***Original Research Article***

# Recent Fecal Biomarker for Inflammatory Bowel Disease, Neutrophil Gelatinase Associated Lipocalin


## Abstract

**Background:** In the field of IBD Many biomarkers have been studied to find the ideal noninvasive biomarker that best correlate with disease severity and prognosis.

NGAL is a biomarker that have been studied and measured either in serum or fecal in different pathological conditions AKI, IBD, autoimmune disorder

In our study we aim to evaluate the accuracy of fecal NGAL levels in correlation with endoscopic scoring, and common serum inflammatory markers in patients with IBD.

**Methods:** In this randomized prospective controlled study Fecal NGAL levels were measured using Elisa technique in 30 patients with ulcerative colitis 10 patients with Crohn's disease and 20 healthy subjects. the results were correlated to colonoscopic severity index and commonly used serum inflammatory markers (highly sensitive CRP, ESR, white blood cell count).

**Results:** Fecal NGAL levels (median and interquartile range) were significantly elevated in active ulcerative colitis (UC) 6.05 (3.6–15.1) mg/kg and Crohn's disease (CD) 4.9 (1.5– 7.7) mg/kg, compared to healthy controls (HC) 0.3 (0.1–0.4) mg/kg. Sensitivity and specificity were 94.7% and 95.7%, respectively, for distinguishing between active IBD and HC.

**Conclusions:** Fecal NGAL is noninvasive biomarker that strongly correlate to different parameters of disease activity in IBD patients. As existing biomarkers are expressed mainly in granulocytes, NGAL's epithelial localization may give supplementary diagnostic information.

**Keywords:** Fecal Biomarker, Inflammatory Bowel Disease, Neutrophil Gelatinase Associated Lipocalin.

## Introduction

Inflammatory bowel disease (IBD) is a group of autoimmune disease with gastrointestinal and extra gastrointestinal manifestation with different disease activity and presentations [1].

Colonoscopy remains the golden standard for diagnosis and follow up but in some conditions like Crohn's of small intestine will be difficult for regular follow up, colonoscopy is invasive procedure which not very well tolerated by the patients [2].

The non-invasive inexpensive serologic tests are usually used to assess the degree of inflammation and to track the activity of the disease, because no single method proved to be effective in measuring the activity of the disease other biomarkers alone or in combination may improve the accuracy in diagnosing and monitoring IBD [3].

Fecal calprotectin is well-established IBD biomarker that have good correlation with inflammation in IBD and, important for clinical practice but this test has variable sensitivity and specificity that has been reported in meta-analyses

NGAL's small size (25 kDa) protein that show relative stability and expressed in different cells including neutrophilic granulocytes, and epithelium of the gastrointestinal, respiratory, and urogenital tracts and strongly expressed in the intestinal epithelial cell layer during inflammation LCN2, the coding gene for NGAL, is one of the most over- expressed genes in the colonic mucosa in ulcerative colitis (UC) and CD compared with healthy individuals [4, 5]. The mucosal distribution of inflammation makes this protein markedly different from the most studied fecal IBD biomarkers [4].

## Patients and Methods:

This randomized prospective controlled study was carried out at Endoscopy unit of internal medicine department at Tanta University Hospital after approval from Ethical Committee and obtaining informed written consent. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki (6th revision, 2008).

Forty adult cases with definitive diagnosis of UC or Crohn's disease (confirmed by clinical, endoscopic, and histological workup) were included.

Patients with comorbid disease that may affect laboratory data and inflammatory mediator such as liver cirrhosis, renal failure, pregnant female, patient with diabetes were excluded.

Subjects were divided into 3 groups as the following: group A (20 healthy cases who had normal colonoscopy serving as control group), group B (10 patients who were previously diagnosed as CD) and group C (30 patients who were previously diagnosed as UC).

**All Cases Were Subjected to the Following:** history taking, complete clinical examination, Laboratory investigations including Complete blood count, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) levels and liver and kidney function tests.

Fecal NGAL estimation using ELISA technique using a sterile container Fresh fecal sample Then, the test samples were isolated soon after collection then analyzed within 2hours.

Colonoscopy with biopsy: Endoscopy were performed by experienced gastroenterologists blinded to the results of the NGAL High-definition video scope using cope (epk©i.scan 5000) was used in all examinations (Pentax medical. Japan). Biopsies were obtained from inflamed or healed colonic mucosa or from random sites if inflamed area wasn't found the endoscopic disease activities were assessed Montreal classification of severity of IBD (0: Inactive disease and normal mucosa, 1: Mild, 2: moderate, 3: Severe disease)

**Statistical analysis:**

Statistical analysis of the present study was conducted, using SPSS version 26 (IBM®, USA). Quantitative variables were expressed as mean and standard deviation (SD) and compared using F test. Quantitative non- parametric data (e.g., NGAL) were presented as median and interquartile range (IQR). Categorial variables were expressed as frequency and percentage and analyzed by Chi-square test. The overall diagnostic performance was assessed by ROC curve analysis. P value ≤ 0.05 was considered statistically significant.

## Results

The incidence was higher in the third decade of life and decrease incidence with advanced aging. There were 3 female patients and 7 male patients in the CD group and in the UC group 14 patients were female and 16 patients were male with male predominance was high in the studied groups. **Table 1**

**Table 1: Comparison between the three studied groups according to demographic data.**

| | | Control (n=20) | | CD (n=10) | | UC (n=30) | | Test of sig. | P |
|---|---|---|---|---|---|---|---|---|---|
| | | No. | % | No. | % | No. | % | | |
| **Sex** | Female | 8 | 40.0 | 3 | 30.0 | 14 | 46.7 | $X^2$=0.853 | 0.471 |
| | Male | 12 | 60.0 | 7 | 70.0 | 16 | 53.3 | | |
| **Age (years)** | Min. – Max. | 18.0 – 57.0 | | 25.0 – 45.0 | | 18.0 – 50.0 | | F=1.046 | 0.358 |
| | Mean ± SD. | 34.65 ± 11.69 | | 31.10 ± 5.43 | | 30.90 ± 8.55 | | | |

Abdominal pain and diarrhea were the main presentations of CD, one patient was presented by fever or bleeding per rectum. In the UC group the main presentations were diarrhea and fresh bleeding per rectum, 2 patients presented by fever and 8 patients presented by abdominal pain. **Table 2**

**Table 2: The main clinical presentation of the two studied groups.**

| | CD (n=10) | | UC (n=30) | |
|---|---|---|---|---|
| | No. | % | No. | % |
| **Bleeding per rectum** | 1 | 10.0 | 20 | 66.7 |
| **Diarrhea** | 4 | 40.0 | 30 | 100.0 |

| | | | | |
|---|---|---|---|---|
| **Fever** | 1 | 10.0 | 2 | 6.7 |
| **Abdominal pain** | 10 | 100.0 | 8 | 26.7 |

Levels of ESR was markedly elevated in patient with IBD in comparison to control group (p-value <0.001). **Table 3**

CRP level was important inflammatory marker that was significantly elevated in the diseased group in comparison to control group suggesting that CRP is an important marker in diagnosis and monitoring of disease severity (p-value < 0.001). **Table 3**

Hemoglobin level was severely decreased in the affected groups in comparison to the control group suggesting that iron deficiency anemia is an important predictor of IBD (p-value < 0.001). **Table 3**

leukocytosis was found in patient with IBD and WBCs were significantly elevated in the affected group in comparison to control group suggesting that elevation of WBCs count act as important biomarker that correlate with severity of inflammation (p-value<0.001). **Table 3**

NGAL level as inflammatory marker that was significantly elevated in IBD in comparison to control group suggesting the important role of NGAL as inflammatory biomarker in diagnosis and monitoring the disease severity of IBD (p-value <0.001). **Table 3**

**Table 3: Comparison between the three studied groups**

| | **Control (n=20)** | **CD (n=10)** | **UC (n=30)** | **F** | **P** |
|---|---|---|---|---|---|
| **ESR 1st hour (mm/hour)** | 10.0 – 30.0 | 40.0 – 90.0 | 40.0 – 100.0 | **45.499*** | **<0.001*** |
| | 17.0 ± 6.16 | 54.50 ± 19.64 | 52.27 ± 15.03 | | **p1<0.001*** **p2<0.001*** |
| **ESR 2nd hour (mm/hour)** | 20.0 – 40.0 | 50.0 – 120.0 | 70.0 – 120.0 | **114.434*** | **<0.001*** |
| | 27.80 ± 7.64 | 70.80 ± 21.34 | 80.57 ± 10.80 | | **$p_1$<0.001*** **$p_2$<0.001*** |
| **CRP (mg/L)** | 1.0 – 6.0 | 10.0 – 96.0 | 16.0 – 106.0 | **13.955*** | **<0.001*** |
| | 3.40 ± 1.57 | 40.50 ± 29.23 | 31.40 ± 25.25 | | **$p_1$=0.001*** **$p_2$<0.001*** |
| **Hemoglobin (g/dl)** | 11.0 – 16.0 | 10.0 – 12.50 | 7.0 – 11.0 | **25.711*** | **<0.001*** |
| | 12.02 ± 1.14 | 10.69 ± 0.74 | 10.03 ± 0.89 | | **$p_1$=0.002*** **$p_2$<0.001*** |
| **WBCs** | 4.0 – 10.0 | 7.0 – 13.0 | 8.0 – 16.0 | **23.415*** | **<0.001*** |
| | 5.74 ± 1.37 | 9.67 ± 2.52 | 10.04 ± 2.61 | | **$p_1$<0.001*** **$p_2$<0.001*** |

| | | | | | |
|---|---|---|---|---|---|
| **NGAL (mg/kg)** | 0.34 – 0.89 | 0.77 – 2.32 | 0.47 – 3.92 | **51.319*** | **<0.001*** **$p_1$=0.005*** **$p_2$<0.001*** |
| | 0.43 ± 0.15 | 1.34 ± 0.52 | 2.49 ± 0.95 | | |

**$p_1$:** p value for comparing between Control and CD
**$p_2$:** p value for comparing between Control and UC

Colonoscopy had been done to all patient and colonoscopic finding as regard distribution of the lesion and area affected of the colon was studied and illustrated that in CD group, 3 patients with mild disease,6 patients with moderate disease and one patient with sever disease. In UC group, 8 patients with mild disease, 20 patients with moderate disease and 2 patients with severe disease. **Table 4**

**Table 4: Colonoscopic finding using Montreal classification in the studied groups**

| | **CD (n=10)** | | **UC (n=30)** | |
|---|---|---|---|---|
| | **No.** | **%** | **No.** | **%** |
| **Mild (E1)** | 3 | 30.0 | 8 | 26.7 |
| **Moderate (E2)** | 6 | 60.0 | 20 | 66.7 |
| **Severe (E3)** | 1 | 10.0 | 2 | 6.7 |

The inflammatory marker ESR&CRP were elevated with elevation of NGAL level suggesting significant correlation between NGAL level and inflammatory mediator. There was a significant positive correlation between WBCs count and NGAL level in the two patients' groups (CD and UC). The severity of the disease was increased with elevation of NGAL level suggesting that a significant correlation is present between NGAL level and colonoscopic severity. **Table 5**

**Table 5: Correlation between NGAL level and different parameters in each group.**

| | **NGAL** | | | |
|---|---|---|---|---|
| | **CD (n=10)** | | **UC (n=30)** | |
| | **r** | **P** | **r** | **p** |
| **ESR 1st hour** | 0.951 | **<0.001*** | 0.460 | **0.010*** |
| **ESR 2nd hour** | 0.894 | **<0.001*** | 0.366 | **0.047*** |
| **CRP** | 0.757 | **0.011*** | 0.544 | **0.002*** |
| **WBCs** | 0.740 | **0.014*** | 0.580 | **0.001*** |
| **Colonoscopy** | 0.658 | **0.038*** | 0.473 | **0.008*** |

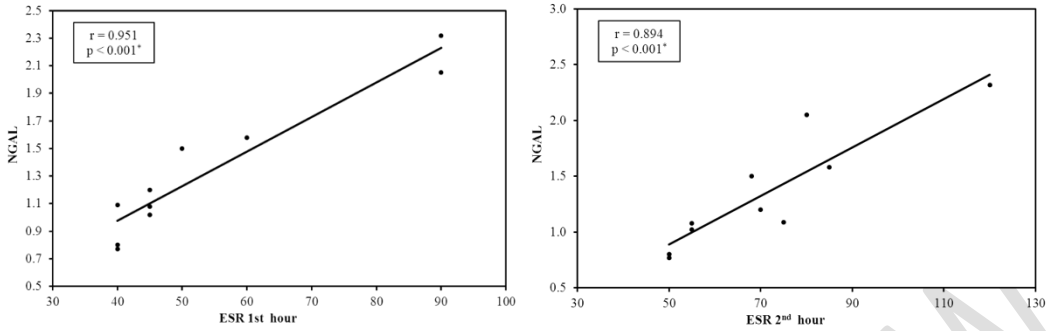


**Figure 1: Correlation between NGAL level and ESR first and second hour level in CD group.**

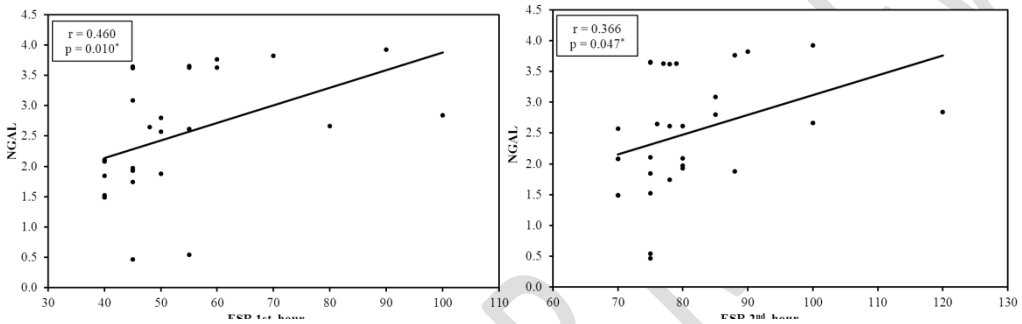


**Figure 2: Correlation between NGAL level and ESR first and second hour level in UC group.**

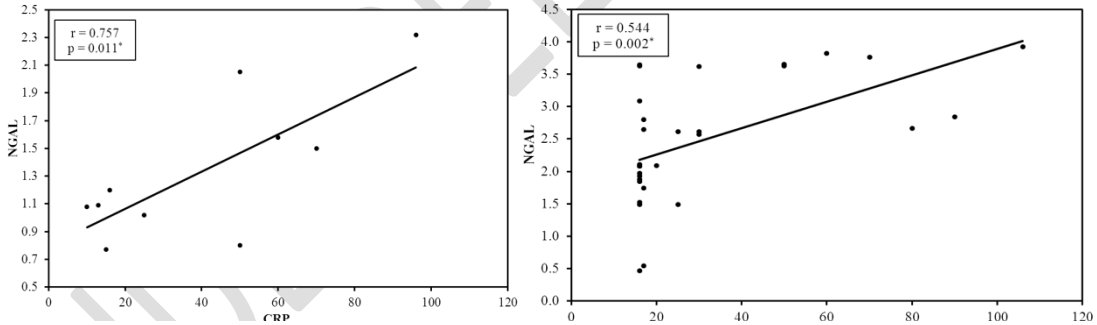


**Figure 3: Correlation between NGAL level and CRP level in UC AND CROHNS group.**

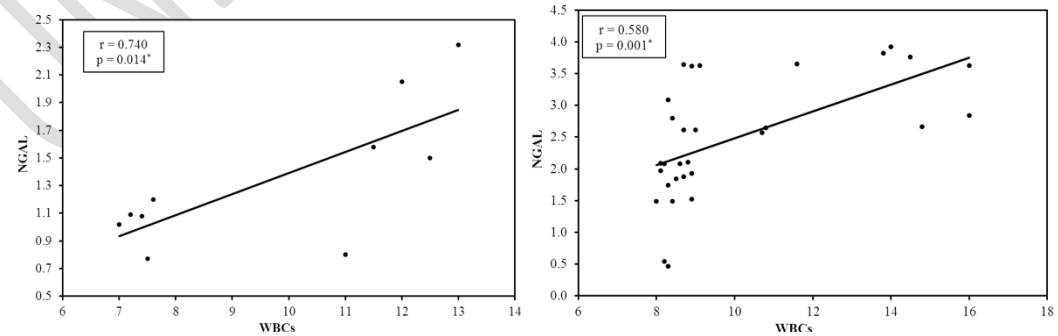


**Figure 4: Correlation between NGAL level and WBCs count in uc and CD group**

**Table 6: Correlation between NGAL level and colonoscopic severity.**

| | NGAL | | | |
|---|---|---|---|---|
| | CD (n=10) | | UC (n=30) | |
| | r | p | r | p |
| **Colonoscopy** | 0.658 | 0.038* | 0.473 | 0.008* |

The Cut off value of NGAL for diagnosis of CD was >0.767 mg/kg with sensitivity 90 % and specificity 90 %. Area under the curve (AUC) was 0.983 in the ROC curve analysis with 81.8% positive predictive value (PPV) and 94.7% negative predictive value (NPV). The accuracy of NGAL measurement for diagnosis of CD was 90 %. **Table 7 Figure 5**

The Cut off value of NGAL for diagnosis of UC was >0.54 mg/kg with sensitivity 93.33 % and specificity 90 %. Area under the curve (AUC) was 0.992 in the ROC curve analysis with 93.3 % positive predictive value (PPV) and 90% negative predictive value (NPV) The accuracy of NGAL measurement for diagnosis of UC was 92%. **Table 7 Figure 6**

**Table 7: Agreement (sensitivity, specificity) for NGAL to diagnose CD and UC patients (n = 10) from control (n = 20)**

| | AUC | p | 95% C.I | Cut off | Sensitivity | Specificity | PPV | NPV | Accuracy |
|---|---|---|---|---|---|---|---|---|---|
| **CD** | 0.983 | <0.001* | 0.947 – 1.018 | >0.767 | 90.0 | 90.0 | 81.8 | 94.7 | 90 |
| **UC** | 0.992 | <0.001* | 0.976 – 1.008 | >0.54 | 93.33 | 90.0 | 93.3 | 90.0 | 92.0 |

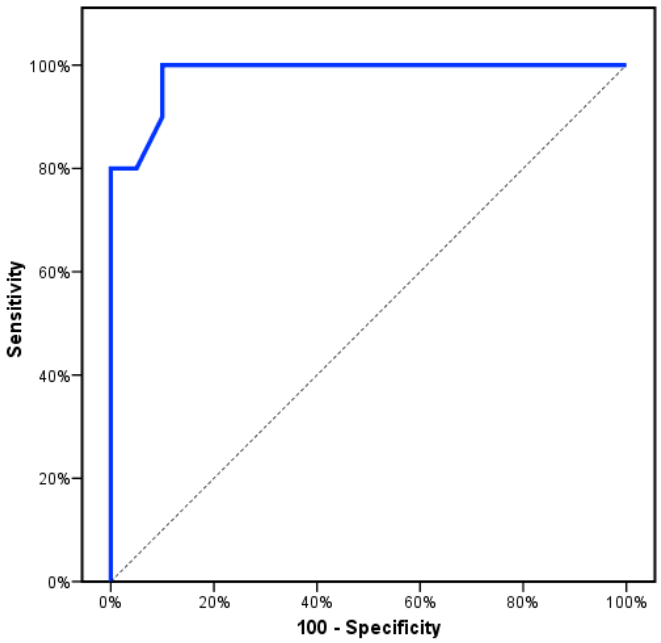


**Figure 5: ROC curve for NGAL to diagnose CD patients (n = 10) from control (n = 20)**

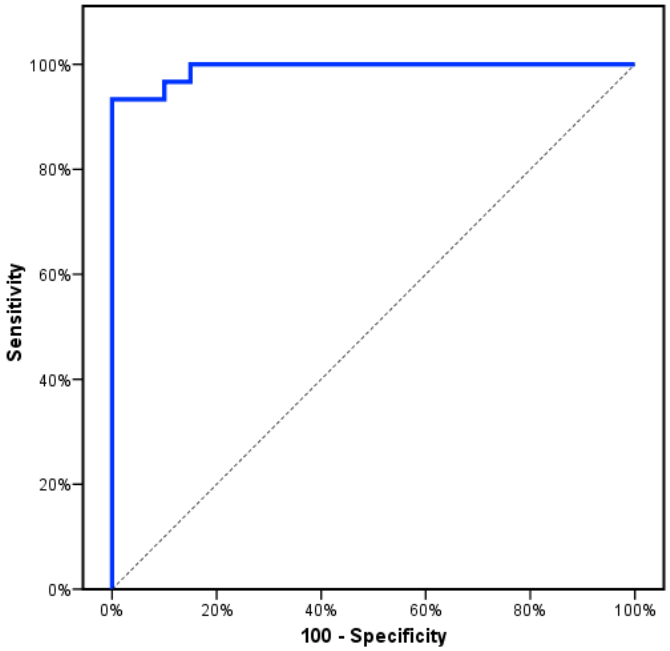


**Figure 6: ROC curve for NGAL to diagnose UC patients (n = 30) from control (n = 20)**

## Discussion

There is a global increase in the Screening and diagnosis of inflammatory bowel disease, IBD is a group of disease characterized by remission and relapse, patients with IBD requires frequent

monitoring and screening by different stereological, Fecal biomarkers and colonoscopy. fecal and serological biomarkers are easily used, easily reproducible, objective and less invasive methods. Some studies found that NGAL is present in the colonic epithelium with high expression, suggesting that NGAL evaluation may help in the assessment of the inflammatory bowel disease activity [6].

Our study aimed to correlate the frequently used serological biomarkers with disease activity and to determine specificity and sensitivity of Fecal NGAL in correlation with the frequently used serological biomarkers and colonoscopic disease activity

The existing fecal biomarkers calprotectin and lactoferrin are molecules mainly found in granulocytes [6]. We suggest that an additional biomarker should reflect different aspects of the inflammatory process than the existing ones. In this respect, fe-NGAL could be a good candidate marker, and previous studies from our laboratory [1]. The expression of NGAL is potently regulated in the colonic epithelium during inflammation in addition to being expressed in granulocytes. Thus, fe-NGAL may be a more sensitive test than calprotectin in a more chronic inflammatory setting with low numbers of infiltrating granulocytes. Moreover, fe-NGAL should be studied in pediatric IBD, where meta-analyses show that calprotectin may have a relatively low specificity.

In our study we measure the level of Fecal NGAL and serum inflammatory markers (ESR, CRP, WBCs) of 40 patients with IBD with variable degree of severity and 20 healthy subject we correlate between the NGAL level and another inflammatory biomarker and colonoscopic findings.

WBCs count was statistically significantly increased in patients with IBD than in control group. In agreement with our study, a study performed by Ashraf M. Okba1 et al. [7] who, showed that

significantly higher WBC in active UC group compared to both inactive UC patients and controls group (P = 0.000).

As regard ESR level and CRP, in our study it was statistically significantly increased in patients than in control group and in agreement with our study, a study performed by Sheng-Qiang Gao, et al. [8] showed that ESR and CRP level was significantly increased in CD patients than control group (P ≤0.001).

Correlation between Fecal NGAL level and colonoscopic findings was done in our study and showed that Fecal NGAL has strong correlation with colonoscopic findings, and this was in agreement with the results of Silje Thorsvik, et al. [9] Who showed that Fecal NGAL has excellent correlation with colonoscopic findings.

Fecal NGAL was measured in our study and was found to be significantly increased in patients with IBD than in control group.

In the ROC analysis of our material, we found AUC was 0.983 with sensitivity and specificity for Fecal NGAL of 90 % and 90 %, respectively, for distinguishing between CD and heathy control.

In agreement with our study, a study per formed by Silje Thorsvik, et al. [9] who showed that fecal NGAL is markedly raised in active UC and CD compared with irritable bowel syndrome (IBS) and healthy controls with sensitivity and specificity for Fecal NGAL of 95.7 % and 94.7 %, respectively.

Also, in agreement with our study, a study per formed by Dilyara Mukhametova, et al. [10] showed that fecal NGAL is markedly raised in active IBD compared to the healthy controls. The discrepancy in the cut-off value of NGAL between different studies may be due to different populations and different number of cases and controls.

Altogether, these findings show that fe-NGAL is a very promising biomarker of IBD. The use of fe-NGAL as a biomarker for IBD was suggested by Nielsen et al. [4].

The limitations of the study were small sample size and being single center study. Further studies on large number of patients with lower GIT symptoms to determine any role of serum NGAL in organic colonic diseases other than UC including CRC in non-UC patients. Also, further studies to evaluate the role of NGAL in assessing response to therapy for UC.

## Conclusions:

In conclusion, serum NGAL is a valuable non- invasive marker in assessment of UC activity and correlated positively with inflammatory markers (CRP and ESR) and markers of disease activity (Mayo and UC histopathology grading).

**COMPETING INTERESTS DISCLAIMER:**

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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