Non-coding RNA Research 9 (2024) 318–329

Contents lists available at ScienceDirect

Non-coding RNA Research

journal homepage: www.keaipublishing.com/en/journals/non-coding-rna-research

Original Research Article

# Expression profile of serum LncRNAs *MALAT-1* and *CCAT-1* and their correlation with Mayo severity score in ulcerative colitis patients can diagnose and predict the prognosis of the disease

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ARTICLE INFO

*Keywords:*
Ulcerative colitis
*MALAT-1*
*CCAT-1*
Mayo score

ABSTRACT

*Background:* Ulcerative colitis (UC) has emerged as an accelerated-incidence chronic condition. UC has been identified as a precancerous lesion for colorectal cancer. Up-to-date genomic research revealed the value of many noncoding RNAs (ncRNAs) in UC pathogenesis, diagnosis, and prognosis.
*Aim:* The present study was aimed at measuring both *MALAT-1* and *CCAT-1* in the sera of UC patients as diagnostic and prognostic biomarkers and correlating them with the Mayo score which is a novel predictive indicator of malignant transformation as well as with clinicopathological characteristics of the disease.
*Patients and methods:* Sixty-six UC patients and 80 healthy individuals participated in this study, the serum fold changes of *MALAT-1* and *CCAT-1* were measured by using quantitative real-time PCR (qRT-PCR).
*Results:* The current study findings include overexpressed lncRNAs *MALAT-1* and *CCAT-1* in the sera of ulcerative colitis patients [(median (IQR) = 2.290 (0.16–9.36), mean ± SD = 3.37 ± 3.904 for *MALAT-1,* and median (IQR) = 7.305 (0.57–16.96), mean ± SD = 6.81 ± 4.002 for *CCAT-1* than controls, ROC curve analysis reported that these genes could predict UC. Both genes were positively correlated with each other which enforces their synergistic effects. Both genes are diagnostic for UC patients.
We related studied genes to the severity of the disease. In addition to a significant positive correlation between each gene with ESR and Mayo score, we further classified the patients according to severity (according to Mayo score to remission, mild, moderate, and severe groups) with the following results; lower levels of *MALAT-1* and *CCAT-1* were significantly associated with mild disease and increased gradually with more severe forms of the disease ($p < 0.05$). Linear regression analysis with Mayo Score as a dependent variable revealed that only the predictive power of *CCAT-1* and ESR are significant. Moreover, ROC curve analysis when compared to that of the Mayo score revealed that *CCAT-1* reached 99 % accuracy. In summary, both genes are prognostic factors for UC patients.
*Conclusion:* *MALAT-1* and *CCAT-1* are diagnostic and prognostic serum biomarkers of ulcerative colitis.

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https://doi.org/10.1016/j.ncrna.2024.01.012
Received 1 November 2023; Received in revised form 18 January 2024; Accepted 20 January 2024
Available online 22 January 2024

## 1. Introduction

Ulcerative colitis (UC) is a chronic idiopathic inflammation of intestinal mucosa with recurrent episodes of exacerbations. Ulcerative colitis is a subtype of inflammatory bowel disease (IBD) that is characterized by inflammation of the mucosa and sub-mucosa starting in the rectum and extending to proximal segments of the colon, causing ulcers to develop [1,2].

As in recent decades, the prevalence of IBD has risen, mainly in developing countries, the disease has turned out to be a global alarm. In Mediterranean countries, the prevalence of patients with IBD was estimated at 5 per 100,000 in urban areas [3]. In the past few years, a remarkable increase in the incidence of UC in the African population and Egypt has been noted [4] UC mainly affects young populations with a peak age of diagnosis ranging from 15 to 30 years of age [5]. A rise in disease prevalence tends to be caused by alterations in lifestyles especially changes in eating patterns such as a preference for fast food, increased carbohydrate use, and a decrease in the daily intake of alimentary fibers [6].

The etiology of IBD is not fully comprehended however it is suggested to be an interaction between genetic predisposition and environmental influences thus leading to intestinal microbiome dysbiosis ending in inflammation of the intestinal mucosa [7]. The cancerous transformation of colonic mucosa is a major consequence of IBD. Patients with long-standing UC are at a higher risk of developing colitis-associated colorectal cancer compared to the normal population [8].

Long noncoding RNAs (lncRNAs) are RNAs transcripts of 200 nucleotides length or more that don't code for protein but they are gene expression regulators through different molecular pathways [9]. In IBD, lncRNAs were reported to be connected to apoptotic pathways of intestinal epithelial cells, lipid metabolism pathways, and regulation of immune system response to IBD-enhanced intestinal inflammation [10].

*MALAT-1* is an abbreviation of Metastasis Associated lncRNA that was first discovered in lung adenocarcinoma but lately, it has been discovered to be present in all tissues and cells with a regulatory function at transcriptional and post-transcriptional levels. *MALAT1* is located in chromosome 11q13.1 [11,12]. *MALAT-1* is significantly overexpressed in the inflammatory intestinal mucosa of UC patients when compared to the healthy intestinal mucosa and was also reflected in plasma levels [13]. Also, *MALAT-1* expression has been upregulated in colorectal cancer [14]. *MALAT-1* has been reported to take part in colorectal cancer pathogenesis by suppressing multiple microRNAs (miRNAs). Therefore, *MALAT-1* can be a potent biomarker for colorectal cancer prediction and diagnosis [15].

The Colon Cancer–Associated Transcript–1 lncRNA (*CCAT-1*) has been documented to be upregulated in cancerous cells of the colon and to have a reported role in colorectal cancer development and prognosis. *CCAT1* gene is located in 'gene desert' on chromosome 8q.24.21 [16,17]. Also, it was stated that *CCAT-1* endorsed inflammation and cell chemotaxis in mammalian intestinal epithelial cells. *CCAT-1* has been reported to be upregulated in IBD tissues more than healthy adjacent tissues and may be allied with the initiation of IBD [17].

Despite the discrete genomic locations of *MALAT1* and *CCAT1*, they are functionally related regarding ulcerative colitis pathogenesis, as they not only closely related to inflammatory mucosal cells of IBD and cancer cells of colorectal cancer [13,15], but also related to the integrity of intestinal barrier functions through two different mechanisms [15,16].

In addition, according to the authors of a recent review study (2022) [17], the best method for diagnosing ulcerative colitis is to use enteroscopy in conjunction with composite histopathological biomarkers (≥2 biomarkers) to assess the severity and minimize the grey area for each biomarker.

Furthermore, no study correlates these markers with the severity of UC disease. Therefore, the present study aims to measure both CCAT-1 and MALAT-1 in UC patients' sera as easily accessible, non-invasive diagnostic and prognostic biomarkers and correlate them with the Mayo score, which is a novel predictive indicator of malignant transformation [18], as well as with clinicopathological characteristics of the disease. The goal is to establish a basis for using these two biomarkers as composite biomarkers in combination with endoscopy for the future diagnosis and prognosis of ulcerative colitis."

## 2. Subjects and methods

### 2.1. Participants and ethics declaration

The population of the current study (66 ulcerative colitis patients, and 80 controls) was collected from the Internal Medicine Department and from Tropical Department outpatients' clinics and inpatients sections, Faculty of Medicine, Fayoum University over a period from Jan 2022 to Sep 2022. RNA extraction and all clinical tests were done in the Medical Biochemistry and Molecular Biology Department as well as in the Microbiology Department, Faculty of Medicine, Fayoum University. After the Ethical approval of this study was obtained from the Fayoum Ethical Committee (no R209, session, 89), we began to collect venous blood samples from all patients and controls after they were assigned written consent. Current study as per the Declaration of Helsinki.

### 2.2. Diagnosis, Inclusion and exclusion criteria

The final diagnosis depended on full history, clinical signs, and symptoms, radiological and colonoscopic examination, pathological analyses of colonoscopic biopsies collected from the rectum, sigmoid, left colon, transverse colon, right colon, and ilium confirm the diagnosis and determine the extent of the disease (proctitis, left-sided or pancolitis).

Thirteen patients were newly diagnosed cases with no therapy initiated and fifty-three patients were chronic patients who received different combinations of the following therapeutic medicine (aminoacylate, oral steroids, immunosuppressant, and monoclonal antibodies). Inclusion criteria were adult, with the determined extent of the disease by colonoscopy and determined severity score by Mayo score. Exclusion criteria are patients aged less than 18, with current infection, autoimmune disease, or malignancy anywhere in the body, also patients with a history of cancer.

### 2.3. Mayo-score

The Mayo Score (Table 1) was created to serve as an activity index of ulcerative colitis in clinical assessment. Four items were assessed and were all scored on a scale of 0–3, for an overall score of 12. Mayo Score items should include an evaluation of two patient-reported findings; Stool frequency and rectal bleeding, and two physician-reported findings; the Endoscopic aspect of the intestinal mucosa, and the physician's Global Assessment.

We classified patients according to Mayo score and the extent of the disease **(Table 2)** [18,19]

### 2.4. Blood samples

Six milliliters of whole blood were drawn via venous puncture into two tubes, the first tube contained salts of EDTA as anticoagulant and was used for CBC and ESR estimation, the second tube was left at room temperature for 15 min to allow blood coagulation and then centrifuged at 4000xg to promote serum separation, collected sera were stored at −80c for further processing.

**Table 1**
Mayo score items.

| Item | Variety | Score (0–3) |
|---|---|---|
| **Patient-reported findings** | | |
| Stool frequency/day | The normal number of stools for this patient | 0 |
| | 1–2 stools more than normal | 1 |
| | 3–4 stools more than normal | 2 |
| | 5 or more stools more than normal | 3 |
| Rectal bleeding | None | 0 |
| | Blood flecks in the stool less than half the time | 1 |
| | All stools contain blood | 2 |
| | The existence of pure blood | 3 |
| **Physicians reported findings** | | |
| Endoscopic aspect of the intestinal mucosa | Normal or inactive colitis | 0 |
| | Mild colitis is characterized by mild erythema and a decrease in vascularity | 1 |
| | Moderate colitis: visible erythema and erosions | 2 |
| | Spontaneous bleeding in severe colitis | 3 |
| Physician's Global Assessment | Normal | 0 |
| | Mild colitis | 1 |
| | Moderate colitis | 2 |
| | Severe colitis | 3 |

**Table 2**
Classification of the patients according to Mayo score and extent of the disease.

| Classification according to Mayo score | | | | |
|---|---|---|---|---|
| Degree of severity | Remission | Mild | Moderate | Severe |
| Mayo score | <2 | 2–4 | 5–7 | >7 |
| Number of patients | 3 (4.5 %) | 13 (19.7 %) | 18 (27.3 %) | 32 (48.5 %) |
| **Classification according to the Extent of the disease** | | | | |
| Extent | Procitis | | Left-sided | Pancolitis |
| Parts involved | Rectum and Sigmoid colon | | Rectum, sigmoid, and descending colon | All colon |
| Number of patients | 13 (19.7 %) | | 25 (37.9 %) | 28 (24.4 %) |

### 2.5. Total RNA extraction, reverse transcription, and quantitative real-time PCR (qPCR) for detection of LncRNAs in the sera of studied groups

We extracted RNA via a Qiagen extraction kit as directed by the producer's instructions, and the RNA purity was determined using a NanoDrop 1000 (Thermo Fisher Scientific). Reverse transcription was performed using purified RNAs and the RT2 first strand kit (Qiagen) according to the producer's instructions. Fold changes of the studied lncRNAs *MALAT-1* and *CCAT-1* in the serum were calculated using the $2^{-\Delta\Delta Ct}$ equation and Ct values of patients, and controls for both studied genes (*MALAT-1*, *CCAT-1*) and housekeeping gene (*GAPDH*) that obtained from PCR. Primers of involved genes are settled in Table 3 below. We used customized primers for *MALAT-1*, *CCAT-1*, *GAPDH*, and Maxima SYBR Green PCR kit (Thermo, USA) according to the manufacturer's protocol. The real-time PCR mixture was 20 μl (10 μl master mix, 1 μl forward primer, 1 μl reverse primer, 2.5 μl cDNA, and 5.5 μl RNAase-free water), Operating Rotor gene Q System (Qiagen) with the following conditions: 95 °C for 10 min, followed by 40 cycles at 95 °C for 15 s and 60 °C for the 60s [20].

### 2.6. Estimation of sample size

The sample size of this study is 66 (the available cases), We used the G*power program for the different two tails tests used in our statistical analysis (*t*-test Wilcoxon-Mann-Whitney, F test, Z tests as regression, Spearman correlation test) using the medium effect of Cohen, the power of sample ranged from 0.74 to 0.989), the critical F was 3.62.

### 2.7. Statistical analysis

The Statistical Package for Social Sciences (SPSS) version 24 was used in investigating data. The minimum and maximum, mean, median, standard deviation (SD), and standard error of the mean (SEM) are representative quantitative data. The frequency and percentage represented the categorical data. Nonparametric data were compared by Chi-squared test. The Spearman correlation coefficient was used for correlations among two markers and between markers and other quantitative variables. Wilcoxon-Mann-Whitny test was conducted to compare the two groups and (2 groups) or the Kruskal Wallis test (more than 2 groups). The linear regression analysis was constructed to identify the significant predictors of ulcerative colitis among all significant parameters revealed from the comparison between UC and healthy subjects with the Mayo Score as a dependent variable. The receiver operating characteristic (ROC) curve was conducted with the area under the curve (AUC) analysis to detect the best cutoff value of the two analyzed markers for UC detection. All tests are considered statistically significant when the P value is $\leq 0.05$.

## 3. Results

### 3.1. Basic features of ulcerative colitis patients and controls

The mean age of the ulcerative colitis patients' group was 33.3 ± 9.7 years and the healthy subjects' group, was 31.3 ± 10 years. Both groups were matched regarding age and sex. The male gender was more prevalent in both the UC group (65.15 %) and controls (65 %) than the female gender (**Table 4**). Fig. 1 shows the variable frequency histogram

**Table 4**
Demographic characteristics and some serological test parameters of ulcerative colitis patients' group and healthy subjects. (Independent *t*-test for quantitative variables and Chi-square ($\chi 2$) test for qualitative variables).

| Parameter | Control (n = 80) | UC (66) | P value |
|---|---|---|---|
| **Age (years)** | 31.3 ± 10 | 33.3 ± 9.7 | 0.207 |
| **Gender F, n (%)** | 28 (35 %) | 23 (34.85 %) | 0.583 |
| **M, n (%)** | 52 (65 %) | 43 (65.15 %) | |
| **Hb (mg/dL)** | 13.2 ± 1.3 | 11.4 ± 1.8 | **< 0.001** |
| **HCT** | 44.3 ± 2.9 | 34.5 ± 6 | **< 0.001** |
| **TLC (thousands)** | 5.9 ± 1.6 | 8.4 ± 4.2 | **< 0.001** |
| **neutrophil %** | 53.9 ± 9 | 61 ± 13.3 | **< 0.001** |
| **Platelets (thousands)** | 296.8 ± 59.2 | 313.5 ± 89 | 0.179 |
| **CRP** | 9.9 ± 2.7 | 31.4 ± 24.9 | **< 0.001** |
| **ESR** | 1 ± 0.4 | 14.5 ± 17.5 | **< 0.001** |
| **Albumin** | 3.8 ± 0.3 | 3.8 ± 0.7 | 0.612 |

UC, ulcerative colitis, Hb: hemoglobin, HCT: hematocrit, TLC: total leucocytic counts, CRP, C reactive protein, ESR: erythrocyte sedimentation rate.

**Table 3**
Primers used in PCR.

| Target gene | Forward nucleotide sequences | Reverse nucleotide sequences |
|---|---|---|
| *MALAT-1* | 5′-CTTCCCTAGGGGATTTCAGG-3′ | 5′-TAGTTGGCATCAAGGCACTG-3′ |
| *CCAT-1* | 5′-TCACTGACAACATCGACTTTGAAG- 3′ | 5′-GGAGAAAACGCTTAGCCATAC AG-3′ |
| *GAPDH* | 5′-CTGACTTCAACAGCGACACC-3′ | 5′-TAGCCAAATTCGTTGTCATACC-3′ |

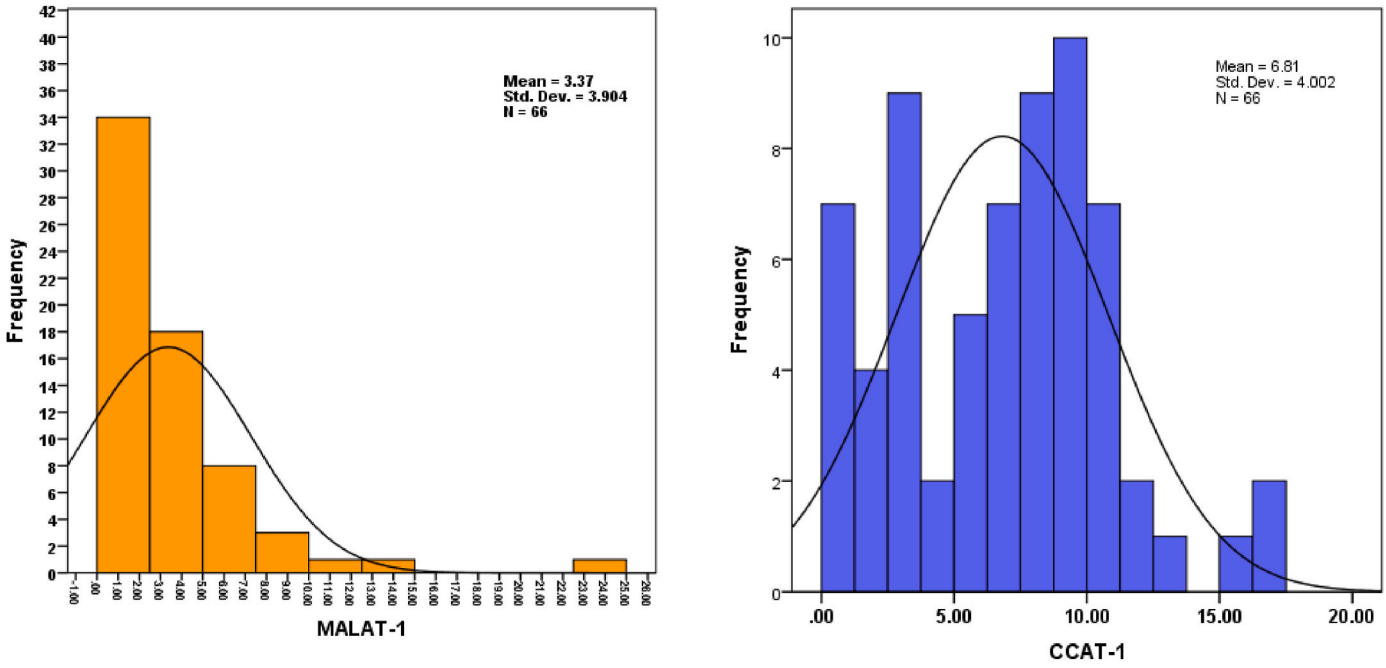



**Fig. 1.** Variable frequency histogram and distribution curve of *MALAT-1* and *CCAT-1* of UC patients.

and distribution curve of *MALAT-1* and *CCAT-1* of UC patients.

In terms of laboratory data, Hemoglobin (Hb) and Hematocrit levels (HCT) were significantly lower in the UC group than in controls ($P < 0.001$). While, total leucocytic count (TLC), neutrophils, Erythrocyte Sedimentation Rate (ESR), and *C*-reactive protein (CRP) were significantly higher in the UC group than in the controls ($P < 0.001$). As regards albumin and platelet counts, there were no significant differences observed between the two groups ($P > 0.05$) (Table 4). Lower Hb and HCT in the UC group than controls which reflects a chronic rectal bleeding state while higher TLC, neutrophils, CRP, and ESR mirror inflammation of bowel mucosa **(Table 4)**.

### 3.2. Upregulated serum levels of MALAT-1 and CCAT-1 in UC patients than healthy controls and the assessment of their diagnostic values by ROC curve analysis

*MALAT-1* and *CCAT-1* levels in the serum of both UC patients (n = 66) and healthy controls (n = 80) were measured by RT-qPCR. Mann–Whitney-U test was used to analyze the differences in serum levels of *MALAT-1* and *CCAT-1* between the UC and control groups. It was observed that serum levels of *MALAT-1* and *CCAT-1* were significantly higher in the UC group than in the control group (Table 5, Fig. 2, $P < 0.001$).

Diagnostic values of serum *MALAT-1* and *CCAT-1* for UC were assessed by ROC curve analysis. In ROC curve analysis, true positive cases were UC patients and true negative cases were healthy controls. As shown in Table 6, Fig. 3 regarding *MALAT-1,* the area under the curve was 0.697, and a 95 % confidence interval of 0.586–0.808 ($P < 0.0001$) with Sensitivity (94.57 %) and Specificity (79.25 %). Regarding *CCAT-1*, the area under the curve was 0.902, and a 95 % confidence interval of 0.831–0.972 ($P < 0.0001$) with Sensitivity (99.25 %) and Specificity (98.7 %). On comparison between the target genes' ROC curves and the severity score (Mayo score) Roc curve analysis, we can conclude that *CCAT-1* reaches a total accuracy of 99 %.

**Table 5**
Comparison between ulcerative colitis patients' group and healthy subjects' group regarding *MALAT-1* and *CCAT-1* (Mann–Whitney-U test).

| Studied gene | Median | IQR | Mean ± SD | P vs. control |
|---|---|---|---|---|
| ***MALAT-1*** | 2.290 | 0.16–9.36 | 3.37 ± 3.904 | **< 0.001** |
| ***CCAT-1*** | 7.305 | 0.57–16.96 | 6.81 ± 4.002 | **< 0.001** |

**IOR**, inter-quartile range; ***MALAT-1,*** Metastasis-associated lung adenocarcinoma transcript 1; ***CCAT-1***, Colon cancer–associated transcript–1. The control value was 1 by the $2^{-\Delta\Delta Ct}$ equation.

### 3.3. Correlation of both MALAT-1 and CCAT-1 and age, Mayo-score, and some serological parameters in the ulcerative colitis group

As shown in Table 7 and Fig. 4, there was a significant positive correlation between *MALAT-1* and *CCAT-1* with $r = 0.620$ ($P < 0.001$). Regarding *MALAT-1*, there were positive correlations with TLC, ESR, and Mayo-score with $r = 0.329$ ($P = 0.007$), $r = 0.288$ ($P = 0.019$), and $r = 0.415$ ($P = 0.001$), respectively. While, *CCAT-1* was positively correlated with ESR and Mayo-score ($r = 0.303$ ($P = 0.014$), $r = 0.566$ ($P = 0.001$) respectively). There were no significant correlations between *MALAT-1* and *CCAT-1* and other parameters such as Hb, HTC, Neutrophils, CRP, and disease duration.

### 3.4. Analysis of MALAT-1 and CCAT-1 values regarding the endoscopic determination of the type of ulcerative colitis

Values of both *MALAT-1* and *CCAT-1* regarding the endoscopic determination of the type of ulcerative colitis were analyzed by the Mann–Whitney-U test and Kruskal Wallis test (Table 8, Fig. 5). Lower levels of *MALAT-1* and *CCAT-1* were significantly associated with the milder type (proctitis) and increased gradually with more aggressive types (left-sided and pancolitis).

### 3.5. Analysis of MALAT-1 and CCAT-1 values regarding the disease severity of ulcerative colitis

Values of both *MALAT-1* and *CCAT-1* regarding the disease severity of ulcerative colitis were analyzed by the Mann–Whitney-U test and Kruskal Wallis test (Table 9, Fig. 6A and 6B)**. *Lower levels of MALAT-1 and CCAT-1 were significantly associated with mild disease and increased gradually with more severe forms of the disease.***

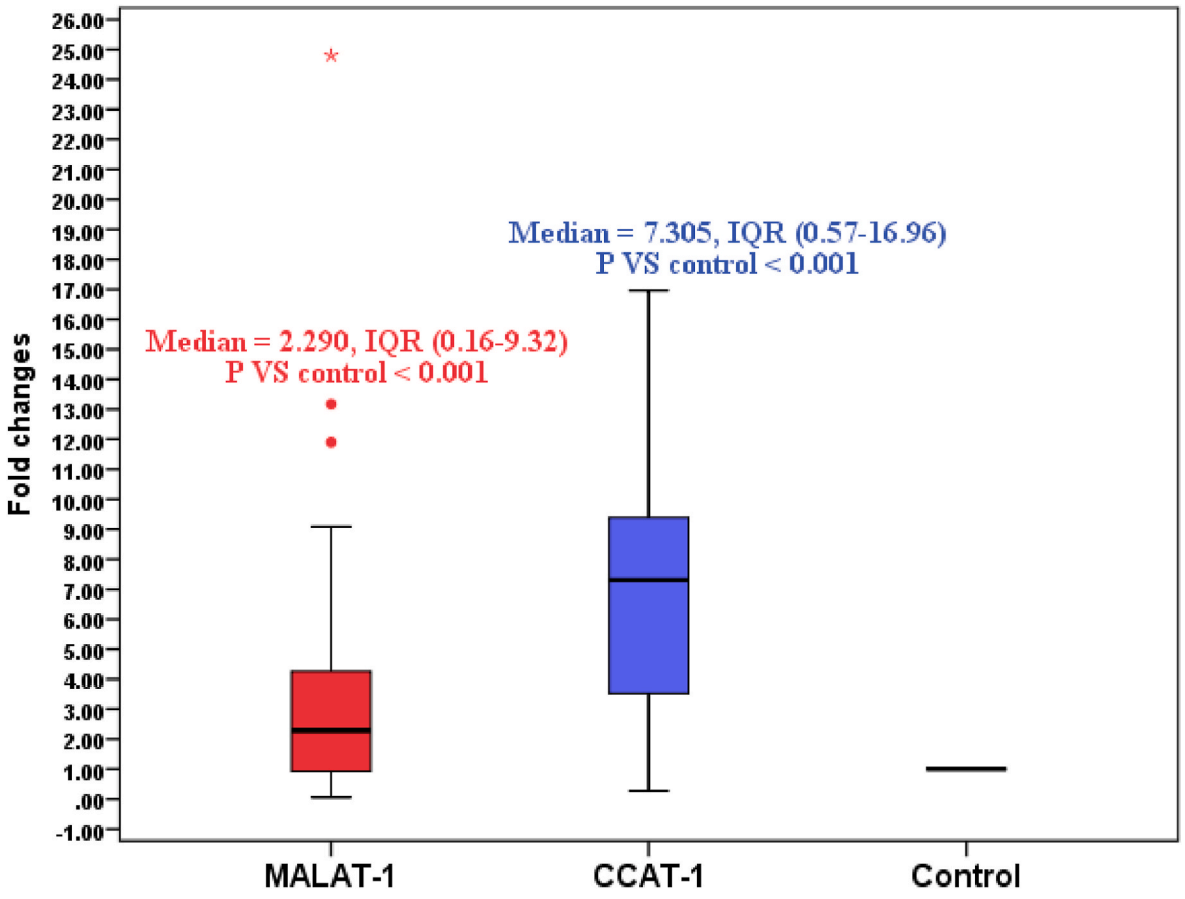



**Fig. 2.** Boxplots represent the fold changes of *MALAT-1* and *CCAT-1* in the ulcerative colitis (UC) group in comparison with the healthy control group.

**Table 6**
ROC curve analysis represents the sensitivity and specificity of *MALAT-1*, *CCAT-1*, and Mayo-score regarding comparing ulcerative colitis patients' values and normal healthy values.

| UC patients vs. healthy subjects | | | | | | |
|---|---|---|---|---|---|---|
| Studied gene | AUC 95 % CI | p-value | Cut-off point | Sensitivity (%) | Specificity (%) | Total accuracy |
| ***MALAT-1*** | 0.697 (0.586–0.808) | **<0.0001** | 2.04 | 94.57 | 79.25 | 86.91 % |
| ***CCAT-1*** | 0.902 (0.831–0.972) | **<0.0001** | 4.06 | 99.25 | 98.7 | 98.97 % |
| **Mayo Score** | 1 (1.0 0–1.00) | **<0.0001** | 1 | 100 | 100 | 100 % |

### *3.6. Association of ulcerative colitis patients' clinical characters with MALAT-1 and CCAT-1 (Mann–Whitney-U test and Kruskal Wallis test)*

*MALAT-1* values were significantly higher in ulcerative colitis patients with positive family history (median (IQR) = 4.01 (0.26–11.27) than in patients with negative family history (Median (IQR) = 1.98 (0.16–7.55) with P value 0.031. Also, *CCAT-1* was significantly higher in patients with positive family history (median (IQR) = 8.22 (0.89–19.47) versus 4.06 (0.43–14.77) with P value 0.012.

Association analysis between treatment categories and target genes revealed that usage of immunosuppressants was associated with lower levels of *MALAT-1* and *CCAT-1* (P = 0.028 and 0.049 respectively).

On the other hand, no significant association between *MALAT-1* and *CCAT-1* and other clinical characteristics such as sex, smoking, coffee consumption, diabetes, hypertension, extraintestinal manifestations, and treatment (P > 0.05) (Table 10).

### *3.7. Linear regression analysis: the predictor variables are MALAT-1, CCAT-I, HTC, Hb, TLC, neutrophil percentage, and ESR, and the dependent variable is Mayo Score*

The linear regression analysis regarding all parameters that have a significant difference between ulcerative colitis patients and normal healthy subjects (*MALAT-1*, *CCAT-1*, HTC, Hb, TLC, neutrophil percentage, and ESR) as predictor variables and Mayo Score as a dependent variable. R is 0.641, the adjusted R squire is 0.339 and the R squire of change is 0.410 with high significance of associated changes (P < 0.001). Only the predictive power of CCAT-1 and ESR are significant (B = 0.362, P = 0.001& B = 0.038, P = 0.044 respectively) (see Table 11).

## 4. Discussion

Inflammatory bowel diseases (IBD) particularly ulcerative colitis (UC) have emerged as one of the world's fastest-growing chronic conditions with an accelerated estimated incidence. UC has been identified as a precancerous lesion for colorectal cancer. Enduring inflammation of the colon epithelia has been linked to the initiation of colorectal cancer

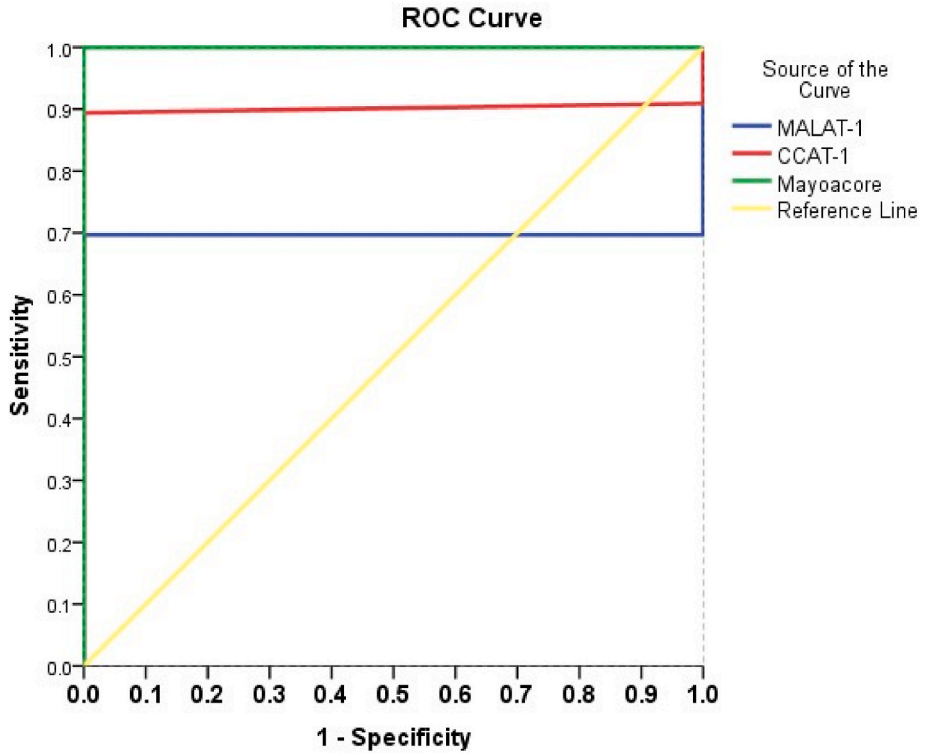



**Fig. 3.** ROC curve analysis represents the sensitivity and specificity of *MALAT-1*, *CCAT-1*, and Mayo-score regarding comparing ulcerative colitis patients' values and normal healthy values.

**Table 7**
Spearman correlation of both *MALAT-1* and *CCAT-1* and age, Mayo-score, and some serological parameters in the ulcerative colitis group.

| Variable | *MALAT-1* | | *CCAT-1* | |
|---|---|---|---|---|
| | r | P value | r | P value |
| *CCAT-1* | 0.620 | <0.001 | | |
| **Age (years)** | −0.187 | 0.132 | −0.053 | 0.675 |
| **Hb (mg/dL)** | −0.014 | 0.909 | −0.044 | 0.724 |
| **HTC** | −0.040 | 0.748 | −0.19 | 0.880 |
| **TLC** | **0.329** | **0.007** | 0.192 | 0.122 |
| **Neutrophils** | 0.107 | 0.393 | 0.019 | 0.879 |
| **ESR** | **0.288** | **0.019** | **0.303** | **0.014** |
| **CRP** | 0.034 | 0.785 | 0.116 | 0.355 |
| **Mayo-score** | **0.415** | **0.001** | **0.566** | **0.001** |
| **Disease duration** | 0.008 | 0.951 | 0.123 | 0.324 |

Hb: hemoglobin, HTC: hematocrit, TLC: total leucocytic counts, CRP, C reactive protein, ESR: erythrocyte sedimentation rate.

[21]. Recent studies revealed a different expression pattern of ncRNAs between healthy, inflamed, adenocarcinoma and cancerous tissue of colonic tissues suggesting that ncRNAs may be promising prognostic and diagnostic markers and therapeutic targets [21,22].

The specific cause of IBD including UC is not fully comprehended; however, it was well documented that the intact intestinal barrier that segregates between intestinal lumen and intestinal epithelial cells is critical for preserving intestinal hemostasis. Failures in barrier function result in contact of epithelial cells with bowel pathogenic organisms and toxic substances fostering a response of inflammation in the intestine. Inflammation and oxidative damage in the epithelium of the intestines is believed to play a role in colorectal cancer development. Thus, investigating the biological processes that trigger the loss of barrier function is critical to discovering novel targets for therapy for inflammatory bowel disease [23].

The purpose of our study is to evaluate the validity of two intestinal barrier functions related to lncRNAs (*MALAT-1* and *CCAT-1*) as diagnostic biomarkers, prognostic biomarkers, and targets of therapy in UC patients by measuring their levels in the serum of UC patients in comparison with controls, relating these genes to the severity score of the disease (Mayo score) and types of the disease, by proposing their possible functions and their association to current medical therapy.

Our most significant results were upregulated lncRNAs *MALAT-1* and *CCAT-1* in the serum of ulcerative colitis patients [(median (IQR) = 2.29 (0.16–9.36), mean ± SD = 3.37 ± 3.904 for *MALAT-1,* and median (IQR) = 7.305 (0.57–16.96), mean ± SD = 6.81 ± 4.002 for *CCAT-1* than controls, ROC curve analysis reported that these genes could predict UC with cutoff 0.697, AUC 2.04, sensitivity 94.57 % and specificity reaches 79.25 % for *MALAT-1*, and cutoff 0.902, AUC 4.06, sensitivity 99.25 % and specificity reaches 98.7 % for *CCAT-1*. Also, significantly higher *MALAT-1* and *CCAT-1* were accompanying patients with positive family history, and they were positively correlated with each other which enforces their synergistic effects. Both genes are diagnostic for UC patients.

We related studied genes to the severity of the disease. In addition to the significant positive correlation between each gene with ESR and Mayo score, we further classified the patients according to severity (according to Mayo score to remission, mild, moderate, and severe groups) with the following results; lower levels of *MALAT-1* and *CCAT-1* were significantly associated with mild disease and increased gradually with more severe forms of the disease ($P < 0.05$). Moreover, ROC curve analysis when compared to that of the Mayo score revealed that *CCAT-1* reached 99 % accuracy. Furthermore, lower levels of *MALAT-1* and *CCAT-1* were significantly associated with the milder type (proctitis) and increased gradually with more aggressive types (left-sided and pancolitis) ($P < 0.05$). Linear regression analysis with Mayo Score as a dependent variable revealed that only the predictive power of *CCAT-1* and ESR are significant. Association analysis between treatment categories and target genes revealed that usage of immunosuppressants was associated with lower levels of *MALAT-1* and *CCAT-1* ($P = 0.028$ and 0.049 respectively). In summary, both genes are prognostic factors for UC patients.

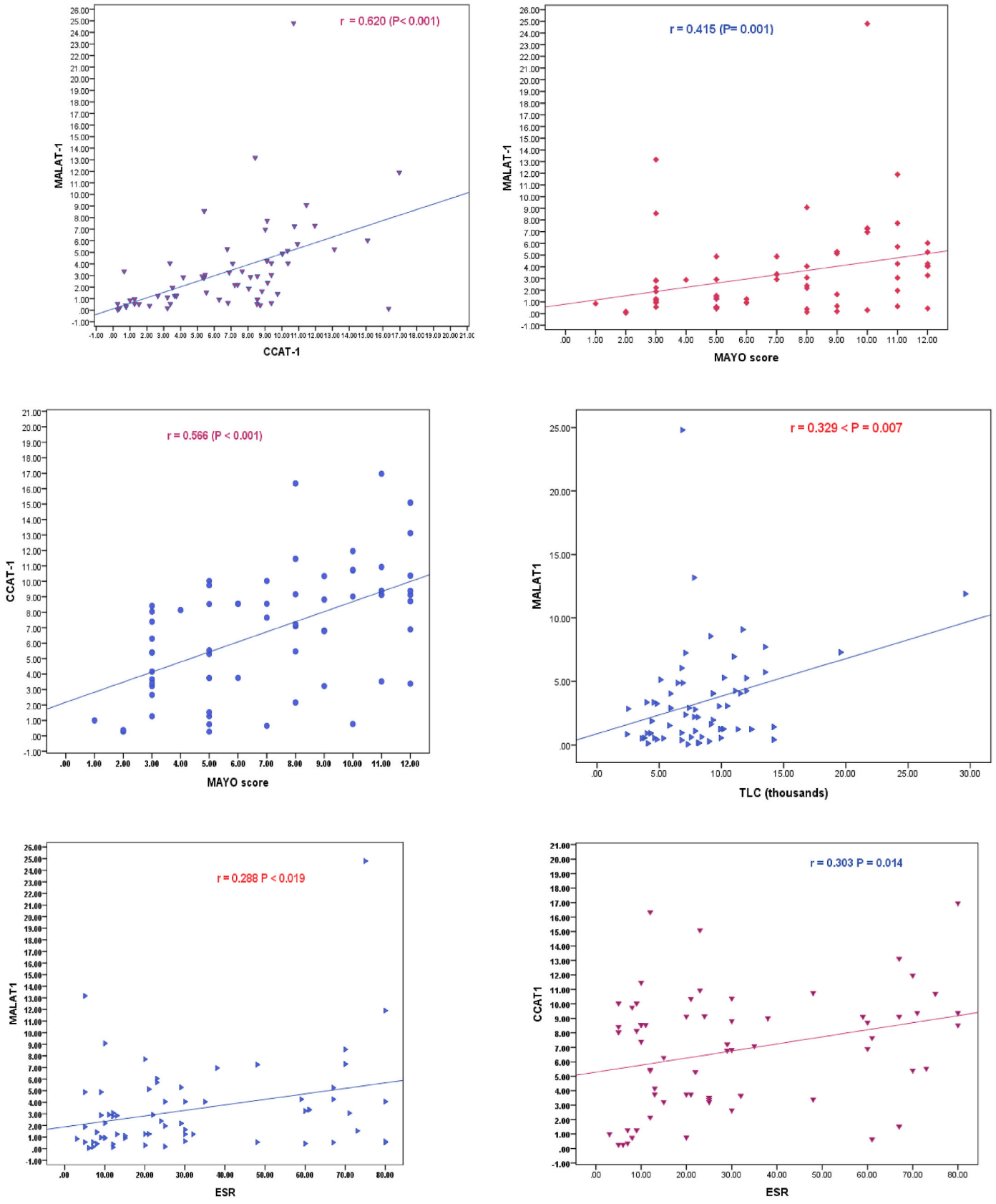



**Fig. 4.** Spearman correlation of both *MALAT-1* and *CCAT-1* and each of them with TLC, ESR, and Mayo score in the ulcerative colitis group.

**Table 8**
Statistical analysis of the values of both *MALAT-1* and *CCAT-1* regarding the endoscopic determination of the type of ulcerative colitis. (Mann–Whitney-U test and Kruskal Wallis test).

| Variable | Proctitis )n = 13) Median (IQR) | Left side (n = 25) Median (IQR) | Pancolitis (n = 28) Median (IQR) | P value |
|---|---|---|---|---|
| ***MALAT-1*** | 0.59 (0.01–5.87) | 2.03 (0.-5.26) | 4.0 (0.1–13.23) | **0.007**[a] < **0.001**[b] < **0.001**[c] |
| ***CCAT-1*** | 1.56 (0.21–11.45) | 7.23 (0.13–11.45) | 9.11 (6.18–13.31) | < **0.001**[a] < **0.001**[b] < **0.001**[c] |

[a] Significance of Left side vs. Proctitis.
[b] Significance of left side vs. pancolitis.
[c] Significance of proctitis vs. pancolitis.

For verification of these findings, we referred to previous literature, as for *MALAT-1*, according to ***Zhu et al 2020***., approximately 50 % of UC patients had increased *MALAT-1* and *ANRIL* levels in the mucosa of the colon of UC patients compared to controls, which was mirrored in plasma levels of *MALAT-1*. They referred to this finding as the variability in disease activity, the disease phenotype that affects disease liability to develop cancer [24]. ***Zhang et al 2020*** found that *MALAT-1* is overexpressed in the chemically induced colitis model and its increase was associated with inhibited cell viability, and promotion of apoptosis and inflammation [25].

***Ma et al 2022*** found that the lncRNA *MALAT-1* plays an unexpected role in preventing an effective and defensive Th17 response. Adaptive Th17 cells are the primary source of IL-17A. IL-17A endorses an intact epithelial barrier and limits the spreading of inflammatory response in case of tissue injury. *MALAT-1* genetic ablation increases IL-17A in Th17 cells thus improving disease consequences in colitis-induced models. From the previous findings, we could conclude that increased *MALAT-1* expression is a risk factor for developing ulcerative colitis and provokes the potential usage of this gene as diagnostic, prognostic, and target for genetic therapy [26].

Concerning *CCAT-1*, ***Ma et al., 2019*** investigated the connection between *CCAT-1* and inflammatory pathways in colorectal cancer cell lines and colonic biopsies from IBD patients. They discovered that *CCAT-1* levels were elevated in cancer cell lines and IBD patients. They also discovered that *CCAT-1* negatively affected miR-185–3p transcription. The function of miR-185–3p is to bind to the 3′ UTR of MLCK mRNA decreasing its expression. As a result, *CCAT-1* promotes MLCK expression by inhibiting miR-185–3p. MLCK is involved in the phosphorylation of the myosin light chain and the ensuing distribution of tight junction proteins, which results in increased cell membrane permeability. That looked at the critical mechanism underlying epithelial barrier disruption in response to inflammatory stimulation [17].

In the current study, there was a strong significant positive correlation between *MALAT-1* and *CCAT-1* with $r = 0.620$ ($P < 0.001$) which suggests their synergistic effect. But whether their functions in intestinal barrier permeability are related or not needs more research.

IBD therapies are divided into two categories: drug treatment and

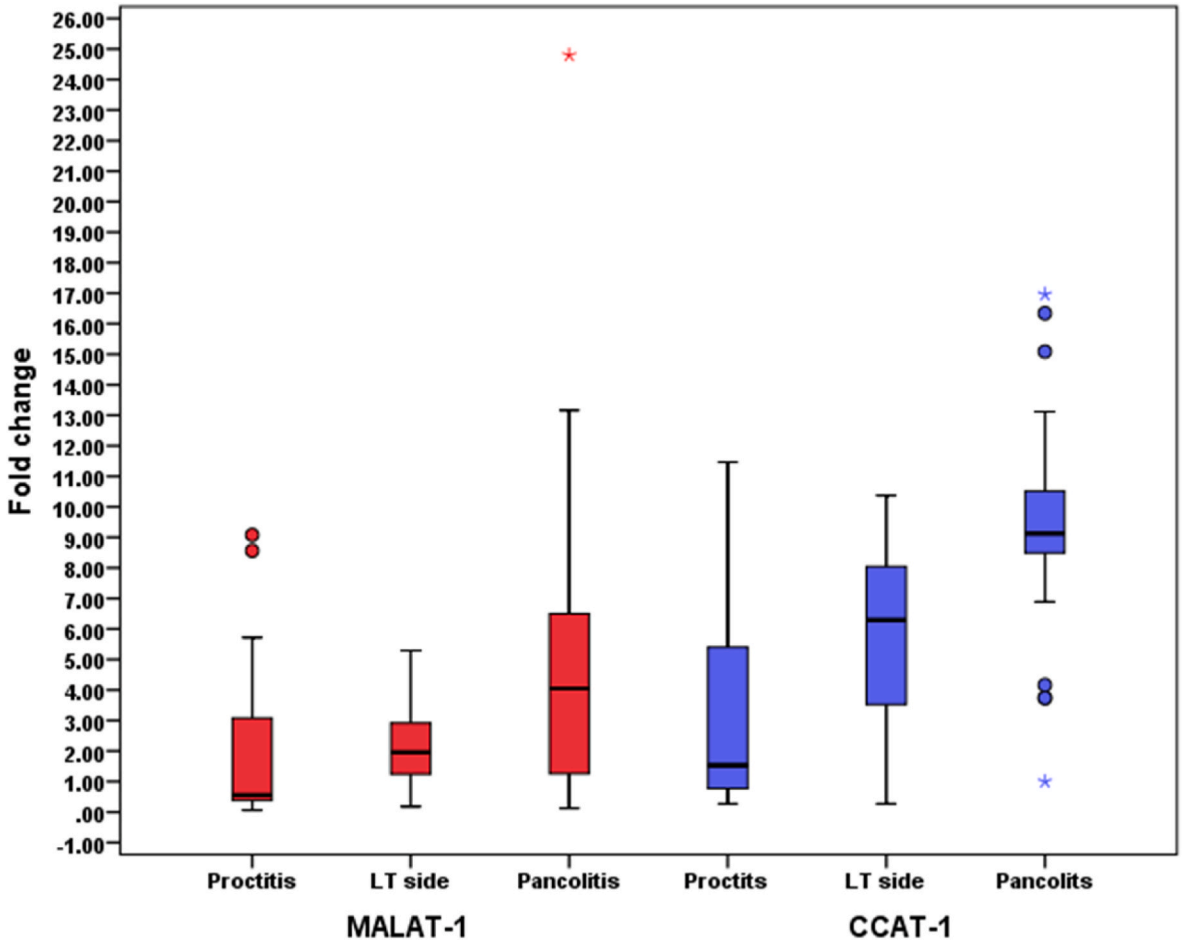
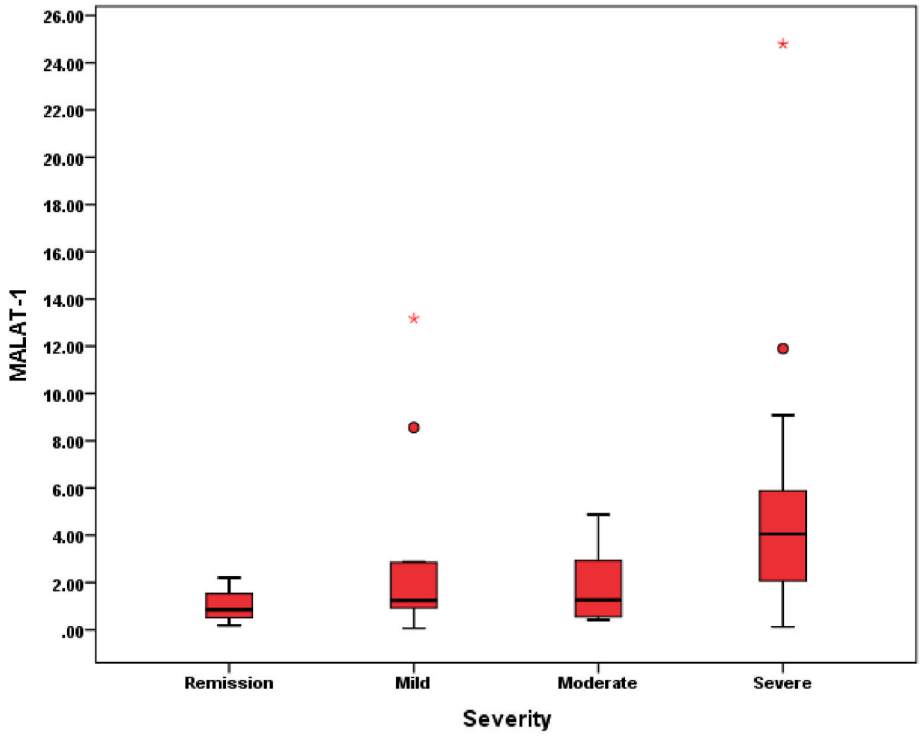



**Fig. 5.** Fold changes of *MALAT1* And *CCAT1* in different types of UC.

**Table 9**
Statistical analysis of the values of both *MALAT-1* and *CCAT-1* regarding the disease severity of ulcerative colitis. (Mann–Whitney-U test and Kruskal Wallis test).

| Variable | Remission (3) | Mild (13) | Moderate (18) | Severe (32) | P value |
|---|---|---|---|---|---|
| ***MALAT-1*** | 0.83 (0.03–.61) | 1.26 (0.02–3.13) | 1.54 (0.29–4.89) | 4.23 (0.01–9.05) | 0.090[a]<br>**0.05**[b]<br>**< 0.001**[c,e,f]<br>0.093[d] |
| ***CCAT-1*** | 3.4 (0.97–7.61) | 4.1 (0.11–8.54) | 5.52 (0.21–10.01) | 9.28 (2.04–15.11) | **0.05**[a]<br>**0.041**[b]<br>**< 0.001**[c,e,f]<br>**0.01**[d] |

[a] Significance of Remission vs. Mild.
[b] Significance of Remission vs. Moderate.
[c] Significance of Remission vs. Severe.
[d] Significance of Mild vs. Moderate.
[e] Significance of Mild vs. Severe.
[f] Significance of Moderate vs. Severe.




A

**Fig. 6A.** Boxplots represent the fold changes of *MALAT-1* in ulcerative colitis (UC) patients' group in different severity grades.

surgery. Aminosalicylates, steroids, immunosuppressant drugs, and monoclonal antibodies are some of the most commonly used medications. Even so, these treatment options have side effects that deteriorate life quality and are insufficient for the achievement of complete recovery. Retrieval of the intestinal mucosa is viewed as the major medicinal therapy goal to lower the frequencies of hospital admissions, surgery complications, and disabilities and to reduce colorectal cancer development risk. So, by controlling levels of target genes, we could introduce new therapeutic elements that aim at the recovery of the intestinal barrier and preventing future hazards [21].

The strengths of our study were that it is the first study to demonstrate fold changes of MALAT-1 and CCAT-1 in the sera of UC patients and relate their levels to severity scores and types of the disease. We also demonstrated a strong significant positive correlation between MALAT-1 and CCAT-1 with $r = 0.620$ ($P < 0.001$). Additionally, there was a strong positive correlation with the Mayo score, with $r = 0.415$ ($P = 0.001$) for MALAT1 and $r = 0.566$ ($P = 0.001$) for CCAT1. Moreover, ROC curve analysis revealed a sensitivity of 94.57 % and a specificity of 79.25 % for MALAT-1, and a sensitivity of 99.25 % and a specificity of 98.7 % for CCAT-1. These findings suggest their utility as non-invasive biomarkers that can be used in conjunction with endoscopy as composite biomarkers, reducing the grey zone of each biomarker and providing information about the severity and type of the disease.

Currently used biomarkers include fecal calprotectin (FC), serum *C*-reactive protein (CRP), and serum proteinase 3 antineutrophil cytoplasmic antibodies (pANCA). While FC and CRP are sensitive, convenient, and noninvasive biomarkers, they have certain limitations, including low specificity for both. FC also lacks patients' compliance, and CRP is useful for short-term prognosis rather than long-term follow-up. Regarding pANCA, it is used in the differentiation between UC and Crohn's disease, making it specific rather than sensitive as a biomarker [17].

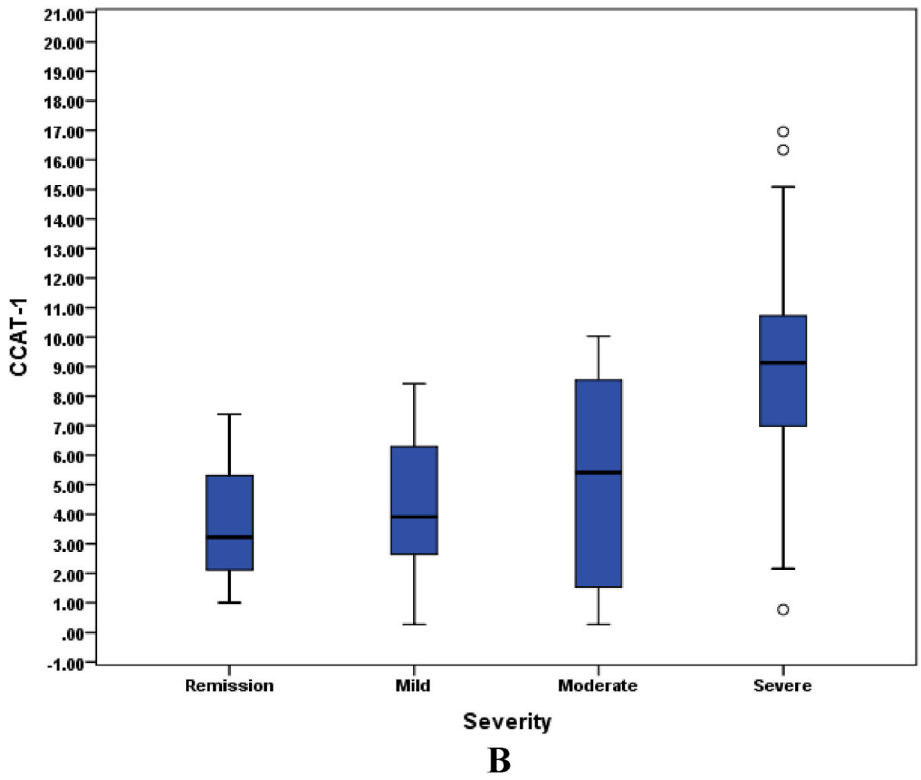



**Fig. 6B.** Boxplots represent the fold changes of *CCAT-1* in the ulcerative colitis (UC) patients' group in different severity grades.

**Table 10**
Analysis of the details of ulcerative colitis patients' clinical characteristics concerning family history, sex, Smoking, Coffee consumption, diabetes, hypertension, extraintestinal manifestations, and treatment and its association with *MALAT-1* and *CCAT-1* (Mann–Whitney-U test and Kruskal Wallis test).

| Variable | | N (%) | *MALAT-1* | | *CCAT-1* | |
|---|---|---|---|---|---|---|
| | | | Median (IQR) | P | Median (IQR) | P |
| **F H** | **Yes** | 17 (25.75) | 4.01 (0.26–11.27) | **0.031** | 8.22 (0.89–19.47) | **0.012** |
| | **No** | 49 (74.25) | 1.98 (0.16–7.55) | | 4.06 (0.43–14.77) | |
| **Gender** | **Female** | 28 (27.68) | 2.35 (0.16–10.55) | 0.685 | 7.3 (0.45–17.05) | 0.801 |
| | **male** | 42 (72.72) | 2.28 (0.19–9.36) | | 7.14 (0.57–16.89) | |
| **Smoking** | **Yes** | 18 (5.68) | 2.08 (0.16–10.24) | 0.372 | 6.59 (0.66–17.25) | 0.641 |
| | **No** | 48 (94.32) | 2.29 (0.13–9.33) | | 7.30 (0.45–16.02) | |
| **C C** | **Yes** | 11 (16.67) | 3.22 (0.02–6.77) | 0.091 | 6.89 (0.59–20.54) | 0.106 |
| | **No** | 55 (83.33) | 2.18 (0.16–9.36) | | 7.30 (0.31–15.88) | |
| **Diabetic** | **Yes** | 7 (10.60) | 2.91 (0.14–10.25) | 0.113 | 7.11 (0.44–19.11) | 0.209 |
| | **No** | 59 (89.40) | 3.01 (0.16–9.33) | | 8.01 (0.09–15.48) | |
| **HTN** | **Yes** | 3 (4.54) | 2.29 (0.09–9.39) | 0.669 | 7.05 (0.49–18.78) | 0.803 |
| | **No** | 63 (95.46) | 2.29 (0.16–9.36) | | 7.305 (0.57–16.55 | |
| **E I M** | **No** | 44 (66.67) | 2.29 (0.09–12.01) | 0.087 | 6.22 (0.33–17.02) | 0.177 |
| | **MS** | 8 (12.12) | 2.59 (0.15–8.77) | | 7.11 (0.41–16.55) | |
| | **MS & eye** | 11 (16.67) | 2.29 (0.16–9.28) | | 5.94 (0.57–18.24) | |
| | HB | 3 (4.54) | 3.05 (0.11–10.33) | | 7.30 (0.55–15.98) | |
| **Treatment** | **No** | 11 (16.67) | 3.49 (0.18–10.33) | **0.028** | 8.04 (0.65–12.15) | **0.049** |
| | **AS** | 4 (6.06) | 3.22 (0.19–8.44) | | 6.89 (0.59–18.71) | |
| | **AS & OS** | 17 (25.76) | 3.79 (0.19–10.13) | | 5.85 (0.57–9.77) | |
| | **AS &MCA** | 6 (9.09) | **2.05 (0.08–5.94)** | | **4.13 (0.43–10.31)** | |
| | **AS & IS** | 19 (28.79) | **1.02 (0.02–3.16)** | | **3.25 (0.33–6.54)** | |
| | **OS & MCA** | 5 (7.57) | **2.11 (0.15–5.05)** | | **5.08 (0.27–9.41)** | |
| | **OS & MCA** | 4 (6.06) | 3.49 (0.22–9.59) | | 6.89 (0.37–19.22) | |

C C: Coffee consumption MS: musculoskeletal HTN: Hypertension.
E I M: Extra intestinal manifestations HB Hepatobiliary.
AS: Amino-salicylates OS: Oral steroid.
MCA: Monoclonal Antibodies IS*:* immunosuppressant.

The advantages of using MALAT1 and CCAT1 as new serum biomarkers for the diagnosis and prognosis of ulcerative colitis over the currently established biomarkers are their high sensitivity (reaching 95 % for MALAT1 and 99 % for CCAT1) and high specificity (99 % for CCAT1).

Limitations of this study include the relatively small sample size and that the patients are from the same geographical area. Also, this study lacks the functional relationship between these two genes which should be examined in a larger study.

**Table 11**
Linear regression analysis, the predictor variables are *MALAT-1*, *CCAT-1*, HTC, Hb, TLC, Neutrophil percentage, and ESR, and the dependent variable is Mayo Score.

| R | R Square | Adjusted R Square | Std. An error in the Estimate | Change Statistics | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | R Square Change | F Change | df1 | df2 | Sig. F Change |
| 0.641[a] | 0.410 | 0.339 | 2.73819 | 0.410 | 5.765 | 7 | 58 | **< 0.001** |

| Model | Unstandardized Coefficients | | Standardized Coefficients | t | P value. |
|---|---|---|---|---|---|
| | B | Std. Error | Beta | | |
| **(Constant)** | 4.165 | 3.490 | | 1.194 | 0.238 |
| **MALAT-1** | −0.029 | 0.103 | −0.034 | −1.963 | 0.780 |
| **CCAT-1** | 0.362 | 0.103 | 0.430 | 2.855 | **0.001** |
| **HTC** | 0.027 | 0.117 | −0.098 | −0.602 | 0.637 |
| **Hb** | 0.055 | 0.390 | 0.014 | 0.693 | 0.284 |
| **TLC** | −0.097 | 0.090 | 0.121 | 1.087 | 0.282 |
| **Neutrophil%** | −0.002 | 0.028 | −0.010 | −0.216 | 0.930 |
| **ESR** | 0.038 | 0.019 | 0.282 | 1.845 | **0.044** |

a. Dependent Variable: MAYO score.
b. Predictors: (Constant), MALAT-1, CCAT-1, HTC, Hb, TLC, Neutrophil %, ESR.
Dependent Variable: Severity (Mayo Score).

## 5. Conclusion

*MALAT-1* and *CCAT-1* are diagnostic and prognostic serum biomarkers of ulcerative colitis and were strongly positively correlated to the Mayo score, they could be potential targets of therapy to help in the retrieval of healthy intestinal mucosa.

## Funding

This study received no funding.

## Ethics approval and consent to participate

Each step involving human biological material in the study was carried out following the ethical guidelines of the institutional and national ethics committees. Following an explanation of all study targets and methods, everyone who participated agreed upon informed consent. The ethical permission for the study was received from the Faculty of Medicine, Fayoum University Ethics Committee. This research protocol adhered to the Declaration of Helsinki's ethical standards and recommendations.

## Patient consent for publication

The Participants' consent was obtained.

## CRediT authorship contribution statement

**Marwa A. Ali:** Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization. **Olfat G. Shaker:** Methodology, Investigation, Data curation. **El Shimaa Gomaa Ali:** Methodology. **Eman M. Ezzat:** Writing – original draft, Investigation. **Abeer A. Khalifa:** Data curation. **Essam A. Hassan:** Data curation, Conceptualization. **Marwa A. Habib:** Conceptualization. **Heba Mostafa Ahmed:** Writing – review & editing. **Asmaa F.A. Dawood:** Writing – review & editing. **Esam Ali Mohamed:** Visualization, Supervision.

## Declaration of competing interest

No conflict of interest related to this study.

## Acknowledgments

The authors gratefully acknowledge the assistance of all paramedical personnel in completing and finalizing the scientific work.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ncrna.2024.01.012.

## References

[1] T. Sairenji, K.L. Collins, D.V. Evans, An update on inflammatory bowel disease, W. B. Saunders, Prim. Care Clin. Off. Pract. 44 (4) (2017) 673–692, https://doi.org/10.1016/j.pop.2017.07.010. Dec. 01.
[2] A. Kaur, P. Goggolidou, Ulcerative colitis: understanding its cellular pathology could provide insights into novel therapies, J. Inflamm. 17 (1) (2020), https://doi.org/10.1186/s12950-020-00246-4. BioMed Central Ltd., Apr. 21.
[3] E.F. Mostafa, A. Metwally, S.A. Hussein, Inflammatory Bowel Diseases Prevalence in Patients Underwent Colonoscopy in Zagazig University Hospitals, 2018 [Online], http://mis.zu.edu.eg/ajied/home.aspx.
[4] A. Moussa, N. Eltaweel, M. Elbadry, Epidemiological and Clinical Characteristics of Ulcerative Colitis in Upper Egypt: A Single Center Study, 2021 [Online], https://aeji.journals.ekb.eg/.
[5] B.C. Da Silva, A.C. Lyra, R. Rocha, G.O. Santana, Epidemiology, demographic characteristics and prognostic predictors of ulcerative colitis, WJG Press, World J. Gastroenterol. 20 (28) (2014) 9458–9467, https://doi.org/10.3748/wjg.v20.i28.9458. Jul. 28.
[6] S. Esmat, M. El Nady, M. Elfekki, Y. Elsherif, M. Naga, Epidemiological and clinical characteristics of inflammatory bowel diseases in Cairo, Egypt, World J. Gastroenterol. 20 (3) (2014) 814–821, https://doi.org/10.3748/wjg.v20.i3.814.
[7] H. Wang, et al., Circulating MicroRNA223 is a new biomarker for inflammatory bowel disease, Medicine 95 (5) (2016), https://doi.org/10.1097/MD.0000000000002703.
[8] A.G. Long, E.T. Lundsmith, K.E. Hamilton, Inflammation and colorectal cancer, Current Medicine Group LLC 1, Current Colorectal Cancer Reports 13 (4) (2017) 341–351, https://doi.org/10.1007/s11888-017-0373-6. Aug. 01.
[9] M.K. Ray, C.G. Fenton, R.H. Paulssen, Novel long non-coding RNAs of relevance for ulcerative colitis pathogenesis, Noncoding RNA Res 7 (1) (Mar. 2022) 40–47, https://doi.org/10.1016/j.ncrna.2022.02.001.
[10] R. Yarani, A.H. Mirza, S. Kaur, F. Pociot, The emerging role of lncrnas in inflammatory bowel disease, Exp. Mol. Med. 50 (12) (2018), https://doi.org/10.1038/s12276-018-0188-9. Nature Publishing Group, Dec. 01.
[11] J. Roh, et al., MALAT1-regulated gene expression profiling in lung cancer cell lines, BMC Cancer 23 (1) (2023), https://doi.org/10.1186/s12885-023-11347-7. Dec.
[12] L. Sun, P. Zhang, W. Lu, LncRNA MALAT1 regulates mouse granulosa cell apoptosis and 17 β-estradiol synthesis via regulating miR-205/CREB1 Axis, BioMed Res. Int. 2021 (2021), https://doi.org/10.1155/2021/6671814.
[13] M. Zhu, J. Xie, LncRNA MALAT1 promotes ulcerative colitis by upregulating lncRNA ANRIL, Dig. Dis. Sci. 65 (11) (Nov. 2020) 3191–3196, https://doi.org/10.1007/s10620-020-06093-w.
[14] W.W. Xu, J. Jin, X. yu Wu, Q.L. Ren, M. Farzaneh, MALAT1-related signaling pathways in colorectal cancer, Cancer Cell Int. 22 (1) (2022), https://doi.org/10.1186/s12935-022-02540-y. BioMed Central Ltd, Dec. 01.
[15] L. Wang, K.B. Cho, Y. Li, G. Tao, Z. Xie, B. Guo, Long noncoding RNA (LncRNA)-mediated competing endogenous RNA networks provide novel potential biomarkers and therapeutic targets for colorectal cancer, Int. J. Mol. Sci. 20 (22) (2019), https://doi.org/10.3390/ijms20225758. MDPI AG, Nov. 02.
[16] Z. Liu, Q.J. Chen, S.S. Hann, The functions and oncogenic roles of CCAT1 in human cancer, Biomed. Pharmacother. 115 (2019), https://doi.org/10.1016/j.biopha.2019.108943. Elsevier Masson SAS, Jul. 01.

[17] D. Ma, et al., CCAT1 lncRNA promotes inflammatory bowel disease malignancy by destroying intestinal barrier via downregulating miR-185-3p, Inflamm. Bowel Dis. 25 (5) (Apr. 2019) 862–874, https://doi.org/10.1093/ibd/izy381.
[18] A.N. Naegeli, et al., Full, partial, and modified permutations of the Mayo score: characterizing clinical and patient-reported outcomes in ulcerative colitis patients, Crohns Colitis 360 3 (1) (2021), https://doi.org/10.1093/crocol/otab007. Jan.
[19] W. Xu, et al., The Mayo endoscopic score is a novel predictive indicator for malignant transformation in ulcerative colitis: a long-term follow-up multicenter study, Front Surg 9 (2022), https://doi.org/10.3389/fsurg.2022.832219. Mar.
[20] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method, Methods 25 (4) (2001) 402–408, https://doi.org/10.1006/meth.2001.1262. Dec.
[21] L. Lin, et al., Which long noncoding RNAs and circular RNAs contribute to inflammatory bowel disease? Cell Death Dis. 11 (6) (2020) https://doi.org/10.1038/s41419-020-2657-z. Springer Nature, Jun. 01.
[22] J. Rao, L. Shao, M. Lin, J. Huang, L. Fan, Lncrna uca1 accelerates the progression of ulcerative colitis via mediating the mir-331-3p/brd4 axis, Int. J. Gen. Med. 14 (2021) 2427–2435, https://doi.org/10.2147/IJGM.S304837.
[23] F. Genua, V. Raghunathan, M. Jenab, W.M. Gallagher, D.J. Hughes, The role of gut barrier dysfunction and microbiome dysbiosis in colorectal cancer development, Front. Oncol. 11 (2021), https://doi.org/10.3389/fonc.2021.626349. Frontiers Media S.A., Apr. 15.
[24] M. Zhu, J. Xie, LncRNA MALAT1 promotes ulcerative colitis by upregulating lncRNA ANRIL, Dig. Dis. Sci. 65 (11) (2020) 3191–3196, https://doi.org/10.1007/s10620-020-06093-w. Nov.
[25] B. Zhang, T. Li, C. Wang, J. Han, B. Wang, G. Sun, LncRNA MALAT1: a potential therapeutic target in DSSinduced ulcerative colitis progression in vitro, Trop. J. Pharmaceut. Res. 19 (9) (2020) 1871–1877, https://doi.org/10.4314/tjpr.v19i9.11. Sep.
[26] S. Ma et al., "Chromatin-associated lncRNA Malat1 Regulates Th17 Effector Program and Promotes Intestinal Inflammation", doi: 10.1101/2022.03.21.485192..