
P1		**0.645**	**0.452**	**0.506**	**F 3**	**0.04***
P2			**0.063**	**0.076**		
P3				**1.000**		
BUN (mg/dL)	**14.3 ± 1.85**	**14.8 ± 2.12**	**14.3 ± 1.81**	**14.4 ± 2.19**	**F 0.15**	**0.930**

***Data are expressed as mean ± SD, median (IQR), or number (%). F: One way ANOVA, X^2: Chi-square* H:Kruskal-Wallis test**
IHD: ischemic heart disease, GI: Gastrointestinal, Others*: Hepatitis, Neuritis, Myositis, BMI: Body Mass Index, HbA1C: Hemoglobin A1C, INR: International Normalized Ratio, BUN: Blood Urea Nitrogen, ALT: Alanine Transferase, AST: Aspartate Transferase, ALP: Alkaline Phosphatase. P1: P value between mild group and (moderate group, severe group and critical group), P2: P value between moderate group and (severe group and critical group), P3: P value between severe group and critical group.

Fecal calprotectin (mcg/g) at baseline was significantly higher in (the moderate group, severe group, and critical group) than mild group (P value <0.001), considerably higher in (the severe and critical group) than moderate (P value <0.001) and significantly higher in critical group than severe group (P value <0.001). Fecal calprotectin (mcg/g) after 21 days was significantly higher in (the moderate group, severe group, and critical group) than mild group (P value <0.001),

considerably higher in (severe and critical group) than moderate (P value <0.001) and significantly higher in critical group than severe group (P value <0.001). Fecal calprotectin status was significantly higher at baseline than after 21 days among the four groups (P value <0.001, as presented in **Tab 2 and Fig 1.**

Table 2. Comparison of the fecal calprotectin status and COVID-19 severity in four groups of this study.

COVID-19 severity		All cases	Mild Group (N=14)	Moderate Group (N=12)	Severe Group (N=12)	Critical Group (N=12)	Test	P value
Fecal Calprotectin (mcg/g) (At baseline)	No (%)	50 (100)	14 (28)	12 (24)	12 (24)	12 (24)	F 10463.4	<0.001*
	Fecal Calprotectin	145.15 ± 64.11	69.96 ± 2.06	114.42 ± 1.56	171.17 ± 2.98	237.58 ± 3.23		
	P1			<0.001*	<0.001*	<0.001*		
	P2				<0.001*	<0.001*		
	P3					<0.001*		
Fecal Calprotectin (mcg/g) (After 21 days)	No (%)	34 (100)	14 (41.17)	10 (29.42)	7 (20.58)	3 (8.82)	F 3638.9	<0.001*
	Fecal Calprotectin	60.16 ± 33.04	34.25 ± 1.31	56.1 ± 1.29	79.71 ± 1.8	149 ± 4.58		
	P1			<0.001*	<0.001*	<0.001*		
	P2				<0.001*	<0.001*		
	P3					<0.001*		
Test		t 7.11	t 54.69	t 94.19	t 73.27	t 39.5		
P		<0.001*	<0.001*	<0.001*	<0.001*	<0.001*		

Data are expressed as mean ± SD or number (%). F: One-way ANOVA, X^2: Chi-square.
A P-value of comparison between the four groups regarding the mean Fecal Calprotectin Level. (%), P1: P value between mild group and (moderate group, severe group and critical group), P2: P value between moderate group and (severe group and critical group), P3: P value between severe group and critical group.

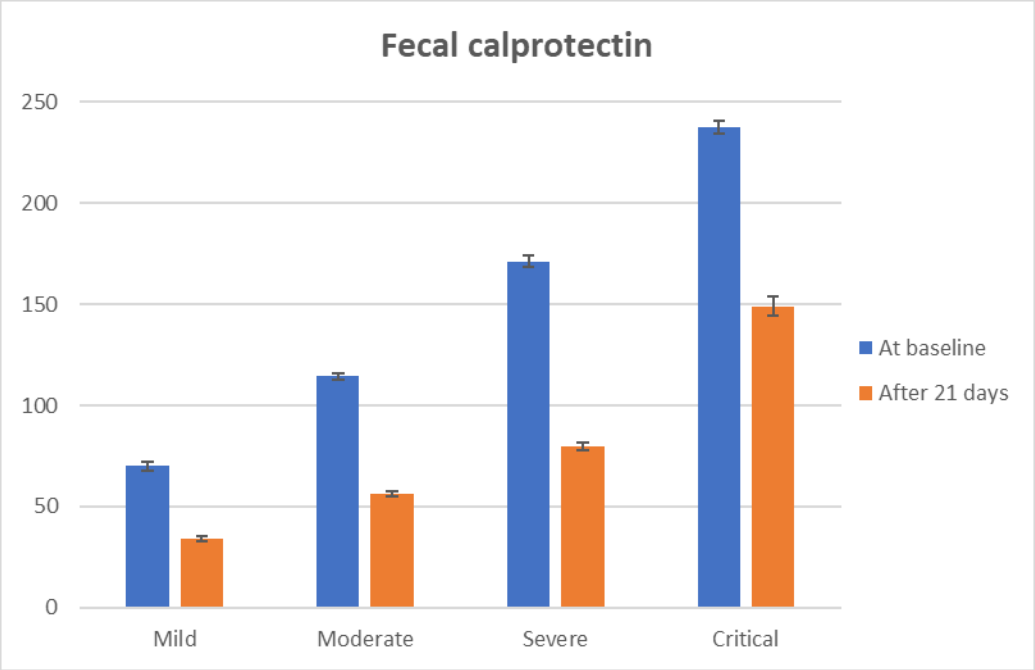

Figure 1. Comparison of the fecal calprotectin status in four studied groups.

The O2 Saturation and SBP were significantly lower in (the moderate group, severe group, and critical group) than in the mild group (P value <0.001), significantly lower in (the severe and critical group) than moderate (P value <0.001) and significantly lower in critical group than severe group (P value <0.001). Respiratory rate, ESR, CRP, ferritin, D-Dimer, and calprotectin were significantly higher in (the moderate group, severe group, and critical group) than in the mild group (P value <0.001), significantly higher in (severe and critical group) than moderate (P value <0.001) and significantly higher in critical group than severe group (P value <0.001). The temperature was insignificantly different between the moderate and severe groups and between the severe and critical groups. The temperature was significantly higher in (the moderate group, severe group, and critical group) than in the mild group (P value was significantly higher in the

critical group than the moderate group (P value =0.001). Heart Rate was insignificantly different between the mild group (the moderate group and the severe group) and between the moderate group (the severe group and the critical group). Heart Rate was significantly higher in the critical group than in (the mild group and severe group) (P value =0.001 and 0.036, respectively). SBP was insignificantly different between the mild group and the moderate group. SBP was significantly lower in (the severe group and critical group) than in the mild group (P value <0.001), was significantly lower in (the severe group and critical group) than in the moderate group (P value <0.001), and was significantly lower in critical group than severe group (P value =0.005), were presented in **Table 3.**

Table 3. Comparison of the clinical and biochemical parameters between the four study groups at baseline (Start of study).

Parameters	Mild Group (N=14)	Moderate Group (N=12)	Severe Group (N=12)	Critical Group (N=12)	Test	P
O_2 Saturation (%)	94.9 ± 1.61	87.1 ± 2.57	76.8 ± 3.02	64.5 ± 3.94		
P1		**<0.001***	**<0.001***	**<0.001***	F=268.2	**<0.001***
P2			**<0.001***	**<0.001***		
P3				**<0.001***		
Temperature (ºC)	37.2 ± 0.3	38.2 ± 0.23	38.4 ± 0.23	38.6 ± 0.21		
P1		**<0.001***	**<0.001***	**<0.001***	F=84.14	**<0.001***
P2			0.242	**0.001***		
P3				0.153		
Respiratory Rate (breaths/ min)	14.3 ± 1.77	18.8 ± 2.09	23.4 ± 3.53	26.5 ± 2.75		
P1		**<0.001***	**<0.001***	**<0.001***	F=55.15	**<0.001***
P2			**<0.001***	**<0.001***		

	P3			**0.027***		
Heart Rate (Pulse/min)	81.9 ± 5.33	89.7 ± 2.93	87.6 ± 24.95	102.8 ± 8.85		
P1		0.452	0.696	**0.001***	F=5.58	**0.002***
	P2		0.981	0.087		
	P3			**0.036***		
SBP (mm Hg)	120.7 ± 6.46	114.2 ± 5.97	101.3 ± 7.72	91.7 ± 6.15		
P1		0.070	**<0.001***	**<0.001***	F=49.31	**<0.001***
	P2		**<0.001***	**<0.001***		
	P3			**0.005***		
ESR (mm/hr)	29.9 ± 5.37	48 ± 3.67	84.6 ± 5.32	90.7 ± 3.89		
P1		**<0.001***	**<0.001***	**<0.001***	F=501.66	**<0.001***
	P2		**<0.001***	**<0.001***		
	P3			**0.013***		
CRP (mg/L)	12 ± 2.57	29.2 ± 4.47	69.4 ± 5.12	90.6 ± 4.6		
P1		**<0.001***	**<0.001***	**<0.001***	F=919.73	**<0.001***
	P2		**<0.001***	**<0.001***		
	P3			**<0.001***		
Ferritin (ng/mL)	123.4 ± 8.1	296.4 ± 12.86	366.6 ± 13.4	578.3 ± 21.88		
P1		**<0.001***	**<0.001***	**<0.001***	F=2114.53	**<0.001***
	P2		**<0.001***	**<0.001***		
	P3			**<0.001***		
D-Dimer (ng/mL)	173 ± 13.78	288.1 ± 12.2	388.8 ± 19.92	462.2 ± 17.3		
P1		**<0.001***	**<0.001***	**<0.001***	F=800.25	**<0.001***
	P2		**<0.001***	**<0.001***		

			P3	**<0.001***		
Calprotectin	70.7 ±2.81	114.23 ±4.31	170.36±4.86	237.27 ±5.34		
P1		**<0.001***	**<0.001***	**<0.001***	F=85.25	**<0.001***
	P2		**<0.001***	**<0.001***		
		P3		**<0.001***		

Data are expressed as mean ± SD.F: One-way ANOVA, X^2: Chi-square. ESR: Erythrocyte sedimentation rate, CRP: C-Reactive Protein, SBP: Systolic Blood Pressure, Fecal Calprotectin Level. p- value of comparison between the four groups at baseline. (%), P1: P value between mild group and (moderate group, severe group and critical group), P2: P value between moderate group and (severe group and critical group), P3: P value between severe group and critical group.

The COVID-19 mortality within 21 days from the start of the study was significantly higher in the critical group than in the mild group, moderate group, and severe group. Duration of hospital stay from the start of the study was significantly higher in (the moderate group, severe group, and critical group) than in the mild group (P value <0.001), considerably higher in (the severe and critical group) than in the moderate (P value <0.001) and significantly higher in critical group than severe group (P value <0.001). SARS-CoV-2 RNA PCR negativity before day 21 was insignificantly different among the four groups, as presented in Table 4.

Table 4. Comparison of outcome parameters between the four studied groups.

Parameters	Mild Group (N=14)	Moderate Group (N=12)	Severe Group (N=12)	Critical Group (N=12)	Test	P-value
COVID-19 mortality within 21 days from the start of study N (%)	0 (0%)	2 (14.29%)	5 (35.71%)	9 (64.29%)	X^2 18.597	**<0.001***
Duration of hospital stay from the start of study: (days)	0 ± 0	5.7 ± 1.5	13.8 ± 3.49	19.6 ± 2.75		
P1		**<0.001***	**<0.001***	**<0.001***	F 183.33	**<0.001***
	P2		**<0.001***	**<0.001***		
		P3		**<0.001***		
SARSCoV-2 RNA PCR negativity	12 (85.71%)	8 (57.14%)	7 (50%)	2 (14.29%)	X^2 7.180	0.066

before day-21 N (%)						

Data are expressed as mean ± SD or number (%). F: One-way ANOVA, X^2: Chi-square. P1: P value between mild group and (moderate group, severe group and critical group), P2: P value between moderate group and (severe group and critical group), P3: P value between severe group and critical group.

In univariate regression, total protein, creatinine, temperature, respiratory rate, heart rate, and SBP were independent predictors of the severity of COVID-19 (P value <0.05). In Multivariate regression, total protein, creatinine, temperature, respiratory rate, heart rate, and SBP weren't independent predictors of COVID-19, as presented in **Table 5**.

Table 5. Univariate and multivariate regression of (total protein, creatinine, O2 saturation, temperature, respiratory rate, heart rate, SBP, ESR, CRP, ferritin, D-dimer, and duration of hospital stay) versus severity of COVID-19.

	Univariate			Multivariate		
	Odds ratio	**95% CI**	**P**	**Odds ratio**	**95% CI**	**P**
Total protien	0.111	0.020 – 0.606	**0.012***	0.0052	0 – 20.972	0.214
Creatinine (mg/dL)	0.001	0- 0.239	**0.014***	0	2.327– 199259.29	0.311
O_2 saturation	0	---	0.998	---	---	---
Temperature	391.79	9.444 – 16253.1	**0.002***	0.857	0.003–244.09	0.957
Respiratory rate	2.145	1.3706 – 3.357	**<0.001***	1.187	0.452–3.122	0.728
Heart rate	1.087	1.012 – 1.168	**0.023***	0.919	0.472–1.787	0.802
SBP	0.692	0.555 – 0.862	**0.001***	0.703	0.477–1.036	0.075
ESR	4.972	---	0.997	---	---	---
CRP	5.977	---	0.998	---	---	---
Ferritin	5.134	---	0.998	---	---	---
D-dimer	2.475	---	0.998	---	---	---
Duration of hospital stay	4.533	0.7534 – 27.275	0.098	---	---	---

*Significant as P value≤0.05, CI: Confidence interval.

Fecal calprotectin can significantly predict the severity of COVID-19 (P <0.001 and AUC = 1) at cut-off >118 with 100% sensitivity, 100% specificity, 100% PPV and 100% NPV. CRP can significantly predict the severity of COVID-19 (P <0.001 and AUC = 1) at cut-off >38 with 100% sensitivity, 100% specificity, 100% PPV and 100% NPV. ESR can significantly predict the severity of COVID-19 (P <0.001 and AUC = 1) at cut-off >54 with 100% sensitivity, 100% specificity, 100% PPV and 100% NPV. Albumin can't insignificantly predict COVID-19 severity (P =0.244 and AUC = 0.595) at cut-off >4.1 with 45.83% sensitivity, 61.54% specificity, 52.4% PPV and 55.2% NPV. WBCs can't insignificantly predict COVID-19 severity (P =0.724 and AUC = 0.530) at cut-off ≤8.4 with 41.67% sensitivity, 65.38% specificity, 52.6% PPV and 54.8% NPV, as presented in **Table 6**.

Table 6: Role of (Fecal Calprotectin, CRP, ESR, albumin, and WBCs) in the prediction of severity of COVID-19 disease.

	Cut-off	Sensitivity	Specificity	PPV	NPV	AUC	P value
Fecal calprotectin (mcg/g) (At baseline)	>118	100%	100%	100%	100%	1	**<0.001***
CRP	>38	100%	100%	100%	100%	1	**<0.001***
ESR	>54	100%	100%	100%	100%	1	**<0.001***
Albumin	>4.1	45.83%	61.54%	52.4%	55.2%	0.595	0.244
WBCs	≤8.4	41.67%	65.38%	52.6%	54.8%	0.530	0.724

PPV: positive predictive value, NPV: negative predictive value, AUC: area under the curve.

4. Discussion

Although fecal calprotectin has prognostic value in assessing inflammatory bowel disease (IBD), the relationship of fecal calprotectin level with the severity of COVID-19 disease is not determined yet. Fecal calprotectin (FC) has emerged as a reliable fecal biomarker, enabling the detection of intestinal inflammation in IBD and infectious colitis **[15]**.

Previous research suggests that SARS-CoV-2 has an affinity for binding to cells within the gastrointestinal tract, specifically the epithelial cells of the small and large intestines. Specific receptors, such as ACE2 and the transmembrane serine protease 2, are believed to facilitate this interaction **[16,17]**.

A study conducted in Austria by Effenberger M. et al. revealed a significant correlation between Fecal Calprotectin (FC) concentration and serum interleukin-6 (IL-6) concentration ($p<0.001$) in patients with SARS-CoV-2 infection. However, no significant correlation was observed between FC concentration and C reactive protein (CRP) or ferritin. These findings suggest that SARS-CoV-2 infection triggers an inflammatory response in the gut, as evidenced by diarrhea, elevated FC levels, and a systemic IL-6 response **[3]**. Furthermore, our clinical and laboratory parameters analysis in patients with SARS-CoV-2 infection demonstrated that FC levels exhibited significant correlations with ESR, CRP, ferritin, and D-dimer. Critically ill patients showed significantly higher levels of these parameters than severe, moderate, and mild patients at baseline. Additionally, oxygen saturation (O2 Sat) and systolic blood pressure (SBP) were significantly lower in critically ill patients compared to severe, moderate, and mild patients at baseline.

Our research indicates that fecal calprotectin levels were considerably higher in critically ill patients than severely ill patients, who had higher levels than moderately sick patients at the study's start and after 21 days (p-value <0.001). This finding aligns with the observations of Effenberger M. et al., who provided evidence of intestinal inflammation caused by SARS-CoV-2, as individuals with current or resolved diarrhea exhibited higher concentrations of fecal calprotectin compared to those without diarrhea **[3]**.

A comprehensive meta-analysis revealed a significantly higher incidence of severe disease among patients exhibiting gastrointestinal (GI) symptoms than those without GI symptoms **[18]**. Notably, a recent extensive retrospective cohort study involving 29,393 COVID-19 patients demonstrated a substantial 50% increase in the risk of severe illness associated with GI symptoms. Moreover, patients presenting with both GI symptoms and fever exhibited a markedly elevated risk of severe COVID-19, with an 85% increase observed **[19]**.

In this study, we observed that higher levels of FC were associated with critical cases, resulting in increased mortality rates and extended hospital stays. Notably, this finding aligns with previous research by Nobel et al., indicating that COVID-19 patients with gastrointestinal symptoms tend to experience longer durations of illness **[20].**

Severe COVID-19 infection is associated with increased inflammatory markers like D-dimer, ESR, CRP, ferritin, fibrinogen, and pro-inflammatory cytokines such as IL-6 **[21].** A serial measuring of inflammatory markers might help evaluate and monitor disease severity. It is noted that specific serological markers like IL-6, CRP, ferritin, and ESR are raised to a greater extent in severe COVID-19 disease than in mild ones **[22].** These results were consistent with our data regarding the inflammatory markers. The higher values in the four groups at the study's baseline had significantly reduced after seven days and after 21 days.

Our findings on gastrointestinal (GI) symptoms, fecal calprotectin (FC) levels, and the severity of COVID-19 infection diverge from certain other studies, including those based on US data that did not establish a correlation between GI symptoms and adverse outcomes **[23,25]**. Notably, a US case-control study reported a significantly lower short-term mortality rate among patients with digestive symptoms **[24].**

While the underlying reasons for these discrepancies remain unclear, potential explanations include variations in reporting practices, patient populations, and distinct viral strains **[26,27]**. Additionally, patients with digestive symptoms experience a significantly prolonged viral clearance compared to those solely exhibiting respiratory symptoms **[28]**.

The strength of our study is that it includes four groups of different stages of COVID-19 severity to compare fecal calprotectin levels, inflammatory markers, and clinical follow-up at baseline after 21 days.

Our study has some limitations. The sample size is small, and we did not measure inflammatory cytokines (IL-6, TNF, IL-1b). Therefore, we could not assess the link between fecal calprotectin and cytokine levels. Further studies are recommended to confirm our findings.

5. Conclusion

Our findings indicate a substantial correlation between fecal calprotectin levels and the severity of COVID-19. Notably, fecal calprotectin levels were considerably higher in the critical group compared to other groups, both at baseline and after 21 days. Moreover, the percentage of COVID-19 mortality within 21 days was significantly higher in critical patients than in other groups who manifested GI symptoms with or without respiratory symptoms. Consequently, fecal calprotectin is a potential marker for assessing the severity of COVID-19 disease.

Footnotes.

Mohamed Emara (professor of gastroenterology, hepatology, and infectious diseases), Nabila Hassan (professor of gastroenterology, hepatology, and infectious diseases), and Amany Mohamed(assistant professor of community medicine) were the peer reviewers.

E- Editor: Salem Youssef Mohamed, Osama Ahmed Khalil, Amany Mohammed.

Ethical considerations

The Zagazig institutional review board approved the study (ZU-IRB#9084-8-8-2021). All individual participants in the study provided written informed consent.

Ethical approval: All procedures involving human participants followed the institutional and national research committee's moral standards, the 1964 Helsinki Declaration, and its later amendments or comparable ethical standards. All authors declare that consent was obtained from the patient (or other approved parties) to publish this study.

Data and materials availability: The datasets used or analyzed during the current study are available from the corresponding author upon reasonable request.

Competing interests: The authors declare that they have no competing interests.

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This work was done according to the **STROBE** guidelines.

Authors' contributions

AH conceived the research concept, while AG and AA conducted the clinical examinations and monitored the patients. AG and RA collaborated to gather the necessary laboratory data. All authors actively participated in analyzing and interpreting the patient information and composing the manuscript. All authors thoroughly reviewed and approved the final version of the manuscript.

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