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Significance of serological biomarkers in follow up of Inflammatory Bowel Disease patients. A Clinical, Endoscopic and Histopathologic study

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Abstract: The aim of this work is to investigate sensitivity and diagnostic accuracy of serum procalcitonin, serum iron and Red cell distribution width (RDW) for differentiating active from remission stages of inflammatory bowel diseases (IBD).60 Patients with confirmed IBD diagnosis (Crohn's and ulcerative colitis) were divided into 2groups (30 remission and 30 exacerbation) classified according to Truelove and Witts index in ulcerative colitis and Harvey Bradshaw index in crohn's disease. Routine diagnostic investigations, serum Procalcitonin and serum iron level were withdrawn. Serum procalcitonin was done by commercially available ELISA kits. The study showed significant difference between the 2 groups as regards serum iron where median in remission was 52 mcg/dl while in activity was 26 mcg/dl (P value:0.033), RDW (P value:0.014) and serum procalcitonin where mean in remission was 0.62 ng/ml while in activity was 0.98 ng/ml (P value:0.029). There was a positive significant correlation between CRP and degree of disease activity (P value: 0.01). There is negative non-significant correlation between serum iron and degree of disease activity (P value: 0.17). There is positive non-significant correlation between serum procalcitonin (P value:0.08), RDW (P value:0.28) and degree of disease activity. RDW, serum procalcitonin and serum iron in IBD patients need to be included in further studies with larger sample size for the possibility to be used as future laboratory markers for disease activity.

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Keywords: Procalcitonin; serum iron; RDW; Crohn's disease; Ulcerative Colitis; IBD

1. Introduction

Ulcerative colitis (UC) and Crohn's disease (CD) are inflammatory bowel diseases (IBD) that are generally complicated by systemic or local infection. Clinical, endoscopic, histological, and radiological investigations are typically necessary to make an accurate diagnosis and assessment of disease activity **[1].** IBD have always seemed to be rare in the Middle East and Northern Africa. No accurate registry or cohort of patients had ever studied the exact prevalence of CD and UC in these populations. In Mediterranean countries, the prevalence of UC was estimated at 5/100000 in urban areas [**2**]. However, marked increase in the frequency of IBD was noted in the last 10 years ratio of 6:1 for UC to CD in a recent Egyptian study [3].

Although some clinical activity indices are commonly used in IBD, specific and sensitive laboratory markers that correlate with disease activity and associated complication are still lacking **[4].** Traditional markers, such as C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and white blood cell (WBC) count, are still the most common markers used in clinical practice **[5].** C-reactive protein (CRP) is a widely used marker of inflammation and it has been shown to correlate with disease activity, especially in CD patients **[6]**. It increases rapidly during inflammatory processes and resolves early after amelioration of the inflammation. However, in UC patients, CRP response is usually moderate **[7].**

Anemia is the most prevalent extra intestinal complication of IBD **[8].** Serum iron was previously included in a number of studies as inflammatory marker of IBD. Red Cell Distribution Width (RDW) is a quantitative measurement of anisocytosis. Pro-inflammatory cytokines have been reported to inhibit

the maturation of erythrocytes, which is caused by erythropoietin. Thus, inflammation causes immature red blood cells to be released into the peripheral circulation, which may result in anisocytosis **[9].**

Procalcitonin (PCT), a pro-hormone of 116 amino acids, is the precursor for the calcium homoeostasis hormone, calcitonin which is found in the thyroid C cells and the pulmonary endocrine cells. It has been found to circulate at very low concentrations in normal serum **[10].** Procalcitonin plays a major role in systemic inflammation and induces a dose-dependent increase in TNFα secretion **[11].** Plasma level of PCT increases during bacterial infections and sepsis **[12].** There are some data showing that serum PCT level is a useful marker in many inflammatory disorders. A previous study showed that plasma concentrations of PCT appear to reflect the derangement in gut barrier function in patients with acute pancreatitis **[13],** and colorectal surgery [**14].** Several studies evaluated the role of procalcitonin in patients with inflammatory bowel diseases [**15 -19].** According to current evidence in the scientific literature, the clinical significance of measuring procalcitonin for diagnosing and monitoring IBD disease is rather elusive, and its association with disease severity is still confined to a limited number of studies [**20].**

2. Methodology

This case control study was conducted in IBD clinic at tropical medicine department, Ain Shams University, Cairo, Egypt. Sixty Patients with confirmed IBD (Crohn's (14), ulcerative colitis (43) and (3) with unclassified IBD) from January 2017 till January 2018 were included. They were divided into group A: which included 30 patients with IBD in remission (controls) and group B 30 patients with IBD in activity (cases) classified according to Truelove and Witts index in ulcerative colitis and Harvey Bradshaw index in crohn's disease. Ethical approval for study protocol was acquired from local institutional ethical committee.

Inclusion criteria:

1. Age between 18 to 60 years, including males and females.
2. Accepted participation in the current study and aim of the intervention, the expected outcome and possible complications and signed a written consent form.
3. Patients confirmed as IBD were included in the study: The diagnosis of IBD was established by a typical history, appropriate endoscopic and radiologic imaging studies as well as histopathological evaluations.

Exclusion criteria:

1-Patients proved to have inter current infection by stool analysis and culture.

2- Patients on iron therapy for any purpose.

3- Patients with other autoimmune diseases.

4- Patients with history or clinical suspicion of TB.

5- Patients with malignancy or terminal diseases.

Criteria of activity

Ulcerative colitis patients were categorized into mild, moderate and severe based on the Truelove and Witts' severity index **[21].**

Crohn's disease patients were classified according to Harvey Bradshaw index **[22]:**

The Harvey-Bradshaw index was devised in 1980 as a simpler version of the CDAI for data collection purposes. It consists of only clinical parameters.

A score of less than 5 is generally considered to represent clinical remission **[23].**

The diagnosis of IBD was confirmed by a typical history, appropriate endoscopic and radiologic imaging studies as well as histopathological evaluations. UC was diagnosed when there was evidence of a diffuse mucosal disease of colon with different proximal extensions from the rectum with or without backwash ileitis, superficial inflammation up to presence of ulcerations with histopathological evidence of ulcerative colitis like crypt abscess, cryptitis. CD was diagnosed if skip lesions were found at endoscopy; a cobblestone appearance; mucosal ulceration or aphthous lesions were found. Furthermore, radiologic evidence of skip lesions; fistulizing or stricturing diseases were suggestive of crohn's disease. All cases with crohn's disease were confirmed by histopathological examination and presence of non caseating granuloma.

Simplified Geboe's score **[24]** was applied for evaluation of histopathological activity in cases diagnosed as UC.

Endoscopic examinations were performed by 2 senior experts. Patholgical examination was done by a single gastrointestinal pathology expert. All patients were subjected to clinical evaluation and investigatory workup including CBC with RDW, ESR, CRP, serum albumin, stool analysis. In addition to the base line investigations, serum procalcitonin and serum iron level were assayed. Serum procalcitonin was done by commercially available ELISA kits**.**

Type of assay: sandwich enzyme-linked immune-sorbent assay technology.

Sampling:

Serum: samples were allowed to clot for 2 hours at room temperature or overnight at 4°C before centrifugation for 20 minutes at approximately 1000×g. The supernatant was collected and the assay was carried out immediately.

Assay Procedure

1. Standard, test sample and control (zero) wells on the pre-coated plate were set respectively, and then, record their positions. It was recommended to measure each standard and sample in duplicate. Washing plate was done 2 times before adding standard, sample and control (zero) wells.
2. Aliquot 0.1ml of 2000pg/ml, 1000pg/ml, 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.2pg/ml, standard solutions into the standard wells.
3. Addition of 0.1 ml of Sample / Standard dilution buffer into the control (zero) well was done.
4. Addition of 0.1 ml of properly diluted sample (Human serum, plasma, tissue homogenates and other biological fluids.) into test sample wells was done.
5. The plate was sealed with a cover and incubated at 37 °C for 90 min.
6. The cover was removed and the plate content was discarded, the plate was clapped on the absorbent filter papers or other absorbent material. The wells were not let completely dry at any time. The plate was not washed.
7. Addition of 0.1 ml of Biotin- detection antibody working solution into the above wells (standard, test sample & zero wells) was done. Addition of the solution at the bottom of each well without touching the side wall was done.
8. The plate was sealed with a cover and incubated at 37°C for 60 min.
9. The cover was removed, and the plate was washed 3 times with Wash buffer.
10. Addition of 0.1 ml of SABC working solution into each well was done, the plate was covered and incubated at 37°C for 30 min.
11. The cover was removed and plate washed 5 times with Wash buffer, and each time the wash buffer was let to stay in the wells for 1-2 min.
12. Addition of 90 µl of TMB substrate into each well was done, the plate was covered and incubated at 37°C in dark within 15-30 min. (Note: This incubation time is for reference use only, the optimal time should be determined by end user.) And the shades of blue can be seen in the first 3-4 wells (with most concentrated PCT standard solutions), the other wells show no obvious color.
13. Addition of 50 µl of Stop solution into each well and mixing thoroughly were done. The color changed into yellow immediately.
14. Reading of the O.D. absorbance at 450 nm in a microplate reader immediately after adding the stop solution was done.

Calculation of results:

For calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The PCT concentration of the samples can be interpolated from the standard curve.

Data Management and Analysis:

The collected data was revised, coded, tabulated and introduced to a PC using Statistical package for Social Science **(SPSS 20).** Data was presented and suitable analysis was done according to the type of data obtained for each parameter.

i. Descriptive statistics:

1. Mean, Standard deviation (± SD) and range for parametric numerical data, while Median and Interquartile range (IQR) for non parametric numerical data.
2. Frequency and percentage of non-numerical data.

ii. Analytical statistics:

1. **Student T** Test was used to assess the statistical significance of the difference between two study group means.
2. **Mann Whitney Test (U test)** was used to assess the statistical significance of the difference of a non parametric variable between two study groups.
3. **Chi-Square test** was used to examine the relationship between two qualitative variables.
4. **Fisher's exact test:** was used to examine the relationship between two qualitative variables when the expected count is less than 5 in more than 20% of cells.
5. **The ROC Curve (Receiver Operating Characteristic)** provides a useful way to evaluate the Sensitivity and specificity for quantitative Diagnostic measures that categorize cases into one of two groups.
6. **Correlation analysis (using Spearman's method):** To assess the strength of association between two quantitative variables. The correlation coefficient denoted symbolically "rs" defines the strength (magnitude) and direction (positive or negative) of the linear relationship between two variables.

- r= 0-0.19 is regarded as very weak correlation
- r=0.2-0.39 as weak correlation
- r=0.40-0.59 as moderate correlation
- r=0.6-0.79 as strong correlation
- r= 0.8-1 as very strong correlation

3. Results:

Regarding demographic features; that the mean age of IBD patients in remission is 35.6 years while mean age in activity is 33.7 years. There is no statistical significant difference between patients in remission and activity as regards sex. There is a significant difference between remission and activity as regards diagnosis where 90% of patients in activity have UC, while patients in remission 53.3% of

patients have UC and 40% have Crohn's. There is no statistical significant difference as regards disease onset between the 2 groups. **(Table 1)**

The comparison between patients in remission and activity as regards clinical findings revealed a significant difference between the 2 groups as regards pulse where only 6.7% of patients in remission have tachycardia versus 26.7% of patients in activity (P=0.038)**.**

Table 1: Demographic data of IBD patients

		Remission		Activity		test of significance	
		Mean/Median/N	SD/IQR/%	Mean/Median/N	SD/IQR/%	p value	sig.
Age		35.67	10.85	33.77	10.41	0.492 (T)	NS
Gender	Male	14	46.7%	14	46.7%	1 (C)	NS
	Female	16	53.3%	16	53.3%		
Diagnosis	UC	16	53.3%	27	90.0%	0.003 (F)	S
	Crohn's	12	40.0%	2	6.7%		
	UC & Crohn's Unclassified	2	6.7%	1	3.3%		
Onset of the disease (years)		5	2 - 9	4	2 - 8	0.415 (M)	NS

F: Fisher exact test C: Chi square test T: t test M: Mann Whitney

The correlation between laboratory data and patients in remission and activity showed a significant difference as regards hemoglobin level where 20% of patients in remission have hemoglobin <10.5 while 46.7% of patients in activity have hemoglobin level <10.5. Also there is significant difference as regards ESR where in 26.7% of patients in remission have ESR >30 versus 73.3% of patients in activity **(Table 2).**

Table 2: Comparison between patients in remission and activity regarding laboratory data.

		Remission		Activity		test of significance	
		N / Mean	% / SD	N / Mean	% / SD	p value	sig.
Hb	normal	24	80.0%	16	53.3%	0.028 (C)	S
	<10.5	6	20.0%	14	46.7%		
WBC	normal	28	93.3%	29	96.7%	0.492 (F)	NS
	elevated	0	0.0%	1	3.3%		
	reduced	2	6.7%	0	0.0%		
PLt	normal	28	93.3%	24	80.0%	0.254 (F)	NS
	elevated	2	6.7%	6	20.0%		
ESR	normal	22	73.3%	8	26.7%	<0.001 (C)	S
	>30	8	26.7%	22	73.3%		
Albumin		3.73	0.66	3.52	0.29	0.118 (T)	NS

F: Fisher exact test, C: Chi square test, T: t test

As regards the current treatment**;** there is significant difference only in surgical treatment where 33.3% of patients in remission underwent surgery while only 6.7% of patients in activity did (**Table 3)**.

Table 3: Comparison between patients in remission and activity as regards the current treatment

		Remission		Activity		Chi square test	
		N	%	N	%	p value	sig.
surgery	no	20	66.7%	28	93.3%	0.010 (C)	S
	yes	10	33.3%	2	6.7%		
steroid	no	10	33.3%	6	20.0%	0.243 (C)	NS
	yes	20	66.7%	24	80.0%		
5-ASA	no	6	20.0%	5	16.7%	0.739 (C)	NS
	yes	24	80.0%	25	83.3%		
Immunosupp	no	14	46.7%	12	40.0%	0.602 (C)	NS
	yes	16	53.3%	18	60.0%		
Biological	no	22	73.3%	20	66.7%	0.573 (C)	NS
	yes	8	26.7%	10	33.3%		

F: Fisher exact test C: Chi square test T: t test

Regarding the extent of disease, 63% of US patients in activity have extensive colonic involvement, 22.2% have rectosigmoidal affection and 14.8% have left sided affection.

While in Crohn's disease patients in activity were only 2 and both of them had ileocolonic affection.

Endoscopic findings revealed significant difference between endoscopic pattern in remission and in activity as regards vascular pattern where 44.4% of patients in remission have partial to complete loss of vascular pattern in contrast to 60.7% of patients in activity. Also there is significant difference as regards bleeding on endoscopic examination where 77.8% of patients in remission have no bleeding where 57.1% of patients in activity have mucosal bleeding. There is significant difference as regards erosions and ulcers where 55.6% of patients in remission have no erosions and ulcers in contrast to92.9% in activity **(Table 4).**

Table 4: Comparison between patients in activity and remission (UC) as regards endoscopic findings:

		Remission		Activity		test of significance	
		N	%	N	%	p value	sig.
Vascular pattern	normal	10	55.6%	11	39.3%	0.022 (F)	S
	partial loss	0	0.0%	9	32.1%		
	complete loss	8	44.4%	8	28.6%		
Bleeding	no	14	77.8%	12	42.9%	0.020 (C)	S
	mucosal	4	22.2%	16	57.1%		
	mild luminal	0	0.0%	0	0.0%		
	mod/ severe luminal	0	0.0%	0	0.0%		
Erosions & ulcer	no	10	55.6%	2	7.1%	0.001 (F)	S
	erosions	0	0.0%	5	17.9%		
	sup ulcer	8	44.4%	20	71.4%		
	deep ulcer	0	0.0%	1	3.6%		

F: Fisher exact test C: Chi square test

Regarding correlation of serum activity markers with scoring of disease activity there is significant correlation (positive) between CRP and degree of disease activity. There is significant correlation (negative) between serum albumin and degree of disease activity. There is correlation (positive) between serum procalcitonin and RDW and degree of disease activity. There is non-significant (negative) correlation between serum iron and degree of disease activity **(Tble 5).** There is significant difference in ESR level with disease activity where 37.5% of patients in mild activity have ESR >30, 80% of patients in moderate activity have ESR >30 and 100% of patients in severe activity have ESR >30.

Table 5: correlation of CRP, serum iron, procalcitonin, serum albumin and RDW with scoring of disease activity.

N= 30		CRP	S. Iron	procal	RDW	Albumin
Scoring	rs	0.44	-0.25	0.32	0.20	-0.36
	p value	0.01	0.17	0.08	0.28	0.05
	sig.	S	NS	NS	NS	S

We found that CRP at cut off >6 was able to differentiate activity from remission with sensitivity of 56.6% and specificity of 66.67%**.** RDW at cut off >15.7 was able to differentiate activity from remission with sensitivity of 66.7% and specificity of 93.33%**.** Serum iron at cut off <= 21 mcg/dl was able to differentiate activity from remission with sensitivity of 50% and specificity of 93.33%. Procalcitonin at cut off >0.7 (ng/ml) was able to differentiate activity from remission with sensitivity of 56.7% and specificity of 73.3%**.** The ability of RDW and serum iron to differentiate activity from remission was statistically significant **(Table 6).**

Regarding correlation of serum activity markers with microscopic Geboe's score; only Procalcitonin showed a statistical significant correlation with esinophils (Grade 2A) (P value= 0.035).

Although there was no statistical significant correlation between serum procalcitonin and other grades, yet there is noticeable increase in the mean±SD of procalcitonin with increased severity of each grade **(Figures 1-6), (Table 7).**

Table 6: The ability of serum markers to discriminate activity from remission

Activity marker	AUC	95% CI	Cutoff point	sensitivity	Specificity	p value	sig.
CRP	0.601	0.467 to 0.725	>6 *	56.6	66.67	0.171	NS
RDW	0.806	0.683 to 0.896	>15.7 *	66.7	93.33	0.0001	S
Serum iron	0.66	0.562 to 0.777	<=21 *	50	93.33	0.03	S
procalcitonin	0.623	0.489 to 0.745	>0.7 *	56.7	73.33	0.101	NS

Table 7: correlation of procalcitonin with Geboe's score

		Procalcitonin				ANOVA or T-Test	
		N	Mean	±	SD	F or T	P-value
Grade 0 Architectural changes And MNC	No abnormalities	1	0.100	±	0.000	F= 1.466	0.281
	Presence of architectural changes	3	0.917	±	0.711		
	Presence of architectural changes and chronic mononuclear cell infiltrate	8	1.275	±	0.667		
Grade 1 Basal plasma cells	Mild increase	5	1.010	±	0.906	T= -0.308	0.764
	Marked increase	7	1.143	±	0.597		
Grade 2A Esinophils	No increase	6	0.675	±	0.567	T= -2.440	0.035*
	Mild increase	6	1.500	±	0.603		
Grade 2B Neutrophils in LP	Mild increase	7	1.036	±	0.844	T= -0.288	0.779
	Marked increase	5	1.160	±	0.537		
Grade 3: cryptitis	None	1	0.100	±	0.000	F= 1.209	0.343
	< 50% crypts involved	7	1.107	±	0.706		
	> 50% crypts involved	4	1.300	±	0.663		
Grade 4 Crypt abscess and surface ulcerations	None	1	0.100	±	0.000	F= 1.729	0.232
	Marked attenuation	6	0.992	±	0.697		
	Probable crypt destruction: probable erosions	5	1.400	±	0.616		

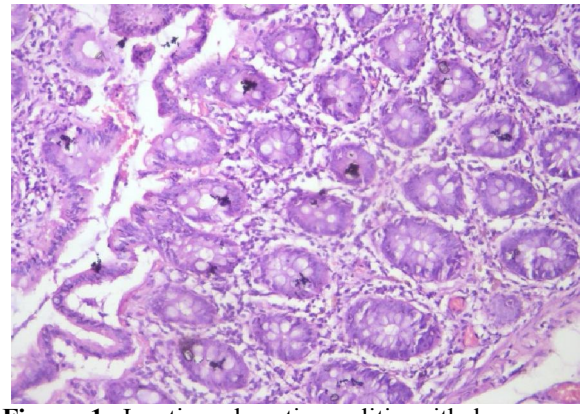


Figure 1: Inactive ulcerative colitis with low serum calcitonin level (0.100) showing intact epithelium, absent crypt distortion, mild mononuclear inflammatory infiltrate, absent cryptitis and crypt abscess. (H & Ex200)

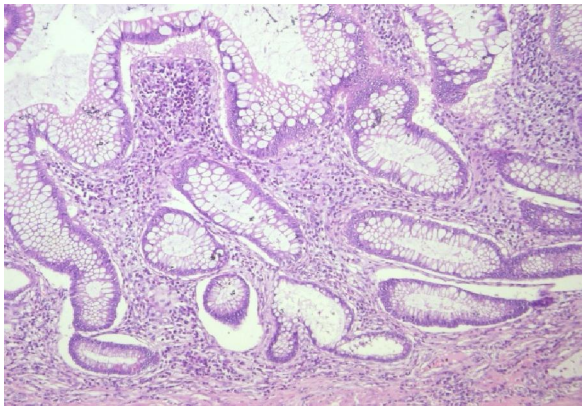


Figure 2: a case of active Ulcerative colitis with crypt distortion, chronic mononuclear infiltrate, mild esinophils) (Geboes score 0.2) with moderately elevated procalcitonin level (0.675) (H & Ex200)

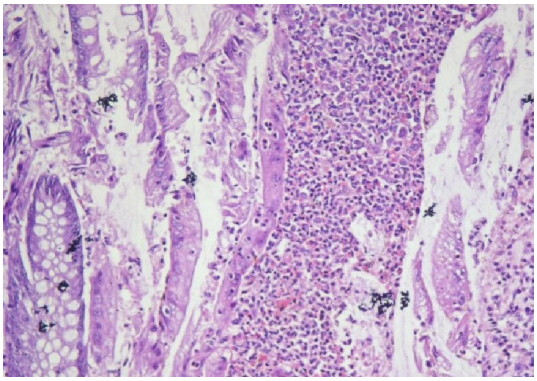


Figure 3: Active ulcerative colitis showing cryptitis (Geboes score 3.2) and dense neutrophilic infiltrate in lamina propria (Geboes score 2B.2). Serum procalcitonin in this case was markedly elevated (1.500) (H & Ex200)

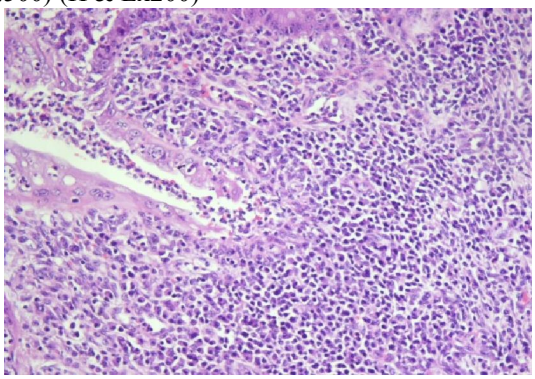


Figure 4: Active ulcerative colitis with evident cryptitis showing neutrophils infiltrating and destroying epithelium with subsequent formation of crypt abscess "Geboes score 4.3". The lamina propria shows heavy mononuclear inflammatory cells with marked increase in basal plasma cells (Geboes score 1.2). Serum procalcitonin level was high (1.400) (H & Ex200)

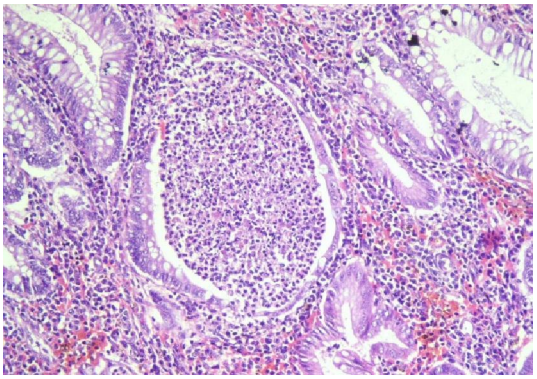


Figure 5: show crypt destruction by dense neutrophilic infiltrate and crypt abscess formation. "Geboes score 4.3". serumprocalcitonin level was markedly elevated (1.700) (H & Ex200)

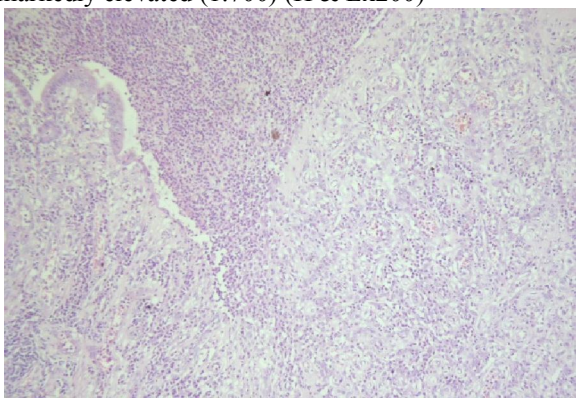
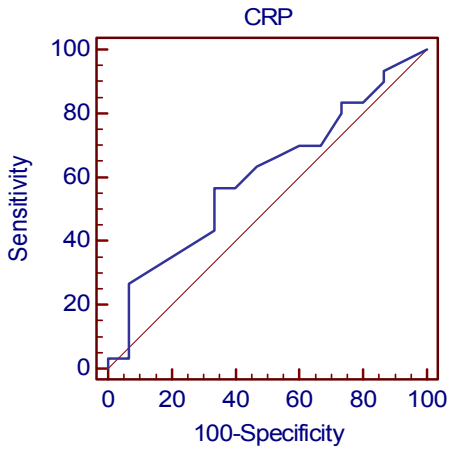


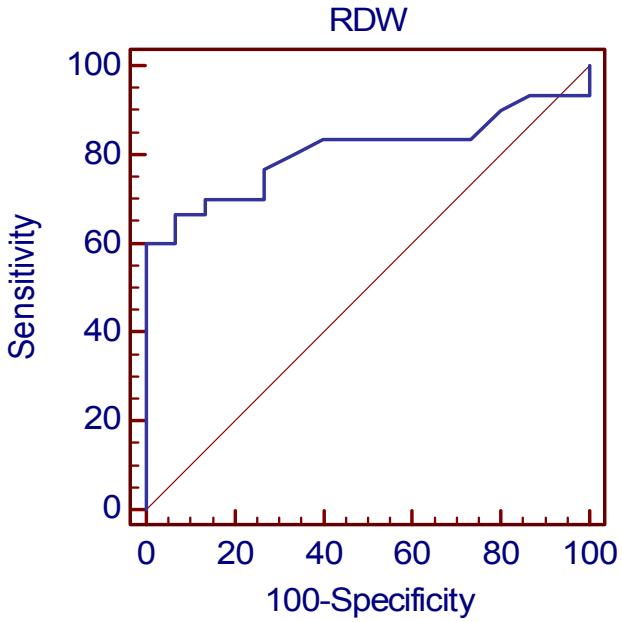
Figure 6: show marked surface epithelial ulcerations covered by fibrin purulent exudates (Serum proclacitonin was markedly elevated (1.701) (H & Ex200)



AUC	95% CI	Cutoff point	sensitivity	Specificity	p value	sig.
0.601	0.467 to 0.725	>6 *	56.6	66.67	0.171	NS

Curve 1: ROC curve of the ability of CRP to differentiate activity from remission.

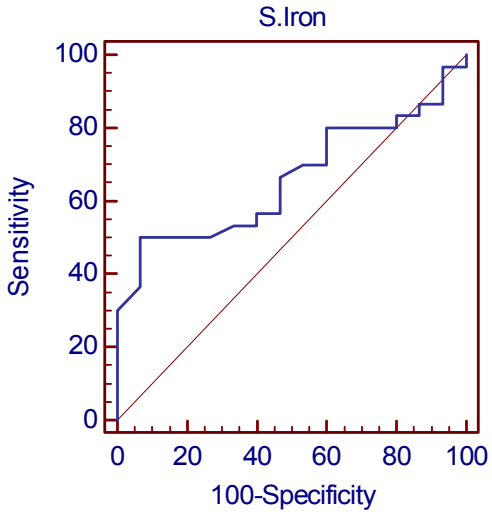
Shows that CRP at cut off >6 was able to differentiate activity from remission with sensitivity of 56.6% and specificity of 66.67%.



AUC	95% CI	Cutoff point	sensitivity	Specificity	p value	sig.
0.806	0.683 to 0.896	>15.7 *	66.7	93.33	0.0001	S

Curve 2: ROC curve of the ability of RDW to differentiate activity from remission.

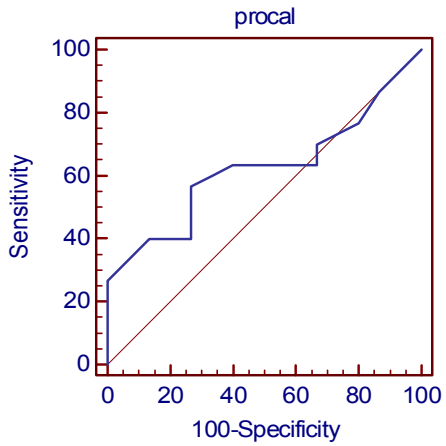
Shows that RDW at cut off >15.7 was able to differentiate activity from remission with sensitivity of 66.7% and specificity of 93.33%.



AUC	95% CI	Cutoff point	sensitivity	specificity	p value	sig.
0.66	0.562 to 0.777	<=21 *	50	93.33	0.03	S

Curve 3: ROC curve of the ability of serum iron to differentiate activity from remission.

Shows that serum iron at cut off <= 21 was able to differentiate activity from remission with sensitivity of 50% and specificity of 93.33%.



AUC	95% CI	Cutoff point	sensitivity	specificity	p value	sig.
0.623	0.489 to 0.745	>0.7 *	56.7	73.33	0.101	NS

Curve 4: ROC curve of the ability of procalcitonin to differentiate activity from remission.

Shows that procalcitonin at cut off >0.7 was able to differentiate activity from remission with sensitivity of 56.7% and specificity of 73.3%.

4. Discussion

Endoscopy with biopsy and histological evaluation remains the gold standard method for detecting and quantifying bowel inflammation. This technique is expensive, invasive and not well tolerated by patients as the need for repeated examinations affects their quality of life. Thus, it is clear that a simple, rapid, sensitive, specific, inexpensive, and non-invasive marker to detect and monitor intestinal inflammation, both in general but especially in IBD, is needed **[25].**

According to the available scientific literature, the clinical significance of procalcitonin for diagnosing IBD or monitoring disease activity remains elusive, and its association with disease severity is confined to a limited number of case-control studies, with low sample size **[20].**

Also previously published studies indicate that RDW increases during inflammation **[26].** Impaired iron absorption or increased loss of iron was found to correlate with disease activity and markers of inflammation in inflammatory bowel disease (IBD) **[27].**

This study aimed to evaluate sensitivity and specificity of serum procalcitonin, serum iron and red cell distribution width (RDW) as novel biological markers of activity in IBD patients in relation to clinical, biological, endoscopic and radiological findings.

This study included 60 patients diagnosed as IBD, among them 43 were diagnosed as ulcerative colitis and 14 Crohn's disease while 3 were unclassified, this was similar to results observed from different parts of the world, where UC is much more common than CD. In an Egyptian study (**3**), the ratio of patients diagnosed with UC to patients diagnosed with CD was approximately 6:1. In northeastern Poland, this ratio was approximately 15:1, and an increase in the total number of cases diagnosed with IBD has been reported **[28].**

This study population included 32 female and 28 male with no significant differences between genders of the active and remission groups. In literature the incidence of IBD in established populations is similar in men and women but is influenced by race and ethnicity [**29].**

The mean age of patients in activity and in remission is 35.6 and 33.7 respectively (4^{th} decade). It was reported that the peak age at onset for CD was between 20 and 30 years and that for UC was between 30 and 40 years **[30].**

As regards disease presentation in the 2 study groups, patients in activity have more frequent abdominal pain (P =0.003), diarrhea (P <0.001), bleeding per rectum (P<0.001), tenesmus (P=0.037) and tachycardia (P= 0.038) than patients in remission, this agrees with the clinical indices of severity for IBD. The cardinal symptom of UC is bloody diarrhea, sometimes accompanied with abdominal pain or frequent bowel movements.

The cardinal symptoms of CD are similar to UC. Patients frequently present with chronic abdominal pain and/or diarrhea **[31].**

The mean ESR levels in our study were significantly higher in patients with active IBD and disease activity compared to those in remission (P <

0.001). These results were similar to the that of **Ipek et al. [26].** On contrary, **Koido et al., [17]** found the ESR in severe UC was not significantly higher than in mild-to-moderate UC (P = 0.0697).

Regarding the serum procalcitonin level, it was found to be significantly higher in patients with IBD activity with a mean of 0.98 ng/ml than those in remission with a mean of 0.62 ng/ml (P value 0.029). This was similar to a study by **Oussalah et al., [16]** that showed that the median value of serum procalcitonin was significantly higher in patients with active IBD (0.1 ng/mL; IQR 25–75th, 0.07 to 0.21) in comparison with those with inactive disease (0.07 ng/ml; IQR 25–75th, 0.06 to 0.08) (P = 0.02).). Similarly, in the study of **Koido et al., [17],** PCT was ~3-fold higher in patients with active disease. Also**, Nishio et al., [19]** found that PCT was ~3-fold higher in patients with active disease. In contrast **Chung et al., [18], Ge et al., [32] and Hosomi et al., [33]** found that PCT was non-significantly higher in patients with active disease.

Regarding the correlation between procalcitonin and the degree of disease activity in both UC & Crohn's disease we found that there is positive non-significant correlation between serum procalcitonin and degree of disease activity (r =0.32) (P value 0.08), Also we found that at a cut off value >0.7 ng/ml serum procalcitonin was able to differentiate activity from remission with sensitivity of 56.7% and specificity of 73.3%. This agreed with **Oussalah et al., [16]** who found that, in patients with CD (n=30), procalcitonin was strongly correlated with disease activity marker namely, CDAI (Crohn's Disease Activity Index) (r = 0.545, P = 0.002), In patients with UC (n = 27), procalcitonin was correlated with the SCCAI (Simple Clinical Colitis Activity Index) (r = 0.423, P = 0.03)**. Oussalah et al. [16],** by using ROC analysis, were able to identify a serum procalcitonin cut-off of 0.14 ng /ml as having a high accuracy (sensitivity, 100%; specificity, 95%) for detecting severe forms of CD as defined by a CDAI ≥300.

In contrast, **Thia et al., [34] and Oruc et al [15]** found non significant correlation between Procalcitonin levels and CD and UC activity with poor accuracy. But procalcitonin may be used as predictor for bacterial infection in IBD patients **[20].**

As regards serum iron in IBD patient, we found that there is a significant difference between the 2 groups (P value 0.033), and that there is a negative non-significant correlation between serum iron and degree of disease activity (r = -0.25) (P value 0.17). Iron deficiency anemia and anemia of chronic disease are the most common type of anemia in IBD patients **[35]**. Some authors reported impaired intestinal iron absorption correlated with disease activity and markers of inflammation in patients with CD**.** In a study by **Bengi et al [35]** on 257 CD and 208 UC patients, anemia was detected at a higher rate in those with active disease and this authors reported that that the frequency of anemia increases with increased clinical activity in IBD**.** ROC curve shows that serum iron at cut off ≤ 21 mcg/dl was able to differentiate activity from remission with sensitivity of 50% and specificity of 93.33%.

As regards CRP, we found that there is no significant difference between the 2 groups as regards CRP (P value 0.176), in contrast to **Ipek et al. [26]** who found that CRP levels were significantly increased in patients with active UC compared to the patients in remission (P <0.001). There is remarkable heterogeneity in the CRP response between CD and UC. Whereas CD is associated with a strong CRP response, UC has only a modest to absent CRP response **Vermeire et al. [36]** which justifies our results as most of the study population was Ulcerative colitis.

As regards serum albumin, we found that there is negative significant correlation between serum albumin and degree of disease activity (P= 0.05) (r = -0.36), this is consistent with the fact that Albumin is a typical example of a negative acute phase reactant and decreased levels may be found during inflammation. However, other conditions such as malnutrition and malabsorption also cause low albumin levels **[36].**

5. Conclusion

New serum biomarkers for IBD activity and differentiation between exacerbation and remissions as RDW, Serum procalcitonin and serum iron could be used efficiently after more large scale studies validation.

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