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Serum Osteoprotegerin (OPG) as a Potential Biomarker for Disease Activity in Ulcerative Colitis

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ABSTRACT

Background: Inflammation and ulceration of the intestinal mucosa occur repeatedly in patients with ulcerative colitis (UC), leading to numerous bouts of bleeding per rectum.

Objective: To evaluate serum osteoprotegerin (OPG) level in cases who had ulcerative colitis and its correlation with disease activity.

Patients and Methods: This case control was conducted at the National Liver Institute, Menoufia University on 89 patients. They were divided into two groups and were matched in age and gender: Group I: 50 patients with ulcerative colitis diagnosed utilizing colonoscopy and histopathological examination. Group II: 39 individuals who had normal colonoscopic findings (grossly and histopathologically) as a control group.

Results: Differences in OPG readings between the control group and UC subgroups were statistically significant. The mean OPG level in patients with extraintestinal manifestations (EIM) was 596 ± 365 pg/ml, while the mean OPG level in patients without EIM was 384 ± 333 pg/ml. OPG levels were not significantly linked to the prevalence of extraintestinal symptoms. The concentration of OPG was positively correlated with disease severity. **Conclusion:** The present study suggested that serum osteoprotegerin can be considered as a novel biomarker for assessing ulcerative colitis activity.

Keywords: Osteoprotegerin (OPG), Biomarker, Ulcerative Colitis.

INTRODUCTION

Inflammation and ulceration of the intestinal mucosa occur repeatedly in patients with ulcerative colitis (UC), leading to numerous bouts of bleeding per rectum [1]. Normal progression involves the entire colon and begins in the rectum. The development of ulcerative colitis is typically the result of a combination of hereditary and environmental factors [2].

Endoscopy along with mucosal biopsy for histopathology is the gold standard for diagnosing UC. Diagnostic imaging and laboratory testing can narrow down a patient's potential conditions [3]. Patients with severe ulcerative colitis are less likely to die if their disease activity is detected at an early stage [4].

Commonly used noninvasive diagnostics for primary diagnosis and reliable monitoring of disease activity in UC include erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), white blood cells (WBC), fecal calprotectin, as well as PMN-elastase. Nevertheless, a perfect test has not yet been created. So, using a second serum marker is helpful in both anticipating the severity of a disease and making an accurate diagnosis [2].

Osteoprotegerin (OPG), or tumor necrosis factor receptor superfamily member 11B (TNFRSF11B), plays a crucial function in many NF-kappa B signaling pathways. OPG participates in a wide variety of biological processes, including those that regulate cell differentiation, survival, and apoptosis [5]. It plays a pivotal role in bone metabolism, inflammation, cancer, and other areas. Osteoclastogenesis is triggered by a complex composed of receptor activator of NF-kappa B (RANK) and receptor activator of NF-kappa B ligand (RANKL). Inflammatory pathways are also affected by the interactions between OPG, RANKL, and RANK. Several pathways are involved in T-lymphocyte and dendritic cell (DC) survival are activated after RANKL-RANK binding [6].

Recent research has linked OPG, specifically in the setting of inflammatory bowel disorders, to pro-inflammatory processes, contradicting earlier assertions that OPG plays a significant role in bone metabolism and is critical for accurate identification of the inflammatory bowel diseases (IBD) [7, 8].

Aim of present work was evaluation of serum osteoprotegerin (OPG) level in ulcerative colitis patients and correlate it to disease activity.

PATIENTS AND METHODS

A case control study was conducted at the National Liver Institute, Menoufia University on 89 subjects divided into two groups and were matched in age and gender: **Group I:** 50 patients with ulcerative colitis diagnosed grossly on colonoscopy as well as histopathological examination. **Group II:** 39 individuals who had normal colonoscopic findings grossly and histopathologically as a control group.

These 39 normal individuals had undergone colonoscopy for colorectal cancer screening, bleeding per rectum and anemia with unknown cause and were found to have normal colonoscopic findings**.** Exclusion criteria included pregnancy, malignancy, collagen diseases as rheumatoid arthritis and systemic lupus erythematosus, Crohn's disease and bone disease. Detailed history was noted and the patients were

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carefully examined with emphasis for any signs suggestive of gastrointestinal pathology.

Laboratory investigations included complete blood picture, urea, creatinine, serum alanine aminotransferase (AST) and alanine aminotransferase (ALT), serum albumin, serum bilirubin, serum gamma-glutamyl transferase (GGT), prothrombin time and international normalised ratio (INR), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), antinuclear antibody (ANA), anti-double-stranded DNA.

Colonoscopy and biopsy:

A complete colonoscopy was done, including intubation of the ileum. Specimens were obtained from at least 2 biopsy sites in the ileum and rectum, among the 5 analyzed bowel locations [9]. Using a combination of clinical, endoscopic, and histological findings, we determined that the patient had ulcerative colitis according to the criteria established by the European Crohn's and Colitis Organization (ECCO)[10].

- **Disease severity:** was assessed by Truelove and Witt's severity index.
- **Clinical response:** to be at follow up a drop of 3 points on the Mayo scale.
- **Clinical remission:** The Mayo score less than 3.
- **Mucosal healing:** Subscore of 0 or below on the Mayo endoscopy, with a corresponding decrease of at least 1 point from pre-test.

Administrative and Ethical Design:

Official approval was obtained from the Faculty of Medicine, Menoufia University. The National Liver Institute at Menoufia University granted their official clearance, and the Institute's Ethics Committee gave their stamp of approval (Institutional Research Board IRB, (NLI IRB 00003413 FWA0000227)). Each patient who participated in the study provided his(her) written consent. The study was conducted according to the Declaration of Helsinki.

Statistical Analysis

Information was compiled, tabulated, and analysed statistically with SPSS 22. (SPSS Inc. Chicago, IL, U.S.A). Statistical analyses, such as: quantitative differences between the two groups were determined using the independent t-test and the Mann Whitney test. Chi-square test was employed to determine statistical significance between qualitative variables. Quantitative data were expressed as mean ± SD (Standard deviation). Receiver operating characteristic (ROC) analysis: It is graphical plot of sensitivity against one minus the specificity (false positive rate) for different cutoffs. P value < 0.05 was considered significant.

RESULTS

Table 1 summarizes the demographic characteristics of both groups in our study. Smoking was more prevalent among the healthy group. The average duration of disease activity was 6.4 ± 1.9 years. According to Truelove and Witts Criteria, 19 (39%) patients were diagnosed with mild disease activity. The associated extraintestinal manifestations are shown (Table 2).

Table (1): Patients' Characteristics (N = 89)

	UC (N = 50)	Control (N = 39)	*P* value
Age, years			0.139 [a]
Mean ± SD	41.2 ± 15.1	46.3 ± 16.8	
Range	19 – 67	19 – 70	
BMI, kg/m^2			0.227 [a]
Mean ± SD	31.8 ± 5.3	30.5 ± 5	
Range	22.4 – 39.7	22.3 – 39.9	
Gender			0.880 [b]
Male	29 (58%)	22 (56.4%)	
Female	21 (42%)	17 (34.6%)	
Smoking			0.019 [b]
Yes	3 (6%)	9 (23%)	
No	47 (94%)	30 (77%)	

UC: ulcerative colitis; BMI: body mass index

[a] Independent sample t test; [b] Chi-square test.

Table (2): Characteristics of UC Group (N = 50)

Parameter		Value
Disease Duration, years	Mean ± SD	6.4 ± 1.9
	Range	2.2 – 9.5
Extraintestinal Manifestations	None	39 (78%)
	Ankylosing Spondylitis	2 (4%)
	Cirrhosis	2 (4%)
	Systemic Lupus Erythematosus	1 (2%)
	Erythema Nodosum	3 (6%)
	Pyoderma Gangrenosum	1 (2%)
	Uveitis	2 (4%)
Truelove and Witts Criteria	Mild	19 (38%)
	Moderate	14 (28%)
	Severe	17 (34%)

As shown in **Table 3**, the average overall Mayo score was 4.16 ± 0.8, 8 ± 1.4, and 11.5 ± 0.5 in the mild, moderate, and severe groups, respectively. When comparing OPG readings taken from the control group and UC subgroups, a large and statistically significant difference was found, but OPG levels were not linked to the existence of EIM in a statistically significant way **(Table 4).**

Table (3): Components of Mayo Score in UC Group

Score	Stool Frequency	Rectal Bleeding	Mucosal Appearance at Endoscopy	Physician's Global Assessment
0	8 (16)	7 (14)	5 (10)	6 (12)
1	12 (24)	13 (26)	10 (20)	8 (16)
2	12 (24)	9 (18)	16 (32)	13 (26)
3	18 (36)	21 (42)	19 (38)	23 (46)

Table (4): Comparing OPG Levels (pg/ml) in UC Groups and Control

		Mean± SD
Disease Severity	Mild UC	135.3±33.7
	Moderate UC	288.6±77.4
	Severe UC	864.8±200
	Control	60.5±9.8
	***P* value* 0.0001**	
Extraintestinal Manifestations	No	383.7±332.9
	Yes	596.3±364.8
	***P* value 0.83**	

UC: Ulcerative colitis

As shown in **Table 5**, a statistically significant difference was observed in laboratory measurements between control group and UC subgroups except ASCA levels, which were similar across groups.
Furthermore, a statistically significant difference was observed between mild, moderate and severe cases of UC (using Bonferroni post-hoc test) except ASCA levels which had equal levels across the UC subgroups.
OPG levels were found to significantly correlate with other laboratory parameters including Hgb, WBC count, platelet count, ESR, CRP, albumin, and ANCA (Table 6).

Table (6): Correlation between OPG and Laboratory Measurements

Lab Parameters	Correlation Coefficient	*P* value*
Hgb	- 0.751	< 0.001
WBC Count	0.878	< 0.001
Platelet Count	0.678	< 0.001
ESR	0.860	< 0.001
CRP	0.843	< 0.001
Albumin	- 0.633	< 0.001
ANCA	0.841	< 0.001
ASCA	- 0.073	0.613

* Pearson test.

Receiver operating characteristic (ROC) analysis revealed that OPG is an excellent diagnostic test since area under the curve (AUC) equaled 0.965 ± 0.017, and *P* value was less than 0.0001. At a cut-off point of 97pg/ml, the test was found to have a sensitivity of 98% and a specificity of 87.2% **(Figure 1).**

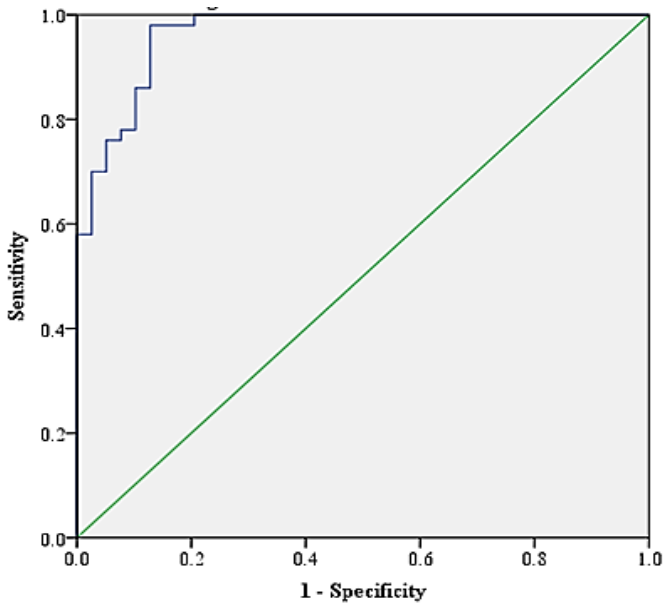

Figure (1): ROC Curve of OPG

By running a Spearman test, OPG levels were found to have a significant positive correlation with the severity of UC assessed by the Truelove and Witts criteria (r = 0.927, *P* < .001) (**Figure 2)**. OPG levels were also found to have a significant positive correlation with the Mayo endoscopic subscore (r = 0.655, *P* < .001), (**Figure 3)**.

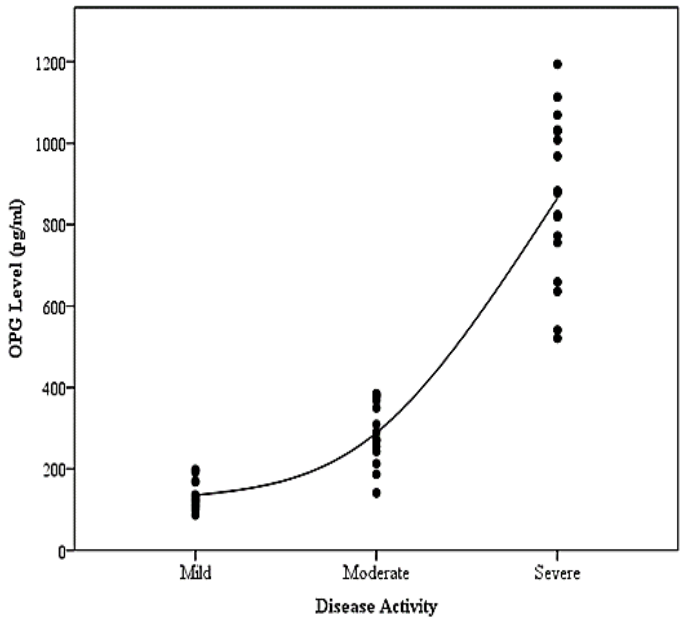

Figure (2): Correlation between OPG and Disease Activity

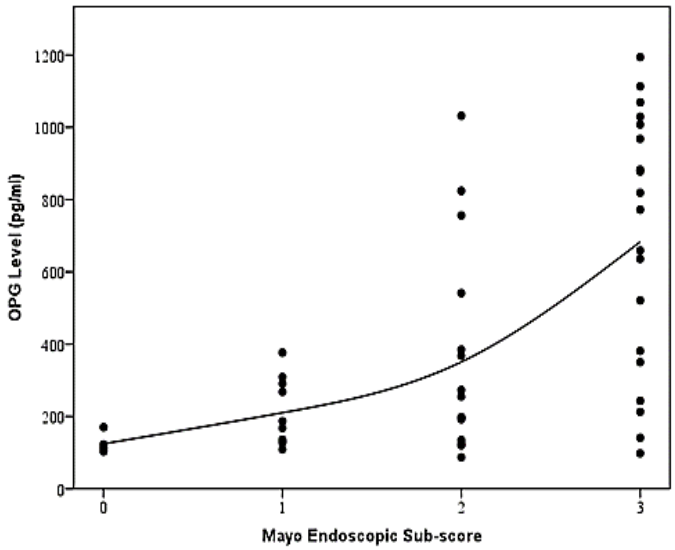

Figure (3): Correlation between OPG and Mayo Endoscopic score

DISCUSSION

Fifty patients who had ulcerative colitis (UC group) as well as 39 healthy controls (control group) had their serum osteoprotegerin levels measured and compared to disease activity.

Comparison of demographic characteristics of both groups revealed that none of the groups differed from one another significantly in terms of age, gender, or body mass index. However, smoking was more prevalent among the healthy group. Our results showed that age was slightly different between UC and control groups, which disagrees with literature [11], as previous studies reported that UC was significantly associated with age. This disagreement may be due to the predominance of males in our study, as it was found that UC increased with age in postmenopausal females. **Bertl *et al.*** [12] reported in their case control study that IBD cases were predominantly males and more smokers, but cases who had IBD and controls did not differ significantly as regards age and BMI as in our study. In accordance with our results, **Vahid *et al.*** [13] reported that in terms of age, gender, body mass index, and smoking status, no significant change was found between the UC and control groups.

In the current study, we assessed diseased activity using Truelove and Witts score. The average duration of disease activity was 6.4 ± 1.9 years, ranging from 2.2 to 9.5 years. In all patients with UC, 11 (22%) patients had associated extraintestinal manifestations, including ankylosing spondylitis (4%), primary biliary cirrhosis (4%), systemic lupus erythematosus (2%), erythema nodosum (6%), pyoderma gangrenosum (2%), and anterior uveitis (4%). According to Truelove and Witts Criteria, 19 (39%), 14 (28%), and 17 (34%) patients were diagnosed with mild, moderate, and severe disease activity, respectively. In the present study, the average overall Mayo score of mild, moderate, and severe groups was significantly different. The level of the OPG was slightly less than in other studies which may be explained by the shorter duration of the disease in the present study. **Miznerova *et al.*** [14] reported that the mean duration of disease was around 10 years. As regards severity in UC, 10 (33.3%) patients were mild, 12 (30%) were moderate and 8 (26.7%) were severe. Also, **Krela-Kaźmierczak *et al.*** [15] reported that the mean disease duration of UC was around 8 years.

The serum level of OPG differed significantly between IBD and the control group. Furthermore, positive correlations between OPG and Truelove and Witts UC severity scores and between OPG and Mayo endoscopic subscores were found. This coincides with the study by **Murad *et al.*** [16] who revealed that serum osteoprotegerin levels significantly correlated with Mayo score for ulcerative colitis. As well, this coincides with **Krela-Kaźmierczak *et al.*** [15] who demonstrated that the level of OPG varied remarkably between IBD and control groups. The present study also comes in agreement with the systematic review by **De Voogd *et al.*** [7] who reported that OPG serum level was significantly elevated in patients with UC and correlated positively with disease severity.

In line with findings of current study, **Salama *et al.*** [17] found that OPG, ESR, and CRP levels were considerably higher in patients with active illness compared to controls. Also, the present work showed that the hemoglobin level was significantly lower in cases with IBD. Furthermore, **Stanisławowski *et al.*** [18] reported a statistically significant rise in the mean blood OPG level between UC patients and controls. The study also reported that hemoglobin decreased in UC cases and CRP was significantly elevated in UC cases.

To test the diagnostic value of OPG in order to prove OPG's diagnostic worth for UC, a receiver operating characteristic (ROC) analysis was performed. The OPG was found to be an excellent diagnostic test where area under the curve (AUC) was over 0.9. The sensitivity of the test was 98% at a cutoff of 97pg/ml, while the specificity was 87.2%. This was similarly reported by **Murad *et al.*** [16] who showed that OPG's AUCs were more than 0.9 (P 0.001), indicating higher sensitivity and specificity.

In addition, the present study showed that OPG levels were found to significantly correlate with other laboratory parameters including Hgb, WBC count, platelet count, ESR, CRP, albumin, and ANCA. In agreement with the present study, **Sylvester *et al.*** [19] revealed that significant correlations were found between OPG, albumin and CRP. Also, **De Voogd *et al.*** [7] reported a positive relationship between OPG and platelet and leukocyte counts and a negative relationship with erythrocyte and hemoglobin counts.

CONCLUSION

The present study demonstrated that serum osteoprotegerin was significantly elevated in patients with UC and its concentration was positively correlated with disease severity. Hence, serum osteoprotegerin can be considered as a novel biomarker for assessing severity of ulcerative colitis in Egyptian patients.

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Competing interests: Nil.

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