Med. J. Cairo Univ., Vol. 92, No. 2, Accepted 28/4/2024
DOI: 10.22608/MJCU. 597-607, June 2024
www.medicaljournalofcairouniversity.net

KIF9-AS1 as a Potential Biomarker in Ulcerative Colitis Disease

AHMED E. TAHA, M.D.*; SAMAR E. ABDEL-WAHAB, M.Sc.**;
HEND A. TAMIM, M.D.*** and MOHAMED S. ABDEL-BARY, M.D.*

The Department of Endemic Medicine, Faculty of Medicine, Cairo University, General Practitioner**, Ministry of Health and Clinical Pathology Department***, Faculty of Medicine, Cairo University*

Abstract

Background: Ulcerative colitis (UC) is a chronic inflammatory illness of the colon & one of the two main Inflammatory bowel disease (IBD) types. Unfortunately, The majority of cases with UC exhibit severe symptoms & signs as a result of the absence of sensitive biomarkers that enable early detection.

Aim of Study: To assess the role of KIF9-AS1 as a potential biomarker in the identification of UC disease.

Patients and Methods: This research involved 68 individuals who were subdivided into four groups to evaluate the value of KIF9-AS1 in diagnosis of UC: Group (1): Included 17 normal subjects as a control group; Group (2): Involved17 cases with Irritable bowel syndrome (IBS); Group (3): Included 17 patients with active UC; Group (4): involved 17 cases with UC in remission. All cases were exposed to clinical examination, full history taking, laboratory investigations as CBC, CRP, FC & KIF9-AS1 and colonoscopy.

Results: The study demonstrated that WBC, CRP & FC were significantly elevated in ulcerative colitis groups than normal and IBS groups (p<0.001) with a cut-off value 3.8 for CRP and 303 for FC so that it may be considered a diagnostic factor for ulcerative colitis disease. KIF9-AS1 level between the four studied groups showed slightly higher level in UC patients but with no statistically significant variance (p<0.05).

Conclusion: Plasma samples from cases with UC contained a greater concentration of KIF9-AS1 than those from healthy controls. Though, it was not statistically significant. Also, Other inflammatory markers such as WBCs, CRP & FC were statistically significant to be greater in active UC cases than UC cases in remission and controls. So, they can be simple diagnostic markers for active UC patients.

Key Words: *Ulcerative colitis – KIF9-AS1 – Disease activity.*

Introduction

IDIOPATHIC inflammatory disorders of the rectum, ileum, and colon are referred to as inflammatory bowel disease (IBD). Identified forms of inflammatory bowel disease (IBD) include ulcerative colitis (UC) and Crohn's disease (CD) **[1]**. IBD is a universal ailment characterized by an unprecedented rise in both incidence and prevalence. An increasing body of evidence indicates that immunological, genetic, and modifiable environmental factors are strongly related to the development of inflammatory bowel disease (IBD) in susceptible hosts. This predisposition leads to immunological responses targeting a specific subset of intestinal commensal microbiota **[2]**.

UC is confined to the rectum & colon, with superficial inflammation confined to the mucosa & submucosa being its defining characteristic. Rectal hemorrhage, abdominal pain, diarrhea, & superficial mucosal ulceration are all symptoms of UC. Radiological examinations, biopsy histology, endoscopic characteristicsand clinical symptoms are all considered to diagnose UC with precision **[3]**.

Regrettably, sensitive biomarkers for early detection are lacking in UC, resulting in most cases with UC presenting with severe disease & the absence of valuable diagnostic markers for these conditions. Consequently, it is essential to identify potential biomarkers for UC **[3]**.

ncRNAs, containing long noncoding RNA (lncRNA), microRNA (miRNA), transfer RNA, circular RNA (circRNA), ribosomal RNA, & small nucleolar RNA, exert an influence on every phase of gene expression, commencing with mRNA translation and extending through transcription and mRNA stability **[4]**. Consistently, prior research has revealed the crucial functions that ncRNAs play in the pathogenesis of diseases **[5]**.

Inflammation in human diseases has been traced back to dysregulated expression or dysfunction of ncRNAs. Particularly crucial for the regulation of gene expression in cells are three types of ncRNAs: lncRNAs, miRNAs, & circRNAs **[6]**.

Correspondence to: Dr. Ahmed E.Taha,
E-Mail: a.tahaa85@cu.edu.eg

As of now, a growing body of research has indicated that ncRNAs have the potential to function in novel biomarkers for disease [7]. Long non-coding RNAs (lncRNAs), classified as ncRNAs exceeding 200 nucleotides in length, possess the capability to regulate gene expression via transcriptional control, post-transcriptional control, chromatin alteration, & genomic imprinting [8].

Consequently, lncRNAs have the potential to serve as diagnostic biomarkers for a variety of diseases; however, their function & mechanism necessitate additional research. According to a prior investigation, inflamed CD and UC showed differential expressions of 745 and 438 long non-coding RNAs, respectively, in comparison to healthy people [9].

Based on the available information, KIF9-AS1, LINC01272, and DIO3OS have yet to be investigated. Recent investigations have centered on non-coding RNAs (ncRNAs), specifically long non-coding RNAs (lncRNAs), which are primarily transcribed by RNA polymerase (Pol) II/Pol I, with a subset also transcribed by RNA Pol III [3].

Long non-coding RNAs (lncRNAs) have the capacity to modulate the expression of genes that code for proteins both during and after transcription, and they may also influence physiological processes [10,11].

Furthermore, long non-coding RNAs (lncRNAs) play pivotal roles in the control of gene expression and are involved in apoptosis, cell differentiation, and cell cycle progression. In inflammation, however, the precise functions of lncRNA remain unclear. Further investigation is necessary for understanding the lncRNA regulatory network in its entirety, as well as to identify the biological and molecular mechanisms that underlie the effects of lncRNAs [12,13,14].

Patients and Methods

This case control study involved68 subjects among them, 17 apparently healthy sex as well as age matched volunteers who were coming for routine checkups were included in the study as a control group. The study was performed at the Gastrointestinal endoscopy & Liver unit in Kasr Al-Einy (GIELUKA), Faculty of Medicine, Cairo University at the period from January 2021 to August 2023. The laboratory work-up and analysis of data were performed at the Clinical Pathology Department, Faculty of Medicine, Cairo University. Informed consent was taken from all cases who participated in our research.

Patients aged greater than 18 years of both genders with UC confirmed by histopathology of colonic biopsies taken during colonoscopy procedures were involved in the study. All degrees of disease activity and severity were included.

Patients younger than eighteen years, pregnant females, cases with other autoimmune diseases, patients with neoplastic disorder and patients receiving chemotherapy or immunosuppressive therapy were excluded from the research.

After obtaining Institutional Review Board approval of the Cairo University Hospital with a code of MS-166-2022, the study group were distributed into four groups: Group (1): 17 volunteers as a control group Group (2): 17 patients with irritable bowel syndrome, diagnosed by recurrent abdominal pain on at least 1 day/week in the last 3 months associated with 2 or more of the following: Change in stool frequency, related to defecation, change in the form of stool (according to Rome IV classification) and with normal level of fecal calprotectin. Group (3): 17 patients with ulcerative colitis confirmed by mucosal affection in colonoscopic assessment and histopathology of colonic biopsy and in active condition confirmed by elevated CRP and/or symptoms such as fever and diarrhea and with a Mayo score >3. Group (4): 17 ulcerative colitis patients in remission indicated by normal CRP and relief of symptoms such as fever or diarrhea for more than 8 weeks and confirmed by mucosal healing in colonoscopic assessment and histopathology of colonic biopsy and with a Mayo score < /= 3.

Activity of UC was calculated according to Mayo score. The Mayo Score evaluates the severity of UC using both endoscopic and clinical factors. This score has been employed several clinical trials and practices since it was first suggested by Schroeder et al., for use in a study of 5-ASA medicines for UC. Stool frequency, rectal bleeding, abnormalities on flexible proctosigmoidoscopy or colonoscopy, as well as doctor global evaluation all contribute to a patient's overall Mayo Score, which can range from 0 to 12 [15].

Mayo score less than 3 indicates clinical remission. Normal mucosa or inactive UC receives a score of 0 on the endoscopic portion of the Mayo Score, whereas mild disease characterized by mild friability, diminished vascular pattern, as well as mucosal erythema receives a score of 1. A score of 2 shows a mild disease with mild degree of friability and erosions but no ulceration or spontaneous bleeding; a score of 3 implies ulceration as well as spontaneous bleeding [16].

All the participants were subjected to comprehensive history taking concerning age, residency, occupation, history of pregnancy as well as lactation, special habits, family history of UC, associated medical or surgical conditions, history of drugin take, presence of diarrhea, stool frequency, blood in stools, abdominal pain, weight loss, fever, bleeding per rectum, urgency, extra intestinal manifestations e.g. ophthalmological symptoms, rheumatological symptoms and dermatological symptoms. Complete general and abdominal examination were

done. Complete blood picture, C-reactive protein (CRP), Fecal calprotectin testing.

Colonoscopy was done for patients having UC at the Gastrointestinal Endoscopy and Liver Unit Kasr Al-Ainy University (GIELUKA) Cairo University. Based on the Montreal classification of IBD, ulcerative proctitis (E1), left-sided UC (E2), as well as severe UC (E3) were determined by the extent of colitis during the initial colonoscopy **[17].**

Colonoscopic biopsies were taken from ulcerative colitis patients & sent for histopathology to confirm the diagnosis.

Determination of serum levels of KIF9-AS1 lncRNA was done as follows: Four ml of blood was collected on EDTA vacutainer and the mononuclear layer was obtained by Ficoll separation. Detection and quantification of KIF9-AS1 lncRNA level were done through the extraction of RNAs, followed by reverse transcription of all RNA using a reverse transcription kit (Thermo Scientific Revert Aid First Strand cDNA Synthesis Kit, cat no. K1622) to generate specific cDNAs, Quantification of cDNAs by quantitative real-time PCR (RT-PCR) using the corresponding primers and probe (Thermofisher cat no. 4370048).

In this study, KIF9-AS1 lncRNA was detected by TaqMan® Gene Expression Assay (Applied Biosystems, cat no. 4448892). GAPDH was used as the control for normalization (Applied Biosystems, cat no. 4453320).

Statistical methods:

The statistical package for the Social Sciences (SPSS) version 28 (IBM Corp., Armonk, NY, USA) was utilized to code and input the information. For quantitative variables that followed a normal distribution, the data were summarized via the mean and standard deviation; for non-normally distributed variables, the median and interquartile range; and for categorical variables, the frequencies (number of cases) and relative frequencies (percentages). Analysis of variance (ANOVA) was employed to compare groups for normally distributed quantitative variables using the multiple comparisons post hoc test. In the case of non-normally distributed quantitative variables, the non-parametric Kruskal-Wallis test and Mann-Whitney test were utilized. To compare categorical data, the chi-squared test ($\chi 2$) was applied. Instead, exact tests are utilized when the expected frequency is below five. To establish relationships between quantitative variables, the Spearman correlation coefficient was utilized. A ROC curve was generated through area under curve analysis to determine the optimal cutoff values for fecal calprotectin, CRP, and WBC in order to detect active UC. *p*-values that were below 0.05 were deemed to indicate statistical significance.

Results

In the current study, we have compared demographics and characteristics of the studied groups and have observed that the control and IBS groups had significantly higher ages than ulcerative colitis groups ($p<0.001$), while no statistical difference has been found when we compared the four groups regarding gender, marital status, occupation, smoking habits and type 2 diabetes as shown in (Table 1).

Patients who had hypertension have shown a weak significant difference ($p=0.048$) in control and IBS group in comparison to UC groups as shown in (Table 1).

Regarding symptoms, abdominal pain was found in 12 patients (70.6%) in IBS group, 9 cases (52.9%) in active UC, 3 cases (17.6%) in remission UC groups and 3 subjects (17.6%) in normal group. Fever was observed more in active UC (9 cases, 52.9%), diarrhea was observed more in all cases of active UC (100%) and in IBS group in 9 cases (52.9%), while it was less in remission UC (found in 5 cases (29.4%) and least in normal subjects found in 2 cases (11.8%). Rectal bleeding was mostly observed in active UC in 14 cases (82.4%). To justify, diarrhea and BPR have shown a statistically high significance in active UC group more than the other groups ($p<0.001$) as shown in (Table 1). Fever has shown a weak significant difference in active group than the other groups as shown in (Table 1).

Laboratory tests that were done to our four groups have shown that WBCs were significantly higher in ulcerative colitis groups than normal and IBS groups ($p<0.001$), that it may be considered a non-specific diagnostic factor for ulcerative colitis disease (As illustrated in Table 1).

Severity of ulcerative colitis between active state and remission state was assessed by Mayo score and has shown a higher significant value in active ulcerative colitis group ($p<0.05$) as follows: Mild cases were observed in 5 cases (29.4%) in active UC, 17 cases (100%) in UC in remission state. Moderate cases were observed in 7 cases (41.2%) in active UC, no cases in remission, severe cases were 5 cases (29.4%) in active UC & no cases in remission UC state ($p<0.01$) as in (Table 2).

Ulcerative colitis in remission group had more duration than active ulcerative colitis group with statistically significant difference ($p<0.001$) as shown in (Table 3).

The median Mayo score in active colitis group was 8 while it was zero score in remission UC group, the score in first quartile was 6 in active UC group with zero score in remission UC group also, in the third quartile it was 9 in active UC group and zero in remission UC group with highly significant difference ($p<0.001$) as shown in (Table 3).

Table (1): Comparison between four groups of study subjects regarding demographic data, chronic illness and laboratory values.

	Group (1) Normal		Group (2) IBS		Group (3) UC/Active		Group (4) UC/remission		*p*-value
	No.	%	No.	%	No.	%	No.	%	
Age (years)	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD	*p*
	49.53	8.12	52.71	12.17	30.24	8.71	35.06	10.38	<0.001
Sex:									
Female	9	52.9	9	52.9	8	47.1	8	47.1	1
Male	8	47.1	8	47.1	9	52.9	9	52.9	
Marital status:									
Single	5	29.4	5	29.4	8	47.1	7	41.2	0.635
Married	12	70.6	12	70.6	9	52.9	10	58.8	
Occupation:									
Yes	12	70.6	12	70.6	8	47.1	14	82.4	0.164
No	5	29.4	5	29.4	9	52.9	3	17.6	
HTN:									
Yes	5	29.4	8	47.1	1	5.9	7	41.2	0.048*
No	12	70.6	9	52.9	16	94.1	10	58.8	
Diabetes mellitus:									
Yes	3	17.6	6	35.3	3	17.6	5	29.4	0.633
No	14	82.4	11	64.7	14	82.4	12	70.6	
Smoking:									
Yes	6	35.3	9	52.9	7	41.2	9	52.9	0.659
No	11	64.7	8	47.1	10	58.8	8	47.1	
Abdominal Pain:									
Yes	3	17.6	12	70.6	9	52.9	3	17.6	0.002
No	14	82.4	5	29.4	8	47.1	14	82.4	
Fever:									
Yes	0	0	5	29.4	9	52.9	2	11.8	0.001
No	17	100	12	70.6	8	47.1	15	88.2	
Diarrhea:									
Yes	2	11.8	9	52.9	17	100	5	29.4	<0.001*
No	15	88.2	8	47.1	0	0.0	12	70.6	
BPR:									
Yes	0	0	1	5.9	14	82.4	2	11.8	<0.001*
No	17	100	16	94.1	3	17.6	15	88.2	
Hb: (g/dL)	12.79	1.05	12.42	1.34	11.56	1.84	12.77	1.60	0.063
WBC: (x1000)	5.26	1.08	5.76	1.16	8.98	2.27	7.75	2.36	<0.001*
PLT: $x10^9$/L	314.76	55.68	299.35	44.86	295.41	88.48	295.47	68.02	0.806

p>0.05 = Non-significant.
**p*<0.001: Highly significant.
IBS: Inflammatory bowel syndrome.
UC : Ulcerative colitis.
Hb : Hemoglobin concentration.
WBC: White blood cells.
PLT : Platelet count.

Table (2): Severity between the ulcerative colitis groups according to Mayo score.

	Active UC		UC in remission		*p*-value
	Count	%	Count	%	
Severity:					
Mild	5	29.4	17	100	<0.01
Moderate	7	41.2	0	0.0	
Severe	5	29.4	0	0.0	

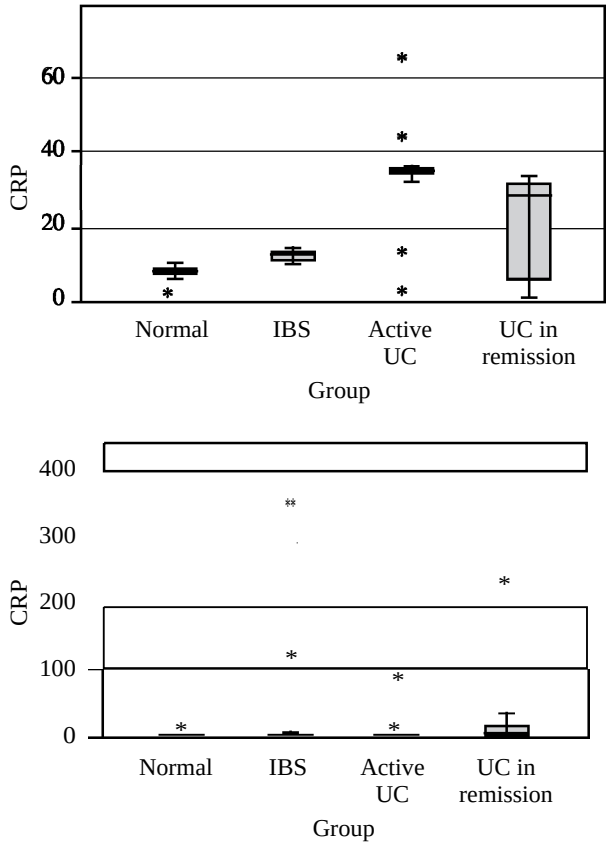
Table (3): Comparison between duration and Mayo score in ulcerative colitis patients.

	Active UC	UC in remission	*p*-value
Duration (months):			
Median	7.00	18.0	<0.001*
1st quartile	4.00	12.0	
3rd quartile	11.0	24.0	
Mayo score:			
Median	8.00	0.00	<0.001*
1st quartile	6.00	0.00	
3rd quartile	9.00	0.00	

Regarding UC in remission group, 13 patients (76.5%) were treated using medical treatment and immune suppression, while only 4 patients (23.5%) were treated using biological treatment.

When we compared the studied groups regarding CRP and FC, ulcerative colitis groups have shown a higher significant difference than normal and IBS groups (p<0.001), in comparison between both groups, IBS values were higher than control, but it didn't reach significance. On the other hand, KIF9-AS1 has shown insignificant difference between the four groups (p>0.05) (as illustrated in Fig. 1).

CRP & FC were found to have a cut-off value calculated using area under ROC curve to give a value of 33.8 with sensitivity 76.5% and specificity 100% and a value of 303 with sensitivity 64.7% & specificity 100%, respectively, While WBCs showed no cut-off value during the study. This comparison has been done between UC patients in active state and in remission one as shown in (Fig. 2).



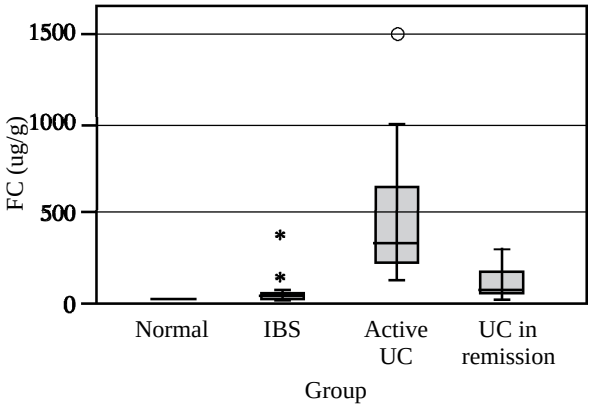


Fig. (1): Comparison of CRP, Fecal Calprotectin (ug/g) and KIF9-AS1 in the four studied groups.

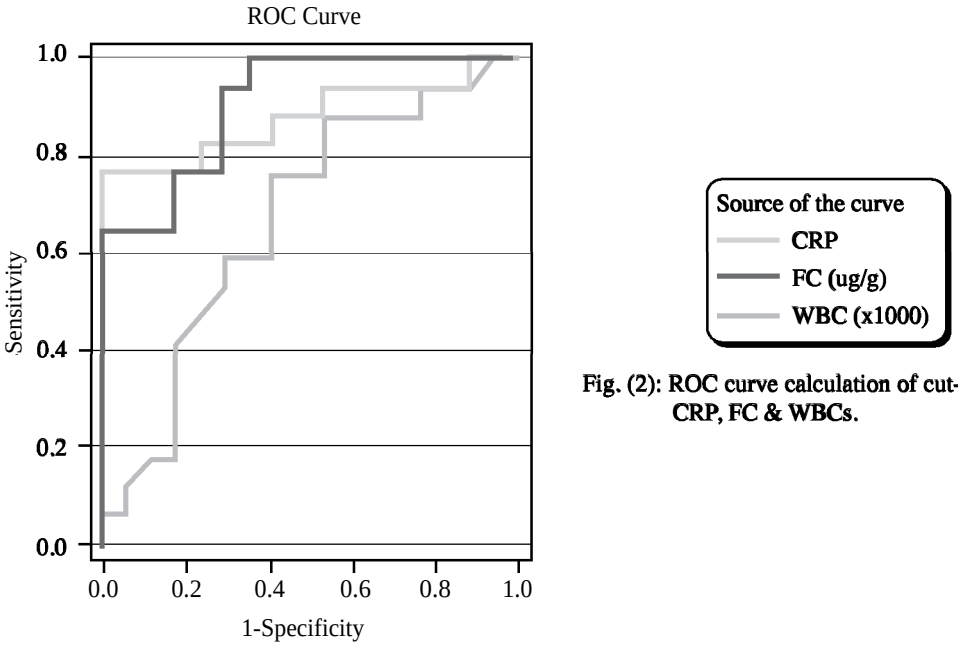


Fig. (2): ROC curve calculation of cut-off value of CRP, FC & WBCs.

Regarding KIF9-AS1, there was no relation between different studied parameters (demographics, symptoms and severity) and KIF9-AS1 either in cases with active ulcerative colitis and cases with UC in remission (p>0.05). (As in Tables 6,7,8).

Also As illustrated in Table (5) there was no relation between age, laboratory findings, duration and Mayo score, and KIF9-AS1 either in cases with active ulcerative colitis and those with UC in remission (p>0.05).

Table (4): Comparison of KIF9-AS1 between different grades of Mayo score in ulcerative colitis patients.

KIF9-AS1	Active UC (17)			UC in remission (17)			p-value
	Median	Minimum	Maximum	Median	Minimum	Maximum	
Mayo score:							
Mild	0.56	0.35	0.79	2.91	0.14	30.52	0.858
Moderate	0.66	0.12	4.09				
Severe	0.33	0.32	2.59				

Table (5): Correlation coefficient between different studied parameters and KIF9-AS1 in active ulcerative colitis patients and UC patients in remission.

KIF9-AS1	Active UC		UC in remission		Active & remisson UC	
	r	p	r	p	r	p
Age	-0.174	0.505	0.044	0.866	0.054	0.763
HB (g/dL)	-0.330	0.196	-0.205	0.430	-0.074	0.678
WBC (x1000)	0.304	0.235	-0.145	0.580	-0.115	0.517
PLT (x109/L)	-0.125	0.632	0.179	0.491	0.079	0.657
CRP	0.372	0.142	0.262	0.309	-0.048	0.786
FC (ug/g)	0.021	0.937	-0.044	0.866	-0.253	0.149
Duration (months)	0.068	0.794	0.037	0.888	0.218	0.216
Mayo score	0.198	0.447	0.177	0.496	-0.206	0.0242

r: Correlation coefficient.

Table(6): Relation between different studied parameters and KIF9-AS1 in patients with active ulcerative colitis.

KIF9-AS1 Active colitis	Median	1st quartile	3rd quartile	p-value
Sex:				
Female	*0.50*	*0.33*	*2.59*	*0.815*
Male	*0.64*	*0.44*	*0.74*	
Occupation:				
Yes	*0.48*	*0.33*	*1.19*	*0.370*
No	*0.66*	*0.53*	*0.79*	
Hypertension:				
Yes	*0.32*	*0.32*	*0.32*	*0.235*
No	*0.64*	*0.41*	*1.23*	
Diabetes mellitus:				
Yes	*0.33*	*0.32*	*97.80*	*0.676*
No	*0.64*	*0.46*	*0.79*	
Smoking:				
Yes	*0.35*	*0.32*	*1.67*	*0.315*
No	*0.69*	*0.53*	*0.79*	
Fever:				
Yes	*0.71*	*0.33*	*1.67*	*0.673*
No	*0.51*	*0.41*	*0.73*	
Diarrhea:				
Yes	*0.63*	*0.35*	*0.79*	
BPR:				
Yes	*0.59*	*0.33*	*1.67*	*1.000*
No	*0.63*	*0.46*	*0.79*	
Severity:				
Mild	*0.63*	*0.50*	*0.79*	*0.714*
Moderate	*0.66*	*0.46*	*0.76*	
Severe	*0.33*	*0.32*	*1.67*	

- Active UC patients: Patients that are recently discovered to have UC and haven't started treatment yet.

Table (7): Relation between different studied parameters and KIF9-AS1 in patients with ulcerative colitis in remission.

KIF9-AS1 Ulcerative colitis in remission	Median	1st quartile	3rd quartile	p-value
Sex:				
Female	*1.02*	*0.43*	*4.83*	*0.370*
Male	*12.05*	*0.58*	*24.33*	
Occupation:				
Yes	*2.78*	*0.40*	*16.58*	*0.091*
No	*30.52*	*1.02*	*231.00*	
Hypertension:				
Yes	*4.80*	*0.46*	*30.52*	*0.417*
No	*2.79*	*0.40*	*16.58*	
Diabetes mellitus:				
Yes	*4.80*	*1.02*	*17.53*	*0.646*
No	*2.79*	*0.41*	*19.70*	
Smoking:				
Yes	*4.83*	*1.02*	*22.81*	*0.139*
No	*0.58*	*0.35*	*12.05*	
Fever:				
Yes	*115.57*	*0.14*	*231.00*	*1.000*
No	*4.80*	*0.43*	*17.53*	
Diarrhea:				
Yes	*4.80*	*0.43*	*17.53*	*0.959*
No	*2.93*	*0.43*	*19.70*	
BPR:				
Yes	*0.58*	*0.14*	*1.02*	*0.235*
No	*4.83*	*0.43*	*22.81*	
Treatment:				
B	*0.76*	*0.43*	*7.53*	*0.541*
M	*10.69*	*0.69*	*24.33*	

Table (8): Relation between different studied parameters and KIF9-AS1 in all patients with ulcerative colitis.

KIF9-AS1 Ulcerative colitis	Median	1st quartile	3rd quartile	*p*-value
Sex:				
Female	0.76	0.35	4.80	0.695
Male	0.74	0.47	12.05	
Occupation:				
Yes	0.61	0.35	7.53	0.534
No	0.78	0.58	3.34	
Hypertension:				
Yes	2.91	0.41	24.03	0.347
No	0.69	0.40	4.09	
Diabetes mellitus:				
Yes	2.91	0.34	57.67	0.460
No	0.69	0.43	4.09	
Smoking:				
Yes	1.35	0.39	20.17	0.422
No	0.69	0.40	2.59	
Fever:				
Yes	0.71	0.32	2.59	0.383
No	0.76	0.43	16.58	
Diarrhea:				
Yes	0.64	0.35	2.59	0.231
No	2.93	0.43	19.70	
BPR:				
Yes	0.59	0.33	1.35	0.051
No	2.79	0.46	17.53	
Severity:				
Mild	0.91	0.43	17.53	0.163
Moderate	0.66	0.46	0.76	
Severe	0.33	0.32	1.67	
Treatment:				
Biological	0.63	0.35	0.79	0.085
Medical	4.80	0.43	17.53	

Discussion

Ulcerative colitis (UC) is a prominent phenotype of inflammatory bowel disease (IBD) characterized by persistent inflammation of the colon and rectum. Although UC is prevalent, its etiology remains inadequately understood. The pathogenesis of UC is a multifaceted process involving a combination of environmental, intestinal microbiome, nutritional, and genetic factors **[18]**.

While genetic factors may contribute to a certain extent, they can only account for a relatively minor proportion (7.5-22%) of the risk associated with UC **[19]**. Genome-wide association studies (GWAS) identified a number of non-coding regions of the genome associated with IBD risk **[20]**. Regarding IBD, neither the role of LncRNAs nor their contribution to the disease's progression have been fully investigated **[21,22]**.

RNAs longer than two hundred bases are referred to as LncRNAs, and they are inadequately conserved **[23]**. Their functions in regulating gene expression remain poorly understood **[24]**. It occurs on 98% of them, while polyadenylation is not ensured in all cases. In roughly 25% of all identified long non-coding RNAs, at least two distinct alternative spliced isoforms have been identified **[25]**.

Our study has aimed to assess the role of KIF9-AS1 lncRNA as a diagnostic biomarker in ulcerative colitis by comparing its level between ulcerative colitis patients in activity and in remission, IBS patients and normal subjects.

When we compared the four groups regarding gender, marital status, occupation and smoking habits, they have shown no statistically significant difference ($p>0.05$).

This has been like El-Matary et al., **[26]** who have shown that there wasn't significant variance among UC cases and controls in employment or marital statusand to the study of Russelet al., **[27]** who have shown no correlationamong smoking status & the frequency of diarrhea in UC.

Also, Elamir et al., **[28]** have demonstrated no significant variance between UC patients and controls as regarding sex ($p=0.868$).

While our study didn't agree with Bastida et al., **[29]**.

Who revealed that current smoking status protects against UC as they used different subjective parameters to assess the severity as hospital stay and steroids intake, while we used a more specific Mayo score. Also, we didn't agree with the study of Zhai et al., **[30]**.

Who have stated that, compared to nonsmokers, current smokers with UC are more probable to have a milder illness and require fewer oral corticosteroids to manage their illness.

The current study has shown that the control and IBS groups had significantly higher ages than UC groups ($p<0.001$), this differed from Elamir et al., **[28]**.

Who showed no significant difference ($p=0.106$). This may be attributed to most of the control group who visited our clinic had older ages while the ulcerative colitis patients were middle aged.

Regarding hypertension in groups (1, 2, 3, and 4), patients who had hypertension were more in control groups with a weak significant difference ($p=0.048$). Conversely, He et al., **[31]**.

Research has demonstrated that UC is correlated with an increased risk of developing hypertension in comparison to the general population. This may be attributed to older ages in our control group while the patients who have visited our clinic from active and remission UC groups were middle aged While regarding the diabetic patients in our study in groups 1, 2, 3 and 4, patients who had type 2 diabetes have shown no statistically significant distinction (p>0.05). This didn't agree with Jess et al., *[32]* who found an enhancedrisk of type 2 diabetes in cases with UC, but patients in this study used steroids for longer time than our patients and the study was a cohort study in which they followed the patients for longer periods (from 1995 until 2014). Also, their research population was restricted to individuals older than thirty years.

In our study, abdominal pain was found in (70.6%) in IBS group and (52.9%) in active UC cases and less observed in remission UC cases and normal subjects. Fever was found more in active UC (52.9%) than other studied groups, diarrhea was found more in all cases of active UC and in 52.9% of IBS group, while the least was in remission UC and normal subjects, respectively (p<0.001). Rectal bleeding was more observed in active UC group (82.4%) with statistically highly significance (p<0.001) in comparison with the other groups.

This has been similar to the study of Malik & Aurelio, *[33]* who revealed that Common presenting complaints of UC include abdominal pain, bloating, diarrhea, fever & fatigue

Our study has shown that WBCs, CRP and FC have been significantly elevated in UC groups than normal and IBS groups (p<0.001) so that they may be considered simple diagnostic factors for UC disease with cut-off value (3.8) for CRP and (303) for FC with sensitivity 64.7% & specificity of 100%, while WBCs didn't show a cut-off value. IBS values have been higher than control, but they didn't reach significance. However, heamoglobin concentration and platelets count didn't show significance in comparison between the four groups (p>0.05). UC patients values have been higher than control ones, but also they didn't reach significance.

Our study has been like Elamir et al., *[28]* who have shown a statistically significant variance among UC & control groups regarding Total leucocytic count (p<0.001) & CRP (p<0.001) while it has been different regarding haemoglobin & Platelets number with a statistically significant variance among UC & control groups (p<0.001).

Also, our study has shown similar results regarding FC as the study of Zittan et al., *[34]* who have shown that FC is the most promising non-invasive evaluation for monitoring the endoscopic activity of UC and Leighton et al., *[35]* who have shown that FC is reliable for the detection of colonic mucosal inflammation in UC.

In this study, UC in remission group has shown more duration than active UC group with greatly statistically significant distinction (p<0.001)

In the present study, when we compared the studied groups regarding KIF9-AS1, no significant distinction among the four groups was detected (p>0.05). Also, the study has shown no relation between different studied parameters (age, symptoms, disease severity, laboratory findings, duration and Mayo score) and KIF9-AS1 either in cases with active UC or in remission (p>0.05)

In contrast to our findings, Wang et al. *[36]* observed a statistically significant increase in KIF9-AS1 mRNA expression levels among cases with UC in comparison to healthy controls. 0.872 (P0.0001) was the area under the ROC curve comparing the expression of KIF9-AS1 in patients with UC to that of healthy controls. In summary, our research provides helpful insights into long non-coding RNAs, a class of molecules that may serve as biomarkers and play a role in immune cell migration, activation, & intestinal barrier function. However, further research is necessary to establish the biological significance of KIF9-AS1 expression through in vivo & in vitro experiments.

References

1- SARTOR R.B.: Genetics and environmental interactions shape the intestinal microbiome to promote inflammatory bowel disease versus mucosal homeostasis. Gastroenterology, 139 (6): 1816-1819, 2010.

2- VAN LIMBERGEN J., RADFORD-SMITH G. and SATSANGI J.: Advances in IBD genetics. Nature reviews Gastroenterology & Hepatology, 11 (6): 372, 2014.

3- WANG S., HOU Y., CHEN W., WANG J., XIE W., ZHANG X. and ZENG L.: KIF9 AS1, LINC01272 and DIO3OS lncRNAs as novel biomarkers for inflammatory bowel disease. Molecular medicine reports, 17 (2): 2195-2202, 2018.

4- XIE N. and LIU G.: ncRNA-regulated immune response and its role in inflammatory lung diseases. American Journal of Physiology-Lung Cellular and Molecular Physiology, 309 (10): L1076-L1087, 2015.

5- BAYOUMI A.S., SAYED A., BROSKOVA Z., TEOH J.P., WILSON J., et. al.: Crosstalk between long noncoding RNAs and microRNAs in health and disease. International journal of molecular sciences, 17 (3): 356, 2016.

6- HAYES E.L. and LEWIS-WAMBI J.S.: Mechanisms of endocrine resistance in breast cancer: An overview of the proposed roles of noncoding RNA. Breast Cancer Research, 17: 1-13, 2015.

7- KHALIL A.M., GUTTMAN M., HUARTE M., GARBER M., RAJ A., et al.: Many human large intergenic noncoding RNAs associate with chromatin-modifying complexes

and affect gene expression. Proceedings of the National Academy of Sciences, 106 (28): 11667-11672, 2009.

8- GUTTMAN M. and RINN J.L.: Modular regulatory principles of large non-coding RNAs. Nature, 482 (7385): 339-346, 2012.

9- MIRZA A.H., BERTHELSEN C.H., SEEMANN S.E., PAN X., FREDERIKSEN K.S., et al.: Transcriptomic landscape of lncRNAs in inflammatory bowel disease. Genome medicine, 7: 1-22, 2015.

10- BIERHOFF H., SCHMITZ K., MAASS F., YE J. and GRUMMT I.: Noncoding transcripts in sense and antisense orientation regulate the epigenetic state of ribosomal RNA genes. In Cold Spring Harbor symposia on quantitative biology (Vol. 75, pp. 357-364). Cold Spring Harbor Laboratory Press, January 2010.

11- EADES G., ZHANG Y.S., LI Q.L., XIA J.X., YAO Y. and ZHOU Q.: Long non-coding RNAs in stem cells and cancer. World journal of clinical oncology, 5 (2): 134, 2014.

12- WANG K.C. and CHANG H.Y.: Molecular mechanisms of long noncoding RNAs. Molecular cell, 43 (6): 904-914, 2011.

13- LIU X., LI D., ZHANG W., GUO M. and ZHAN Q.: Long non-coding RNA gadd7 interacts with TDP-43 and regulates Cdk6 mRNA decay. The EMBO journal, 31 (23): 4415-4427, 2012.

14- LAKHOTIA S.C.: Long non-coding RNAs coordinate cellular responses to stress. Wiley Interdisciplinary Reviews: RNA, 3 (6): 779-796, 2012.

15- SCHROEDER K.W., TREMAINE W.J. and ILSTRUP D.M.: Coated oral 5-aminosalicylic acid therapy for mildly to moderately active ulcerative colitis. New England Journal of Medicine, 317 (26): 1625-1629, 1987.

16- PAINE E.R.: Colonoscopic evaluation in ulcerative colitis. Gastroenterology report, 2 (3): 161-168, 2014.

17- SATSANGI J., SILVERBERG M.S., VERMEIRE S. and COLOMBEL J.: The Montreal classification of inflammatory bowel disease: Controversies, consensus, and implications. Gut, 55 (6): 749-753, 2006.

18- TAKU K., BRITTA S., CHEN W.S., FERRANTE M., SHEN B., BERNSTEIN C.N. and TOSHIFUMI H.: Ulcerative colitis (primer). Nature Reviews: Disease Primers, 6 (1), 2020.

19- TURPIN W., GOETHEL A., BEDRANI L. and CROITORU, MDCM K.: Determinants of IBD heritability: Genes, bugs, and more. Inflammatory bowel diseases, 24 (6): 1133-1148, 2018.

20- SARAVANARAJAN K., DOUGLAS A.R., ISMAIL M.S., OMOROGBE J., SEMENOV S., et al.: Genomic profiling of intestinal T-cell receptor repertoires in inflammatory bowel disease. Genes & Immunity, 21 (2): 109-118, 2020.

21- MOMOZAWA Y., DMITRIEVA J., THÉÂTRE E., DEFFONTAINE V., RAHMOUNI S., et al.: IBD risk loci are enriched in multigenic regulatory modules encompassing putative causative genes. Nature communications, 9 (1): 2427, 2018.

22- LIN L., ZHOU G., CHEN P., WANG Y., HAN J., et al.: Which long noncoding RNAs and circular RNAs contribute to inflammatory bowel disease?. Cell Death & Disease, 11 (6): 456, 2020.

23- JOHNSSON P., LIPOVICH L., GRANDÉR D. and MORRIS K.V.: Evolutionary conservation of long non-coding RNAs; sequence, structure, function. Biochimica et Biophysica Acta (BBA)-General Subjects, 1840 (3): 1063-1071, 2014.

24- STATELLO L., GUO C.J., CHEN L.L. and HUARTE M.: Gene regulation by long non-coding RNAs and its biological functions. Nature reviews Molecular cell biology, 22 (2): 96-118, 2021.

25- ZACHAROPOULOU E., IOAKEIM S., TZOUVALA M., KARAMANOLIS G., VEZAKIS A. and GAZOULI M.: Correlation of polymorphisms in long non-coding RNAs with the pathogenesis of inflammatory bowel diseases. Digestive and Liver Disease, 50 (6): 624-626, 2018.

26- EL-MATARY W., DUFAULT B., MOROZ S.P., SCHELLENBERG J. and BERNSTEIN C.N.: Education, employment, income, and marital status among adults diagnosed with inflammatory bowel diseases during childhood or adolescence. Clinical Gastroenterology and Hepatology, 15 (4): 518-524, 2017.

27- RUSSEL M.G., VOLOVICS A., SCHOON E.J., VAN WIJLICK E.H., LOGAN R.F., et al.: Inflammatory bowel disease: Is there any relation between smoking status and disease presentation?. Inflammatory bowel diseases, 4 (3): 182-186, 1998.

28- ELAMIR A., SHAKER O., KAMAL M., KHALEFA A., ABDELWAHED M., et al.: Expression profile of serum LncRNA THRIL and MiR-125b in inflammatory bowel disease. Plos one, 17 (10): e0275267, 2022.

29- BASTIDA G. and BELTRÁN B.: Ulcerative colitis in smokers, non-smokers and ex-smokers. World journal of gastroenterology: WJG, 17 (22): 2740, 2011.

30- ZHAI H., HUANG W., LIU A., LI Q., HAO Q., et al.: Current smoking improves ulcerative colitis patients' disease behaviour in the northwest of China. Gastroenterology Review/Przegląd Gastroenterologiczny, 12 (4): 286-290, 2017.

31- HE J., ZHANG S., QIU Y., LIU F., LIU Z., et al.: Ulcerative colitis increases risk of hypertension in a UK biobank cohort study. United European gastroenterology journal, 11 (1): 19-30, 2023.

32- JESS T., JENSEN B.W., ANDERSSON M., VILLUMSEN M. and ALLIN K.H.: Inflammatory bowel diseases increase risk of type 2 diabetes in a nationwide cohort study. Clinical Gastroenterology and Hepatology, 18 (4): 881-888, 2020.

33- MALIK T.F. and AURELIO D.M.: Extraintestinal manifestations of inflammatory bowel disease, 2021.

34- ZITTAN E., KELLY O.B., GRALNEK I.M., SILVERBERG M.S. and HILLARY STEINHART A.: Fecal cal-

protectin correlates with active colonic inflammatory bowel disease but not with small intestinal Crohn's disease activity. JGH Open, 2 (5): 201-206, 2018.

35- LEIGHTON J.A., HELPER D.J., GRALNEK I.M., DOTAN I., FERNANDEZ-URIEN I., et al.: Comparing diagnostic yield of a novel pan-enteric video capsule endoscope with ileocolonoscopy in patients with active Crohn's disease: A feasibility study. Gastrointestinal endoscopy, 85 (1): 196-205, 2017.

36- WANG S., HOU Y., CHEN W., WANG J., XIE W., ZHANG X. and ZENG L.: KIF9 AS1, LINC01272 and DIO3OS lncRNAs as novel biomarkers for inflammatory bowel disease. Molecular medicine reports, 17 (2): 2195-2202, 2018.

الدور المحتمل للحمض النووى الريبى الطويل KIF9-AS1 كمرقم حيوى فى مرض التهاب القولون التقرحى

التهاب القولون التقرحى هو شكل من أشكال الانتكاس المزمن لمرض التهاب الأمعاء ويتميز بالتهاب الغشاء المخاطى المستمر فى الطبقات الداخلية من القولون والمستقيم. صعوبة اكتشاف وتشخيص المرض فى بدايته لعدم وجود تحاليل خاصة لتشخيصه كما انفحص المنظار مكلف وله بعض المضاعفات. لذلك هناك حاجة ماسة لوجود تحاليل سهلة وخاصة لتشخيص مرض المرض.

من المعروف أن الالتهاب يؤدى إلى زيادة دلالات الالتهابات و تشير مجموعة متزايدة من الأدلة إلى أن نسبة بعض الاحماض النووية الريبية الطويلة ومنها KIF9-AS1 ترتفع مع مرض القولون التقرحى.

ولذلك، فى هذه الدراسة، هدفنا هو معرفة دورKIF9-AS1 كمرقم حيوى فى تشخيص مرض القولون التقرحى.

أجريت الدراسة على ٦٨ مريضاً انقسموا إلى اربعة مجموعات وهم (١٧ شخصا طبيعياً و١٧ شخصاً يعانون من التهاب القولون العصبى و١٧ مريضاً يعانون من التهاب القولون التقرحى النشط وقد حصلوا على درجة مايو اكثر من ٣ بالمنظار و١٧ مريضاً يعانون من التهاب القولون التقرحى الخاملو وقد حصلوا على درجة مايو٣ او اقل بالمنظار).

وجدنا أن أن مرضى القولون العصبى والقولون التقرحى النشط يعانون من الام البطن اكثر من مرضى القولون التقرحى الخامل والاشخاص الطبيعيين. لاحظنا ايضا ان معظم المرضى الذين يعانون من مرضى القولون التقرحى النشط يعانون من ارتفاع بدرجة الحرارة ودم مع البراز مقارنة بالمرضى الذين يعانون من القولون التقرحى غير النشط ومرضى القولون العصبى وأن كل مرضى القولون التقرحى النشط كانوا يعانون من الاسهال وكان ذلك اكثر من مجموعتى اضطراب القولون العصبى والقولون التقرحى الخامل.

كما وجد ان قيم كرات الدم البيضاء وبروتين سى التفاعلى (CRP) وقيم تحليل البراز Fecal calprotectin وتحليل AS1-KIF9 مرتفعة فى مجموعتى القولون التقرحى مقارنة بالمجموعتين الاخرين.

كما كانت هناك قيمة فاصلة لكل من البروتين سى التفاعلى أكثر من ٣,٨، وقيمة فاصلة ل Fecal calprotectin اكثر من ٣٠٣ تشير إلى وجود مرض نشط.

واخيرا فمن المحتمل ان يكون اداة تشخيصية جيدة لتشخيص مرض القولون التقرحى ولكن ما زلنا بحاجة لاستكمال الابحاث العلمية لمعرفة مدى تاثيره.