Evaluating Serum Calprotectin and Serum Oncostatin M Levels as Diagnostic Markers in Crohn's Disease and Ulcerative Colitis Iraqi Patients.

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Abstract:

Introduction: Inflammatory bowel disease (IBD), comprising Crohn's disease (CD) and ulcerative colitis (UC), presents as chronic gastrointestinal inflammation. Timely diagnosis and effective monitoring are crucial for better outcomes.
This study aims to explore serum calprotectin and oncostatin M as potential biomarkers for diagnosing and monitoring CD and UC. Calprotectin, released during inflammation, and oncostatin M, an immune-response cytokine, have shown promise, but their precise role in IBD remains unclear.
Methodology: Using a cross-sectional observational design, the study included 93 IBD patients on biological treatment (50 CD, 43 UC) at Baghdad Teaching Hospital. Demographic data and disease characteristics were collected via interviews, and blood samples were analyzed using specific ELISA kits for calprotectin and oncostatin M levels.
Results: The results demonstrated significantly elevated serum levels of both biomarkers in IBD patients, increasing with disease activity. Significant distinctions were observed among different disease statuses in UC and CD patients.
Conclusion: These findings suggest that serum calprotectin and oncostatin M have potential as practical and non-invasive biomarkers for diagnosing and monitoring IBD. However, further research is required to validate their clinical utility and optimize IBD management.

Keywords: Inflammatory bowel disease, Crohn's disease, ulcerative colitis, serum calprotectin, serum oncostatin M.

تقييم مستويات سيروم كالبروتيكتين ومستويات سيروم أونكوستاتين إم كعلامات تشخيصية في مرض كرون والتهاب القولون التقرحي لدى المرضى العراقيين

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الخلاصة:

المقدمة: تُعد أمراض الالتهاب الأمعائي، والتي تشمل مرض كرون والتهاب القولون التقرحي، مرضًا مزمنًا يتميز بالتهاب المجرى الهضمي. التشخيص السريع والرصد الفعال أمران بالغي الأهمية لتحقيق نتائج أفضل. يهدف هذا البحث إلى استكشاف الكالبروتكتين والأونكوستاتين أم كمؤشرات محتملة للتشخيص والرصد لمرض كرون والتهاب القولون التقرحي. أظهر الكالبروتكتين، وهو مركب يتم إفرازه أثناء الالتهاب، والأونكوستاتين أم، وهو سيتوكين مرتبط بالاستجابة المناعية، وعدد من الوعود، ولكن الدور الدقيق لهما في أمراض الالتهاب الأمعائي لا يزال غير واضح.

المنهجية: باستخدام تصميم مراقبة عرضية مقطعية، شملت الدراسة 93 مريضًا بأمراض الالتهاب الأمعائي يخضعون للعلاج البيولوجي (50 حالة من مرض كرون و43 حالة من التهاب القولون التقرحي) في مستشفى بغداد التعليمي. تم جمع البيانات الديموغرافية والخصائص المرضية من خلال المقابلات، وتم تحليل عينات الدم باستخدام أدوات ELISA المحددة لمستويات الكالبروتكتين والأونكوستاتين أم.
النتائج: أظهرت النتائج زيادة ملحوظة في مستويات الكالبروتكتين والأونكوستاتين أم في مرضى أمراض الالتهاب الأمعائي، وتزايد هذه المستويات مع تصاعد نشاط المرض. لوحظت فروق ملحوظة بين الحالات المرضية المختلفة للتهاب القولون التقرحي ومرض كرون.
الاستنتاج: تشير هذه النتائج إلى أن الكالبروتكتين والأونكوستاتين أم لهما إمكانات كمؤشرات عملية وغير غازية للتشخيص والرصد لأمراض الالتهاب الأمعائي. ومع ذلك، يتطلب المزيد من البحث لتحقيق الصحة السريرية لهذه المؤشرات وتحسين إدارة أمراض الالتهاب الأمعائي.

الكلمات المفتاحية: أمراض الالتهاب الأمعائي، مرض كرون، التهاب القولون التقرحي، الكالبروتكتين السيرومي، الأونكوستاتين أم السيرومي، مؤشرات، التشخيص، الرصد، التهاب المجرى الهضمي، دراسة عرضية مقطعية، اختبار ELISA، نشاط المرض

Introduction

Crohn's disease and ulcerative colitis are chronic inflammatory bowel diseases (IBD) affecting the gastrointestinal (GI) tract[1]. While they share similar symptoms, they have distinct features. Crohn's disease can affect any part of the GI tract, often leading to deep ulcers, fistulas, and strictures[2]. On the other hand, ulcerative colitis primarily affects the colon and rectum, causing inflammation limited to the inner lining[3]. Symptoms for both conditions include abdominal pain, diarrhea, weight loss, and fatigue[4]. Diagnosis involves medical history, physical examination, blood tests, and imaging. Treatment aims to reduce inflammation and maintain remission, often utilizing medications or surgery in severe cases. Regular medical care is crucial for effectively managing these conditions and maintaining a good quality of life[5].

Accurate diagnosis and continuous monitoring play a vital role in managing inflammatory bowel diseases (IBD) like Crohn's disease and ulcerative colitis. A precise diagnosis helps healthcare providers choose the most suitable treatment plan, considering the specific type and severity of the IBD. Regular monitoring is essential for assessing symptom progression, identifying potential complications, and adjusting treatments accordingly to optimize symptom management. It enables early detection of flare-ups and helps prevent serious complications, such as intestinal strictures and fistulas[6]. Usually Erythrocytes Sedimentation Rate and C-Reactive protein are commonly used markers of inflammation and are indicative of disease activity in IBD patients[7, 8]. Additionally, monitoring ensures that patients' response to treatment is assessed over time, allowing for necessary adjustments, and minimizing the risk of treatment resistance. Moreover, it offers psychological support and contributes to medical research, fostering advancements in IBD management and improving overall patient care[9]. Collaborative efforts between patients and healthcare providers, with regular check-ups and open communication, are key to effectively managing IBD and enhancing patients' well-being[10].

Serum calprotectin and serum oncostatin M have emerged as promising biomarkers with potential applications in assessing disease activity and distinguishing between Crohn's disease and ulcerative colitis, both being inflammatory bowel diseases (IBD)[11]. Calprotectin is a protein found in white blood cells, and its levels in the blood are directly related to the extent of inflammation in the gastrointestinal tract. Monitoring serum calprotectin levels can provide valuable insights into IBD activity,

enabling healthcare providers to gauge disease severity and response to treatment[12].

On the other hand, oncostatin M is a cytokine that plays a significant role in the inflammatory process. Elevated serum levels of oncostatin M have been observed in patients with IBD, and its measurement can aid in differentiating between Crohn's disease and ulcerative colitis[13]. As these biomarkers offer non-invasive and easily accessible means of assessing disease activity and distinguishing between the two major forms of IBD, they hold considerable potential for improving clinical management and treatment decisions for patients with Crohn's disease and ulcerative colitis[14]. Further research and validation studies are needed to establish their full utility and integration into routine clinical practice.

The purpose of the article: to review and analyse the current research on the utility of serum calprotectin and serum oncostatin M levels in diagnosing and monitoring Crohn's disease and ulcerative colitis.

Methodology

Ethical consideration: The study adhered to the protocols outlined by the Ethics Committee of the College of Pharmacy at Mustansiriyah University, as indicated by the assigned research number 31, approval number 19, and reference number 72, on July 3rd, 2022. Written informed consent has been obtained from each participant. No incentives were provided, and the participation was entirely voluntary.

Study design: The research employed a cross-sectional observational design to review and analyse the current research on the utility of serum calprotectin and serum oncostatin M levels in diagnosing and monitoring Crohn's disease and ulcerative colitis. The present investigation adhered to the STROBE[15] (The Strengthening the Reporting of Observational studies in Epidemiology) guidelines for reporting cross-sectional observational studies[16].

Setting: The present study was conducted at Baghdad Teaching Hospital, located in Baghdad, Iraq, lasting from July 2022 to February 2023.

Sample size: The G*Power software version 3.1.9.7, with the Research Resource Identifier (RRID) SCR_013726, was utilized to estimate the necessary sample size for the study. The study employed a one-tailed alpha level of 0.05, a confidence interval of 95%, a power of 95%, and an effect size of 0.50. Hence, the minimum sample size required was determined to be 34 (f). The study involved the enrolment of 93 IBD patients on biological treatment (50 with Crohn's disease and 43 with ulcerative colitis).

Inclusion criteria: The study included individuals between the ages of 21 and 57 years who had been previously diagnosed with inflammatory bowel disease (IBD), ulcerative colitis (UC) or Crohn’s disease. These patients received treatment protocols prescribed by physicians at the Gastrointestinal Tract (GIT) Centre in Baghdad Teaching Hospital, located in Baghdad, Iraq.

Exclusion criteria: The study excluded individuals who had the following coexisting conditions: rheumatoid arthritis, systemic lupus erythematosus, diabetes mellitus; cardiovascular, hepatic, or renal diseases; organ transplant recipients; patients with any type of cancer.

Bias: This study utilizes random selection, a rigorous and systematic approach, to ensure that each participant from the IBD population has an equal opportunity to be included in the sample. The aforementioned concept forms the foundation of probability sampling and holds significant importance in probability methodologies and the ability

to generalize findings. The utilization of random selection effectively mitigates the presence of sampling selection bias.

Data collection: Demographic data such as age, gender, weight, height, working status, disease duration, smoking status, income, marital status, family history, and presence of extra-intestinal manifestations were collected via direct patient interviews using a patient data chart specially designed for this study. The body mass index (BMI) was computed by dividing the weight in kilograms by the square of the height in meters.

Sample preparation: From each patient, a 5 ml venous blood sample was taken and allowed to clot. Later the clot was removed by centrifuging at 2,000-3,000 rpm for 20 minutes. The resultant collected supernatant was kept in deep freeze (-80°C) till the time of analysis of biomarkers.

Serum Oncostatin M measurement

Calprotectin measurements were performed using the Human Oncostatin M (OSM) ELISA (Double Antibody Sandwich ELISA technique) Kit (Melsin Medical Co., Limited, China) as outlined in Table 1. Microelisa Stripplate was utilized. Standard and testing sample wells were prepared. Standard wells received 50µl of standard, while testing sample wells got 10µl of sample and 40µl of diluent. HRP-coupled reagent (100µl) was added, incubated at 37°C for 60 minutes under adhesive film. Wells were washed, then filled with 400µl of Wash Solution. Complete liquid removal was vital. Remaining cleanse solution was removed. Wells were dried, treated with 50µl each of chromogen solutions A and B, mixed and incubated (37°C, 15 mins), shielded from light. Stop Solution (50µl) was added, changing well hue. Optical Density (OD) at 450 nm was read within 15 mins using microtiter plate reader.

Serum calprotectin measurement

Calprotectin measurements were performed using the Human calprotectin (CAL) ELISA (Double Antibody Sandwich ELISA technique) Kit (MyBioscore.Inc., USA) as outlined in Table 1. The procedure involved: taking the ELISA Kit out of the refrigerator 20 minutes before the test and letting it reach room temperature. Diluting the concentrated washing buffer by a factor of 1:25 with double-distilled water. Adding 1.0ml of Standard Diluent to the lyophilized standard vial and waiting 30 minutes. After complete dissolution, combining and labeling the tube slightly. Withdrawing Enzyme Conjugate solution proportional to the number of wells and diluting it with Enzyme Diluent in a 1:100 ratio, prepared 30 minutes in advance and not to be reused for additional testing. Subtracting the values of the blank well from the OD values of each sample and standard.

Statistical analysis

Statistical analysis in this study was conducted using GraphPad Prism version 8 (RRID:SCR_002798). The Shapiro-Wilk test was selected as the normality test. The data are presented in the form of mean ± standard deviation. The standard error of the mean (SEM) is a statistical measure that quantifies the variability or uncertainty associated with the estimate of the mean of a population based on a sample. The data were subjected to statistical analysis using an unpaired Student's t-test and a two-way analysis of variance (ANOVA). After the analysis of variance (ANOVA) indicated significant differences among the data sets, a post-hoc test known as Tukey's multiple-comparisons test was employed to compare the datasets. A p-value below the threshold of 0.05 was considered statistically significant.

Table 1: Summary of kits and equipment

Diagnostic kits	Supplier	Cat. No.
Human Oncostatin M (OSM) ELISA KIT	Melsin Medical Co., Limited, China	EKHU-2288
Human calprotectin (CAL) ELISA Kit	MyBioSource, USA	MBS2601681
HumaReader HS	HUMAN	REF16670

Results

A total of 93 IBD patients were included in the analysis. Of these, 50 patients diagnosed with Crohn's disease, and 43 patients diagnosed with UC, as presented in Figure 1. The demographic and disease characteristics of the two treatment groups are summarized in Table 2. There were no significant differences in age, gender distribution, disease duration, between the UC and CD groups ($p > 0.05$).

Table 2: Demographic characteristics of IBD patients.

Demographic Data		Ulcerative Colitis	Crohn's Disease
Number of patients		43 (46.24%)	50 (53.76%)
Age (years)		33.51 ± 10.90	31.86 ± 10.59
Gender	Male	26 (60.46%)	34 (68%)
	Female	17 (39.54%)	16 (32%)
BMI (kg/m^2)		23.95 ± 4.61	23.11 ± 4.28
Marital status	Single	15 (34.88%)	19 (38%)
	Married	28 (65.11%)	31 (62%)
Employment state	Studying	10 (23.25%)	15 (30%)
	Employee	9 (20.93%)	11 (22%)
	Retired	1 (2.32%)	2 (4%)
	unemployed	15 (34.88%)	17 (34%)
	free business	8 (18.60%)	5 (10%)
Income level	<250,000	12 (27.90%)	13 (26%)
	250,000– 500,000	11 (25.58%)	12 (24%)
	500,000 – 1,000,000	18 (41.86%)	19 (38%)
	>1,000,000	2 (4.65%)	6 (12%)
Disease Activity index based on CDAI for CD or PMS for UC	Remission	15 (34.88%)	18 (36%)
	Mild disease	11 (25.58%)	14 (28%)
	Moderate disease	9 (20.93%)	13 (26%)
	Sever disease	8 (18.6%)	5 (10%)
Disease Duration		7 ± 3.73	6.28 ± 4.31

Data expressed as mean ± standard deviations or frequency (percentage, %).
Abbreviations: CD; Crohn's Disease, UC; Ulcerative Colitis, CDAI; Crohn's Disease Activity Index, PMS; Partial Mayo Score.

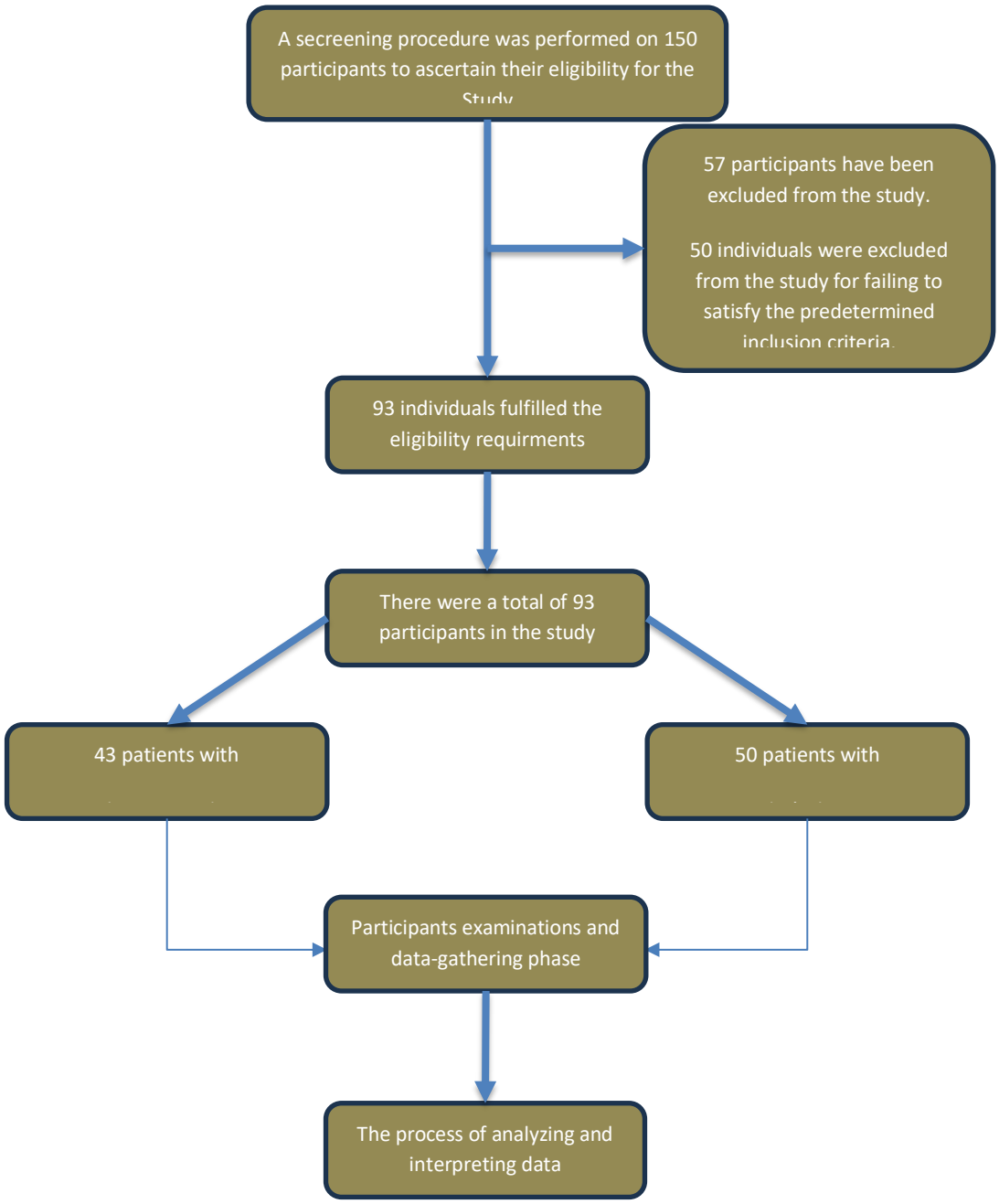

Figure 1: Patients enrolment flow chart

Serum calprotectin level

The results of our study, presented in terms of the mean value plus or minus the standard error of the mean (SEM), reveal a significant variation in outcomes observed among different disease statuses in patients with UC (Ulcerative Colitis).

Serum calprotectin level in Ulcerative colitis (UC)

Starting with UC groups, the mean ± SEM for UC patients in remission, mild disease, moderate disease, and severe disease group were 2223 ± 189.8, 5413 ± 224.9, 8729 ± 421.1 and 9179 ± 164.2 ng/ml, respectively. Regarding the serum calprotectin level in UC patient, all the disease statue showed a

statistically significant distinction ($P < 0.05$), except the patients with moderate and severe disease status, the analysis indicates a non-significant difference ($P > 0.05$). As shown in (table 3) and (figure 2A).

Serum calprotectin level in Crohn's disease (CD)

Regarding CD groups, the mean± SEM for patients in remission, mild disease, moderate disease, and severe disease group were 2661 ± 82.93, 5606 ± 157.1, 8334 ± 165.8 and 9422 ± 273.5 ng/ml, respectively. Regarding the serum calprotectin level in CD patient, all the disease statue showed a statistically significant distinction ($p < 0.05$). As shown in (table 3), (figure 2B).

Table 3: Statistical analysis results of serum calprotectin level (expressed in pg/ml) between Ulcerative colitis (UC) groups and Crohn's Disease (CD) groups. Two-way ANOVA followed by Tukey's multiple comparisons post hoc test. Data expressed as mean ± SEM (Standard Error of Mean).

UC Groups		Mean ±(SEM) for group 1	Mean ±(SEM) for group 2	P Value
Group 1	**Group 2**			
Remission (n=15)	**Mild** (n=11)	2223 ± 189.8	5413 ± 224.9	<0.0001*
Remission (n=15)	**Moderate** (n=9)	2223 ± 189.8	8729 ± 421.1	<0.0001*
Remission (n=15)	**Sever** (n=8)	2223 ± 189.8	9179 ± 164.2	<0.0001*
Mild (n=11)	**Moderate** (n=9)	5413 ± 224.9	8729 ± 421.1	<0.0001*
Mild (n=11)	**Sever** (n=8)	5413 ± 224.9	9179 ± 164.2	<0.0001*
Moderate (n=9)	**Sever** (n=8)	8729 ± 421.1	9179 ± 164.2	0.702
CD Groups		**Mean ±(SEM) for group 1**	**Mean ±(SEM) for group 2**	**P Value**
Group 1	**Group 2**			
Remission (n=18)	**Mild** (n=14)	2661 ± 82.93	5606 ± 157.1	<0.0001*
Remission (n=18)	**Moderate** (n=13)	2661 ± 82.93	8334 ± 165.8	<0.0001*
Remission (n=18)	**Sever** (n=5)	2661 ± 82.93	9422 ± 273.5	<0.0001*
Mild (n=14)	**Moderate** (n=13)	5606 ± 157.1	8334 ± 165.8	<0.0001*
Mild (n=14)	**Sever** (n=5)	5606 ± 157.1	9422 ± 273.5	<0.0001*
Moderate (n=13)	**Sever** (n=5)	8334 ± 165.8	9422 ± 273.5	0.0013*

* = *P<0.05*

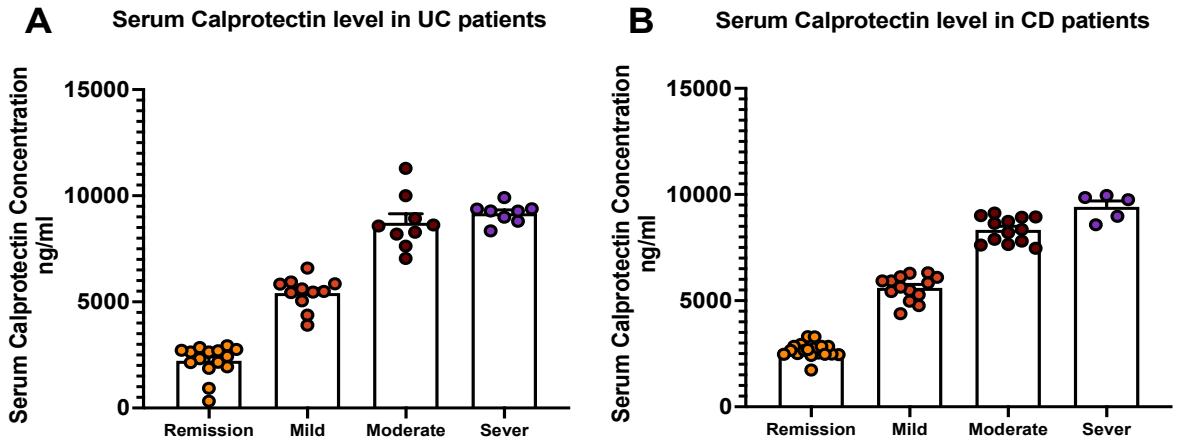

Figure 2: Serum calprotectin level in IBD patients, expressed in ng/ml. (A) Compare serum calprotectin level between Ulcerative Colitis (UC) patients (B) Compare serum calprotectin level between Crohn's Disease (CD) patients, based on disease activity index (CDAI for Crohn's disease or PMS for Ulcerative Colitis).

Serum oncostatin M level

First, and regardless the treatment taken, we evaluate the serum Oncostatin M level in either UC or CD to validate this biomarker usefulness in predicting the disease activity and evaluating the treatment outcome.

Serum oncostatin M level in Ulcerative colitis (UC)

Regarding the serum Oncostatin M in UC, the mean± SEM for patients in remission, mild disease, moderate disease, and severe disease group were 35.47 ± 4.27, 82.64 ± 2.61, 128.6 ± 3.52 and 252.5 ± 9.822 pg/ml, receptively. Regarding the serum Oncostatin M in UC patient, all the disease statue showed a statistically significant distinction ($P < 0.05$). As shown in (table 4) and (figure 3A).

Serum oncostatin M level in Crohn's Disease (CD)

Regarding the serum Oncostatin M in CD, the mean± SEM for patients in remission, mild disease, moderate disease, and severe disease group were 39.67 ± 3.82, 90.79 ± 1.91, 125.8 ± 4.072 and 255.6 ± 15.41 pg/ml, respectively. Regarding the serum Oncostatin M in CD patient, all the disease statue showed a statistically significant distinction ($P < 0.05$). As shown in (table 4) and (figure 3B).

Table 4: Statistical analysis results of serum Oncostatin M level (expressed in ng/ml) between Ulcerative colitis (UC) groups and Crohn's Disease (CD) groups. Two-way ANOVA followed by Tukey's multiple comparisons post hoc test. Data expressed as mean ± SEM (Standard Error of Mean.

UC Groups		Mean ±(SEM) for Group 1	Mean ±(SEM) for Group 2	P Value
Group 1	Group 2			
Remission (n=15)	**Mild** (n=11)	35.47 ± 4.27	82.64 ± 2.61	<0.0001****
Remission (n=15)	**Moderate** (n=9)	35.47 ± 4.27	128.6 ± 3.52	<0.0001****
Remission (n=15)	**Sever** (n=8)	35.47 ± 4.27	252.5 ± 9.82	<0.0001****
Mild (n=11)	**Moderate** (n=9)	82.64 ± 2.61	128.6 ± 3.52	<0.0001****

Mild (n=11)	**Sever** (n=8)	82.64 ± 2.61	252.5 ± 9.82	<0.0001****
Moderate (n=9)	**Sever** (n=8)	128.6 ± 3.52	252.5 ± 9.82	<0.0001****
CD Groups		**Mean ±(SEM) for Group 1**	**Mean ±(SEM) for Group 2**	**P Value**
Group 1	**Group 2**			
Remission (n=18)	**Mild** (n=14)	39.67 ± 3.82	90.79 ± 1.91	<0.0001****
Remission (n=18)	**Moderate** (n=13)	39.67 ± 3.82	125.8 ± 4.072	<0.0001****
Remission (n=18)	**Sever** (n=5)	39.67 ± 3.82	255.6 ± 15.41	<0.0001****
Mild (n=14)	**Moderate** (n=13)	90.79 ± 1.91	125.8 ± 4.072	<0.0001****
Mild (n=14)	**Sever** (n=5)	90.79 ± 1.91	255.6 ± 15.41	<0.0001****
Moderate (n=13)	**Sever** (n=5)	125.8 ± 4.072	255.6 ± 15.41	<0.0001****

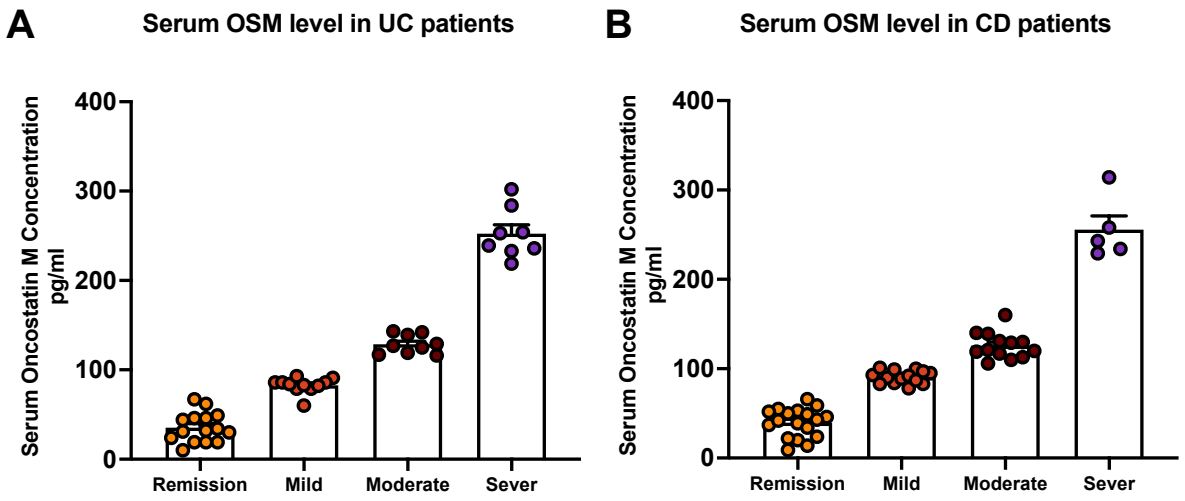

Figure 3: Serum oncostatin M level IBD patients, expressed in pg/ml. (A) Compare serum oncostatin M level between Ulcerative Colitis (UC) patients (B) Compare serum oncostatin M level between Crohn's Disease (CD) patients, based on disease activity index (CDAI for Crohn's disease or PMS for Ulcerative Colitis).

Discussion

The results of this study show that serum calprotectin and oncostatin M levels are significantly elevated in patients with IBD, and the levels are further increased as the disease activity increases. This suggests that both biomarkers can be used as potential indicators of disease activity and can be used to monitor the efficacy of treatment.

The results of this study are consistent with previous studies that have shown that serum calprotectin and oncostatin M levels are elevated in patients with IBD. These biomarkers are also more sensitive than other markers, such as C-reactive protein, in detecting disease activity.

Recently, serum calprotectin has gained more attention as a biomarker for IBD as a blood-based biomarker may be more convenient in routine practice and more

acceptable to patients. Kalla et al. evaluated the serum calprotectin level in a total of 156 patients (n=83 IBD, n=73 non-IBD) using multivariable logistic regression analysis. According to their results, serum calprotectin strongly correlated with fecal calprotectin and was the strongest predictor of IBD diagnosis in comparison with other biomarkers such as C-reactive protein (CRP) and albumin. Serum calprotectin was also capable of predicting treatment escalation and/or surgery in IBD patients[12].

Meuwis et al. aimed to evaluate the value of serum calprotectin as a novel biomarker for CD in 115 patients in comparison with 40 healthy controls. Median serum calprotectin level was 8892 ng/mL in CD patients as in comparison with 1318 ng/mL in healthy controls. Serum calprotectin was significantly higher in active disease than the inactive disease. When serum calprotectin used as a discrete variable appeared complementary to high-sensitivity CRP and faecal calprotectin to predict CD relapse following the infliximab withdrawal. They concluded that serum calprotectin could be used as complementary to faecal calprotectin and high-sensitivity CRP to predict relapse after infliximab withdrawal[17].

Bertani et al. conduct a study with 45 patients with CD and treated with infliximab and one of the measured biomarkers is the oncostatin M, which is considered as a promising biomarker for driving therapeutic choice for CD. Serum level of oncostatin M was lower level in responders and high in non-responders and show a significant difference between the two groups ($p<0.001$)(14). Cao et al, demonstrate that serum level of oncostatin M was elevated in infliximab non-responder IBD patients and lowered in infliximab responder patients. In addition, oncostatin M is a valuable biomarker and can be used in prediction of disease severity[13].

Conclusion

The use of serum calprotectin and oncostatin M levels as biomarkers for IBD has several advantages. First, they are relatively inexpensive and easy to measure. Second, they can be used to monitor the efficacy of treatment and to detect early recurrence of disease.

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