

Serum Interleukin-17 and Matrix Metalloproteinase-9 as Diagnostic Biomarkers in Iraqi Patients with Ulcerative Colitis

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ABSTRACT

Background: Ulcerative colitis (UC) is an immunoinflammatory illness that affects the mucosal membrane of the colon, resulting in erosion and ulcers. Diagnosing ulcerative colitis is difficult and risky. Colonoscopy is the gold standard, but it is invasive, expensive, and can perforate. Serum markers cannot detect mucosal inflammation. creating non-invasive ulcerative colitis biomarkers. **purpose:** To evaluate the levels of Interleukin 17 and Matrix metalloproteinase 9 among the Ulcerative colitis patients in comparison to the healthy control subjects. **Methods:** A case control study was held between September 2025 to march 2026 ,90 participants (60 with Ulcerative colitis and 30 healthy controls) were recruited from a specialized hospital for Gastroenterology and Hepatology in Najaf. The levels of interleukin 17 and Matrix metalloproteinase 9 were estimated using ELISA. **Results:** A Significant difference (P value <0.05) was observed between the ulcerative colitis and healthy control groups in levels of Interleukin 17 and Matrix metalloproteinase 9, the two markers were significantly positively correlated. Age and BMI didn't affect the immunological markers. **Conclusion:** Interleukin 17 and matrix metalloproteinase 9 showed a moderate diagnostic potency as highly sensitive immunological markers but with moderate specificity. The markers can serve as a complementary for colonoscopy rather than as a substitution.

Keywords: Ulcerative colitis, Interleukin 17, Matrix metalloproteinase 9.

Article Information

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INTRODUCTION

Ulcerative colitis (UC) is an immunoinflammatory disease that effects the mucosal membrane of the colon, resulting in erosion and atrophic ulcer ⁽¹⁾. Genetic predisposition and environmental factors play a role in diverse symptoms characterized by frequent abdominal pain accompanied by bloody diarrhea, urgency and other symptoms that primarily effect individuals aged 15-30 and 50-70, significantly impacting health, quality of life and increasing development of colorectal cancer ⁽²⁾. The immune systems excessive response to commensal microbiota and dietary antigen leads to continuous inflammation. Dysregulation of innate and adaptive immune cells response accompanied by altered cytokine balance, leads to intestinal barrier dysfunction and disease development ⁽³⁾. Ulcerative colitis requires various medical intervention for the induction and maintenance of remission with surgical intervention being require in refractory

cases, the occurrence of inflammatory bowel disease increased over past decade, with the global prevalence of UC reported approximately at 5 million cases by 2023. UC incidence increasing in Arab region, including Iraq especially in young male, while higher incidence rate in north America, Europe ⁽⁴⁾. Diagnosis of ulcerative colitis present significant challenges that can negatively impact patient outcome ⁽⁵⁾. Current diagnostic method such colonoscopy is gold standard but is invasive, costly and carries risks such as perforation. Current serum biomarkers lack sensitivity and specificity and don't reflect mucosal inflammation. highlighting need new, non-invasive biomarker to enhance diagnosis of UC ⁽⁶⁾.

Interleukin-17 (IL-17), It is a proinflammatory cytokine that plays important role in chronic inflammatory processes and support the pathogenesis of allergic conditions, tissue remodeling, malignancies, and

autoimmune diseases⁽⁷⁾. Also, the production of chemokines, like CXCL1, CXCL2, and IL-8/CXCL8 These chemokines act as signaling molecules that guide immune cells to areas where they are needed, thereby playing a vital role in the immune response

It is secreted by many immune cell populations from both the adaptive and innate immune systems, notably Th17 cells, CD8+ T cells, $\gamma\delta$ T cells, natural killer T cells, and innate lymphoid cells. The role of IL17 in UC is complex its might have potential protective role and maintaining epithelial barrier integrity in patients with UC and this process is multifaceted involving many cytokines and immune responses⁽⁸⁾.

Matrix metalloproteinase 9, Matrix metalloproteinase 9 (MMP-9) play an important role in pathophysiology of ulcerative colitis, influencing inflammation and tissue remodeling, angiogenesis, wound healing. Matrix metalloproteinase 9 contributes to breakdown of the extracellular matrix, which can exacerbate the intestinal permeability and inflammation. MMP9 is secreted by various cells such as macrophage and neutrophil, fibroblast and endothelial cell⁽⁹⁾. MMP9 has pivotal role in ulcerative colitis development their ability to degrade of extracellular matrix facilitates tissue remodeling and has important role in cancer development, influencing tumor invasion and spread⁽¹⁰⁾. This suggests it's a potential usefulness diagnostic biomarker for ulcerative colitis.

This study aimed to evaluate the diagnostic potency of these two markers (IL-17 and MMP-9) in the confirmation of Ulcerative colitis diagnosis and determine their capability to serve as a non-invasive substitution for the colonoscopy.

METHODS

Study Design and subjects' selection

It is case control study conducted in Najaf Governorate between September 2025 and march 2026. The sample comprised 90 participants (60 patients with mild to moderate

ulcerative colitis, as confirmed by gastroenterologist and 30 apparently healthy control) recruited during their visit to the specialized hospital for Gastroenterology and Hepatology in Najaf/Iraq. The age ranged from 16 yrs. To 70 yrs. (age, sex and BMI matching were conducted between the UC patients and healthy controls to reduce the potential cofounding factors).

Any patient with one or more of these conditions was excluded (Age < 16 years or older than 70 years' old, Patients with Crohn's disease, diabetes, and other autoimmune disease, Patients with infectious GIT diseases, Pregnant or breastfeeding women, Malignances).

Ethics and Permission

The College of Medicine's ethical council of the University of Kufa granted approval to this investigation. Additionally, before the initiating of the study project, permission was obtained from Najaf health directorate, Najaf specialized hospital for Gastroenterology and Hepatology, and verbal informed consent had been obtained from all participants before sample collection, coupled with the approval of the institutional ethics committee. All patients had been About the study and they permitted the researcher to give them a questionnaire and to have the blood sample.

The ethics letter code is MEC-48

Sample Collection and Preparation

The patients were diagnosed with ulcerative colitis by the gastroenterologist in remission state. They were on specific drugs (most of them on Mesalazine and few on biologic drugs). Blood samples (5 ml) were collected in gel tubes from all 90 participants, the whole blood sample was allowed to clot at room temperature for at least 10 minutes, then it was centrifuged for 5-10 minutes with 3000 RPM, the serum portion was divided to four Eppendorf tubes (0.5 ml), were stored in the deep freezer at (-80 c°) for later use when sample collection is completed. The interleukin 17 and MMP-9 levels were measured using ELISA kits from (BT-Lab

company/China, Cat. No E0936Hu) according to the manufacturer's instruction. The assay sensitivity was 15.12ng/L).

Statistical Analysis

Microsoft excel 2019 was utilized for data entry, The statistical analysis was conducted using IBM SPSS version 26 (Chicago, USA). with significant level of P-value <0.05

Data distribution

Shapiro wilk test was utilized to test the normality of the data, with p value <0.05 is considered as a non-normal distribution, The immunological markers' results were non normally distribution. Statistical tests and process, Median and interquartile were used for baseline descriptive, The raw data were log transformed first and then converted into ranks. Residuals were created to handle the non-parametric nature of the data while adjusting the results for the confounders (age and BMI), Next a preliminary linear regression was performed to assess the effect of age and BMI as covariant on the results of the immunological markers

Mann Whitney U test was performed for pairwise comparison (UC vs controls) . Spearman's Rho correlation was utilized to demonstrate the inter-relationship among the markers and the intra-relationship with age and BMI. Finally, Receiver operating Characteristic (ROC) curve was constructed to determine the optimal cutoff, diagnostic sensitivity and specificity for each marker among the ulcerative colitis patients.

RESULTS

The immunological markers distribution across the study groups (UC, controls)

Since the data were skewed to the right and contained extreme values, the data are presented as Median (IQR) rather than Mean $\pm$ SD. The UC exhibited higher median levels of the markers (MMP-9, IL-17) while the control had the lowest levels. The results are shown in table 1(A, B).

Pairwise comparison (intragroup difference)

Mann-Whitney U test revealed that Ulcerative colitis had significantly higher levels (P value < 0.05) in comparison to the control group, based on the markers level. The results are shown in table 2

Inter-markers relationship

Data Adjusting for covariates (Age and BMI)

To confirm the independence of the markers (their levels are not affected by the confounders e.g. Age and BMI) a linear regression model was employed on the ranked (log transformed data). Neither Age (P value > 0.05) nor BMI (P value > 0.05) significantly affected any of the marker's level as shown in table 3. this confirms that the results of the markers were primarily due to the disease status.

Spearman correlation was performed to observe the inter-marker relationship and the Intra-relationship of the markers with BMI and Age, there was a significant positive correlation between IL-17 with MMP-9 (R=.366, P= .000). Age and BMI showed a non-significant weak positive association with MMP-9 and IL-17, and with one another. As shown in table 4 and figures 1 and 2.

Diagnostic Power of the markers

The diagnostic accuracy of the two markers for ulcerative colitis diagnosis was assessed using the ROC curve analysis, they showed moderate diagnostic utility (moderate specificity), thus the markers can't be reliable for exclusion of UC cases (false positive). The detailed results of ROC curve is reported in table 5 and illustrated in figure 3

Table 1.A: The clinical profile (IL-17) level distribution across the study groups (UC, controls).

Variable, descriptive results	Ulcerative colitis (UC)	Controls
IL-17		
Median (IQR)	136.96 (102.1-165.2)	55.8 (50-68.2)
Minimum	74	37.3
Maximum	283.4	91.3

Table 1.B: The clinical profile (MMP-9) level distribution across the study groups (UC, controls).

Variable, descriptive results	Ulcerative colitis (UC)	Controls
MMP-9 Median (IQR)	374.7 (331 - 490.7)	194.1 (172.6 – 234.7)
Minimum	219	149
Maximum	781.8	304

Table 2: Intragroup Pairwise comparison of the markers level (IL-17, MMP-9) using U test.

Markers	IL-17	MMP-9
Mann Whitney	9.000	45.000
P value	.000*	.000*
P value is significant at < .05		

Table 3: Linear regression of the study immunological markers (IL-17, MMP-9) against the cofounder variables (Age, BMI), with P value of 0.05 considered as significant.

Study group	Markers	Standardized Coefficients	
		Beta	P-value
Age	IL-17	.002	.986
	MMP-9	.026	.780
BMI	IL-17	.056	.956
	MMP-9	.117	.206

Table 4: Spearman correlation to explore the relationship among the markers (MMP-9, IL-17) with each other and their relationship with BMI and Age

Groups		IL-17 (ng/l)	MMP-9 (ng/l)	Age(years)	BMI
IL-17 (ng/l)	®		.537**	.002	.005
	P value	.	.000	.981	.954
MMP-9 (ng/l)	®	.537**		.037	.120
	P value	.000	.	.689	.193
Age(years)	®	.002	.037		.095
	P value	.981	.689	.	.302
BMI	®	.005	.120	.095	
	P value	.954	.193	.302	.
®: Correlation coefficient					

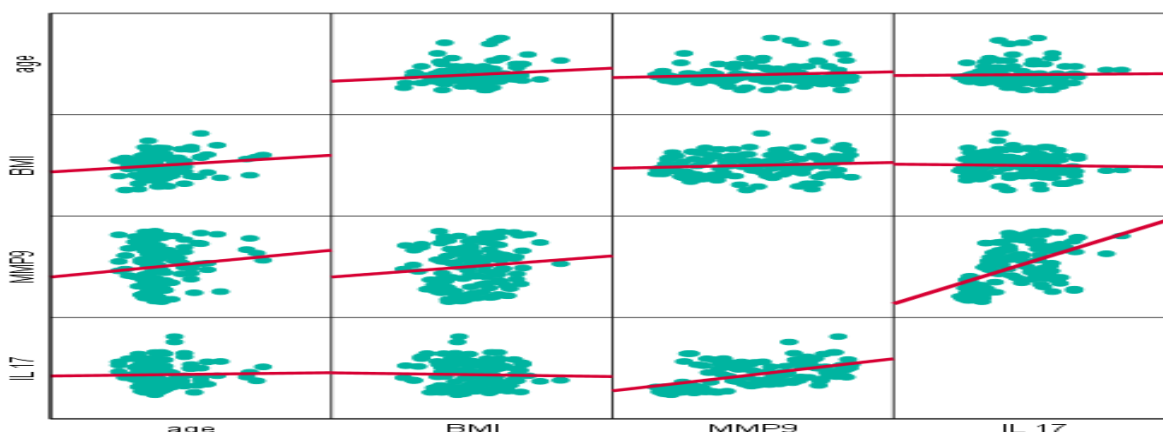


Figure 1 A: scatterplot matrix shows the correlation between the markers (MMP-9, IL-17) level with each other and with BMI and Age.

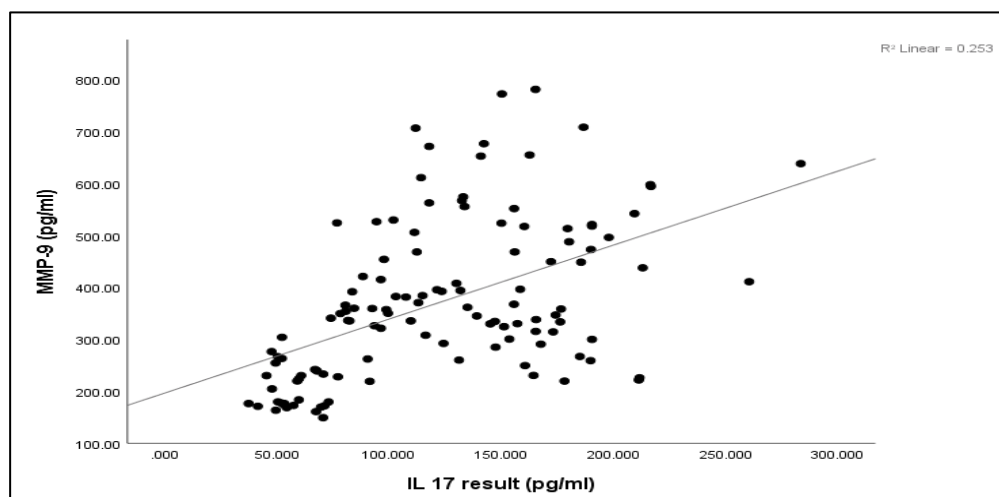


Figure 2: scatterplot represents spearman correlation showing a significant proportional correlation between the levels of MMP-9 (Y axis) and IL-17 (X axis).

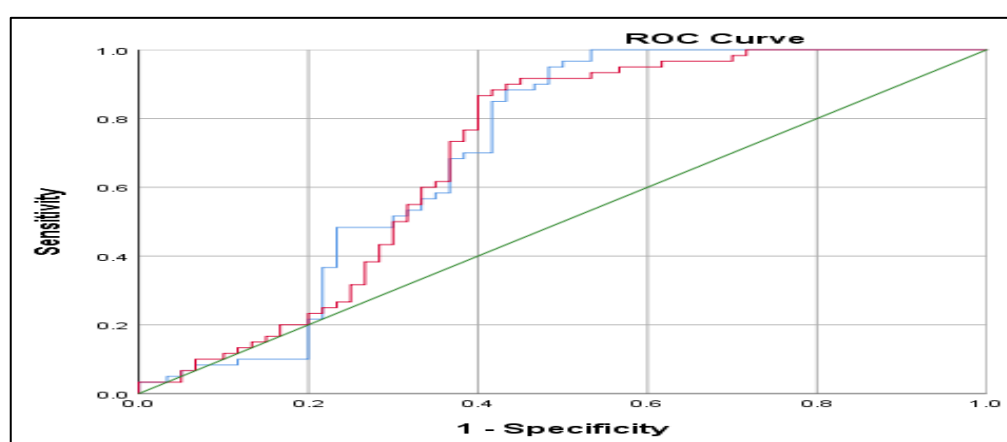


Figure 3: Receiver operating characteristics curve of Matrix Metalloproteinase 9 (Red), Interleukin 17 (Blue), with an optimal reference line (Green). The highest sensitivity (Y axis) and specificity (X axis).

Table 5: The receiver operating characteristics curve results of the immunological markers (MMP-9, IL-17) and their diagnostic accuracy (Sensitivity, specificity) and the optimal cutoff in diagnosis of Ulcerative colitis patients

Markers	AUC	Specificity (%)	Sensitivity (%)	Optimal Cutoff	Asymptotic 95% CI	
					Lower Bound	Upper Bound
MMP9	.696	48.3%	100%	207.1	.597	.795
IL17	.702	47%	100%	73.485	.603	.801

CI: Confidence Interval, AUC: Area Under Curve

DISCUSSION

Colonoscopy is considered the gold standard for diagnosis and evaluation of the mucosal damage in inflammatory bowel diseases, but there are multiple hurdles related to it including (requires specialized personnel, high risk for perforation and other complications, logistical limitations).

Primary Immunological Markers Findings

The current study focused on two immunological markers (MMP-9, IL-17) across the study groups (UC, controls), the markers showed a significant difference between the groups, the levels were much higher in UC group patients in comparison to the apparently healthy control subjects who showed a lower serum level.

The T-helper 17/Interleukin-17 Axis in the Iraqi Clinical Context.

The current study results showed a significant high level of interleukin 17 in both UC cohorts but not in the control group which is the core cytokine produced by T-helper 17, act as a bridge between the two arms of immunity (innate and adaptive). The significant elevation suggests that there is may reflect persistent immune activation secondary to mucosal dysbiosis. Our findings are consistent with the results of the meta-analysis in 2024 ⁽¹¹⁾ and studies conducted in Iraq during 2024-2025 ^(12, 13) and Egypt ⁽¹⁴⁾, where they reported that IL-17 is not only elevated during UC but also correlated with the severity and disease extension (e.g. pancolitis) which is consistent with the elevated IL17 levels observed in the current study. On the other hand, ⁽¹⁵⁾ reported that IL-17 showed no significant association with UC pathology without discussing the possible cause.

MMP-9 and its Role in Intestinal Permeability and Neural Sensitization

The matrix metalloproteinase 9 (MMP-9) showed a significantly high concentration in the serum of the UC cohort in comparison to the control groups due to mucosal degradation

triggered by the pathology of the disease, this is in agreement with the previous studies done by ⁽¹⁶⁻¹⁸⁾ that showed the same findings. The ulcerative colitis MMP-9 levels in the current study was elevated while in another study done by ⁽¹⁹⁾ consisted of two cohorts, the first cohort consisted of 66 UC patients the serum NGAL-MMP-9 levels were significantly increased at baseline in UC patients versus HC (103.8 versus 42.4 ng/mL; $P < 0.0001$), while in the other cohort ($n=132$), NGAL-MMP-9 levels were increased at baseline in active UC patients versus HC (86.5 versus 60.4 ng/mL; $P = 0.10$). In spite of the common knowledge in most of the papers regarding MMP-9 and their elevation in the active phase of ulcerative colitis, other data from researches such as ⁽²⁰⁾ reported that MMP-9 remains high and not returned to the baseline levels even during the healing phase (deep mucosal healing) which make it unreliable to confirm the complete healing alone. The suggested opinion for these unusual results is due to the variable specificity of the tests used to yield the results and the test dynamics as well.

Independence of Biomarkers from Age and Body Mass Index: A Multivariate Analysis

The use of multivariate regression analysis is considered a major strength of the study. Adjusting for age and BMI improved the validity of the immunological findings. No significant associations were found between age/BMI and marker levels. The elevated IL-17 and MMP-9 levels appeared to be associated with UC rather than demographic factors. Such as adiposity or chronological aging this is in alignment with data from the meta-analysis by ⁽²¹⁾ that showed BMI is significantly related to Crohn disease but not UC risk. This independence of the markers is important for the role of these biomarkers, so the patient doesn't need any nutritional status adjustment prior testing for the correct diagnosis.

The possible confounders (Age and BMI) in our study didn't show any significant impact on the levels of the three markers,

indicating that the levels were driven only by the disease status which is inconsistent with previous research by^(22, 23), who reported a significant correlation between IL-17 and BMI due to the adipokinin released by the adipocytes which in turn stimulate the differentiation of T-helper 17 that secrete high levels of IL-17 (false positive) which eventually increase the inflammation.

Pathophysiological synergic effect: The Interleukin-17 /matrix metalloproteinase-9 (MMP-9) Cascade.

There was a significant positive correlation that has been demonstrated between the two markers (MMP-9, IL-17), this is an indicator of the underlying pathophysiology where these markers work simultaneously to result in the disease and elucidate the proposed feedback loop, starting with the IL-17 that serve as a regulator and stimulator for the epithelium to release the MCP-1⁽²⁴⁾, that eventually secrete the cytolytic/histolytic MMP-9^(25, 26). this cytokine-proteases axis could serve as a rational target for future therapeutic intervention in UC.

Limitation of the study

Despite the bright sides of the current study, but there were some limitations that need to be taken in consideration in the future studies due to their impact on the outcomes, these are:

Homogeneity of the confounders

The multivariate regression showed that age and BMI had no effect on the markers level, but the included age groups didn't include pediatrics (<16 yrs. Old) or senile (>70 yrs. Old) and for BMI the study lacked extreme groups neither extremely slim individuals with low (<15) nor those with morbid obesity with BMI (>40), hence the independence of the markers from age and BMI can't be generalized for the entire lifespan.

The study is **single center-based** study an **only one-time random** sample was obtained and processed

CONCLUSION

1.MMP-9 and IL-17 serve as a unit during the inflammatory response in UC, without being affected by age or BMI

2.The markers (MMP-9 and IL-17) showed moderate specificity in UC cases, so colonoscopy is still needed to exclude the false positive cases

RECOMMENDATION

People with extreme age groups, BMI should be included to show the effects of the confounders on the marker level and demonstrate the effect of BMI on IL-17 level Multiple centers in variable cities should be implicated

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Ethical approval

The present study which is conducted by (Zainab Haider Abdulmajeed, Hawraa Ameer Mubark) was approved by the local Department of Medical Microbiology committee.

Statement of Permission and Conflict of Interests

The others declare that there is No conflict of interests.

REFERENCES

1. Kokorev A, Korepin S, Okhotnikova K, Nemchaninova D, Ivanov S. FEATURES OF CLINICAL AND ENDOSCOPIC MANIFESTATIONS OF ULCERATIVE COLITIS. Un-ther-journal [Internet]. 4 March 2024 [cited through 13 May 2026]; 5(4):156-67.
2. Yücel İ. Ulcerative Colitis and Nursing. Health and Technology Journal (HTechJ). 2023;1(5):455-60.
3. Kałużna A, Olczyk P, Komosińska-Vashev K. The Role of Innate and Adaptive Immune Cells in the Pathogenesis and Development of the Inflammatory Response in

- Ulcerative Colitis. *Journal of Clinical Medicine*. 2022;11(2):400.
4. Le Berre C, Honap S, Peyrin-Biroulet L. Ulcerative colitis. *The Lancet*. 2023;402(10401):571-84.
 5. Gracie D, Ford A. IBS-like symptoms in patients with ulcerative colitis. *Clinical and Experimental Gastroenterology*. 2015:101.
 6. Nooredinvand HA, Poullis A. Emerging role of colorectal mucus in gastroenterology diagnostics. *World J Gastroenterol*. 2022;28(12):1220-5.
 7. Julius S, University Ix KI. Interleukin-17 in Health and Disease: A Double-Edged Sword in Immunity. *Research Output Journal of Public Health and Medicine*. 2024;4:42-6.
 8. Reynolds JM, Martinez GJ, Nallaparaju KC, Chang SH, Wang Y-H, Dong C. Cutting Edge: Regulation of Intestinal Inflammation and Barrier Function by IL-17C. *The Journal of Immunology*. 2012;189(9):4226-30.
 9. Velasquez M, O'Sullivan C, Brockett R, Mikels-Vigdal A, Mikaelian I, Smith V, et al. Characterization of Active MMP9 in Chronic Inflammatory Diseases Using a Novel Anti-MMP9 Antibody. *Antibodies*. 2023;12(1):9.
 10. Guntarno NC, Rahaju AS, Kurniasari N. The Role of MMP-9 and VEGF in the Invasion State of Bladder Urothelial Carcinoma. *The Indonesian Biomedical Journal*. 2021;13(1):61-7.
 11. Li J, Tian H, Jiang HJ, Han B. Interleukin-17 SNPs and serum levels increase ulcerative colitis risk: a meta-analysis. *World J Gastroenterol*. 2014;20(42):15899-909.
 12. Alrikabi MH. Investigation of IL-23, IL-17 and calprotectin levels in blood of Iraqi patients with ulcerative colitis. *Iraqi journal of biotechnology*. 2023;22(1).
 13. Abdul-Hussein SS, Ali EN, Alkhalidi NMF, Zaki NH, Ad'hiah AH. Roles of IL-17A and IL-23 in the Pathogenesis of Ulcerative Colitis and Crohn's Disease. *Iraqi Journal of Science*. 2021;62(8):2526-35.
 14. Menesy A, Hammad M, Aref S, Abozeid FAM. Level of interleukin 17 in inflammatory bowel disease and its relation with disease activity. *BMC gastroenterology*. 2024;24(1):135.
 15. Al-Abassi HM, Nazal MF, Ad'hiah AH, Mushe'al KI, Mubarak IH, Alqaisy IA, et al. Serum Profile of Cytokines in Iraqi Inflammatory Bowel Disease Patients. *Mustansiriya Medical Journal*. 2015;14(2):11-6.
 16. Yablecovitch D, Kopylov U, Lahat A, Amitai MM, Klang E, Ben-Ami Shor D, et al. Serum MMP-9: a novel biomarker for prediction of clinical relapse in patients with quiescent Crohn's disease, a post hoc analysis. *Ther Adv Gastroenterol*. 2019;12:1756284819881590.
 17. Czajkowska A, Guzinska-Ustymowicz K, Pryczynicz A, Lebensztejn D, Daniluk U. Are Matrix Metalloproteinase-9 and Tissue Inhibitor of Metalloproteinase-1 Useful as Markers in Diagnostic Management of Children with Newly Diagnosed Ulcerative Colitis? *J Clin Med*. 2022;11(9).
 18. Khudhur NE, Falih ES, Hachim SK. Association of MMP-9 and PAD-4 according to UC extension and severity. *Perinatal Journal*. 2026;34(1):616-24.
 19. de Bruyn M, Arijs I, Wollants WJ, Machiels K, Van Steen K, Van Assche G, et al. Neutrophil gelatinase B-associated lipocalin and matrix metalloproteinase-9 complex as a surrogate serum marker of mucosal healing in ulcerative colitis. *Inflamm Bowel Dis*. 2014;20(7):1198-207.
 20. de Bruyn M, Ferrante M. Failure of MMP-9 Antagonists in IBD: Demonstrating the Importance of Molecular Biology and Well-Controlled Early Phase Studies. *Journal of Crohn's and Colitis*. 2018;12(9):1011-3.
 21. Rahmani J, Kord-Varkaneh H, Hekmatdoost A, Thompson J, Clark C, Salehisahlabadi A, et al. Body mass index and risk of inflammatory bowel disease: A

systematic review and dose-response meta-analysis of cohort studies of over a million participants. *Obes Rev.* 2019;20(9):1312-20.

22. Park SH, Lee KA, Choi JH, Park S, Kim DW, Jung SY. Impact of Obesity on the IL-6 Immune Marker and Th17 Immune Cells in C57BL/6 Mice Models with Imiquimod-Induced Psoriasis. *Int J Mol Sci.* 2023;24(6).

23. Chehimi M, Vidal H, Eljaafari A. Pathogenic Role of IL-17-Producing Immune Cells in Obesity, and Related Inflammatory Diseases. *Journal of Clinical Medicine.* 2017;6(7):68.

24. Lin C-Y, Tsai C-H, Tang C-H, Lo Y-S, He X-Y, Yang S-Y, et al. MCP-1 controls IL-17-promoted monocyte migration and M1 polarization in osteoarthritis. *International Immunopharmacology.* 2024;132:112016.

25. Chen X, Chang L, Li X, Huang J, Yang L, Lai X, et al. Tc17/IL-17A Up-Regulated the Expression of MMP-9 via NF- κ B Pathway in Nasal Epithelial Cells of Patients With Chronic Rhinosinusitis. *Front Immunol.* 2018;9:2121.

26. Zhang F, Tanaka H, Kawato T, Kitami S, Nakai K, Motohashi M, et al. Interleukin-17A induces cathepsin K and MMP-9 expression in osteoclasts via celecoxib-blocked prostaglandin E2 in osteoblasts. *Biochimie.* 2011;93(2):296-305.