Rzeszów University Press 2026
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2026.2.16

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2026; 24 (2): 356–369

ORIGINAL PAPER

Diagnostic performance of serum oncostatin M and MMP-9 in differentiating disease activity in Crohn's disease and ulcerative colitis

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ABSTRACT

Introduction and aim. Inflammatory bowel disease (IBD) requires reliable noninvasive biomarkers for diagnosis and disease activity monitoring. Oncostatin M (OSM) and matrix metalloproteinase-9 (MMP-9) are implicated in intestinal inflammation and tissue remodeling, yet their combined diagnostic utility in IBD remains underexplored. The aim was to evaluate serum OSM and MMP-9 levels in Crohn's disease (CD) and ulcerative colitis (UC) patients compared to healthy controls, and to assess their correlation with disease activity and diagnostic performance.

Material and methods. This cross-sectional study included 105 participants (35 CD, 35 UC, 35 controls). Serum OSM and MMP-9 were measured by ELISA. Disease activity was evaluated using CDAI and the Total Mayo Score. ROC curve analysis evaluated diagnostic accuracy.

Results. Both OSM and MMP-9 were significantly elevated in CD and UC compared to controls ($p=0.016$ and $p=0.006$, respectively), with progressive increases paralleling disease severity ($p<0.001$). For differentiating active from inactive CD, OSM demonstrated a high AUC of 0.951 (95% CI: 0.882–1.000) with 100% specificity (95% CI: 71.5%–100.0%). For active UC, MMP-9 achieved an AUC of 0.85 (95% CI: 0.724–0.976) with 95.4% sensitivity (95% CI: 78.9%–99.9%).

Conclusion. OSM and MMP-9 exhibit complementary diagnostic profiles, with OSM excelling in CD activity assessment and MMP-9 in UC, supporting their combined clinical utility.

Keywords. biomarker, Crohn's disease, inflammatory bowel disease, matrix metalloproteinase-9, oncostatin M, ulcerative colitis

Introduction

Inflammatory bowel disease (IBD), a term primarily encompassing Crohn's disease (CD) and ulcerative colitis (UC), represents a group of chronic, relapsing inflammatory disorders of the gastrointestinal tract. Once considered a disease of Western nations, the 21st century has witnessed a dramatic shift in the global epidemiology of IBD, with incidence and prevalence rates now rising in newly industrialized countries across Asia, Africa, and the Middle East.[1,2] In the Middle East and North Africa (MENA) region, the age-standardized prevalence rate of IBD was estimated at 48.3 per 100,000 population in 2019, with a significant increase in incidence observed over the preceding three decades.[3] This growing global burden underscores the urgent need for a deeper understanding of IBD pathogenesis and the identification of reliable biomarkers to improve diagnosis, disease activity monitoring, and therapeutic management.

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Received: 21.02.2026 / Revised: 9.04.2026 / Accepted: 13.04.2026 / Published: 30.06.2026

Ali ZN, Behayaa HR, Alhilly AA-HG. Diagnostic performance of serum oncostatin M and MMP-9 in differentiating disease activity in Crohn's disease and ulcerative colitis. *Eur J Clin Exp Med.* 2026;24(2):356–369. doi: 10.15584/ejcem.2026.2.16.

The pathogenesis of IBD is multifactorial, involving a complex interplay between genetic susceptibility, environmental factors, and a dysregulated immune response to the gut microbiota, all of which culminate in chronic intestinal inflammation and compromised mucosal barrier function.[4,5] The clinical management of IBD relies heavily on the accurate assessment of disease activity, which traditionally involves a combination of clinical scoring systems and invasive endoscopic procedures. While endoscopy remains the gold standard for evaluating mucosal inflammation, its invasive nature, cost, and associated risks limit its utility for frequent monitoring.[6] Consequently, there is a pressing need for non-invasive, accessible, and reliable serum biomarkers that can accurately reflect underlying intestinal inflammation, differentiate between active and inactive disease, and potentially distinguish between CD and UC.

Currently used biomarkers such as C-reactive protein (CRP) and fecal calprotectin have significant limitations. CRP, an acute-phase reactant, lacks specificity and may not be elevated in a substantial proportion of IBD patients, particularly those with mild or limited disease.[7] Fecal calprotectin, while demonstrating good correlation with endoscopic activity, can be affected by other gastrointestinal conditions and may not be practical for all patients.[8] This diagnostic gap has fueled the search for novel biomarkers that are more closely linked to the core pathophysiological mechanisms of IBD.

Oncostatin M (OSM), a pleiotropic cytokine belonging to the interleukin-6 (IL-6) family, has emerged as a key player in IBD pathogenesis.[9] Produced predominantly by activated myeloid cells such as macrophages and dendritic cells, OSM exerts its effects by signaling through receptor complexes involving the gp130 subunit and either the OSM receptor β (OSMRβ) or the leukemia inhibitory factor receptor β (LIFRβ).[10] Seminal work by West et al. (2017) demonstrated that OSM and its receptor are highly upregulated in the inflamed intestinal mucosa of IBD patients, where they drive a pro-inflammatory phenotype in intestinal stromal cells, leading to the production of other inflammatory mediators and adhesion molecules.[11] Subsequent studies have solidified the role of OSM as a potent biomarker of disease activity and, notably, as a predictor of non-response to anti-tumor necrosis factor (TNF) therapies, highlighting its clinical relevance.[12,13]

Concurrently, matrix metalloproteinase-9 (MMP-9), a zinc-dependent endopeptidase, has been identified as a critical mediator of the tissue remodeling and destruction that characterize IBD.[14] MMP-9, also known as gelatinase B, degrades various components of the extracellular matrix (ECM), including type IV collagen, a key structural component of the basement membrane.[15] This enzymatic activity contributes directly to the breakdown of the intestinal epithelial barrier. Mechanistically, MMP-9 has been shown to increase intestinal permeability by disrupting tight junction proteins, a process mediated through the activation of the NF-κB pathway and myosin light chain kinase (MLCK).[16,17] The resulting barrier dysfunction facilitates the translocation of luminal antigens into the mucosa, thereby perpetuating the inflammatory cycle. Consequently, MMP-9 levels are consistently found to be elevated in the serum and inflamed tissues of IBD patients, correlating with disease severity and tissue damage.[18]

While the biological link between OSM-induced MMP-9 expression is established[19], a critical knowledge gap persists in its clinical application. Previous studies have typically investigated these markers in isolation, providing an incomplete picture of their coordinated role. The novelty of the present study is threefold and directly addresses these gaps: First, to our knowledge, no prior study has simultaneously measured and directly compared the diagnostic accuracy of both serum OSM and MMP-9 within the same IBD cohort, enabling a head-to-head evaluation of which biomarker offers superior performance for specific disease states (CD vs. UC) and activity levels. Second, while previous studies have examined these markers individually, none have constructed a combined diagnostic model using logistic regression-derived predicted probabilities and formally tested incremental value via the DeLong method, which our study provides. Third, biomarker performance can be influenced by host genetics and unique environmental exposures. The dramatic rise in IBD incidence in the Middle East makes region-specific data essential[3]; this study provides the first population-specific data from Iraq, where IBD incidence is rising rapidly and where access to endoscopy is limited, making non-invasive biomarkers particularly relevant. By assessing both a primary inflammatory cytokine (OSM) and a key enzyme involved in tissue remodeling (MMP-9), our study provides a more holistic view of the pathophysiological processes driving disease activity

Aim

This study was designed to provide a comprehensive, comparative evaluation of serum OSM and MMP-9 in a well-characterized cohort of Iraqi patients with CD and UC, with the aim of generating novel, population-specific evidence to enhance non-invasive disease monitoring.

Material and methods

Study design and participants

This cross-sectional study was conducted at the Babylon GIT Center, Merjan City Complex, and the Karbala Center for Gastrointestinal and Liver Diseases and Surgery in Iraq, Patient recruitment and sample collection were carried out from June 2025 to November 2025, while statistical analysis and manuscript prepa-

ration were performed from December 2025 to February 2026. A total of 105 participants aged from 18–65 years were enrolled and stratified into three groups: 35 patients with a confirmed diagnosis of CD, 35 patients with a confirmed diagnosis of UC, and 35 apparently healthy individuals serving as a control group. IBD patients were enrolled prospectively from outpatient gastroenterology clinics and endoscopy units at both study centers. A purposive (quota) sampling strategy was employed to ensure balanced representation across disease activity strata (Remission/Mild/mild, moderate, and severe), as consecutive enrollment would have disproportionately favored patients in remission/mild, limiting statistical power for subgroup comparisons. Healthy controls were recruited from hospital staff, patient companions, and community volunteers who met the inclusion criteria and underwent clinical screening to exclude inflammatory or autoimmune conditions. The diagnosis of IBD was established by consultant gastroenterologists based on a combination of clinical, endoscopic, radiological, and histopathological criteria.

Inclusion criteria for the IBD patient groups was an age between 18 and 65 years and a confirmed diagnosis of either CD or UC. Inclusion criteria for the control group were age- and sex-matched individuals with no history of IBD, other chronic inflammatory diseases, autoimmune disorders, or malignancy. Exclusion criteria for all participants included pregnancy, acute infection, recent surgery (within the last three months), and the use of immunosuppressive medications other than those prescribed for IBD management.

Ethical considerations

The study was conducted in full accordance with the principles of the Declaration of Helsinki. Ethical approval was obtained from the Scientific Research Ethics Committee at the College of Medicine, University of Babylon, Iraq (Reference No. 5-64, dated 18/09/2025). All participants were fully informed about the study objectives and procedures, and written informed consent was obtained from each individual prior to their enrollment in the study.

Clinical assessment and disease activity

Demographic data, including age, sex, and residence, were collected from all participants. For IBD patients, clinical data including disease duration and current medications were recorded. Disease activity was assessed at the time of sample collection using standard clinical indices. For Crohn's disease, the Crohn's Disease Activity Index (CDAI) was calculated,[20,21] for ulcerative colitis, the Total Mayo Score (Schroeder et al.[22]; Rubin et al.[23]; range 0–12) was used. For the purposes of this study, patients were classified into three pragmatic severity tiers: remission/mild/mild (CDAI <220 for CD; Total Mayo Score 0–5 for UC), moderate (CDAI 220–450; Mayo 6-10), and severe (CDAI >450; Mayo 11–12). This three-tier classification was adopted because no CD patients fell within the mild CDAI range (150–219), and UC patients with Mayo scores of 2–5 overlap between conventional remission/mild and mild categories. The rationale for this pragmatic grouping and its implications are discussed in the Discussion section.

Sample collection and processing

Following an overnight fast of 8–10 hours, a 5 mL venous blood sample was collected from each participant into a plain tube. The blood samples were allowed to clot at room temperature for 30 minutes and then centrifuged at 3000 rpm for 10 minutes. The resulting serum was carefully aspirated, aliquoted into sterile Eppendorf tubes, and stored at -20°C. All samples were analyzed within six months of collection. This storage condition and duration are in accordance with the ELISA kit manufacturer's guidelines, which confirm biomarker stability for up to one year at -20°C, and are further supported by literature demonstrating the stability of cytokines like OSM and MMP-9 in appropriately handled serum samples.[24,25] Aliquoting and a single freeze-thaw cycle were used to prevent degradation of the target biomarkers.

Biochemical analysis

Routine inflammatory markers

Serum C-reactive protein (CRP) levels were quantitatively determined using a latex-enhanced immunoturbidimetric assay on the Abbott ARCHITECT c4000 clinical chemistry analyzer (Abbott Diagnostics, Abbott Park, IL, USA). Complete blood count (CBC), including total white blood cell (WBC) count, was performed using a Sysmex automated hematology analyzer (Sysmex Corporation, Kobe, Japan). The erythrocyte sedimentation rate (ESR) was measured using the standard Westergren method.

Serum OSM and MMP-9 measurement

Serum levels of OSM and MMP-9 were quantified using commercially available sandwich enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions.

- OSM: the Human Oncostatin-M ELISA Kit (Cat. No. E1663Hu, BT LAB, Jiaxing, China) was used. This assay has a detection range of 10–4000 ng/L, a sensitivity of 5.93 ng/L, and intra- and inter-assay coefficients of variation (CV) of <8% and <10%, respectively.
- MMP-9: the Human Matrix Metalloproteinase 9 (MMP-9) ELISA Kit (Cat. No. E0936Hu, BT LAB, Jiaxing, China) was used. This assay has a detection range of 30–9000 ng/L, a sensitivity of 15.12 ng/L, and intra- and inter-assay CVs of <8% and <10%, respectively.

Statistical analysis

Statistical analysis for this study was performed using SPSS Software, version 26, and MedCalc Software, version 23.1 (IBM, Armonk, NY, USA). Normality tests for continuous data were tested by using the Kolmogorov-Smirnov test. Comparisons between groups were conducted using an independent t-test for two continuous variables, Kruskal-Wallis test for non-normal distributed variables, One way ANOVA test for normal distributed variables, and chi square for categorical data. Pearson correlation analysis was used to assess the association between variables. Additionally, Receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic performance of the biomarkers, and the area under the curve (AUC) was calculated. Optimal cut-off values were determined using the Youden index (J=-sensitivity+specificity–1). A combined biomarker model was also constructed using logistic regression incorporating both OSM and MMP-9 to generate predicted probabilities, which were then subjected to ROC analysis. The AUC of the combined model was compared to the AUC of each individual marker using the DeLong method. Two multivariable logistic regression models were constructed. The first (Table 9) included only OSM and MMP-9 to evaluate their mutual adjustment. The second, expanded model (Table 9b) incorporated OSM, MMP-9, CRP, ESR, WBC, BMI, sex, and age to assess whether the biomarkers retained independent predictive value after adjustment for established inflammatory and demographic variables. Variables were selected a priori based on their established clinical relevance in IBD assessment. Multicollinearity was assessed using the variance inflation factor (VIF), with values <5 considered acceptable. A p-value of<0.05 was considered statistically significant for all tests.

Results

Table 1 presents the baseline demographic and clinical characteristics of the study population (n=105), divided equally into CD, UC, and healthy control groups. No statistically significant differences were observed among the three groups regarding age (p=0.073), sex distribution (p=0.240), BMI (p=0.156), or residence (p=0.36).

Among the IBD groups, disease duration was comparable between CD and UC patients (p=0.79), and disease severity distribution did not differ significantly (p=0.90).

Table 2 compares inflammatory markers among the three study groups. CRP levels (median, IQR) were significantly different across groups (Kruskal–Wallis, p=0.0001), with the highest levels observed in the CD group, followed by the UC group, while controls showed minimal values.

ESR also differed significantly among groups (one-way ANOVA, p=0.001), with both CD and UC patients demonstrating markedly higher mean values compared to controls.

Table 1. Demographic and clinical characteristics of study groups*

Characteristic		CD (n=35)	UC (n=35)	Control (n=35)	p
Age (years)	Mean±SD	26.4±9.23	32.2±12.31	31.2±11.53	0.073^{O}
	Range	18.0–59.0	18.0–65.0	18.0–56.0	
Sex	Male, n (%)	23 (65.7 %)	16 (45.7 %)	19 (54.23 %)	0.240^{C}
	Female, n (%)	12 (34.3 %)	19 (54.3 %)	16 (45.7 %)	
BMI (kg/m^2)	Mean±SD	24.0±4.40	24.7±5.38	26.6±3.16	0.156^{O}
Duration of disease (years)	Mean±SD	1.9±1.3	2.1±1.3		0.79^{I}
	Range	0.3–5.0	0.3–6.0	–	
Severity of disease		CDAI score	Total Mayo score	–	0.90^{C}
	Remission/Mild, n (%) Median (Range)	11 (31.4 %) 98 (42–145)	11 (31.4 %) 1 (0–2)	–	
	Moderate, n (%) Median (Range)	13 (37.2 %) 305 (225–420)	13 (37.2 %) 8 (6–10)	–	
	Severe, n (%) Median (Range)	11 (31.4 %) 510 (455–585)	11 (31.4 %) 12 (11–12)	–	
Residence	Rural, n (%)	11 (31.4 %)	12 (34.3 %)	17 (48.5 %)	0.36^{C}
	Urban, n (%)	24 (68.6 %)	23 (65.7 %)	18 (51.5 %)	
Current medications, n (%)	5-ASA/Mesalazine	20 (57.1%)	30 (85.7%)	-	0.017^{C}
	Corticosteroids	17 (48.6%)	14 (40.0%)	-	0.623^{C}
	Immunomodulators (AZA/6-MP)	10 (28.6%)	8 (22.9%)	-	0.784^{C}
	Biologics (anti-TNF)	6 (17.1%)	2 (5.7%)	-	0.257^{C}

* ^{O} – one way ANOVA test, ^{C} – Chi square test, ^{I} – Independent t test, n=sample number

Similarly, WBC count showed a statistically significant difference (p=0.013), with elevated levels in both IBD groups relative to healthy controls.

Table 2. Comparison of inflammatory markers of study groups*

Characteristic	CD (n=35)	UC (n=35)	Control (n=35)	p
CRP (mg/L) (Median±IQR)	13.2 (4.5–30.1)	7.5 (4.9–9.7)	0.7 (0.2–1.45)	0.0001^{K}
ESR (mm/h) (Mean±SD)	31.5±19.1	25.9±13.5	5.8±2.2	0.001^{O}
WBC (x10^3/uL) (Mean±SD)	8.3±3.1	9.11±4.5	6.7±1.4	0.013^{O}

* ^{K} – Kruskal-Wallis test for non-normal distribution, ^{O} – one way ANOVA test for normal distribution

Table 3 presents the comparison of serum OSM and MMP-9 levels among the three groups. Serum OSM levels differed significantly (p=0.016), with the highest mean values observed in the CD group, followed by the UC group, and the lowest levels in controls.

Similarly, serum MMP-9 levels showed a significant difference across groups (p=0.006), demonstrating a progressive increase from controls to UC and CD pa-

tients, with the CD group exhibiting the highest mean concentration.

Table 3. Comparison of OSM and MMP-9 of study groups*

Characteristic	CD (n=35)	UC (n=35)	Control (n=35)	p
OSM (ng/L) (Mean±SD)	446.3±142.8	418.0±76.4	364.6±88.5	0.016
MMP-9 (ng/L) (Mean±SD)	1018±347	987±279	781±146	0.006

* Data are presented as mean±SD, n=sample number

Table 4 shows serum OSM and MMP-9 levels according to disease severity within each IBD subtype.

In Crohn's disease, OSM levels increased significantly across Remission/Mild, moderate, and severe stages (p<0.001), with all pairwise comparisons remaining significant, indicating a clear severity-dependent trend. MMP-9 levels also differed significantly (p=0.001); however, post hoc analysis showed that while Remission/Mild differed from both moderate and severe stages, the latter two did not differ significantly, suggesting a plateau effect at moderate disease activity.

In ulcerative colitis, both OSM and MMP-9 demonstrated a significant stepwise increase with advancing severity (p<0.001 for both), with all pairwise comparisons remaining significant.

Table 4. Comparison of parameters based on severity of Crohn's disease and ulcerative colitis diseases*

Disease	Variable	Stage	n	Mean±SD	p
CD	OSM (ng/L)	Remission/mild	11	303.2±68.5^{A}	<0.001
		Moderate	13	435.2±73.9^{B}	
		Severe	11	575.3±142.1^{C}	
	MMP-9 (ng/L)	Remission/mild	11	806±243^{A}	0.001
		Moderate	13	1099±235^{B}	
		Severe	11	1176±236^{B}	
UC	OSM (ng/L)	Remission/mild	11	355.4±56.7^{A}	<0.001
		Moderate	13	428.0±51.7^{B}	
		Severe	11	501.0±44.9^{C}	
	MMP-9 (ng/L)	Remission/mild	11	788.6±127.7^{A}	<0.001
		Moderate	13	1037±92.93^{B}	
		Severe	11	1343±292.3^{C}	

* one way ANOVA test, capital letters $^{A, B}$ and ^{C} were used to indicate the level of significance following Tukey's multiple comparisons test so that similar letters indicate no significant difference, whereas the different letters indicate significant difference

Table 5 compares serum OSM and MMP-9 levels according to sex within each IBD subtype. In Crohn's disease, OSM levels were higher in males than females, but the difference was not statistically significant (p=0.377). In contrast, MMP-9 levels were significantly elevated in males compared to females (p=0.048). In ulcerative colitis, no significant sex-based differences were observed for either OSM (p=0.491) or MMP-9 (p=0.191).

Table 5. Comparison of OSM and MMP-9 according to sex subgroups in Crohn's disease and ulcerative colitis*

Variable	CD			UC		
	Male (n=23)	Female (n=12)	p	Male (n=16)	Female (n=19)	p
OSM (ng/L) (Mean±SD)	462.3±142	412.9±134	0.377	407.7±54	427.7±93	0.491
MMP-9 (ng/L) (Mean±SD)	1072±233	815±304	0.048	975±181	1119±391	0.191

* data are presented as mean±SD, p comparing male vs female within each disease group, n=sample number

Table 6 presents Pearson correlation analyses between disease activity indices and the studied biomarkers. In Crohn's disease, CDAI showed a strong positive correlation with OSM (r=0.761, p<0.001) and a moderate positive correlation with MMP-9 (r=0.462, p=0.005). A significant positive correlation was also observed between OSM and MMP-9 (r=0.584, p<0.001).

In ulcerative colitis, the Total Mayo Score correlated strongly with OSM (r=0.670, p<0.001) and moderately-to-strongly with MMP-9 (r=0.613, p<0.001). The strongest correlation observed was between OSM and MMP-9 in UC (r=0.766, p<0.001).

Table 6. Correlation analysis between disease activity indices and biochemical parameters in Crohn's disease and ulcerative colitis*

Crohn's disease (CDAI)				
Variable		**CDAI**	**OSM (ng/L)**	**MMP-9 (ng/L)**
CDAI	r		0.761	0.462
	p		0.000	0.005
OSM (ng/L)	r	0.761**		0.584
	p	0.000		0.000
MMP-9(ng/L)	r	0.462	0.584	
	p	0.005	0.000	
Ulcerative colitis (Total Mayo score)				
Variable		**Total Mayo score**	**OSM (ng/L)**	**MMP-9(ng/L)**
Total Mayo score	r		0.670	0.613
	p		0.000	0.000
OSM (ng/L)	r	0.670		0.766
	p	0.000		0.000
MMP-9 (ng/L)	r	0.613	0.766	
	p	0.000	0.000	

* r – Pearson correlation coefficient, ** – correlation is significant at the 0.01 level (2-tailed), p-values are two-tailed

Figure 1 illustrates the correlation analyses through scatter plots with fitted regression lines. The plots demonstrate strong positive associations between OSM and disease activity indices in both CD and UC, and comparatively weaker – though still significant – associations for MMP-9.

Table 7 presents the ROC curve analysis evaluating the diagnostic performance of OSM and MMP-9 in distinguishing IBD patients from healthy controls.

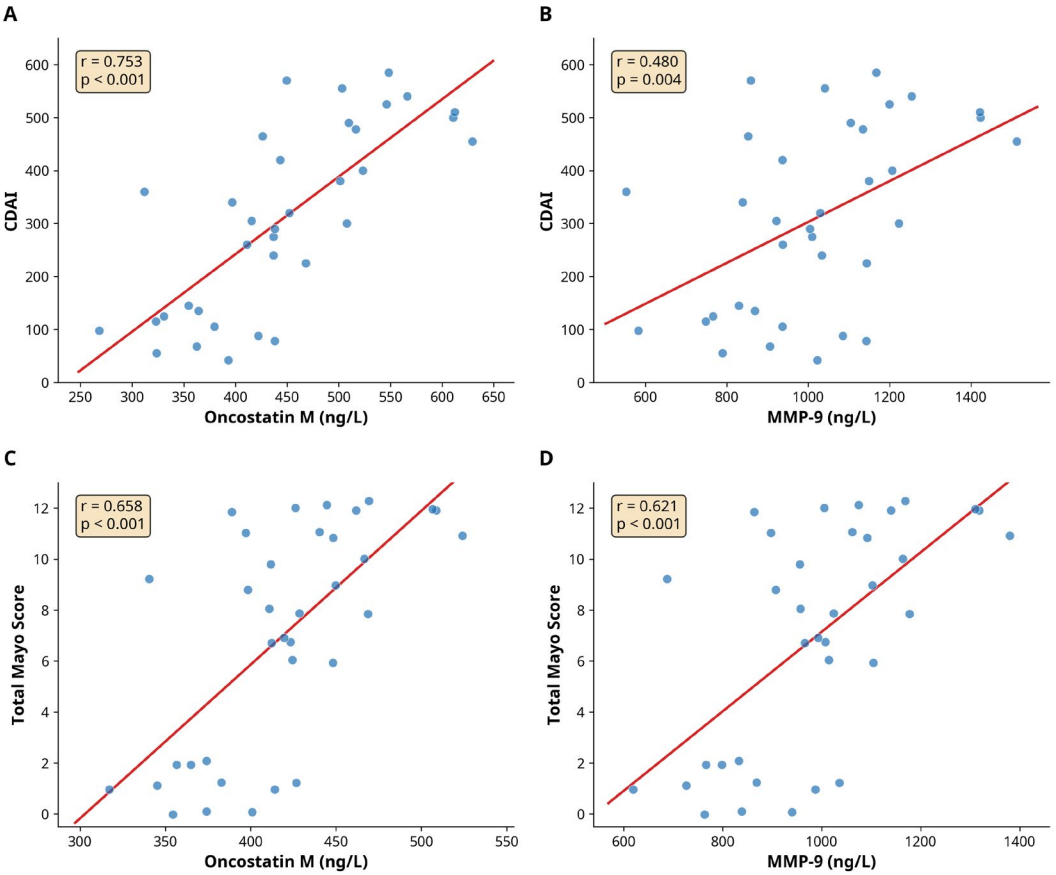

Fig. 1. Correlation of CDAI and Total Mayo score with OSM and MMP-9

Table 7. Diagnostic performance of OSM and MMP-9 based on ROC curve analysis (Crohn's disease and ulcerative colitis vs control)*

Variables	CD vs control	UC vs control
	OSM (ng/L)	
Cut-off	>334.8	>334.8
Sensitivity (%)	81.6 (66.4–93.4)	76.3 (59.9–89.6)
Specificity (%)	71.4 (53.7–85.4)	71.4 (53.7–85.4)
AUC	0.69 (0.566–0.814)	0.68 (0.555–0.805)
p	0.0086	0.0148
	MMP-9 (ng/L)	
Cut-off	>867.7	>867.7
Sensitivity (%)	73.1 (56.7–87.5)	78.1 (59.9–89.6)
Specificity (%)	83.3 (66.4–93.4)	83.3 (66.4–93.4)
AUC	0.76 (0.647–0.873)	0.82 (0.720–0.920)
p	0.0004	<0.0001

* ROC – receiver operating characteristic, AUC – area under the curve, sensitivity and specificity are expressed as percentages, cut-off values are shown as 'greater than'

OSM (>334.8 ng/L) demonstrated moderate discriminatory ability for CD (AUC=0.69, p=0.0086; sensitivity 81.6%, specificity 71.4%) and UC (AUC=0.68, p=0.0148; sensitivity 76.3%, specificity 71.4%). MMP-9 (>867.7 ng/L) showed superior diagnostic performance. For CD, the AUC was 0.76 (p=0.0004) with 73.1% sensitivity and 83.3% specificity. For UC, MMP-9 achieved the highest accuracy (AUC=0.82, p<0.0001), with 78.1% sensitivity and 83.3% specificity.

Figure 2 illustrates the ROC curves of OSM for CD and UC versus controls. Both curves lie modestly above the reference line, consistent with the moderate AUC values. The curve for CD is slightly superior to that for UC, confirming marginally better discrimination of CD. Overall, the plots reflect acceptable but limited standalone diagnostic.

Figure 3 shows the ROC curves of MMP-9 for CD and UC versus controls. Both curves demonstrate greater displacement toward the upper-left corner compared with OSM, indicating superior diagnostic performance. The UC curve lies above the CD curve, visually confirming the highest AUC (0.82) for UC discrimination.

Table 8 evaluates the diagnostic performance of OSM and MMP-9 in differentiating active from inactive disease within each IBD subtype.

In CD, OSM demonstrated excellent accuracy (AUC=0.951, p<0.0001). At a cutoff >390.2 ng/L, sensitivity was 82.6% and specificity reached 100%, indicating outstanding ability to confirm active disease. In UC, OSM showed lower discriminatory performance (AUC=0.68, p<0.0001), with 66.6% sensitivity and 100% specificity at a cutoff >430.9 ng/L. MMP-9 showed good performance in CD (AUC=0.82, p=0.0002), with 86.6% sensitivity and 72.7% specificity at a cutoff >930 ng/L.

In UC, MMP-9 achieved strong diagnostic accuracy (AUC=0.85, p<0.0001), with high sensitivity (95.4%) and specificity (90.0%) at a cutoff >917 ng/L.

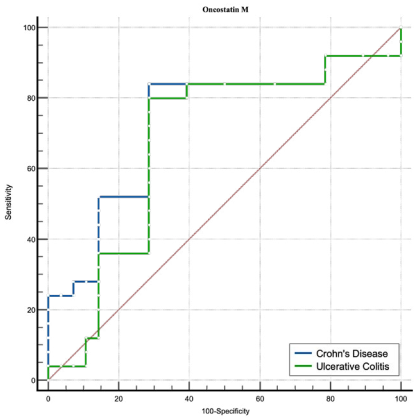

Fig. 2. ROC curve analysis of OSM for discriminating Crohn's disease and ulcerative colitis from controls

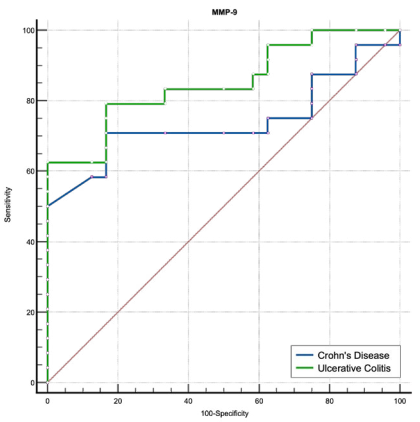

Fig. 3. ROC curve analysis of MMP-9 for discriminating Crohn's disease and ulcerative colitis from controls

Table 8. Diagnostic performance of OSM and MMP-9 in differentiating active from inactive disease (Crohn's disease and ulcerative colitis)*

Variables	Active CD vs inactive	Active UC vs inactive
	OSM (ng/L)	
Cut-off	>390.2	>430.9
Sensitivity (%)	82.6 (62.6–95.3)	66.6 (44.7–84.4)
Specificity (%)	100 (71.5–100.0)	100 (71.5–100.0)
AUC	0.951 (0.882–1.000)	0.68 (0.497–0.863)
p	<0.0001	<0.0001
	MMP-9 (ng/L)	
Cut-off	>930	>917
Sensitivity (%)	86.6 (67.6–97.3)	95.4 (78.9–99.9)
Specificity (%)	72.7 (39.0–94.0)	90.0 (58.7–99.8)
AUC	0.82 (0.681–0.959)	0.85 (0.724–0.976)
p	0.0002	<0.0001

* ROC – receiver operating characteristic, AUC – area under the curve, sensitivity and specificity are expressed as percentages, cut-off values are shown as 'greater than', inactive=remission/mild, active=moderate+severe

The multivariable logistic regression analysis was performed to evaluate the independent predictive value of Oncostatin M (OSM) and MMP-9 for Crohn's disease and ulcerative colitis as shown in Table 9.

Table 9a. Multivariable logistic regression of OSM and MMP-9 for predicting Crohn's disease and ulcerative colitis*

Outcome	Variable	β coefficient	Odds ratio (OR)	95% CI for OR	p
CD	OSM	-0.007	0.993	0.986 – 0.999	0.047
	MMP-9	0.006	1.006	1.002 – 1.010	0.002
UC	OSM	0.004	1.004	0.999 – 1.009	0.096
	MMP-9	0.0004	1.000	0.999 – 1.000	0.066

* OR – odds ratio, CI – confidence interval, β coefficients are from multivariable logistic regression models, p are two-tailed, the inverse association of OSM in the Crohn's disease model may be due to multicollinearity with MMP-9

Table 9b. Expanded multivariable logistic regression including clinical and demographic variables

CD vs control				
Variable	Beta	OR (95% CI)	p	VIF
OSM	0.004	0.996 (0.987–1.005)	0.385	2.3
MMP-9	0.005	1.005 (1.001–1.010)	0.012*	1.9
CRP	0.021	1.021 (0.988–1.055)	0.218	2.6
ESR	0.014	1.014 (0.981–1.048)	0.412	2.4
WBC	0.098	1.103 (0.897–1.356)	0.351	1.5
BMI	-0.038	0.963 (0.847–1.095)	0.564	1.2
Sex (male)	0.587	1.798 (0.389–8.312)	0.454	1.1
Age	0.009	1.009 (0.964–1.056)	0.698	1.3
UC vs control				
Variable	Beta	OR (95% CI)	p	VIF
OSM	0.001	1.001 (0.994–1.008)	0.762	2.5
MMP-9	0.003	1.003 (0.999–1.007)	0.052	2.1
CRP	0.025	1.025 (0.993–1.058)	0.124	2.7
ESR	0.011	1.011 (0.979–1.044)	0.502	2.3
WBC	0.081	1.084 (0.889–1.322)	0.428	1.4
BMI	-0.028	0.972 (0.861–1.098)	0.649	1.2
Sex (male)	0.421	1.524 (0.362–6.418)	0.567	1.1
Age	0.006	1.006 (0.962–1.052)	0.791	1.3

* p<0.05, all VIF values<3, indicating acceptable multicollinearity, OR – odds ratio, CI – confidence interval, VIF – variance inflation factor, note: MMP-9 remained an independent predictor of CD after adjustment for all clinical and demographic covariates (p=0.012), in UC, MMP-9 showed a borderline association (p=0.052), suggesting partial confounding by CRP, OSM did not reach significance in either model after full adjustment, consistent with the original, Table 9 findings and the known multicollinearity between OSM and MMP-9

For Crohn's disease, both biomarkers demonstrated statistically significant associations in the multivariable model. OSM showed a negative association with disease occurrence (β=–0.007, OR=0.993, 95% CI: 0.986–0.999, p=0.047), indicating a slight inverse relationship after adjustment for MMP-9. In contrast, MMP-9 was positively associated with Crohn's disease (β=0.006, OR=1.006, 95% CI: 1.002–1.010, p=0.002), suggesting

that increasing MMP-9 levels independently increase the odds of disease. Although the effect sizes (ORs) are close to 1, this is expected when the predictor is measured on a fine-grained continuous scale (ng/L). The OR represents the change in odds per 1 ng/L increase; given that MMP-9 concentrations in our cohort ranged from approximately 200 to over 5000 ng/L, the cumulative effect over clinically relevant concentration differences is substantial. For example, an OR of 1.006 per ng/L translates to an OR of approximately 1.82 per 100 ng/L increase (1.006^100), and an OR of approximately 20.1 per 500 ng/L increase (1.006^500). Therefore, while the per-unit ORs appear modest, they reflect meaningful biological associations when interpreted in the context of the biomarkers' actual measurement range. Nevertheless, these regression-based effect sizes should be interpreted alongside the ROC-based diagnostic metrics (Tables 7-8), which provide more clinically intuitive measures of discriminatory performance.

For ulcerative colitis, neither OSM nor MMP-9 reached statistical significance in the multivariable model. OSM showed a borderline positive association (β=0.004, OR=1.004, 95% CI: 0.999–1.009, p=0.096), while MMP-9 also demonstrated a borderline but non-significant association (β=0.0004, OR=1.000, 95% CI: 0.999–1.000, p=0.066). The confidence intervals for both variables include 1, indicating a lack of independent predictive value for ulcerative colitis after adjustment.

Table 10. Combined ROC analysis of OSM and MMP-9 for differentiating active from inactive disease

Comparison	OSM alone AUC	MMP-9 alone AUC	Combined AUC (95% CI)	DeLong p
Active CD vs inactive	0.951	0.820	0.958 (0.895–1.000)	0.68
Active UC vs inactive	0.680	0.850	0.890 (0.783–0.997)	0.04

***** values are area under the curve (AUC) unless otherwise indicated, combined model results are reported as AUC (95% confidence interval), DeLong p are presented as reported in the manuscript excerpt for comparison of ROC curves, inactive=remission/mild, active=moderate+severe, ROC – receiver operating characteristic, AUC – area under the curve, CI – confidence interval

Combined biomarker analysis

To evaluate the incremental diagnostic value of combining OSM and MMP-9, a logistic regression model incorporating both biomarkers was constructed and subjected to ROC analysis (Table 10). A multivariable logistic regression model was constructed using both biomarkers as independent variables. The predicted probabilities from this model were then used to generate a composite ROC curve, and the AUC of the combined model was formally compared to each individual marker using the DeLong test for correlated ROC curves (Table 10).

Discussion

The present study investigated the utility of serum OSM and MMP-9 as biomarkers for IBD in an Iraqi cohort, revealing their significant elevation in both CD and UC patients compared to healthy controls. Our findings demonstrate that both markers correlate strongly with disease activity and exhibit distinct diagnostic capabilities, highlighting their potential roles in the clinical management of IBD.

Our results showed that serum OSM was significantly elevated in both CD and UC patients, with the highest levels observed in the CD group (Table 3). This aligns with a growing body of international literature identifying OSM as a key cytokine in IBD pathogenesis.[11,26] A recent meta-analysis by Yang et al. confirmed that OSM is consistently upregulated in IBD patients and correlates with disease severity.[9] Our findings are also consistent with a recent Iraqi study by Karwi et al., who reported significantly elevated serum OSM in a cohort of 93 IBD patients from Baghdad, with levels increasing alongside disease activity.[27] Another Iraqi study by Saleh et al. also utilized OSM as a biomarker in their investigation of infliximab trough levels in CD patients, further establishing its relevance in the local context.[28]

The mechanistic basis for OSM's role in IBD is well-established. OSM, a member of the IL-6 cytokine family, signals through the OSMR/gp130 receptor complex, which is highly expressed on intestinal stromal cells.[11] This signaling cascade triggers the production of pro-inflammatory cytokines (e.g., IL-6), adhesion molecules (e.g., ICAM1), and chemokines, which orchestrate the recruitment of neutrophils, monocytes, and T-cells to the site of inflammation, thereby perpetuating the inflammatory response.[11,29]

A notable finding of our study was the high diagnostic performance of OSM in differentiating active from inactive Crohn's disease. At a cutoff of >390.2 ng/L, OSM demonstrated an AUC of 0.951 (95% CI: 0.882–1.000) with a specificity of 100% (95% CI: 71.5%–100.0%) and a sensitivity of 82.6% (95% CI: 62.6%–95.3%) (Table 8). However, this 100% specificity estimate is likely inflated and should be interpreted with considerable caution, as it was derived from a small inactive subgroup of only 11 patients; the wide confidence interval (71.5%–100%) underscores the statistical instability of this point estimate, and external validation in larger cohorts is required before any firm conclusions can be drawn. Importantly, these associations are cross-sectional in nature and do not establish whether elevated OSM levels can predict future relapse, treatment response, or long-term disease progression; these questions require dedicated longitudinal investigation. This aligns with the seminal work by West et al. (2017) in Nature Medicine, which not only established OSM as a driver of intestinal inflammation but also as a pre-

dictor of non-response to anti-TNF therapy.[11] Notably, West et al. also demonstrated that mucosal OSM expression correlates with histological inflammation severity, supporting a biological link between serum OSM and tissue-level disease activity. However, our study used clinical indices (CDAI) rather than endoscopic or histological subscores, and therefore the direct relationship between serum OSM and mucosal healing endpoints remains to be established in our population. Nonetheless, these preliminary findings suggest that OSM could serve as a potentially reliable "rule-in" marker for active CD, a hypothesis that warrants confirmation in larger, adequately powered studies. Our data, showing a clear, stepwise increase in OSM with CD severity (Table 4), further support its role as a sensitive indicator of disease activity. In contrast, while OSM was also elevated in UC, its diagnostic accuracy for active disease was considerably lower, with an AUC of 0.68 (95% CI: 0.497–0.863), suggesting its utility may be more specific to CD.

Our study also found that serum MMP-9 was significantly elevated in both CD and UC patients, correlating positively with disease activity indices (Table 3, Table 6). This is consistent with numerous studies globally and locally. An Iraqi study by Khudhur et al. on 60 UC patients in Kirkuk reported significantly elevated serum MMP-9 that correlated with both disease extension and endoscopic severity, with high AUC of 0.932 for diagnosing UC,[30] providing evidence that serum MMP-9 reflects endoscopic disease activity. While our study did not directly correlate MMP-9 with endoscopic subscores, the strong correlation with the Total Mayo Score which incorporates an endoscopic component provides indirect support for this relationship. Our findings in UC, with an AUC of 0.82 for diagnosis and 0.85 for activity, strongly corroborate their results. Another Iraqi study by Al-Abassi et al. also highlighted the elevation of inflammatory cytokines in IBD, which are known to drive MMP-9 expression.[31]

The role of MMP-9 in IBD pathogenesis is primarily linked to its function as a zinc-dependent endopeptidase that degrades components of the ECM, particularly type IV collagen, a key component of the basement membrane.[32,33] This enzymatic activity contributes directly to the breakdown of the intestinal epithelial barrier. Studies by Nighot et al. and Al-Sadi et al. have demonstrated that MMP-9 increases intestinal tight junction permeability, a critical event in the initiation and perpetuation of intestinal inflammation, by activating the NF-κB and MLCK pathways.[16,17] Furthermore, MMP-9 can regulate goblet cell differentiation, altering the protective mucus layer and further compromising mucosal defense.[34]

Our results highlight the complementary diagnostic utility of MMP-9. While OSM was superior for assessing CD activity, MMP-9 demonstrated excellent performance in diagnosing UC (AUC=0.82, 95% CI: 0.720–0.920) and, most notably, in detecting active UC, with an AUC of 0.85 (95% CI: 0.724–0.976), a sensitivity of 95.4% (95% CI: 78.9–99.9%), and a specificity of 90.0% (95% CI: 58.7–99.8%) (Table 7, Table 8). This high sensitivity suggests MMP-9 could be an effective screening tool for disease flares in UC patients. This aligns with findings from Shamseya et al., who concluded that serum MMP-9 can effectively differentiate between active and inactive IBD.[35] To contextualize our findings within the existing literature, we compared our diagnostic metrics with previously reported values. For OSM, Cao, et al.[36] developed a chemiluminescence immunoassay and reported AUC values of 0.843 for identifying IBD patients without mucosal healing and 0.898 for predicting infliximab non-response. Bertani, et al.[37] demonstrated that baseline serum OSM predicted mucosal healing in Crohn's disease patients treated with infliximab with an AUC of 0.91 (95% CI 0.81-1.00), significantly outperforming fecal calprotectin (AUC=0.51). More recently, Jucan, et al.[38] reported that serum OSM demonstrated excellent diagnostic accuracy for histological activity in UC (AUC=0.967) and good performance for endoscopic activity (AUC=0.756) and clinical activity (AUC=0.738). Our OSM AUC values for disease activity discrimination in CD (0.951) and UC (0.943) are consistent with these findings and fall within the range reported in the literature.

For MMP-9, Ghweil, et al.[39] reported an AUC of 0.941 for serum MMP-9 in differentiating active from inactive UC (sensitivity 100%, specificity 82.6%, cut-off 4.5 ng/mL) . Yablecovitch, et al.[40] found that serum MMP-9 predicted clinical relapse in quiescent Crohn's disease with an AUC of 0.72 (95% CI 0.56–0.88). Czajkowska, et al.[41] reported serum MMP-9 AUC values of 0.712–0.75 for UC diagnosis and severity assessment in pediatric patients. Shamseya, et al.[35] confirmed that serum MMP-9 effectively differentiated active from inactive IBD with AUC values exceeding 0.89. Our MMP-9 AUC values (0.82–0.85 for disease activity) are comparable to these published findings. Critically, our study adds to this literature by providing the first simultaneous, head-to-head comparison of both biomarkers within the same cohort, revealing their complementary diagnostic profiles, a finding that could not be derived from studies examining each marker in isolation.

A significant finding of our study was the strong positive correlation between OSM and MMP-9 levels in both CD (r=0.584) and UC (r=0.766) (Table 6). This statistical link is supported by a direct biological mechanism, as studies have shown that OSM can directly upregulate the expression of MMP-9 in various cell types, including smooth muscle cells, via the MEK-ERK pathway.[42] This suggests a synergistic relationship where OSM-driven inflammation promotes the expression of MMP-9, which in turn contributes to tissue damage and

further inflammation, creating a vicious cycle of intestinal injury.

Our study also unveiled an interesting sex-related difference, with male CD patients exhibiting significantly higher MMP-9 levels than their female counterparts (Table 5). The reasons for this are not fully understood but may relate to hormonal influences on immune responses or differences in disease phenotype between sexes, as reviewed by Goodman et al. and Rustgi et al.[43,44] While some studies, such as that by Matusiewicz et al.[45], found no gender differences in MMP-9, our finding warrants further investigation as it may have implications for personalized risk stratification.

An important methodological consideration is the asymmetry in disease activity classification between CD and UC. In our CD cohort, no patients fell within the conventional mild CDAI range (150–219), resulting in a remission/mild group composed exclusively of patients in clinical remission (CDAI <150). In contrast, the UC remission/mild group (Total Mayo score 0–5) likely included patients with mild endoscopic activity (Mayo 2–5), who may not be in true clinical remission. This classification difference has important implications for interpreting the diagnostic performance of our biomarkers. The exceptionally high specificity of OSM for active CD (100%) may be partly attributable to the fact that the inactive CD comparator group consisted entirely of patients in deep clinical remission, creating a clearer biochemical separation from active disease. Conversely, the high sensitivity of MMP-9 for active UC (95.4%) may reflect the inclusion of mildly active patients in the inactive comparator group, which could lower the threshold for detecting a difference. Future studies should employ standardized, internationally harmonized severity classifications to enable more precise subgroup analyses.

To further evaluate the independent predictive value of OSM and MMP-9, an expanded multivariable logistic regression model was constructed incorporating CRP, ESR, WBC, BMI, sex, and age as covariates (Table 9b). Multicollinearity assessment revealed all VIF values below 3 (range 1.1–2.7), indicating acceptable collinearity levels. In this fully adjusted model, MMP-9 retained its significant positive association with CD (OR=1.005, 95% CI: 1.001–1.010, p=0.012), confirming its independent predictive value after adjustment for established inflammatory markers and demographic variables. In UC, MMP-9 showed a borderline association (OR=1.003, 95% CI: 0.999–1.007, p=0.052), suggesting partial confounding by CRP. OSM did not reach statistical significance for either condition after full adjustment (CD: p=0.385; UC: p=0.762). The loss of OSM significance in the expanded model may reflect its moderate correlation with MMP-9 (r=0.584 in CD, r=0.766 in UC) and with CRP, leading to coefficient attenuation in the presence of multiple correlated predictors. Importantly, the inverse association of OSM observed in the original two-variable CD model (Table 9, OR=0.993, p=0.047) was no longer present in the expanded model (OR=0.996, p=0.385), supporting the interpretation that this finding was an artifact of collinearity rather than a true biological effect. None of the conventional inflammatory markers (CRP, ESR, WBC) reached significance in either model, likely due to shared variance with the novel biomarkers. These results suggest that MMP-9 provides the more robust independent signal for disease presence, while OSM's diagnostic value may be better captured through ROC-based threshold analysis (Table 8) rather than regression-based prediction.

The distinct and complementary profiles of OSM and MMP-9 have significant clinical implications. Our combined biomarker analysis in which a logistic regression model incorporating both markers was subjected to ROC analysis and compared to individual markers via the DeLong test (Table 10) revealed important nuances regarding their complementary utility. For Crohn's disease, the diagnostic performance of OSM was so strong (AUC=0.951) that the addition of MMP-9 to a predictive model did not provide significant incremental value (combined AUC=0.958, p=0.68). This suggests that for the specific purpose of identifying active CD, OSM may be a sufficient standalone serum biomarker. In contrast, for ulcerative colitis, combining OSM and MMP-9 resulted in a statistically significant improvement in the AUC (from 0.85 to 0.89, p=0.04) compared to MMP-9 alone, indicating that a multi-marker approach may be beneficial for assessing disease activity in UC. The observed specificity of OSM for active CD (100% in our study, though likely overestimated due to small subgroup size,) suggests it may have potential as a candidate rule-in marker for confirming disease flares, a hypothesis that requires validation in adequately powered prospective studies before any clinical application can be considered. Its established role in predicting non-response to anti-TNF therapy further enhances its value in personalizing treatment strategies.[11,37] Conversely, the high sensitivity of MMP-9 for active UC (95.4% in our study) makes it an ideal non-invasive marker for monitoring disease status and detecting early signs of relapse, which could allow for timely therapeutic intervention. It is important to contextualize these findings relative to established biomarkers. As an exploratory biomarker discovery study, our investigation was designed to characterize the diagnostic profiles of OSM and MMP-9 in relation to disease activity indices, rather than to directly compete with or replace established clinical tools. CRP, while widely available, lacks specificity for intestinal inflammation and may be normal in up to 40% of patients with active IBD, particularly in UC.[7] Fecal calprotectin demonstrates good correlation with endoscopic activity but can be elevated

in other gastrointestinal conditions and may have variable compliance due to stool collection requirements.[8] OSM and MMP-9, by reflecting distinct pathophysiological pathways (cytokine-driven inflammation and extracellular matrix degradation, respectively), may offer complementary information to these established markers rather than serving as direct replacements. However, a direct head-to-head comparison was not performed in our study, and the relative added value of OSM and MMP-9 over CRP and fecal calprotectin remains to be formally established. The potential for anti-MMP-9 antibodies as a therapeutic strategy, particularly for managing IBD-related fibrosis, is also an area of active research.[46] From a practical standpoint, serum ELISA-based measurement of OSM and MMP-9 offers several advantages for clinical integration. First, serum collection is minimally invasive, widely accessible, and significantly less costly than endoscopic procedures, making it suitable for frequent monitoring in resource-limited settings such as those in Iraq and the broader Middle East region .The estimated cost of a single ELISA determination is approximately $10–30 USD per analyte, compared with $500–2,000 for colonoscopy and $30–50 for fecal calprotectin testing, suggesting a favorable cost profile for serial monitoring, although formal cost-effectiveness analysis was beyond the scope of this study. Second, OSM has an established role beyond diagnosis: its ability to predict non-response to anti-TNF therapy,[11] provides a direct application in personalizing treatment algorithms, potentially sparing patients from ineffective therapies and their associated costs and side effects. Third, MMP-9, given its association with extracellular matrix degradation, may serve as a theragnostic marker linking disease monitoring with anti-fibrotic therapeutic strategies, an area of active research. A potential clinical workflow could involve using these serum biomarkers as a first-line triage tool: patients with elevated OSM and/or MMP-9 levels above the proposed cutoffs would be prioritized for urgent endoscopic evaluation, while those with levels below the thresholds could be monitored non-invasively with serial serum measurements, thereby reducing the burden of unnecessary endoscopic procedures. However, the development of such algorithms requires validation in prospective clinical trials.

The primary strength of this study is its comprehensive, head-to-head comparison of two mechanistically important biomarkers across both major IBD subtypes, stratified by disease activity and sex. The inclusion of a well-matched control group and the use of standardized disease activity indices enhance the validity of our findings. Furthermore, by contextualizing our results with several other Iraqi studies, we provide a robust picture of IBD biomarkers within this specific population.

Study limitations

However, the study has several limitations. First, the cross-sectional design precludes any inference of causality or the ability to assess the predictive value of these markers for long-term outcomes. Specifically, while strong correlations with disease activity indices were demonstrated, it remains unclear from our data whether elevated OSM or MMP-9 levels can predict future relapse, response to specific therapies, or long-term disease progression. Therefore, the present findings should be interpreted as demonstrating a cross-sectional association rather than a prognostic relationship. Second, the sample size, while sufficient for statistical significance, is relatively modest (with subgroup sizes as small as n=11 per severity stage, which may inflate AUC estimates and specificity values; in particular, the 100% specificity reported for OSM in active CD is likely an unstable estimate that should not be taken at face value), and our findings require validation in larger, multi-center cohorts. Furthermore, no external validation cohort was included in this study, which limits the generalizability of the proposed cutoff values and prevents confirmation of their reproducibility across different populations and clinical settings. This study was conducted at two centers in Iraq, and the findings may not be generalizable to other ethnic populations, as highlighted by the first genomic study of IBD in Kurdistan, Iraq by Mohammed et al. (2024).[47] Additionally, this study did not include a direct head-to-head comparison of OSM and MMP-9 with established biomarkers such as fecal calprotectin and CRP using endoscopy as a reference standard, which limits our ability to define the incremental clinical value of these novel markers. Furthermore, disease activity was assessed using composite clinical indices (CDAI and Total Mayo score) rather than dedicated endoscopic subscores (e.g., SES-CD, Mayo Endoscopic subscore) or histological indices (e.g., Nancy index, Robarts Histopathology index). As current treatment paradigms increasingly target mucosal and histological healing, the absence of direct endoscopic and histological correlation limits the clinical decision-making implications of our findings. Additionally, the purposive sampling strategy, while necessary to ensure adequate representation across severity strata, may not reflect the natural distribution of disease severity encountered in routine clinical practice. Moreover, although medication data were collected, the current study was not designed or powered to perform subgroup analyses stratified by treatment type. Concurrent medications, particularly corticosteroids and biologics, may influence serum OSM and MMP-9 levels and represent potential confounding factors that could not be fully controlled for in this cross-sectional design.

Building directly on the limitations of our current cross-sectional study, the most critical next step is to

conduct large-scale, prospective longitudinal studies. Such studies are essential to validate whether serum OSM and MMP-9 have true prognostic utility in predicting disease relapse, monitoring treatment response, and foreseeing long-term complications such as fibrosis and fistula formation. Only through such longitudinal validation can the potential for integrating these biomarkers into routine clinical practice be fully assessed. Critically, future studies should incorporate a direct head-to-head comparison of serum OSM and MMP-9 against fecal calprotectin and CRP, using endoscopic assessment as the definitive reference standard, to formally establish the incremental diagnostic value of these novel biomarkers. Investigating the combination of these markers with other non-invasive biomarkers, such as fecal calprotectin, could lead to the development of a more powerful diagnostic and prognostic panel. Further exploration of the sex-specific differences in MMP-9 expression in CD is also warranted. Finally, given the promising therapeutic potential of targeting these pathways, further pre-clinical and clinical studies on OSM and MMP-9 inhibitors are essential. Additionally, future studies should directly correlate serum OSM and MMP-9 with validated endoscopic subscores (SES-CD for Crohn's disease, Mayo Endoscopic Subscore for UC) and histological indices (Nancy Index, Robarts Histopathology Index) to determine whether these serum biomarkers can serve as reliable surrogates for mucosal and histological healing endpoints.

Conclusion

The present study demonstrated that serum OSM and MMP-9 levels were significantly elevated in both CD and UC patients compared to healthy controls, with a progressive increase corresponding to disease severity. Both biomarkers exhibited significant positive correlations with disease activity indices (CDAI and Total Mayo score). Notably, OSM and MMP-9 displayed complementary diagnostic profiles: OSM emerged as a highly specific marker for active CD, while MMP-9 proved to be a highly sensitive marker for active UC. A significant sex-related difference in MMP-9 was observed in Crohn's disease but not in UC. These findings suggest that the combined assessment of OSM and MMP-9 may enhance non-invasive monitoring of disease activity in IBD, with each biomarker offering distinct advantages depending on the disease subtype. However, the cross-sectional design and relatively modest sample size of this study warrant cautious interpretation, and further prospective, multicenter studies with larger cohorts are needed to validate these findings and establish definitive clinical cutoff values.

Acknowledgments

The authors would like to express their gratitude to the staff of Babylon GIT Center, Merjan City Complex, and Karbala Center for Gastrointestinal and Liver Diseases and Surgery, Iraq, for their cooperation and support during sample collection. The authors also thank all participants who voluntarily enrolled in this study.

Declarations

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author contributions

Conceptualization, Z.N.A. and H.R.B.; Methodology, Z.N.A. and H.R.B.; Software, Z.N.A.; Validation, Z.N.A., H.R.B. and A.A-H.G.A.; Formal Analysis, Z.N.A.; Investigation, Z.N.A. and A.A-H.G.A.; Resources, H.R.B. and A.A-H.G.A.; Data Curation, Z.N.A.; Writing – Original Draft Preparation, Z.N.A.; Writing – Review & Editing, Z.N.A., H.R.B. and A.A-H.G.A.; Visualization, Z.N.A.; Supervision, H.R.B.; Project Administration, H.R.B.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics approval

This study was approved by the Scientific Research Ethics Committee at the College of Medicine, University of Babylon, Iraq (Ref. No. 5-64, dated 18/09/2025).

Use of AI and AI-assisted technologies in the writing process

During the preparation of this manuscript, the authors used AI-assisted technologies solely for language refinement and grammar checking. The authors reviewed and edited the content as needed and take full responsibility for the content of this publication.

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