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The impact of achieving remission in inflammatory bowel disease on plasma matrix γ-carboxyglutamate protein levels

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Abstract

Matrix γ-carboxyglutamic acid (MGLA) protein is a vitamin K dependent peptide which contributes to the immunomodulatory activity of mesenchymal stromal cells. There is a possible association between MGLA protein and inflammatory bowel disease (IBD) which is divided into Crohn's disease (CD) and ulcerative colitis (UC). However, little is known about the clinical utility of MGLA protein in IBD patients. This study aimed to assess the impact of achieving remission on the serum MGLA protein levels in IBD patients. This prospective observational study included 60 newly diagnosed IBD patients. All patients were subjected to full clinical, laboratory, radiological, and histopathological assessment of IBD at baseline and six months after initiating treatment. Serum MGLA protein level was assessed using an enzyme-linked immunosorbent assay. There were 29 (48.3%) UC cases and 31 (51.67%) CD cases. We observed a significant decrease in serum MGLA protein levels after 6 months of treatment compared to pretreatment values in UC patients (120.490 ± 26.273 vs. 26.320 ± 17.378 nmol/L, p<0.001) and CD patients (125.576 ± 28.208 vs. 28.520 ± 18.443 nmol/L, p<0.001). Serum MGLA protein levels were significantly higher in non-remittent patients compared to remittent UC patients before treatment (142.556 ± 17.096 vs. 110.560 ± 23.659 nmol/L, p<0.001) and after six months of treatment (51.222 ± 4.410 vs. 15.114 ± 3.302 nmol/L, p<0.001). Serum MGLA protein levels were significantly higher in non-remittent patients compared to remittent CD patients before treatment (150.727 ± 7.198 vs. 111.743 ± 25.718 nmol/L, p<0.001) and after six months of treatment (52.182 ± 5.269 vs. 15.506 ± 4.475 nmol/L, p<0.001). This response was irrespective of the therapeutic modality. In conclusion, achievement of remission in IBD patients resulted in a significant decrease in serum MGLA protein levels.

Keywords: Matrix GLA protein; IBD; Fecal calprotectin; Ulcerative colitis; Crohn's disease

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Introduction

Inflammatory bowel disease (IBD) is a chronic non-specific inflammatory disorder of the gastrointestinal tract and is divided into Crohn's disease (CD) and ulcerative colitis (UC).[1] The exact etiology of IBD is not well understood, however, it is currently believed to be a complex interaction between genetics, environmental influences, immunological conditions, and microbial infections.[2,3]

Matrix γ-carboxyglutamic acid (MGLA) protein is an insoluble 12-kDa vitamin K–dependent extracellular protein. It was reported that MGLA protein is synthesized and carboxylated in most human immune system cells which are either engaged in innate or adaptive immune reactions. Therefore, MGLA protein may be a mediator linking inflammatory responses which is a hallmark of IBD.[4,5] Additionally, Shiraishi et al., 2016, reported that vitamin K_2 supplementation improved symptoms of colitis along with Interleukin 6 (IL-6) down-regulation in mice with dextran sodium sulfate (DSS) induced colitis. Furthermore, MGLA protein improved the clinical and histopathological severity of experimentally induced colitis in mice models.[6] All these experimental results indicate that MGLA protein could have an immunomodulatory effect in IBD inflammation which address the need of investigating the MGLA protein involvement in IBD patients,[7,8] however, clinical human studies are still lacking. Therefore, the aim of this study was to assess the impact of achieving remission on the serum MGLA protein levels in IBD patients.

Patients and Methods

This prospective observational study was performed during the period from November 2021 to November 2022. It included 60 newly diagnosed IBD patients, recruited from the Gastroenterology Inpatient Department and Outpatient Clinic of Ain Shams University Hospitals. IBD was diagnosed according to the European Consensus on Crohn's Disease and Ulcerative Colitis (ECCO).[9] We excluded any previously treated IBD patient and any patient with a disease affecting vitamin K levels.

Study procedure

All patients were subjected to history taking and detailed clinical examination. Assessment of disease severity was performed by Truelove-Witts score[10] in UC patients and by Harvey-Bradshaw index (HBI)[11] in CD patients. In addition, data for complete blood picture, liver function tests, renal function tests, C reactive protein (CRP), erythrocyte sedimentation rate (ESR), fecal calprotectin level, intestinal ultrasound, and magnetic resonance enterography (MRE) were retrieved from hospital medical records. Lastly, full colonoscopy (Olympus, Japan) with intubation of terminal ileum and multiple biopsies were taken to confirm the diagnosis, assess the severity and extent of disease by ulcerative colitis endoscopic index of severity (UCEIS) in UC patients and simple endoscopic score for Crohn's disease (SES-CD) in CD patients.[12-14] After 6 months of treatment, all patients were subjected to full reassessment.

Serum level of MGLA protein was assessed using commercial enzyme-linked immunosorbent assay (ELISA) kits for determination of human undercarboxylated matrix Gla Protein (Cat. No E4389Hu, Bioassay Technology Laboratory, Shanghai, China), according to the manufacturer's instructions. The standard curve range was 0.5 nmol/L – 180 nmol/L with a sensitivity of 0.31 nmol/L.

Ethical considerations

The study protocol was reviewed and approved by the Research Ethics Committee of the Faculty of Medicine, Ain Shams University (FMASU MD 245/2020). A written informed consent was obtained from each patient before included in the study.

Statistical Analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS, v.20.0, Chicago, IL, USA) software. Data are presented as number and percentage or mean ± SD, as appropriate. The following statistical tests were used to examine differences for significance, as appropriate: difference of qualitative variables by Chi square test (X^2), differences between

parametric quantitative data by *t* test, and multiple parametric differences by One-way ANOVA followed by the TUKEY post-hoc test for pair-wise analysis. Correlations were tested by Pearson's correlation test. Regression analysis was used to test the relationship between serum MGLA protein and other variables. The receiver operating characteristic (ROC) curve was used to investigate the diagnostic performance of serum MGLA protein for the prediction of remission in IBD patients. A p value of <0.05 was considered significant.

Results

This study included 24 males and 36 females with mean age of 29.35 years. There were 29 (48.3%) UC cases and 31(51.67%) CD cases. Table 1 shows the demographic and clinical characteristics of study groups. Through the post-treatment follow up period of six months, extraintestinal manifestations in UC group were resolved, while in CD group either resolved or improved (Table 2). Moreover, there was an improvement in clinical and endoscopic scores in both groups (Table 3).

Table 1. Characteristics of study patients as presented by study group.

Studied parameters		Ulcerative colitis *n*=29	Crohn's Disease *n*=31
Age (years)	Mean ±SD	28.034 ± 6.652	30.581 ± 7.256
		n (%)	*n* (%)
Gender	Male	9 (31.03%)	15(48.39%)
	Female	20 (68.97%)	16(51.61%)
Medications	Oral	16(55.17%)	11 (35.48%)
	Biological	13 (44.83%)	20 (64.52%)
Remission	Non-remittent	9 (31.03%)	11 (35.48%)
	Remittent	20 (68.97%)	20 (64.52%)

Table 2. Extraintestinal symptoms of the study groups before and post treatment.

Extra-intestinal symptoms		Crohn's Disease		Ulcerative colitis
		Pre-treatment	Post-treatment	Pre-treatment
		n (%)	*n* (%)	*n* (%)
Arthralgia	No	13 (42%)	25 (81%)	20 (69%)
	Yes	18 (58%)	6 (19%)	9 (31%)
Aphthous ulcer	No	15 (48%)	31 (100%)	25 (86 %)
	Yes	16 (52%)	0 (0%)	4 (14%)
Uveitis	No	24 (77%)	31 (100%)	29 (100%)
	Yes	7 (23%)	0 (0%)	0 (0%)
Erythema nodosum	No	31 (100%)	31 (100%)	29 (100%)
	Yes	0 (0%)	0 (0%)	0 (0%)
Pyoderma gangrenosum	No	31 (100%)	31 (100%)	29 (100%)
	Yes	0 (0%)	0 (0%)	0 (0%)

Table 3. Pre and post treatment clinical and endoscopic indices in all study participants.

			Pre-treatment n (%)	Post-treatment n (%)	*p-value
Ulcerative colitis (n=29)	Truelove and Witts Criteria	Mild	0 (0%)	26 (89.66%)	<0.001
		Moderate	7 (24.14%)	3 (10.34%)	
		Severe	22 (75.86%)	0 (0%)	
	UCEIS	Remission	0 (0%)	20 (68.97%)	<0.001
		Mild	10 (34.48%)	6 (20.69%)	
		Moderate	16 (55.17%)	3 (10.34%)	
		Severe	3 (10.34%)	0 (0%)	
Crohn's disease (n=31)	HBI	Remission	0 (0%)	20 (64.52%)	<0.001
		Mild	0 (0%)	6 (19.35%)	
		Moderate	16 (51.61%)	5 (16.13%)	
		Severe	15 (48.39%)	0 (0%)	
	SES-CD	Remission	0 (0%)	20 (64.52%)	<0.001
		Mild	5 (16.13%)	9 (29.03%)	
		Moderate	26 (83.87%)	2 (6.45%)	
		Severe	0 (0%)	0 (0%)	

HBI: Harvey-Bradshaw Index; SES-CD: Simple Endoscopic Score for Crohn Disease, UCEIS: Ulcerative colitis endoscopic index of severity. *$P \leq 0.05$ is significant.

Comparative data analysis of ulcerative colitis patients

Analysis of MGLA protein levels showed no differences between males (123.000±24.062 nmol/L) and females (119.360±27.733 nmol/L, p=0.737). Additionally, MGLA protein levels were not different among patients with UC regarding disease severity at diagnosis (Table 4). We detected that after six months of treatment, MGLA protein levels were significantly lower in UC patients who showed clinically mild disease according to Truelove-Witts criteria (p=0.004) and endoscopic remission according to UCEIS compared to more clinically or endoscopically severe disease (p<0.001, Table 4).

Table 4. Pre- and post-treatment matrix γ-carboxyglutamic acid (MGLA) protein levels according to clinical and endoscopic scores of ulcerative colitis patients.

			n	Mean ± SD	*p*-value
MGLA protein level (nmol \ L) (Pre- treatment)	Truelove and Witts criteria	Moderate	7	112.571±25.767	NS*
		Severe	22	123.009±26.516	
	UCEIS	Mild	10	114.320±25.453	NS#
		Moderate	16	119.500±26.979	
		Severe	3	146.333±9.452	
MGLA protein level (post-treatment) (nmol/L)	Truelove and Witts criteria	Mild	26	23.280 ± 15.598	0.004*
		Moderate	3	52.667 ± 5.033	
	UCEIS	Remission	20	15.114 ± 3.302	<0.001#
		Mild	6	52.167 ± 4.708	
		Moderate	3	49.333 ± 3.786	

*t test, # ANOVA, UCEIS: Ulcerative colitis endoscopic index of severity. $P > 0.05$ is not significant (NS).

Serum MGLA protein levels decreased significantly after six months of treatment compared to pretreatment values (p <0.001). Furthermore, there was a significant decrease in CRP, fecal calprotectin, ESR, TLC, and significant rise in hemoglobin and serum albumin levels post therapy (Table 5).

Serum MGLA protein levels were significantly higher in non-remittent UC patients (n=9) compared to remittent UC patients (n=20) before treatment (142.556 ± 17.096 vs. 110.560 ± 23.659 3.636 nmol/L, p<0.001) and after six months of treatment (51.222± 4.410 vs. 15.114 ± 3.302 nmol/L, p<0.001). Moreover, serum MGLA protein level decreased significantly in non-remittent and remittent groups after treatment (p<0.001).

Table 5. Comparison between pre and post treatment laboratory data, inflammatory markers, and serum matrix γ-carboxyglutamic acid (MGLA) protein level in ulcerative colitis patients.

Studied parameters	Ulcerative colitis		p-value*
	Pre-treatment	Post-treatment	
Total leucocytic count (10^3 /ul)	9.903±3.414	8.241±2.738	0.003
Hemoglobin (g/dl)	9.545±1.030	11.54 ±1.639	<0.001
Platelets (10^3 /ul)	318.82 ±95.731	302.828±100.229	NS
CRP (mg/l)	67.452±31.578	15.414±17.486	<0.001
Fecal calprotectin (ug /g)	646.43 ±1065.939	110.345±60.032	0.011
Albumin (g/dl)	3.776±0.580	4.038±0.414	NS
AST (IU/l)	15.414±10.483	17.517±7.268	NS
ALT (IU/l)	13.483±8.399	12.586±4.136	NS
ESR (mm/h)	44.414±25.763	16.655±17.609	<0.001
MGLA protein (nmol/ l)	120.490±26.273	26.320±17.378	<0.001

Data are presented as mean ± SD. P > 0.05 is not significant (NS).

Comparative data analysis of Crohn's disease patients

Serum MGLA protein levels were significantly higher in female patients compared to male patients in the CD group (136.788 ± 24.976 vs. 113.617 ± 27.200 nmol/L, p=0.020). However, MGLA protein levels were not different among patients with CD regarding disease severity at diagnosis (Table 6). After six months of treatment, MGLA protein levels were significantly lower in CD patients who showed clinical remission according to HBI and endoscopic remission according to SES-CD compared to more clinically or endoscopically severe disease (p<0.001, Table 6).

Table 6. Pre- and post-treatment MGLA protein level according to clinical and endoscopic scores of Crohn's Disease.

			n	Mean ± SD	p-value
MGLA protein level pre-treatment (nmol /L)	HBI	Moderate	16	124.578±31.037	NS*
		Severe	15	126.641±25.896	
	SES-CD	Mild	5	128.304±37.152	NS*
		Moderate	26	125.051±27.059	
MGLA protein level post-treatment (nmol /L)	HBI	Remission	20	15.506 ± 4.475	<0.001#
		Mild	6	53.167 ± 5.037	
		Moderate	5	51.000 ± 5.874	
	SES-CD	Remission	20	15.506 ± 4.475	<0.001#
		Mild	9	53.333 ± 4.500	
		Moderate	2	47.000 ± 7.071	

*t test, # ANOVA, HBI: Harvey-Bradshaw Index; SES-CD: Simple Endoscopic Score for Crohn's Disease.
p> 0.05 is not significant (NS).

There was a significant decrease in TLC, CRP, fecal calprotectin, ESR post treatment compared to pretreatment values, and significant rise in hemoglobin and serum albumin levels compared to pretreatment values (p<0.05 for all). Serum MGLA protein levels showed a significant reduction after six months of treatment compared to pretreatment values (p<0.001, Table 7). In addition, serum MGLA protein levels were significantly higher in non-remittent compared to remittent CD patients before treatment (150.727 ± 7.198 vs. 111.743 ± 25.718 nmol/L, p<0.001) and after six months of treatment (52.182 ± 5.269 vs. 15.506 ± 4.475 nmol/L, p<0.001). Moreover, the decrease in MGLA levels in non-remittent and remittent patients after treatment was significant compared to pretreatment values (p<0.001, Figure 1).

Table 7. Comparison between pre and post treatment laboratory data, inflammatory markers, and serum matrix γ-carboxyglutamic acid (MGLA) protein level in Crohn's disease patients.

Studied parameters	Crohn's disease		*p*-value
	Pre-treatment	Post-treatment	
Total leucocytic count (10^3 /µl)	11.035±2.682	7.742±2.517	<0.001
Hemoglobin (g/dl)	9.394±1.071	11.623±1.573	<0.001
Platelets (10^3//µl)	264.742±85.947	241.226±65.774	NS
CRP (mg/l)	78.226±34.012	11.387±13.160	<0.001
Fecal calprotectin (µg/g)	219.452±122.502	88.161±28.877	<0.001
Albumin (g/dl)	3.290±0.495	4.210±0.485	<0.001
AST (IU/l)	21.387±10.582	16.581±4.904	0.031
ALT (IU/l)	19.581±9.667	19.710±7.067	NS
ESR (mm/l)	55.194±22.305	11.516±8.633	<0.001
MGLA protein (nmol/L)	125.576±28.208	28.520±18.443	<0.001

Data are presented as mean ± SD. P > 0.05 is not significant (NS).

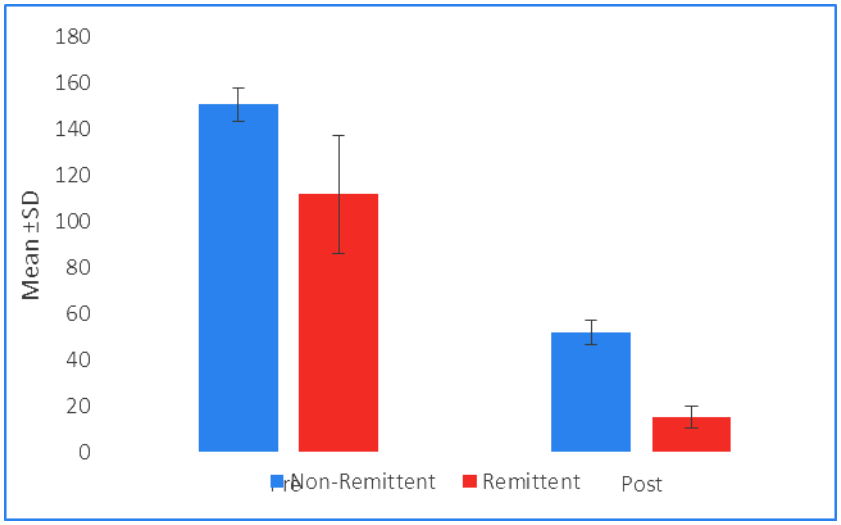

Figure 1. Serum matrix γ-carboxyglutamic acid (MGLA) protein levels in remittent and non-remittent patients with Crohn's disease.

Comparative analysis of MGLA protein levels between patients with ulcerative colitis and Crohn's disease

Serum MGLA protein levels were not different between UC and CD patients before treatment (120.490 ± 26.273 vs. 125.576 ± 28.208 nmol/L, p=0.474) and after treatment (26.320 ± 17.378 vs. 28.520 ± 18.443, *p*=0.637).

Difference in MGLA protein level between oral and biological based treatments

Serum MGLA protein levels were not different between patients who received oral therapy compared to those who received biological therapy in both UC (26.076 ± 16.812 vs. 26.619 ± 18.739, *p*=0.935) and CD (22.615 ± 17.689 vs. 31.768 ± 18.469, *p*=0.191) patients after six months of treatment.

Comparison between MGLA protein level in patients with or without extraintestinal manifestation in the UC and CD patient groups

In general, serum level of MGLA protein was not different among patients with or without extraintestinal manifestation in UC and CD patients before and after treatment except for the pretreatment presence of aphthous ulcers in UC group (Table 8).

Table 8. Comparison of pre and post treatment serum matrix γ-carboxyglutamic acid (MGLA) protein levels and extraintestinal symptoms in study groups.

	Extra-intestinal symptoms		MGLA protein level (nmol/L)			*p*-value
				n	Mean ± SD	
Ulcerative colitis	Pre-treatment	Arthralgia	No	20	120.310 ± 26.4	NS
			Yes	9	120.889 ± 27.5	
		Aphthous ulcers	No	25	124.568 ± 25	0.034
			Yes	4	95.0 ± 12.9	
Crohn's disease	Pre-treatment	Arthralgia	No	13	130.29 ± 29.2	NS
			Yes	18	122.168 ± 27.7	
		Aphthous ulcers	No	15	117.48 ± 28.11	NS
			Yes	16	133.17 ± 26.9	
		Uveitis	No	24	125.010 ± 28.22	NS
			Yes	7	127.514 ± 30.33	
	Post-treatment	Arthralgia	No	25	26.440 ±17.93	NS
			Yes	6	37.183 ± 19.64	

p> 0.05 is not significant (NS).

Comparison of MGLA protein levels in complicated cases:

There were 7 (53.85%) steroids dependent and 6 (46.15%) steroid resistant cases in the UC group. There were 4 (20%) steroid dependent, 5 (25%) steroids resistant, 5 (25%) fistulizing disease, and 6 (30%) stricturing disease in CD group. Pretreatment serum MGLA levels were not significantly different between complicated cases, and it decreased significantly after treatment in both groups (*p*<0.001, Table 9 and 10).

Table 9. Pre- and post-treatment serum matrix γ-carboxyglutamic acid (MGLA) protein levels in complicated cases of ulcerative colitis (UC) disease.

	Steroid dependent n=7	Steroid resistant n=6	*p*-value
Pre-treatment MGLA nmol/L (mean ± SD)	129.00±21.57	129.20±27.70	NS
Post-treatment MGLA nmol/L (mean ± SD)	26.05±18.88	27.28±20.33	NS
p-value	<0.001	<0.001	

p> 0.05 is not significant (NS).

Table 10. Pre- and post-treatment serum matrix γ-carboxyglutamic acid (MGLA) protein levels in complicated cases of Crohn's disease (CD).

	Steroid dependent n=4	Steroid resistant n=5	Fistulizing n=5	Stricturing n=6	*p* value
Pre-treatment MGLA nmol/L (mean±SD)	134.72±21.87	136.80±28.17	119.78±29.17	137.80±21.94	NS
Post-treatment MGLA nmol/L (mean±SD)	15.19±3.20	37.89±18.12	26.99±20.21	41.68±17.39	NS
p value	<0.001	<0.001	<0.001	<0.001	

p> 0.05 is not significant (NS).

Correlation analysis of MGLA protein levels and studies parameters in ulcerative colitis patients:

The Pearson correlation test showed significant positive correlations between post-treatment serum MGLA protein levels and TLC, CRP, fecal calprotectin, and ESR in UC patients, and significant negative correlation with hemoglobin level (*p* <0.001, Table 11). In addition, regression analysis indicated a significant association between serum MGLA protein level and CRP and hemoglobin levels (*p*=0.016 and *p*=0.001, respectively) (Table 12).

Table 11. Correlation between serum matrix γ-carboxyglutamic acid (MGLA) protein levels and other variables in ulcerative colitis (UC) group.

	MGLA protein level (pre-treatment)		MGLA protein level (post-treatment)	
	r	*p*-value	r	*p*-value
Age	-0.130	NS	-0.269	NS
Total leucocytic count	0.165	NS	0.732	<0.001
Hemoglobin	-0.013	NS	-0.887	<0.001
Platelets	-0.340	NS	-0.205	NS
CRP	-0.164	NS	0.920	<0.001
Fecal calprotectin	0.250	NS	0.653	<0.001
Albumin	-0.213	NS	0.102	NS
AST	0.173	NS	-0.030	NS
ALT	0.017	NS	-0.240	NS
ESR	0.285	NS	0.843	<0.001

p> 0.05 is not significant (NS).

Table 12. Regression analysis of serum matrix γ-carboxyglutamic acid (MGLA) protein in ulcerative colitis group.

	Unstandardized Coefficients		Standardized Coefficients	*p*-value
	B	Std. Error	Beta	
Truelove and Witts criteria	-0.515	5.290	-0.009	NS
UCEIS	5.638	2.983	0.221	NS
Total leucocytic count	0.056	0.590	0.009	NS
Hemoglobin	-3.393	1.299	-0.320	0.016
CRP	0.500	0.137	0.503	0.001
Fecal calprotectin	-0.023	0.035	-0.078	NS
ESR	0.051	0.158	0.052	NS

UCEIS: Ulcerative colitis endoscopic index of severity *p*> 0.05 is not significant (NS).

Correlation analysis of MGLA protein levels and studies parameters in Crohn's disease patients

The Pearson correlation test revealed statistically significant positive correlations between pre-treatment serum MGLA protein level and ESR in CD patients (*p*=0.003, Table 13). In addition, there were significant positive correlations between posttreatment serum MGLA protein levels and TLC, CRP, fecal calprotectin, and ESR, and significant negative correlation with hemoglobin and albumin (*p*<0.05, Table 13). In addition, according to regression analysis, there was a significant association between serum MGLA protein level and ESR (*p*=0.031, Table 14).

Table 13. Correlation between serum matrix γ-carboxyglutamic acid (MGLA) protein and other variables in Crohn's disease (CD) group.

	MGLA protein level (pre-treatment)		MGLA protein level (post-treatment)	
	r	*p*-value	r	*p*-value
Age	0.005	NS	0.172	NS
Total leucocytic count	0.343	NS	0.862	<0.001
Hemoglobin	-0.286	NS	-0.802	<0.001
Platelets	-0.163	NS	0.258	NS
CRP	-0.017	NS	0.816	<0.001
Fecal calprotectin	0.107	NS	0.411	0.022
Albumin	-0.178	NS	-0.473	0.007
AST	-0.218	NS	0.117	NS
ALT	-0.056	NS	-0.027	NS
ESR	0.510	0.003	0.804	<0.001

P > 0.05 is not significant (NS).

Table 14. Regression analysis of serum matrix γ-carboxyglutamic acid (MGLA) protein in Crohn's disease (CD) group.

	Unstandardized Coefficients		Standardized Coefficients	*p*-value
	B	Std. Error	Beta	
HBI	5.233	8.158	0.218	NS
SES-Crohn's	0.512	10.151	0.017	NS
Total leucocytic count	0.833	1.194	0.114	NS
Hemoglobin	-1.340	1.742	-0.114	NS

Table 14. Continued.

	Unstandardized Coefficients		Standardized Coefficients	*p*-value
	B	Std. Error	Beta	
CRP	0.357	0.267	0.255	NS
Fecal calprotectin	0.077	0.051	0.120	NS
Albumin	1.379	3.436	0.036	NS
ESR	0.674	0.292	0.315	0.031

HBI: Harvey-Bradshaw Index; SES-CD: Simple Endoscopic Score for Crohn Disease $p > 0.05$ is not significant (NS).

Diagnostic performance of MGLA protein as determined by the receiver operating characteristic (ROC) curve analysis

The ROC curve analysis showed that MGLA protein has an excellent diagnostic performance for the prediction of remission pre and post treatment in UC and CD patients (Table 15 and Figures 2, 3, 4, 5).

Table 15. Receiver operating characteristic (ROC) curve of the pretreatment serum matrix γ-carboxyglutamic acid (MGLA) protein level for the differentiation between remittent and non-remittent cases of ulcerative colitis (UC) patients and of Crohn's disease (CD) patients.

	Cutoff of serum MGLA protein level (nmol/L)	AUC	Sensitivity	Specificity	PPV	NPV	Accuracy	*p-value
Pre-treatment differentiation between remittent and non-remittent cases of UC.	≤140	0.900	95%	77.78%	90.5%	87.5%	90%	<0.001
Post-treatment differentiation between remittent and non-remittent cases of UC.	≤22.49	1.000	100%	100%	100%	100%	100%	<0.001
Pre-treatment differentiation between remittent and non-remittent cases of CD.	≤122.8	0.905	70%	100%	100%	64.7%	90.5%	<0.001
Post-treatment differentiation between remittent and non-remittent cases of CD.	≤23.57	1.000	100%	100%	100%	100%	100%	<0.001

*$p \leq 0.05$ is significant.

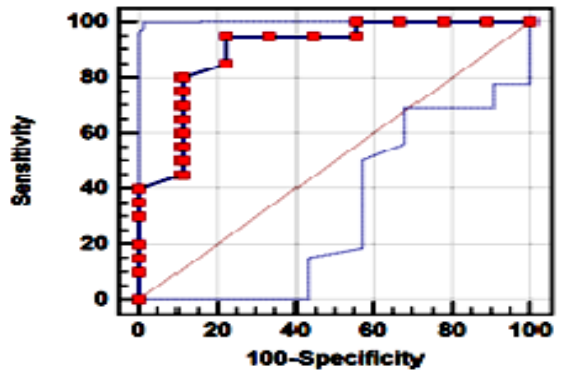

Figure 2. Receiver operating characteristic (ROC) curve of the pretreatment serum matrix γ-carboxyglutamic acid (MGLA) protein level for the differentiation between remittent and non-remittent cases of ulcerative colitis.

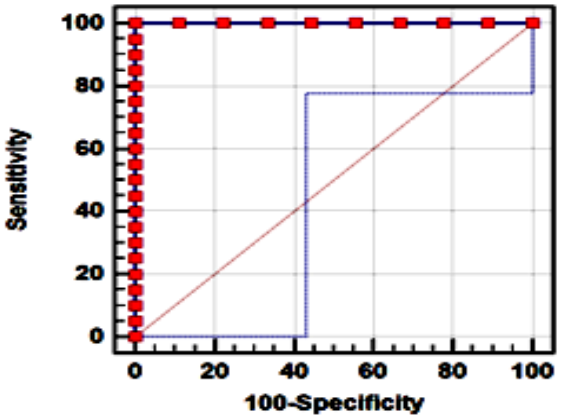

Figure 3. Receiver operating characteristic (ROC) curve of the post-treatment serum matrix γ-carboxyglutamic acid (MGLA) protein level for the differentiation between remittent and non-remittent cases of ulcerative colitis.

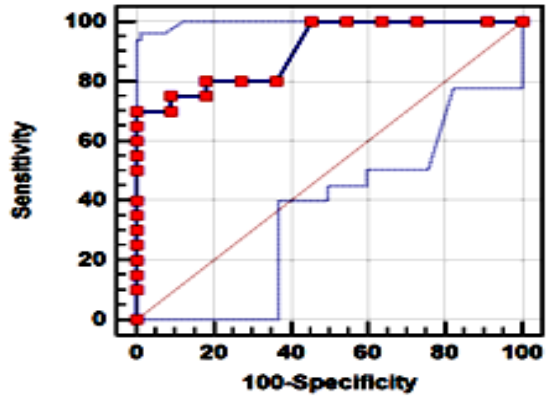

Figure 4. Receiver operating characteristic (ROC) curve of the pretreatment serum matrix γ-carboxyglutamic acid (MGLA) protein level for the differentiation between remittent and non-remittent cases of Crohn's disease.

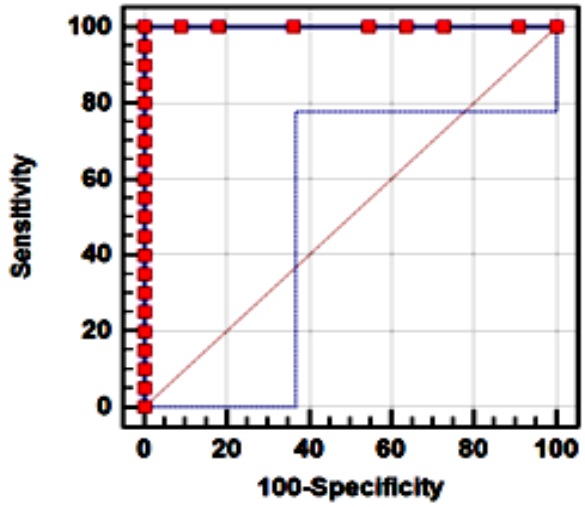

Figure 5. Receiver operating characteristic (ROC) curve of the post-treatment serum matrix γ-carboxyglutamic acid (MGLA) protein level for the differentiation between remittent and non-remittent cases of Crohn's disease.

Discussion

Published experimental results postulate the immunomodulatory role of MGLA protein in IBD disease.[7,8] Therefore, the current study aimed to assess the impact of achieving remission on the serum MGLA protein levels in IBD patients. An earlier study observed that the MGLA protein gene was the fifth highest with an 8.91-fold increase higher in UC patients than in the control group. Additionally, higher MGLA protein mRNA and protein expression in colonic tissue was detected in both UC patients and DSS-induced colitis in a mice model compared to their control groups. Furthermore, the expression of MGLA protein mRNA increased along with the disease severity, while patients in remission had a comparable MGLA protein mRNA expression as the healthy controls.[15]

To the best of our knowledge, this is the first study *assessing* the impact of achieving remission on the serum MGLA protein levels in IBD patients. We observed a significant decrease in serum MGLA protein levels after six months of treatment compared to pretreatment levels in UC and CD patients. In addition, serum MGLA protein levels were significantly higher in non-remittent compared to remittent UC and CD patients before and after six months of treatment. Moreover, there was a significant decrease in MGLA levels in non-remittent and remittent patients after treatment compared to pretreatment values. In addition, this response was irrespective of the therapeutic modality.

Our study findings agreed with those of a previous study, as plasma levels of MGLA protein were significantly higher in IBD cases compared to the control group (629.83 ± 124.20 pmol/mL vs. 546.7 ± 122.09 pmol/mL, $p<0.001$), and there was no difference between CD and UC cases (640.02 ± 131.88 pmol/mL vs. 616.23 ± 113.92 pmol/mL, $p=0.432$). In addition, there was no difference in plasma MGLA protein levels between IBD cases who were treated with biological therapy and those who were treated with other therapeutic modalities (621.37 ± 115.03 pmol/L vs. 648.27 ± 143.36 pmol/L, $p=0.404$).[16]

Both fecal calprotectin and CRP are established markers of IBD activity.[17] This is in line with another study on UC cases which showed that the expression of MGLA protein mRNA increased with the disease severity.[15] Similar to our results, they also observed significant positive correlations between plasma MGLA protein levels with both fecal calprotectin (r = 0.396, $p<0.001$) and hsCRP levels (r = 0.477, $p<0.001$) in IBD patients. Additionally, multiple linear regression analysis showed that MGLA protein levels had a significant association with fecal calprotectin ($p=0.003$).[16] The ROC curve analysis showed that MGLA protein has an excellent diagnostic performance for the prediction of remission pre and post treatment in UC and CD patients. Further studies are needed to determine the most appropriate cut-off value of serum MGLA protein levels. From the current study, we can conclude that achieving remission in IBD patients results in a significant decrease in serum MGLA protein levels.

Author Contributions

All authors certify that they participated in this work including participation in study design, collecting data , analysis and interpretation of data , practical part and writing manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

The study protocol was reviewed and approved by the Research Ethics Committee of the Faculty of Medicine, Ain Shams University (FMASU MD 245/2020).

Informed consent

A written informed consent was obtained from each patient before included in the study.

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